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Food Chemistry 79 (2002) 493–500

Food Chemistry

www.elsevier.com/locate/foodchem

Characteristics of oil–water emulsions stabilised by an industrial α-lactalbumin concentrate, cross-linked before and after emulsification, by a microbial transglutaminase

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Received 8 January 2002; received in revised form 25 March 2002; accepted 25 March 2002

Abstract

Industrial α-lactalbumin concentrate, cross-linked with a microbial transglutaminase, showed lower dilational surface viscosity at a planer oil–water interface than a non-cross-linked α-lactalbumin concentrate. Properties of emulsions containing cross-linked α-lactalbumin were influenced by the sequence of cross-linking and emulsification. Emulsions stabilised by α-lactalbumin concentrate (even without crosslinking) were generally unstable. While cross-linking before emulsification decreased the stability further, the emulsion stability was improved when cross-linking was carried out after emulsification. Results from the sodium dodecyl sulphate (SDS) gel electrophoresis of adsorbed protein suggested that, irrespective of the sequence of cross-linking and emulsification, the adsorbed protein was polymerised too extensively to be resolvable on the gel matrix. Results from the reverse-phase HPLC suggested that the amount of adsorbed protein in emulsions containing protein cross-linking before emulsification was lower than that containing cross-linking after emulsification.

Keywords: Transglutaminase; α-Lactalbumin; Cross-linking; Interfacial viscosity; Emulsion

1. Introduction

Transglutaminase is a transferase that catalyses an acyl transfer reaction between peptide-bound glutamines and a number of primary amines. When the ε-amino group of peptide-bound lysine acts an acceptor in the reaction, the ε- (γ-glutamyl) lysine cross-link is formed (Folk & Chung, 1985). Transglutaminase forms both intra- and inter-molecular covalent bonds between glutamyl and lysine residues of proteins. The cross-linking of proteins in this way offers opportunities for the development of novel protein ingredients with modified functional properties (Motoki, Nio, & Takinami, 1984; Motoki & Seguro, 1998). Milk proteins have shown great tendency towards cross-linking by transglutaminase (Faergemand, Otte, & Qvist, 1997; Faergemand, Murray, Dickinson, & Qvist, 1999; Nonaka et al., 1992; Sharma, Lorenzen, & Qvist, 2001). Caseins, due to their disordered and flexible structures, tend to cross-link more readily than the globular and more compact whey proteins (Ikura, Kometani, Yoshikawa, Sasaki, & Chiba, 1980; Nio, Motoki, & Takinami, 1985; Traore & Meunier, 1992). Although whey proteins have generally been suggested to be as poor substrates, cross-linking can be achieved under certain conditions. Faergemand, Otte, and Qvist (1997) found that partial unfolding by dihiothreitol (DTT) helped in achieving cross-linking of β-lactoglobulin by transglutaminase. Industrial whey protein isolates and α-lactalbumin concentrates, that may already have protein in a partially unfolded state, have been shown to cross-link without the addition of DTT (Faergemand, Otte, & Qvist, 1997; Sharma, Zakora, & Qvist, 2002). Partial unfolding of α-lactalbumin achieved by other means, such as by converting into a molten globule state, also enhanced the cross-linking by transglutaminase

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0308-8146/02/$ - see front matter
PII: S0308-8146(02)00225-X

(Matsumura, Chanyongvorakul, Kumazawa, Ohtsuka, & Mori, 1996; Sharma et al., 2002).

Partial unfolding of protein molecules also occurs when proteins are adsorbed at the oil–water interface (Dalgleish, 1997). Studies on the cross-linking milk proteins after their adsorption at the oil–water interface have been reported (Dickinson, Ritzoulis, Yamamoto & Logan, 1999; Faergemand, Murray, & Dickinson, 1997). Faergemand, Murray, and Dickinson (1997) reported that although β-lactoglobulin was unable to be cross-linked in the bulk solution without partial unfolding, it was easily cross-linked at the oil–water interface.

The adsorption of proteins at the interface and the viscoelastic properties of the interfacial layer are considered important in providing stability to emulsions (Dickinson, 1998, 1999; Walstra, 1983). Some correlations between the viscoelastic properties at the planer oil-water interface and stability of emulsions towards coalescence have been reported (Chen, Dickinson, & Iveson, 1993; Faergemand et al., 1999). Viscoselastic properties of the interfacial layer in a real emulsion are difficult to monitor directly; hence most studies focus on the measurement at a planer oil–water interface. From the limited number of studies on cross-linked proteins, it appears that cross-linking leads to an increase in the viscoelasticity at the oil-water interface. For sodium caseinate and α_{s1} and β-caseins, an increase by 100-fold in the surface shear viscosity was reported when these protein fractions were cross-linked at the oil–water interface (Faergemand et al., 1999).

In general, there is limited published information on the characteristics of emulsions stabilised by proteins cross-linked with transglutaminase. In emulsions stabilised by sodium caseinate and β-lactoglobulin, Faergemand, Otte, and Qvist (1998) found that, while extensive cross-linking reduced the emulsion stability, limited cross-linking was beneficial. In emulsions stabilised by β-casein, Liu and Srinivasan (1999) found that, although emulsifying activity index decreased, the storage stability of emulsions increased with increased polymerisation with transglutaminase. The enhanced emulsion stability was attributed to the enhanced steric stabilisation or stronger cohesive interaction between polymerised β-casein molecules. Dickinson et al. (1999), while studying the emulsion stability of sodium-caseinate-stabilised emulsions, found that cross-linking by transglutaminase prior to emulsification moderately improved the coalescence stability. When the emulsion was cross-linked after emulsification, some enhancements in the short-term stability were noticed but the long-term stability had significantly deteriorated.

In this report, characteristics of oil-water emulsions, stabilised by the industrial α-lactalbumin concentrate, cross-linked before and after emulsification, are investigated.

2. Materials and methods

2.1. Materials

Industrial α-lactalbumin concentrate (PSDI-7200) was supplied by MD Food Ingredients, Denmark. The protein powder contained 93% protein with 75 ± 5% of that being α-lactalbumin. The α-lactalbumin concentrate also contained β-lactoglobulin (10 ± 5%) and non-protein nitrogen (mainly glycomacropeptides, GMPs < 10%).

A Ca^{2+}-independent transglutaminase (Activa MP) was provided by Alsiano Ingredients, Denmark. The transglutaminase was manufactured by Ajinomoto Co., Ltd, Japan from *Streptoverticillium mobaraense* using a fermentation process. The enzyme preparation had an activity of 100 U/g (hydroxamate method), and contained 1% enzyme, with the rest being mainly maltodextrin. The enzyme was used in the original form without any further purification.

n-Tetradecane was obtained from the Sigma Chemical Co., Denmark.

All other chemicals of analytical grade were obtained from the Sigma Chemical Co., Denmark.

2.2. Protein cross-linking

The cross-linking of protein was carried out both before and after emulsification. For cross-linking before emulsification, a solution of 0.5% (w/v) protein in a 20 mM imidazole buffer (pH 7.0) was incubated with transglutaminase (10 U/g protein) for 4 h at 50 °C. The cross-linking reaction was stopped by the addition of 1% (v/v) *n*-ethylmaleimide, NEM (Sharma et al., 2002). For preparation of emulsions, *n*-tetradecane (20%, v/v) was added to the protein solution and the dispersions were homogenised at 50 °C and 16 MPa, using a lab scale jet homogeniser, as described by Burgaud, Dickinson, and Nelson (1990).

For cross-linking after emulsification, emulsions prepared using the method described above, were incubated with transglutaminase (10 U/g protein). The cross-linking reaction was carried out for 4 h at 50 °C, followed by the addition of 1% (v/v) NEM to stop the reaction.

The extent of cross-linking of protein before and after emulsification was monitored by measuring the amount of ammonia produced during the enzymatic reaction (Matsumura et al., 1996).

2.3. Determination of ammonia

The amount of ammonia, in samples cross-linked before and after emulsification, was measured using a Boehringer Mannheim ammonia kit (Boehringer Mannheim GmbH, Mannheim, Germany), following the method described by Sharma et al. (2002).

2.4. Dilational surface viscosity

The dilational surface viscosity of protein samples, before and after cross-linking, was determined using the CIR-100 Interfacial Rheometer (Camtel Ltd, Royston-Hertfordshire, UK). For the formation of the water phase, 15 ml of imidazole buffer, pH 7 was transferred into the CIR-100 measuring dish and the dish was placed in the dish holder. The Pr/Ir Du Noüy ring was slowly lowered until the circular part of the ring just touched the buffer surface. Following this, the oil phase was formed on the water phase by gently transferring 7 ml *n*-tetradecane over the buffer. The oil phase completely covered the Du Noüy ring and provided a layer of approximately 5 mm above the water phase. Protein solution (final concentration of 0.01% w/v) was then injected into the water phase using a Hamilton microlitre syringe. Dilational surface viscosity measurements at the oil–water interface were started immediately after the injection of the protein solution. Measurements were carried out over a period of 22 h at 3 Hz and 20 °C. The data points were collected at every 200 s.

Before each measurement with the protein, a blank measurement was carried out at the oil–water interface without any injection of the protein solution.

2.5. Separation of adsorbed and non-adsorbed protein

The adsorbed and non-adsorbed protein from emulsions was separated following the method described by Faergemand et al. (1998). First, the oil droplets (with adsorbed protein) were separated from the non-adsorbed protein by centrifugation at 10,000 *g* for 15 min at 25 °C. The cream phase was then redispersed in an equal volume of 20 mM imidazole buffer, pH 7.0, and the centrifugation was repeated. The aqueous phases from the centrifugations were collected and analysed for characteristics of non-adsorbed protein. The washed cream phase was redispersed in an equal volume of 4% w/w SDS and 20% w/w glycerol in 0.125 M Tri–HCl buffer, pH 6.8, and then stirred at room temperature for approximately 24 h in order to disrupt the oil droplet surface layer. The dispersion was centrifuged for 15 min at 25 °C and the serum phase, now containing the adsorbed protein, was analysed.

2.6. SDS-PAGE and reverse-phase HPLC

Protein samples, before and after cross-linking, and adsorbed and non-adsorbed protein samples, were analysed by SDS-PAGE and RP-HPLC.

The SDS-PAGE was carried out under dissociating and reducing conditions using the method described by Faergemand, Otte, and Qvist (1997). The separation of protein bands was carried out on a Phastgel Homogeneous 12.5% gel using Phastsystem™ (Pharmacia, Allerød, Denmark). The bands were quantified by analysing scanned images of the gels using Scion Image, version 4.0.1 (Scion Corporation, Maryland, USA). For RP-HPLC, the method followed was as described by Sharma et al. (aubmitted for publication).

2.7. Characterisation of emulsions

2.7.1. Droplet size determination

The droplet size determination of emulsions was carried out using a Malvern MasterSizer Micro Plus (Malvern Instruments Nordic AB, Uppsala, Sweden). Samples were added drop-wise to the small sampling unit of the MasterSizer containing water and measurements were made when the obscuration reading was from 20 to 22. The results were calculated using a Malvern presentation code 5NFD, representing an optical set up of 1.45 for the real part of the particle refractive index, 0.01 for the imaginary part of the particle refractive index and 1.33 for the refractive index of dispersant (water).

2.7.2. Backscattering

Emulsions, immediately after manufacture, were characterised by changes in backscattering profiles with time, using a Turbiscan MA2000 (Formulaction, Toulouse, France). The principle of the operation of the Turbiscan MA2000 is previously described (Mangual, Meunier, Cayre, Puech, & Snabre, 2000). The instrument contains a detection head that moves up and down along a flat-bottomed glass cylindrical cell containing the emulsion. The detection head is composed of a pulsed near infrared light source (wavelength 850 nm) and two synchronous detectors. The transmission detector receives the light that goes through the emulsion (0° from the incident beam), while the backscattering detector receives the light scattered at 135° from the incident beam. The detection head scans the entire length of the sample, acquiring transmission and backscattering data at defined time intervals.

Emulsion (5.5 ml) was carefully transferred to the flat-bottomed measuring cell of Turbiscan (height 100 mm and internal diameter 16 mm) and the cell placed in the Turbiscan. The cell containing the emulsion was scanned for 60-min taking measurements automatically at 2 min intervals. The transmission and backscattering profiles obtained at various time intervals were plotted relative to profile obtained at 0 time (delta transmission and delta backscattering, respectively) to determine the changes occurring during the total measurement time.

2.7.3. Viscosity, creaming and heat stability

Flow curves for emulsions were obtained, within 2–4 h after emulsion formation, with a Bohlin VOR controlled

strain rheometer (Bohlin Rheologi, Lund, Sweden), using C8 cup and bob geometry. Approximately 0.8 ml of emulsion was transferred to the C8 cup and the bob was gently lowered into the cup. The samples were sheared up-sweep, from 0 to 1000 s^{-1}, followed by a down-sweep shearing from 1000 to 0 s^{-1} at 20 °C. The stress data at different shear rates were converted to viscosity values and apparent viscosity at 110 s^{-1} was reported.

For measuring creaming stability, a sample of emulsion (2 ml) was transferred to a graduated centrifuge tube and the tube was centrifuged at 2000 *g* at 20 °C for 20 min. After centrifugation, the height of the cream layer was measured and the subnatant was analysed for droplet size distribution using the Malvern MasterSizer, following the method described above.

For measuring heat stability, 2 ml of emulsion were transferred into a heat-resistant glass vial and the vial was closed with a screw cap. The vial was heated in an autoclave (Heraew, W.C. Heraew Hanau, GmbH, Hanau, Germany) at 121 °C for 15 min. After cooling to room temperature, the sample was examined for visible coagulation and, if stable, was analysed for droplet size distribution using the Malvern MasterSizer, following the method described earlier.

3. Results and discussion

3.1. Dilational surface viscosity

The effect of transglutaminase cross-linking on the dilational surface viscosity at the planer oil–water interface is shown in Fig. 1. The protein, without crosslinking and after crosslinking, was introduced into the bulk phase of the planer oil–water interface to measure the dilational surface viscosity. Protein without crosslinking showed three phases in the adsorption process, as indicated by the changes in its viscosity profile. The initial lag phase (about 2 h) was followed by a phase with steep increase and subsequently by a phase of slow increase in viscosity. It is likely that the three phases for the dilational surface viscosity occur simultaneously during the measurement period (22 h).

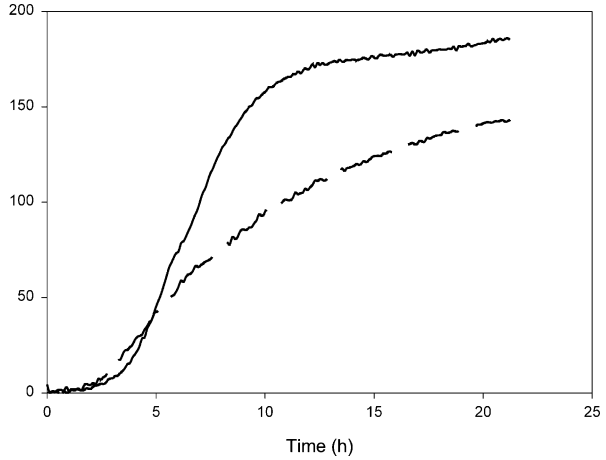

Fig. 1. Effect of cross-linking of industrial α-lactalbumin with transglutaminase on the dilational surface viscosity at the planer oil–water interface. The continuous line represents protein without crosslinking while the dashed line represents crosslinked protein. Protein (0.5% w/v) crosslinking was carried out at 50 °C for 4 h using 10 U/g enzyme/substrate.

When α-lactalbumin was cross-linked, the dilational surface viscosity showed a very gradual increase, without showing the distinction between the three phases noticed for the protein without crosslinking. The final dilational surface viscosity values (i.e. after 20 h of crosslinking) were higher for the protein without crosslinking than for the cross-linked samples. The results for dilational surface elasticity showed similar trends to that of the dilational surface viscosity (elasticity results are not shown).

For the sample without crosslinking, the initial slow or no increase in the dilational surface viscosity may suggest that, initially, the protein either migrated from the bulk phase to near the interface and/or sporadically adsorbed until it formed a cohesive film at the interface, able to display a viscosity value. Once a cohesive film was formed at the interface, there was a rapid increase in the dilational surface viscosity. This increase occurred in approximately 9 h. Once at the surface, some rearrangement, mainly realigning of the adsorbed protein at the interface, converting into favourable thermodynamic positions, as shown by the very slow increase in the dilational surface viscosity, may have occurred (Dalgleish, 1997). As reported before, transglutaminase cross-linking of α-lactalbumin causes the formation of very large aggregates (Sharma et al., 2002). It appears that the larger aggregates formed due to crosslinking are able to form a cohesive film within a short time (~1 h) as shown in Fig. 1. However, it also appears that larger aggregates, adsorbed at the oil–water interface, are fairly rigid and difficult to spread and are not able to increase the strength (i.e. dilational surface viscosity) of the interfacial film as much as the protein without crosslinking.

There is no previous study for direction comparison of the dilational surface viscosity of α-lactalbumin; however, a comparison with a previous study (Faergemand, Murray, & Dickinson, 1997) indicates that the maximum values for the dilational surface viscosity obtained for α-lactalbumin were significantly lower (150–200 μN s/m) than the values obtained for caseins and β-lactoglobulin (600–1000 mN s/m). The differences in values may be mainly attributed to the different types of proteins but also to the different conditions used in the comparative studies. The lower value for dilational surface viscosity for α-lactalbumin (with or without crosslinking) may suggest that there was a low adsorption of

the protein at the interface and/or that the strength of the film formed during emulsification was weak.

3.2. Ammonia

The amount of ammonia released during the Transglutaminase cross-linking reaction of protein is considered to be an indication of the extent of cross-linking (Matsumura et al., 1996; Sharma et al., 2001, 2002). Without cross-linking, the protein solution contained 0.34 mM ammonia (Table 1). Cross-linking of protein before emulsification produced slightly more ammonia (2.45 mM) than cross-linking after emulsification (2.26 mM). Although emulsification leads to partial unfolding and spreading of protein at the oil-water interface (Dalgleish, 1997), the results would suggest that this partial unfolding at the oil-water interface had little effect of the amount of cross-linking. Although there are no published results for a direct comparison, our results are in contrast with the results obtained for β-lactoglobulin by (Faergemand, Murray, & Dickinson, 1997) who, by using dilational surface viscosity measurements, found that β-lactoglobulin at a planer oil–water interface was more susceptible to transglutaminase cross-linking than in the bulk solution. This was attributed to the adsorption-induced exposure of glutamyl and lysine residues of β-lactoglobulin. It appears from our results that, although α-lactalbumin may have partially unfolded at the interface, the actual amount of adsorbed protein may be very small compared to the amount of un-adsorbed protein in the bulk phase. It is also likely that the protein in the commercial α-lactalbumin concentrate was already aggregated and it was difficult to unfold at the interface. Therefore, the cross-linking of the protein did not cause measurable differences for ammonia. Alternatively, it may be speculated that the adsorbed α-lactalbumin is less susceptible to transglutaminase cross-linking. This, however, does not appear to be the case, as the SDS results below do not favour this proposition.

Table 1
Amount of ammonia produced by the cross-linking of α-lactalbumin concentrate

Sample	Ammonia (mM)
Non-cross-linked protein	0.34
Cross-linked protein before emulsification	2.45
Cross-linked protein after emulsification	2.26

Protein cross-linking was carried out either before or after emulsification. Protein dispersion (0.5%, w/v) or emulsion (0.5% protein, 20% *n*-tetradecane, 160 bar at 50 °C) was cross-linked at 50 °C for 4 h using 10 U/g enzyme/substrate.

3.3. SDS-PAGE and RP-HPLC

The SDS-gel profiles of α-lactalbumin concentrate, without and with crosslinking, and unadsorbed and adsorbed protein from emulsions containing protein, with out or with crosslinking are shown in Fig. 2. The protein in emulsions was cross-linked before and after emulsification. Similar to results found previously by Sharma et al. (submitted for publication), cross-linking of α-lactalbumin in solution reduced the amount of protein resolved on the SDS gel (lane 3 vs lane 2). The adsorbed protein from the emulsion containing protein without crosslinking showed predominantly α-lactalbumin (Fig. 2, lane 5) while the adsorbed protein from cross-linked samples, irrespective of the order of cross-linking (i.e. before or after emulsification), showed very little protein as distinct bands (Fig. 2, lanes 7 and 9). It appears that the protein that is cross-linked in the bulk solution and was resolvable in the SDS gel (lane 2), was difficult to resolve once adsorbed at the oil–water interface (lane 7). These results are in general agreement with those obtained by Faergemand et al. (1998) for β-lactoglobulin.

RP-HPLC profiles of adsorbed proteins from samples containing protein, without and with crosslinking, are shown in Fig. 3. Compared with the narrow peak obtained for adsorbed protein from emulsion containing protein without crosslinking, the peaks for the cross-linked samples were broader. The broadening of the

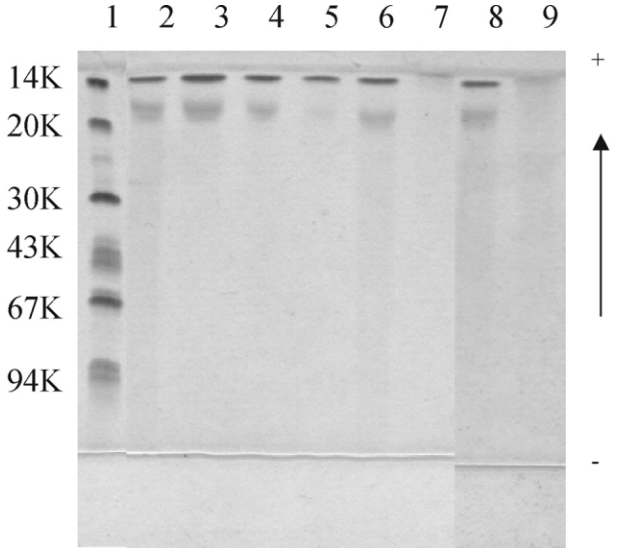

Fig. 2. SDS-PAGE patterns of unadsorbed and adsorbed α-lactalbumin concentrate cross-linked either before or after emulsification. Lane 1: molecular weight standards (from top to bottom: 14000, 20100, 30000, 43000, 67000 and 94000 Daltons, respectively); lane 2: cross-linked protein in solution; lane 3: protein without crosslinking in solution; lanes 4 and 5: unadsorbed and adsorbed protein, respectively, from emulsion containing protein without crosslinking; lanes 6 and 7: unadsorbed and adsorbed protein, respectively, from the emulsion containing protein cross-linking before emulsification; lanes 8 and 9: unadsorbed and adsorbed protein, respectively, from the emulsion containing protein cross-linking after emulsification.

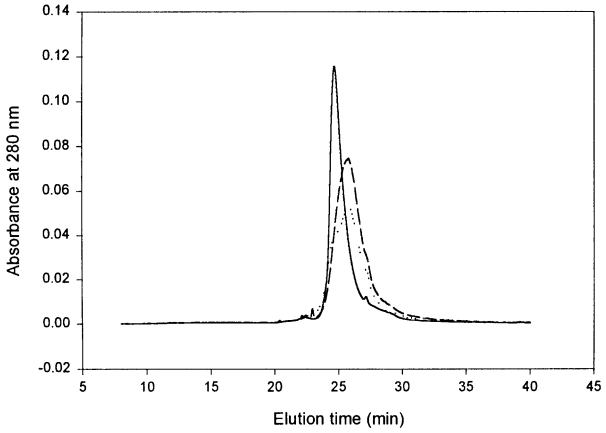

Fig. 3. RP-HPLC profiles of adsorbed protein from emulsions containing α-lactalbumin concentrate crosslinked by transglutaminase. Protein cross-linking was carried out either before or after emulsification. The continuous line represents adsorbed protein from the emulsion containing protein without crosslinking; the dotted line represents the adsorbed protein from the emulsion containing protein cross-linking before emulsification and dashed line represents the adsorbed protein from the emulsion containing protein cross-linking after emulsification.

RP-HPLC peak, after transglutaminase cross-linking, was previously noticed by Sharma et al. (2002) and attributed to the polymerisation of the cross-linked aggregates. Lower height of the peak, for the adsorbed protein from the emulsions containing cross-linking before emulsification, than that from the emulsion containing protein cross-linking after emulsification may suggest that less adsorption of protein occurred when protein was cross linked before emulsification.

By combining the results from SDS-PAGE and RP-HPLC, it can be suggested that adsorbed protein from cross-linked emulsions consisted of very large aggregates of protein that were not resolved in the SDS gel but were apparent in the RP-HPLC. Additionally, the results from RP-HPLC showed that, quantitatively, there was less adsorbed protein when the cross-linking was carried out before emulsification than when there was no cross-linking or the cross-linking was carried out after emulsification.

3.4. Emulsion characteristics

The effects of transglutaminase cross-linking on apparent viscosity, average emulsion droplet diameters, creaming and heat stability are shown in Table 2. The apparent viscosity (at 110 s^{-1}) of the emulsion containing protein without crosslinking was 1.6 mPa. The apparent viscosity of the emulsion decreased (0.8 mPas) when the crosslinking was carried out before emulsification and increased (2.6 mPas) when the crosslinking was carried out after the emulsification. The lower viscosity of the emulsion containing crosslinking before emulsification may be attributed to the reduced amount of adsorbed protein from the sample, as shown by the RP-HPLC results.

Table 2
Characteristics of emulsions prepared from α-lactalbumin concentrate cross-linked by microbial transglutaminase

Emulsion	Apparent viscosity at 110 s^{-1} (mP as)	Volume-surface average diameter, d_{32} (μm)	Cream layer (ml/2.0 ml emulsion)	Heat stability at 121 °C, 15 min
Non-cross-linked protein	1.6	0.59	0.4	Coagulated
Cross-linking before emulsification	0.8	0.54	0.4	Coagulated
Cross-linking after emulsification	2.6	0.56	0.4	Coagulated

Protein cross-linking was carried out either before or after emulsification. Protein dispersion (0.5%, w/v) or emulsion (0.5% protein, 20% *n*-tetradecane, 160 bar at 50 °C) was cross-linked at 50 °C for 4 h using 10 U/g enzyme/substrate.

The volume-surface average diameters (d_{32}) of all emulsions were similar and on centrifugation, all emulsions resulted in the same cream volumes (0.4 ml/2.0 ml) (Table 2). The subnatant layer, after creaming, was translucent, suggesting that almost all oil droplets were separated in the cream phase. Consequently, the droplet size measurements did not give any meaningful results. Upon heating at 121 °C for 15 min, all emulsions showed visible precipitation.

The above results highlight the fact that the emulsions prepared by homogenising oil into α-lactalbumin did not have strong enough steric and/or electrostatic forces to stabilise the oil droplets, thus leading to their rapid aggregation. The control emulsion (emulsion containing protein without crosslinking) itself showed poor stability, possibly due to the highly ordered secondary structure of α-lactalbumin that hinders the unfolding at the oil–water interface. Fang and Dalgleish (1998) have reported that, despite the alterations in the conformation of α-lactalbumin when adsorbed at the oil–water interface, the adsorbed protein retained a large proportion of well-organised structure. It is likely that cross-linking introduced further aggregation to already aggregated structures of α-lactalbumin. Although there are no reports for direct comparison of the emulsifying characteristics of cross-linked α-lactalbumin, the results are in general agreement with the published results for other milk proteins crosslinked with transglutaminase. For emulsions stabilised by sodium caseinate and β-lactoglobulin, Faergemand et al. (1998) found that, while limited cross-linking by transglutaminase improved emulsion stability, extensive cross-linking was detrimental. In the present study, the cross-linking, at least at the oil-water interface, was extensive, as suggested by the absence of resolvable bands in the SDS gel. Faergemand

et al. (1998) also noted that the stability of emulsions stabilised by transglutaminase, cross-linked β-lactoglobulin depended on the protein concentration. At low protein concentration (1.0%), the emulsions cross-linked after homogenisation were more unstable than the emulsions with native protein. Thus, in the present study, the low protein concentration (0.5% w/v) might be a factor for contributing the low stability of emulsions. Comparing the effects of cross-linking sodium caseinate before and after emulsification, Dickinson et al. (1999) found that the cross-linking before emulsification improved the emulsion stability while the cross-linking after emulsification led to mixed results.

3.5. Backscattering and transmission

The destabilisation of an emulsion occurs through reversible or irreversible flocculation of oil droplets. Because of droplet flocculation, changes in light scattering patterns are introduced in an emulsion. To monitor the early manifestation of the destabilisation, transmission and backscattering profiles were obtained for 1 h using the Turbiscan. To determine the creaming profiles, delta transmission and backscattering profiles at the top 4 mm of the emulsion volume in the measuring cell were obtained and plotted against time (Chanamai & McClements, 2000). Although the transmission profiles did not show any significant differences in 1 h (results not shown), changes in the backscattering profiles were discernible (Fig. 4).

In the top 4 mm of the emulsion, delta backscattering increased with time for all emulsions, suggesting that flocculation of oil droplets had occurred. The rate of increase in the delta backscattering of the emulsion containing protein cross-linked before emulsification was higher than those for the other two emulsions. This would indicate that a faster flocculation of oil droplets was occurring in this emulsion than in the other emulsions. The poor emulsion stability of the emulsion containing protein cross-linking before emulsification may be due to the smaller amount of adsorbed protein at the oil-water interface, as also shown by the results from RP-HPLC. The improved stability of the emulsion containing crosslinking after emulsification may be due to strengthening of links among the partially unfolded protein molecules at the interface (Faergemand et al., 1998)

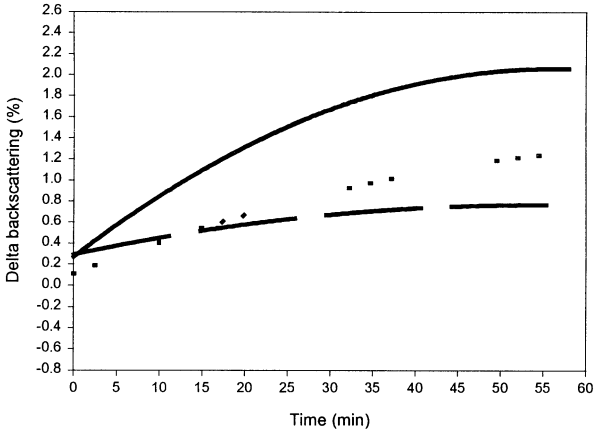

Fig. 4. Delta backscattering profiles of top 4 mm of emulsions containing α-lactalbumin concentrate cross-linked by microbial transglutaminase. Protein cross-linking was carried out either before or after emulsification. The dotted line represents the samples without protein crosslinking; the continuous line represents the sample containing protein crosslinking before emulsification and the dashed line represents the sample containing protein cross-linking after emulsification.

4. Conclusions

Cross-linking of industrial α-lactalbumin with transglutaminase leads to the formation of covalently linked large protein aggregates that are not well resolved in SDS-gels. Cross-linking of industrial α-lactalbumin in the solution reduced the dilational surface viscosity. The properties of emulsions containing cross-linked α-lactalbumin are influenced by the sequence of cross-linking. Protein cross-linking before emulsification leads to poorer emulsion stability than cross-linking after emulsification, due to a lower rate of protein adsorption and restricted ability of protein to unfold at the oil–water interface.

Acknowledgements

We wish to acknowledge the research funding by the Danish Research Council and the Centre for Advanced Food Studies (LMC).

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