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Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions

Sotirios Kiokias, Charikleia Dimakou, Vassiliki Oreopoulou *

Laboratory of Food Chemistry and Technology, School of Chemical Engineering, National Technical University of Athens, Iroon Politechniou, 9, 15780 Athens, Greece

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Abstract

The present paper examines whether certain processing factors may influence the oxidative stability of whey protein oil-in-water emulsions, which are structurally close to innovative industrial products (e.g. "fresh-cheese" and "non-dairy cream" types).

We first checked whether the thermally induced whey protein denaturation might correlate with oxidative changes, in whey protein emulsions prepared with heat pre-treatment at varying temperatures (40–85 °C). The results showed that an increase of whey protein denaturation (in particular at >60 °C temperatures) was associated with a decrease of emulsion oxidative sensitivity, in terms of conjugated diene hydroperoxides production.

In model whey protein emulsions prepared at 70 °C under varying homogenisation pressures (30–600 bars), any effect of the droplet size on the oxidative changes was subsequently investigated. The measurements of conjugated dienes or TBARs did not reveal a clear trend of changes for either of these oxidative indicators with emulsion droplet size.

Keywords: Emulsion; Oxidation; Whey protein; α-Lactalbumin; β-Galactoglobulin; Denaturation; Droplet size

1. Introduction

Both microstructural and oxidative stability of protein-based food emulsions, during storage and consumer use, are highly important issues for the food industry. In many products of this category (e.g. dairy cream, fresh-cheese) milk proteins, such as caseinates or whey protein preparations, are used as emulsifiers to decrease the interfacial tension by adsorbing at the droplet surface during emulsification (Dickinson, 2001; Kiokias, Dimakou, Tsaprouni, & Oreopoulou, 2006). Whey protein concentrate is the main source of the globular whey proteins in food products, serving mainly as emulsifying, gelling and bulking ingredients, which commonly contain a mixture of β-galactoglobulin (~82%), α-lactalbumin, (~15%) and trace amounts of serum albumin, lactose and salts (Donnelly, McClements, & Decker, 1998). According to Hu, McClements, and Decker (2003), whey proteins represent a food additive that can form physically stable emulsions, while they alter the properties of the emulsion droplet interface in a manner that increases oxidative stability.

It is also well known that high temperatures induce denaturation and aggregation of whey proteins (Millqvist-Fureby, Elofsson, & Bergenstahl, 2001), enhancing their adsorption at the interface and resulting to the formation of a thin, gel-liked layer. Kiokias and Bot (2005) have recently investigated the effect of the degree of whey protein denaturation on the emulsion microstructural stability during temperature cycling. However, whether changes of whey protein functionality due to thermal denaturation may also induce oxidative changes in whey protein-stabilised emulsions, is a topic that requires further research, due to its great technological importance.

The initial droplet size distribution is another important factor, which strongly influences both the textural stability

* Corresponding author. Tel.: +30 210 7723166; fax: +30 210 7723163.
E-mail address: vasor@chemeng.ntua.gr (V. Oreopoulou).

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and sensory characteristics of protein-stabilised oil-in-water emulsions. The effect of droplet size on the oxidative sensitivity of protein-stabilised emulsions has mainly been investigated in native emulsion systems (Lethuaut, Metro, & Genot, 2002; Osborn & Akoh, 2004). However, little evidence exists for such an interfacial effect on the oxidative degradation of pre-heated whey protein emulsions, which are closer, in terms of formulation and processing, to innovative industrial products.

Therefore, as compared to most research work in this field, the current study mainly differs in the following respects: (a) it focusses on very concentrated emulsions in terms of both fat and protein phase (30% o/w, 3%, respectively) (b) it is not only based on native emulsions but also involves thermally pre-treated systems. It is the purpose of this paper to pinpoint whether certain processing factors (e.g. thermally-induced protein denaturation or droplet size changes via homogenisation pressure) might influence the oxidative stability of these types of whey protein oil-in-water emulsions, in order to identify the routes by which such undesirable effects might be further avoided.

2. Materials and methods

2.1. Materials

The tested refined sunflower oil was donated by Minerva S.A. (Inofita, Greece). The whey protein concentrates – WPC preparation (Nutrilac, QU 7560) powder was kindly provided by Unilever R&D (Vlaardingen, The Netherlands). All the used chemicals and solvents were of analytical grade.

2.2. Preparation of the emulsions by use of high-pressure homogenisation

Sunflower oil was used to prepare neutral 30% w/w oil-in-water emulsions by mixing the appropriate mass of oil for 1 min in a blender (Waring Commercial, USA), with distilled water containing 3% of whey protein concentrates. Sodium azide was added as an antimicrobial (2 g/kg aqueous phase). First, for the denaturation experiments, a series of pre-heated emulsions was prepared by thermal treatment of the whey protein solution for 15 min at various temperatures between 40 and 85 °C. The pre-emulsion was placed in an ice bath and subjected to sonication (Sonics & Materials inc., model VC 750, Newtown, USA) for 3 min. Then, it passed through a high-pressure valve of a two-stage APV Lab 1000 homogenizer (Albertslund, Denmark) at 300 bars. Samples of 15 ml of each emulsion were transferred to 20 ml cupped vials and placed in a shaking water bath to autoxidise at 35 °C. The pH of the emulsion samples had an initial value of 6.78 ± 0.14 and was measured periodically during the oxidation experiments.

In the next experiments, emulsions of the same formulation were prepared after thermal treatment of the whey protein solution for 15 min at 70 °C and subsequent homogenisation at 30, 60, 100, 300 and 600 bars, to produce emulsions with varying droplet size. Oxidation of the samples was done as previously.

2.3. Methods of oxidative analysis

2.3.1. Measurement of conjugated diene hydroperoxides (CD)

A modification of the method described by IUPAC 2.505 (Paquot & Hautfenne, 1987) has been used for the determination of conjugated diene hydroperoxides. More specifically, the emulsion sample (approximately 0.03 g) was added to a mixture of 10 ml of isooctane/2-propanol (2:1 v/v) and vortexed (1 min). The absorbance was measured at 232 nm using a UV–VIS scanning spectrophotometer (Unicam Helios, Spectronic Unicam EMEA, Cambridge, United Kingdom). A filtration through Macherey-Nagel filters (25 mm, pore 0.2 μm) was applied just before the measurement to remove protein from the sample and thereby diminish its spectrum interference in this region.

The amount of CD in the oxidizing emulsions was calculated by using the relative molecular mass (280 g mol^{-1}) and the molar absorptivity ($e = 26{,}000$) of linoleic acid.

2.3.2. Measurement of thiobarbituric acid-reactive substances (TBARs)

Thiobarbituric acid-reactive substances (TBARs) were determined according to an adapted method of McDonald and Hultin (1987). The emulsion (approximately 0.06 g) was combined with 0.7 ml of water and 2.0 ml of TBA solution (prepared by mixing 15 g trichloroacetic acid, 0.375 g thiobarbituric acid, 1.76 ml of 12 N HCl and 82.9 ml of H_2O) in test tubes and placed in a boiling water bath for 15 min. The tubes were cooled to room temperature for 10 min and then centrifuged (2000*g*) for 15 min. The absorbance was measured at 532 nm. Concentrations of TBARs were calculated from a standard curve prepared with 1,1,3,3-tetraethoxypropane.

2.4. Microstructural analysis

Restricted diffusion-based droplet size measurements were obtained by means of pfg-NMR, using a Minispec MQ20 (Bruker) at Unilever Research and Development Centre (Vlaardingen, Netherlands), according to van Duynhoven et al. (2002).

o/w emulsions were filled to a height of 15 mm in NMR tubes of 10 mm diameter, and thermally equilibrated for 30 min at 20 °C. The filled tubes were inserted into a Bruker Minispec MQ20, operating at a proton resonance frequency of 20 MHz, equipped with a 499-10AVGX-diffusion probe especially designed to have high magnetic field homogeneity of 0.4 ms (dead time of the probe: 0.02 ms, magnet temperature 40 °C). Obtained values of the volume-weighted geometric mean diameter, $d_{3,3}$, and the width σ of the droplet size distribution are converted to the surface-weighted mean diameter, $d_{3,2}$, using the relationship $d_{3,2} = d_{3,3} \cdot \exp(-\sigma^2/2)$.

Confocal scanning laser microscopy (CSLM) images were obtained using a Bio-Rad MRC 1024 equipped with a water-cooled Argon/Krypton laser (488/568/647 nm), combined with a Zeis Axiovert (inverted) microscope. The sample preparation, staining procedure, and experimental conditions have been described extensively by van Dalen (2002).

2.5. HPLC analysis for the determination of whey protein denaturation

A reverse phase HPLC technique was used to determine the degree of whey protein denaturation, based on the method described by Ferreira, Mendes, and Ferreira (2001). The method is based on the analysis of the peak areas of the native proteins, present in the aqueous phase of the emulsion after centrifugation at 18,000*g* for 1.5 h at 35 °C (Sorvall RC-5B, refrigerated Super-speed Centrifuge), and filtration through a 5 μm filter.

A Hewlett-Packard HPLC apparatus was used equipped with a gradient pump (HP 1100) and a diode array detector (Hewlett-Packard, Waldbronn, Germany). A 20 μl sample was injected into a polymeric reversed phase column (PRP-1, 250 mm × 4.1 mm; particle size 10 μm; pore size in particles 10 nm, Hamilton, Reno, Nevada, USA) thermostatted at 50 °C. A gradient elution of A: 0.1% trifluoroacetic acid in water and B: 95% acetonitrile–5% water acidified with 0.1% trifluoroacetic acid was used, as described by Ferreira et al. (2001). The flow rate was maintained at 1 ml/min. The elaboration of the chromatographic data was carried out on a ChemStation for LC 3D software (Agilent Technologies 1999–2000, Waldbronn, Germany).

Each protein was identified by means of the retention time (one peak at ~13 min for α-lactalbumin, two peaks at ~24 and 27.5 min for β-lactoglobulin) and quantified by comparing peak area to the results of a calibration series with pure native standards for α-lactalbumin (α-lb) and β-lactoglobulin (β-lg) with known concentrations in the range 1–5 mg/ml.

2.6. Statistical analysis

Oxidation experiments were carried out in triplicate and, during analysis, each measurement was repeated tree times. Results were averaged ($n = 9$) and statistically analysed with a two-way ANOVA test ($p < 0.05$) by use of the Statistica 7.0 statistical programme. Differences between oxidative indicators for the various treatments were calculated by *post-hoc* comparison of means according to Duncan's multiple range test.

3. Results and discussion

3.1. Microstructural analysis of the tested whey protein emulsions

In the current study, images of emulsions stabilised either by native or fully denatured whey proteins have been produced by use of CSLM microscopy in order to identify any microstructural differences. Therefore, as shown in Fig. 1a for a neutral whey protein emulsion prepared at 40 °C under high-pressure homogenisation (300 bars), a nice structure of generally small and well isolated spherical oil droplets can be observed. In such a system, the electrostatic repulsion between the native protein molecules at the interface prevented flocculation of the droplets, leading to a well-stabilised emulsion, which is in a liquid form, due to the small packing volume of emulsion droplets being insufficient to achieve a yield stress.

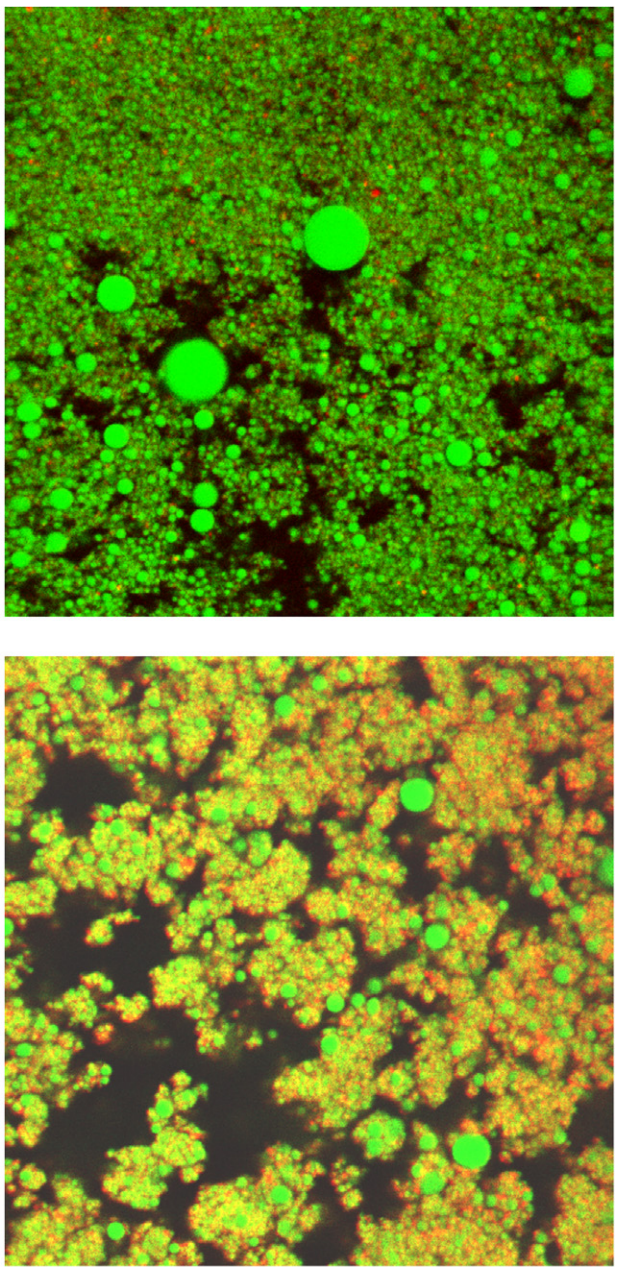

Fig. 1. CSLM picture of 30% whey protein-stabilised o/w emulsions prepared: (a) at 40 °C, and (b) at 85 °C. Fat is stained green, protein is stained red. Protein in the emulsion clearly gets more aggregated upon heating. (Image size is 65 × 65 μm). (For interpretation of the references in colour in this figure legend, the reader is referred to the web version of this article.)

An accurate determination of oil droplet size distribution is desirable in protein-stabilised emulsion gels, as it provides useful information about the quality of the initial emulsification process and the emulsion stability over time. Droplet size measurements in homogenised, heat-treated, whey protein gels are challenging because these systems differ substantially from the archetypal o/w emulsions. In such dispersed systems (pre-heated at >60 °C), a limited protein aggregation upon heating may cause a thicker adsorbed protein layer, whereas bi-molecular bonds formed between emulsion droplets, protein aggregates, aggregates and droplets finally lead to structured emulsion gels. These bi-molecular interactions can develop further during chilled storage, so that the produced emulsions generally mimic the structure of fresh-cheese or spread-type products, in particular if acidification is also involved as a next step in the preparation procedure. Such a system of the present study (thermally treated at 85 °C) can be seen in Fig. 1b.

In this experimental work, a pfg-NMR method, recently developed for application in protein-stabilised gels, has been used for the droplet size measurements. Kiokias, Reszka, and Bot (2004) reported that this specific pfg-NMR technique correlated very well ($r^2 = 0.98$) with the commonly used static light scattering method (SLS) in both native, and pre-heated sunflower oil-based, whey protein emulsions. Therefore, in the current experiments, pfg-NMR was also preferred over the SLS technique due to its rapidness and simplicity, as the analysis is direct without requiring any sample preparation.

Droplets size measurements were carried out in duplicates for neutral emulsions, stabilised with whey protein, heated at 70 °C and homogenised at various pressures. According to the results (Table 1), it is clear that an increase of homogenization pressure, in the range of 30–600 bars, led to a steady reduction of average oil droplet size ($d_{3,2}$ values between ~1.9 and 0.5 μm, respectively), with only a very little change observed at >300 bars. A similar tendency has been reported by Kiokias, Reiffers-Magnani, and Bot (2004) in pre-heated, homogenized, protein-stabilized systems of similar formulation, where the droplet size ($d_{3,2}$) decreased with homogenization pressure (p) according to the equation: $d_{3,2} = p^{-0.4}$.

3.2. Estimation of whey protein denaturation in the emulsions prepared at various temperatures

In their native form, whey proteins have a compact and rigid structure stabilised by intramolecular bonds, namely hydrophobic, hydrogen, electrostatic and disulphide bonds (Kinsella & Whitehaead, 1989). According to the literature, heating at high temperatures induces denaturation, resulting in the unfolding of the whey protein molecules and exposure of their reactive sites (Millqvist-Fureby et al., 2001). Therefore, a limited modification of whey proteins by heat-induced denaturation could lead to improvement or extension of functional properties and thus increase their food applications.

In the present work, the degree of protein denaturation was determined by quantitative HPLC analysis of the amount of native protein in the serum phase of the emulsion gel. Initially, analysis of a sample separated from a native emulsion (following 15 min heating at 40 °C when practically no denaturation occurs) revealed peaks corresponding to β-galactoglobulin (β-gl) and α-lactalbumin (α-lb), as identified by use of pure standards of each protein. No traces of other proteins (serum albumin, immunoglobulins) were identified in these whey protein concentrate preparations. According to the quantification, the native whey protein concentrate preparation consisted of a mixture of 85% β-gl and 15% α-lb. The concentration of each protein, as calculated in the emulsion heated at 40 °C, was selected as a reference to determine the degree of denaturation at each tested temperature (within the range 40–85 °C).

The degree of each whey protein (WP) denaturation at a certain temperature (X °C) was calculated according to the equation

$$\text{Degree of WP denaturation } (X\ ^\circ\text{C}) = (C_{\text{WP-40 }^\circ\text{C}} - C_{\text{WP-}X\ ^\circ\text{C}})/C_{\text{WP-40 }^\circ\text{C}}$$

where $C_{\text{WP-}X\ ^\circ\text{C}}$ is concentration of each native protein (α-lb or β-gl) in serum as a result of HPLC analysis of the emulsion sample subjected to a specific (X °C) temperature.

In this way, the degrees of denaturation were separately calculated for α-lb and β-gl. An example of the decrease in peak area for the native whey proteins with increasing heating temperature is presented in Fig. 2.

Table 1
Effect of droplet size on the oxidative deterioration of 30% sunflower oil-in-water emulsions, after thermal treatment of 3% whey protein for 15 min at 70 °C and homogenising at pressures between 30 and 600 bars

Homogenisation pressure (bars)	Droplet size (μm)	Oxidative factor $OF_{CD} = (CD_{final} - CD_{initial})/CD_{initial}$ after 14 days of oxidation at 35 °C	Oxidative factor $OF_{TBARs} = (TBARs_{final} - TBARs_{initial})/TBARs_{initial}$ after 31 days of oxidation at 35 °C
30	1.89 ± 0.03	0.62 ± 0.24	0.44 ± 0.04
60	1.53 ± 0.05	0.74 ± 0.17	0.51 ± 0.09
100	1.23 ± 0.02	0.64 ± 0.19	0.53 ± 0.37
300	0.63 ± 0.12	0.77 ± 0.17	0.40 ± 0.24
600	0.51 ± 0.07	0.94 ± 0.20	0.47 ± 0.33

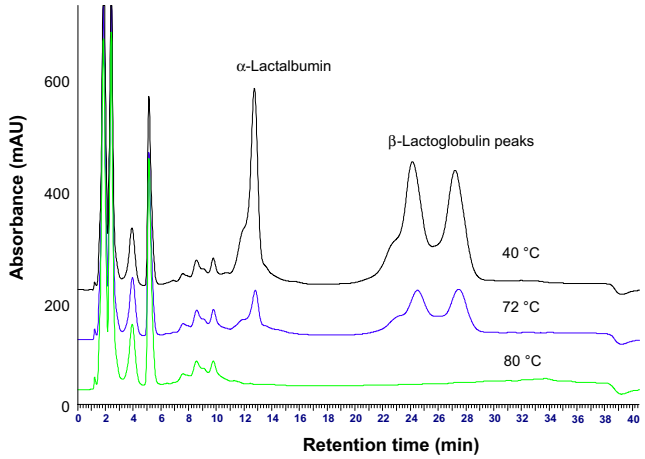

Fig. 2. HPLC chromatogram indicating the decrease of concentrations of the native whey proteins (α-lb, β-gl) in the serum as denaturation progresses with temperature increase.

Fig. 3 shows how denaturation proceeds individually for α-lb and β-gl in the serum phase upon heating in this temperature range. According to the results, very minor changes were observed until 60 °C, where most of each protein remained in its native form. Subsequently, an abrupt increase of the degree of denaturation occurs in the range 60–72 °C, consistent with literature reports indicating that whey protein denaturation takes place mainly within this temperature range (Morr & Ha, 1993; Philips, Whitehead, & Kinsella, 1994). A plateau is then reached for higher temperatures (75–90 °C) as almost all protein has been previously denatured. Interestingly, α-lb concentration decreases faster than β-gl concentration between 60 and 70 °C, in contrast to research evidence that α-lactalbumin is generally more stable against thermally-induced denaturation (Law & Leaver, 2000). Since the patterns of changes for the two proteins, were generally similar, the level of the predominant β-lactoglobulin (85% in the native whey protein sample) was adopted as a reliable measure of the total degree of whey protein denaturation for the remainder of this paper.

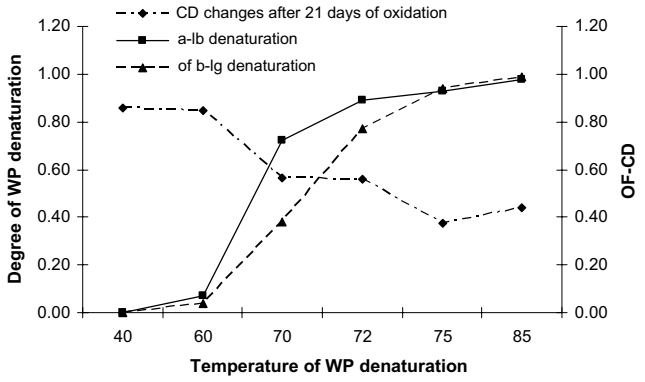

Fig. 3. Correlation between the degree of each whey protein (WP) denaturation* and oxidative factor (OF-CD)** based on conjugated dienes formation after 21 days of autoxidation of 30% sunflower o/w emulsions at 35 °C. *Degree of WP denaturation (X °C) = ($C_{\text{WP at 40 °C}} - C_{\text{WP at } X\text{ °C}}$)/$C_{\text{WP at 40 °C}}$. **$\text{OF}_{\text{CD-}X\text{ °C}} = (\text{CD}_{21\text{ days}} - \text{CD}_{\text{start}})/\text{CD}_{\text{start}}$.

3.3. Association between thermal denaturation and oxidative stability of the whey protein emulsions

A certain body of research evidence has proved that, further to their emulsifying and gelling properties, whey proteins also have a strong antioxidant character, in particular when compared with other emulsifiers (Colbert & Decker, 1991; Hu et al., 2003). Kiokias, Lampa, Tsimogiannis, and Oreopoulou (2005) observed that the native (non-heated) whey protein more effectively inhibited the lipid oxidation of oil in-water emulsions, than did sodium caseinate and Tween 20 emulsifiers.

The present work attempts to relate the associated impairment of whey protein functionality, by thermal denaturation, to the emulsion sensitivity to oxidation. As previously described, the results of HPLC analysis of serum calculate the amount of remaining native whey protein at the interface, thereby allowing the estimation of the depleted protein fraction which progressively migrates from the interface as the denaturation proceeds.

The oxidative status of each emulsion, stabilised with whey protein denatured at a certain temperature (X °C) has been expressed as an oxidative factor – $\text{OF}_{\text{CD-}(X\text{ °C})}$, based on the change of conjugated diene hydroperoxides after 21 days of oxidation over the initial concentration

$$\text{OF}_{\text{CD-}(X\text{ °C})} = (\text{CD}_{21\text{ days}} - \text{CD}_{\text{start}})/(\text{CD}_{\text{start}})$$

Obviously, higher $\text{OF}_{\text{CD-}(X\text{ °C})}$ values reflect a progress of oxidative deterioration in the emulsions. As shown in Fig. 3, an initial stable oxidation rate is followed by an abrupt decrease between 60 and 75 °C. Interestingly, as previously discussed, both α-lb and β-gl are rapidly denatured within the same temperature range. The lower $\text{OF}_{\text{CD-}(X\text{ °C})}$ values at higher temperatures of thermal treatment indicate less oxidative damage in emulsions stabilised with whey protein which is progressively denatured. The pH of the emulsions decreased slightly during oxidation from the initial value of 6.78 ± 0.14 to a final value of 6.43 ± 0.21, independent of the thermal pre-treatment. As the range of variation is small, it is expected not to affect the rate of oxidation, considering also that the measured values are far from the isoelectric point of whey protein concentrates that is between four and five, according to Surh, Ward, and McClements (2006).

According to the Fig. 4, there is a good reversible correlation ($r^2 = 0.90$) between oxidative stability and the degree of whey protein denaturation. Overall, an increase of protein denaturation (in particular from 60 to 75 °C) is associated with a decrease of emulsion oxidative sensitivity.

A partial unfolding of the whey proteins occurs upon heating and during homogenisation (Philips et al., 1994). This involves breaking of physical interactions (loss of secondary structure) so that hydrophobic parts of the molecule are exposed and they facilitate protein anchoring at the interface, enhancing its emulsifying capacity. Mixtures of native and denatured whey proteins have been found to act complementarily in emulsified systems as native whey

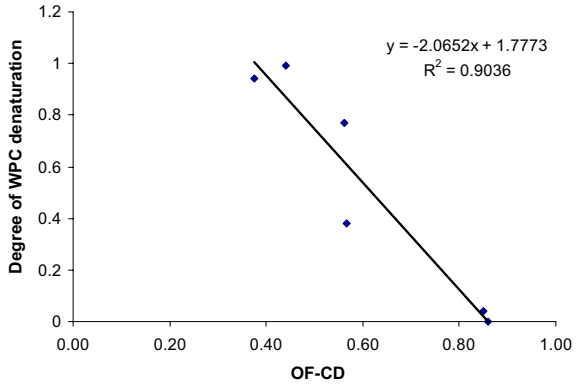

Fig. 4. Correlation between degree of WP denaturation (expressed in terms of β-gl) and oxidative factor (OF-CD) after 21 days of emulsion autoxidation at 35 °C.

proteins move rapidly to the surface, whereas the denatured particles produce a thick membrane at the interface. It should be mentioned that very recent research in emulsions of the same formulation (30% sunflower o/w stabilised by 3% WPC) revealed that the amount of whey protein associated with the fat phase roughly doubled over the temperature range 65–85 °C, (Kiokias & Bot, 2006), in qualitative agreement with relevant findings of Sliwinki, Roubos, Zoet, van Boekel, and Wouters (2003). Therefore, the formation of thicker, viscoelastic protein films at the interface, as denaturation proceeds, may offer a stronger barrier to pro-oxidant agents present in the aqueous phase, preventing them from readily approaching the lipid core of the emulsion droplets. Such an effect may account for the increased oxidative stability of emulsions prepared with denatured protein, under the present experimental conditions.

An alternative explanation can be provided by considering the molecular changes during denaturation of whey protein. Tong, Sasaki, McCements, and Decker (2000) confirmed that protein unfolding and exposure of aromatic amino acids takes place during heating of whey proteins between 60 and 90 °C, a reaction which is enhanced at increasing temperature. When the intramolecular forces between the whey protein molecules are weak (as occurs at neutral pH, under the present experimental conditions), the proteins have time to fully unfold before they aggregate. However, unfolding of whey proteins may induce an increased exposure of reduced sulfyhydryl (S–H) groups from the interior of the molecule. Darka, Mundoma, and Simoyi (1998) have reported that reduced S–H groups can act as strong free radical-scavengers. We can therefore assume that, by increasing the temperature of the heat treatment, a significantly higher proportion of S–H groups is sufficiently exposed to induce a strong antioxidant character and thereby to reduce the oxidation deterioration of the emulsion. At ~80 °C, whey proteins have essentially all their reduced S–H groups in the reactive form, so that an additional increase to 85 °C does not further enhance their antioxidant potential, as was also confirmed by Fig. 3.

3.4. Influence of emulsion droplet size on the oxidative stability of pre-heated whey protein emulsions

In the next experiments, research focusses on emulsions, which were prepared after thermal treatment at 70 °C. As shown by the previous results, in such an emulsion, a significant degree of whey protein denaturation (~40% for β-gl, ~70% for α-lb) may account for a ~30% decrease of emulsion oxidative deterioration as compared with the baseline (non-heated) emulsions.

By using such a model system, we subsequently examined whether the emulsion droplet size (an important microstructural parameter which can be monitored via changes of homogenisation pressure) may further modulate the oxidative resistance. Heated whey protein emulsions were prepared under varying pressures (30, 60, 100, 300, 600 bars) and left to autoxidise at 35 °C in a shaking water bath.

The oxidative status of the emulsions was periodically estimated by measurement of primary (CD hydroperoxides at 232 nm) and secondary (TBARs) oxidation products, after 14 and 31 days of oxidation, respectively.

The results of oxidative factors for both CD and TBARs are presented in Table 1. According to the data no clear effect of droplet size on the oxidative destabilization of the emulsions was observed, in terms of both oxidative indicators.

The literature evidence concerning the influence of oil droplet size and interfacial area on lipid oxidation of emulsions is variable and generally contradictory. Osborn and Akoh (2004) found no effect of droplets size on lipid oxidation of structured lipid-based o/w emulsions, whereas Lethuaut et al. (2002) observed higher oxidation rates when the interfacial area of emulsion was increased (smaller droplets). In a recent work, Dimakou, Kiokias, Tsaprouni, and Oreopoulou (2007) examined the effect of droplet size on oil-in-water emulsions stabilized with protein or Tween 20 emulsifiers and found that the rate of oxidation was not depended on droplet size. Taking into account literature data on activation energy, as well as their results, they discussed that oxidation is controlled by chemical reaction rather than diffusion phenomena, unless experimental conditions imply limitations in oxygen and oxy-radical availability and mobility.

4. Conclusions

The results of this work have indicated that it may be possible to engineer whey protein emulsions with greater oxidative stability by monitoring the temperature of the preparation stage, thereby inducing an "appropriate" degree of whey protein denaturation. On the other hand, no clear effect of droplet size (via varying homogenisation pressure) on the formation of hydroperoxides and TBARs oxidation products was observed under the current experimental conditions.

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