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Transglutaminase Catalyzed Cross-Linking of Sodium Caseinate Improves Oxidative Stability of Flaxseed Oil Emulsion

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ABSTRACT: Sodium caseinate was modified by transglutaminase catalyzed cross-linking reaction prior to the emulsification process in order to study the effect of cross-linking on the oxidative stability of protein stabilized emulsions. The extent of the cross-linking catalyzed by different dosages of transglutaminase was investigated by following the ammonia production during the reaction and using SDS−PAGE gel. O/W emulsions prepared with the cross-linked and non-cross-linked sodium caseinates were stored for 30 days under the same conditions. Peroxide value measurement, oxygen consumption measurement, and headspace gas chromatography analysis were used to study the oxidative stability of the emulsions. The emulsion made of the cross-linked sodium caseinate showed an improved oxidative stability with reduced formation of fatty acid hydroperoxides and volatiles and a longer period of low rate oxygen consumption. The improving effect of transglutaminase catalyzed cross-linking could be most likely attributed to the enhanced physical stability of the interfacial protein layer against competitive adsorption by oil oxidation products.

KEYWORDS: *Sodium caseinate, transglutaminase, cross-linking, emulsion, oxidative stability*

INTRODUCTION

Oxidative stability is an important quality parameter in food emulsions especially in products containing unsaturated fatty acids. Lipid oxidation involves complex radical chain reactions between lipids and oxygen-active species and develops a number of adverse effects on the aroma, flavor, and nutritional value of emulsions.[1] The oxidative stability of an emulsion is dependent on the availability and interaction between reactants: lipids, oxygen, and pro- and antioxidants.[2] Some approaches have been attempted to decrease the oxidative susceptibility and/or to enhance the antioxidant properties of the continuous phase of emulsions, for example, by excluding oxygen from the system, chelating transition metals, or adding antioxidants.[3−5] In the case of a protein stabilized emulsion, the lipid oxidation may be retarded by reinforcing the interfacial protein layer between the oil and the continuous phase against the transfer of oxygen and oxidation products. It has been hypothesized that properties of the interfacial layer such as compactness, thickness, and interfacial rheology are key in influencing the rate of mass transfer to and from the dispersed phase of an emulsion.[6] Emulsion with a thicker interfacial layer of surfactant or protein has been proven to have enhanced oxidative stability.[7,8]

Supply of oxygen may become a limiting factor for the overall oxidation rate. Assuming an efficient transfer of oxygen from the gas to the aqueous phase of the emulsion and a rapid consumption of oxygen in the oil phase in the case of polyunsaturated fatty acids which result in an oxygen gradient across the interface, the diffusion across the layer controls the reaction rate. Real interfacial layers can be mono- or multilayers, but nevertheless, they are very thin. The Fickian diffusion model may not be applicable if the size of the permeant is similar to film thickness.[9] Yet Fickian diffusion has been used to model gas transfer across multilayer interfaces.[10] The system of the present study is further complicated by the viscoelastic nature of the protein layer. In a homogeneous liquid medium, translational diffusion coefficient of solubles is affected by the viscosity of the medium described by the Stokes−Einstein relationship.[11]

The other factors that may affect oxidation are the natural antioxidant activity of the interface forming proteins and electrical charge of the interface.[12,13] Proteins are able to inactivate reactive oxygen species, scavenge free radicals, and chelate transition metals with their histidine, glutamic acid, aspartic acid, and phosphorylated serine and threonine residues.[12] Further on, an electrical charge of the interfacial proteins can influence the affinity of positively charged transition metals to the oil phase. A positively charged interface was found to induce less oxidation because of the electrostatic repulsion between transition metals and the droplet surface.[13]

Transglutaminase introduces inter- or intramolecular cross-links in proteins by catalyzing the acyl transfer reaction between a γ-carboxyamide group of glutamine residues and a ε-amino group of lysine residues.[14] The effect of transglutaminase catalyzed cross-linking on the viscoelastic properties of the interface and on the physical stability of emulsions has been studied earlier,[15−17] but not much information on its effect on oxidative stability is available. Kellerby et al studied the effect of transglutaminase catalyzed cross-linking on lipid oxidation in a sodium caseinate stabilized emulsion.[2] The postemulsification cross-linking was found to take place mainly between the

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adsorbed proteins, resulting in a more cohesive interfacial film as indicated by reduced displacement of the cross-linked interfacial proteins by Tween 20. However, no improvement of oxidative stability was found during the storage for 10 days. For extremely oxygen-sensitive polyunsaturated fatty acids, post-emulsification cross-linking might not be fast enough to retard lipid oxidation, since the oxidation could have deteriorated the oil before any cross-linked interface is formed. Cross-linking before emulsification process could increase both the thickness and the cohesiveness of the interfacial protein layer. Therefore, we assumed that pre-emulsification cross-linking stands a better chance to inhibit lipid oxidation in emulsions.

The aim of this work is to study the possibility of improving the oxidative stability of protein stabilized O/W emulsions by applying a pre-emulsification cross-linking with transglutaminase and whether the possible differences in reaction rates can be linked with oxygen consumption and thus oxygen transfer across the interface. Sodium caseinate, which is known to be reactive toward transglutaminase,[18] was used as the emulsifier in this study.

MATERIALS AND METHODS

Materials. Sodium caseinate was obtained from KasLink Foods (Finland) (protein 94%, lactose 0.1%, fat 1%, ash 3.5%), and flaxseed oil was purchased from Elixi Oil Oy (Somero, Finland), where the fatty acid content was the following: 4% 16:0, 3% 18:0, 12% 18:1, 15% 18:2, and 66% 18:3. Microbial transglutaminase Activa MP (TGase) was purchased from Ajinomoto (Japan) and was further purified as described by Lantto et al.[19] The activity of the enzyme was determined by colorimetric hydroxymate method.[20]

Cross-Linking of Sodium Caseinate. The cross-linking of sodium caseinate was performed prior to emulsification. Sodium caseinate was solubilized in boiling Milli-Q water at a protein concentration of 1%, and the temperature of the solution was cooled to room temperature. The pH was adjusted to 7 using 1.0 M NaOH and 1.0 M HCl. Transglutaminase at dosage of 0, 50, 100, 500 nkat/g sodium caseinate was added after the protein was well dissolved. The samples were incubated at room temperature with mild agitation overnight, and then the transglutaminase was deactivated by heating at 90 °C for 10 min. The control sample without transglutaminase was subjected to the same thermal treatment.

Analysis of the Extent of Cross-Linking. Ammonia production was measured using a kit (R-Biopharm AG). The ammonia that was released from the transglutaminase catalyzed reactions reacted with 2-oxoglutarate to form L-glutamate in the presence of glutamate dehydrogenase (GIDH) and reduced nicotinamide dinucleotide (NADH), whereby NADH was oxidized and was determined by its light absorbance at 340 nm after exactly 20 min of reaction. Two replicates were made for each sample. The cross-linking of sodium caseinate with different dosage of transglutaminase was further studied using a 12% SDS gel (BIO-RAD). A mixture with 5 μL of SDS−PAGE loading buffer (Tris-HCl with β-mercaptoethanol and SDS), 5 μL of water, and 5 μL of sample was heated at 98 °C for 10 min and then loaded into each well.

Preparation of O/W Emulsion. The pH of each sample after the enzyme treatment was readjusted to 7 using 1.0 M NaOH and 1.0 M HCl. Then 90% w/w protein solutions were homogenized with 10% w/w flaxseed oil in two steps. A pre-emulsion was prepared using a stirring-type homogenizer (Heidolph Diax 900, Germany) under constant conditions: 2 times, 2 min at 26 000 rpm at room temperature. The main emulsification was performed by loading 110 mL of pre-emulsion into a pressure homogenizer (MicrofluidicsM-110Y, U.S.) at 0 °C and 40 psig (500 bar). The pre-emulsion was circulated in the homogenizer for 10 min, during which it passes through the chamber 30 times. Sodium azide (0.02 wt %) was added to the emulsions to prevent microbial growth. Immediately after the emulsification process, 100 mL of emulsion was transferred into a 1 L glass bottle and stored in the dark at room temperature with stirring at 300 rpm.

Particle Size Determination. Particle size distribution of the emulsions was measured by laser diffraction (Beckman Coulter LS230, CA). After 0, 5, 10, 15, 20, 25, and 30 days of storage, 1 mL of emulsion sample was transferred into an Eppendorf tube and briefly vortexed before each measurement. The measurement was conducted in an optical model with fluid refractive index 1.33 and the sample refractive index 1.46. The pH of Milli-Q water was adjusted to 7 using 0.1 M HCl and 0.1 M NaOH and used as the measuring medium. The volume-surface mean particle diameter (d32) was determined from the particle size distribution of three batches of emulsions. Two measurements of particle size distribution were conducted from each batch.

Peroxide Value Determination. Peroxide value (POV) was determined spectrophotometrically according to IDF standard 74A:1991. Emulsion (0.3 g) was added to 9.6 mL of chloroform/methanol (7:3) mixture. For color formation, 50 μL of both iron(II) chloride and ammonium thiocyanate solutions were added. The sample was briefly vortexed, reacted in the dark for exactly 5 min, and measured at a wavelength of 500 nm. The experiment was repeated three times.

Headspace Analysis. Fresh emulsion samples (2 mL) were transferred into special 6 mL headspace vials and sealed with silicone rubber Teflon caps with a crimper. The samples were stored in the dark at room temperature with stirring at 300 rpm. The products of lipid oxidation in emulsions stored for 0, 6, 12, 18, 24, and 30 days were analyzed using static headspace gas chromatography (Autosystem XL gas chromatograph equipped with an HS40XL headspace sampler, Perkin-Elmer, Shelton, CT; column NB-54, Nordion) according to the method of Frankel et al. with slight modifications.[5] The emulsions were heated at 80 °C for 30 min. The oven temperature was set to 60 °C, and the run time was 10 min. The analysis was carried out based on the results obtained from three replicates of each emulsion sample.

Oxygen Consumption Measurement. Lipid oxidation in emulsions was studied by monitoring the oxygen consumption in a sealed vial with a single-channel oxygen meter (Precision Sensing GmbH, Regensburg, Germany). The vial (1.84 mL) with a small magnet stirrer at the bottom was completely filled with emulsions after 0, 5, 10, 15, 20, 25, and 30 days of storage and capped. The stirring speed during the measurement was 100 rpm. Consumption of the oxygen was monitored at 5 min intervals for 24 h. The consumed oxygen was plotted against time, and the rate of oxygen consumption was calculated as the slope of the linear part of each plot. The unit was presented in millimole of oxygen consumed in 1 L of emulsions per day. The experiment was repeated three times.

RESULTS

Cross-Linking of Sodium Caseinate by Transglutaminase. The transglutaminase catalyzed reaction results in formation of ε-(γ-glutamyl)lysine cross-link with concomitant release of ammonia.[14] Sodium caseinate was treated with 0−500 nkat TG/g caseinate, and the amount of the produced ammonia was monitored spectrometrically (Figure 1). The extent of cross-linking reaction, as indicated by the ammonia production, increased with increasing dosage of transglutaminase and was the highest when 500 nkat/g transglutaminase was applied.

The cross-linked proteins were further studied using SDS−PAGE electrophoresis (Figure 2). At a dosage of 50 nkat/g, most of the sodium caseinate stayed as monomers and the intensity of the bands of protein polymers with M_w around 97−206 kDa increased only slightly (lane 3). At a transglutaminase dosage of 500 nkat/g, most of the sodium caseinate was cross-linked into polymers and a band of extensively cross-linked sodium caseinate was observed on the top of the lane (lane 5).

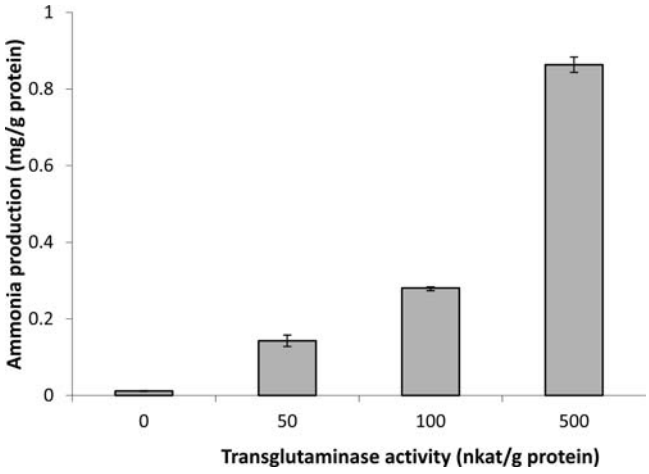

Figure 1. Ammonia produced in the cross-linking reaction between lysyl and glutamyl residues in 1% sodium caseinate solution at different transglutaminase dosages at pH 7 and 20 °C for 20 min. Each point represents the average of two replicates.

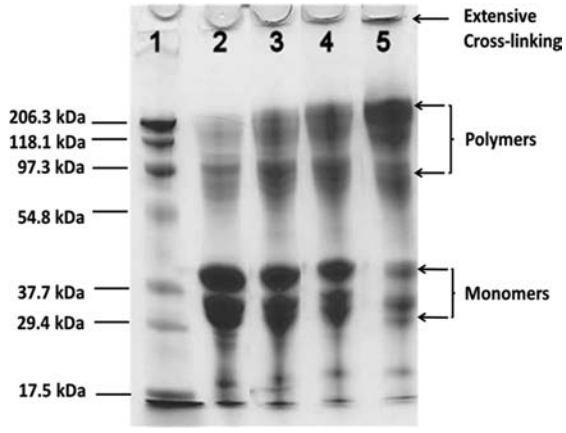

Figure 2. SDS–PAGE of the cross-linked sodium caseiante by transglutaminase. Sodium caseinate was treated with transglutaminase at an enzyme dosage of 50, 100, and 500 nkat/g at room temperature overnight: lane 1, prestained SDS–PAGE standard proteins of 206.3, 118.1, 97.3, 54.8, 37.7, 29.4, and 17.5 kDa migrated from the top to the bottom (BIO-RAD); lane 2, noncross-linked sodium caseinate; lane 3, cross-linked sodium caseinate with 50 nkat/g transglutaminase; lane 4, cross-linked sodium caseinate with 100 nkat/g transglutaminase; lane 5, cross-linked sodium caseinate with 500 nkat/g transglutaminase.

Physical Stability of Emulsions Made of Non-Cross-Linked and Cross-Linked Sodium Caseinate. Emulsions made of the non-cross-linked sodium caseinate (CN) and cross-linked sodium caseinate by 500 nkat/g transglutaminase (TGCN) were prepared under the same homogenization conditions. The lipid oxidation is known to accelerate at the droplet surface, and susbsequently the rate of lipid oxidation increases when more surface area is created with smaller droplet size.[1] Therefore, it is important to investigate the droplet size of CN and TGCN emulsions before discussing the effect of cross-linking. The volume-surface mean particle diameter (d32) of the particles in these two emulsions was followed during the storage time (Figure 3). The pre-emulsification cross-linking did not affect the emulsifying activity of sodium caseinate, since the mean droplet diameter of the fresh TGCN emulsion was the same as that of the CN emulsion (0.16 μm). The physical stability of emulsions was slightly improved by the trans-

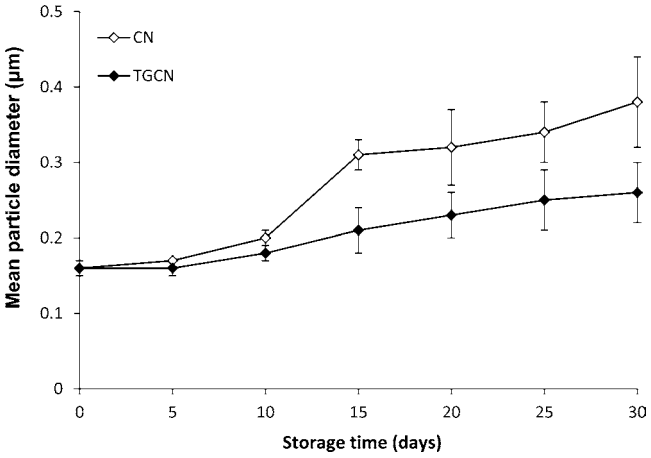

Figure 3. Physical stability of emulsions over 30 days of storage. The emulsions made of noncross-linked and cross-linked sodium caseinate were stored at room temperature in the dark for 30 days. Samples were taken every 5 days, and the volume-surface mean particle diameter (d32) was calculated from volume distribution of three batches of emulsions. Two measurements of particle size distribution were conducted from each batch.

glutaminase treatment. A significant increase of particle size was still observed in both of the emulsions during the storage. After 30 days, the mean droplet diameter increased to 0.26 μm for the TGCN emulsion and 0.38 μm for the CN emulsion because of either droplet flocculation or coalescence.

Effect of Transglutaminase Catalyzed Cross-Linking on the Oxidative Stability of Emulsions. The oxidative stability of the emulsions stabilized by the non-cross-linked sodium caseinate and the cross-linked sodium caseinate was further studied. The emulsions made of sodium caseinate cross-linked by different dosage of transglutaminase exhibited very similar stability against lipid oxidation in the peroxide value measurements (data not shown). Therefore, only the emulsion made of sodium caseinate cross-linked by the highest enzyme dosage (500 nkat/g) was compared with the emulsion with the unmodified protein.

Peroxide value is a measure commonly used to describe the quality of oil, but instead of measuring the rate of hydroperoxide formation, it is measuring the concentration of hydroperoxides in the oil phase, which depends on their decomposition rate in further steps of the reaction. The rate of peroxide decomposition may be of importance in aqueous systems, where it can be catalyzed by the metals present. The peroxide values were measured in order to study the reaction rates (Figure 4). The concentration of fatty acid hydroperoxides stayed at a relatively low level during the first 15 days of storage for both CN emulsion and TGCN emulsion, whereas the formation of peroxides in the CN emulsion accelerated after the induction phase and reached a considerably higher level (386 mequiv/kg oil) after 26 days compared to the TGCN emulsion (50 mequiv/kg oil).

Further, the volatile compounds produced during the storage were analyzed by headspace gas chromatography (Figure 5). Since the flaxseed oil used in this study contains a mixture of different unsaturated fatty acids, there was a complex mixture of secondary oxidation products formed during the lipid oxidation. For this reason, whole chromatograms are shown with two identified peaks for propanal and hexanal, respectively, and other peaks corresponding to the unidentified oxidation

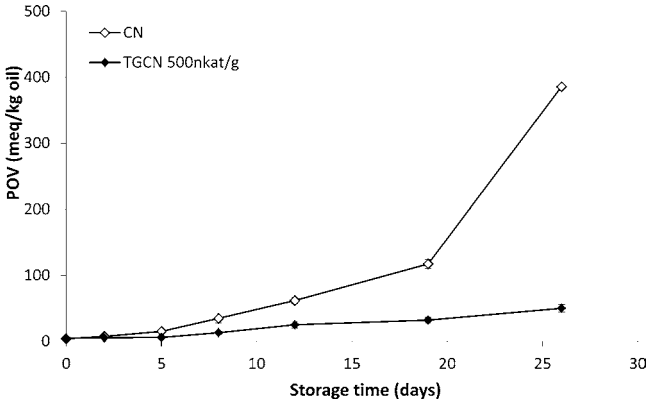

Figure 4. Oxidation measured as peroxide values of flaxseed oil emulsions as stored without light exposure at 20 °C with mixing and large airspace to ensure supply of oxygen to continuous phase.

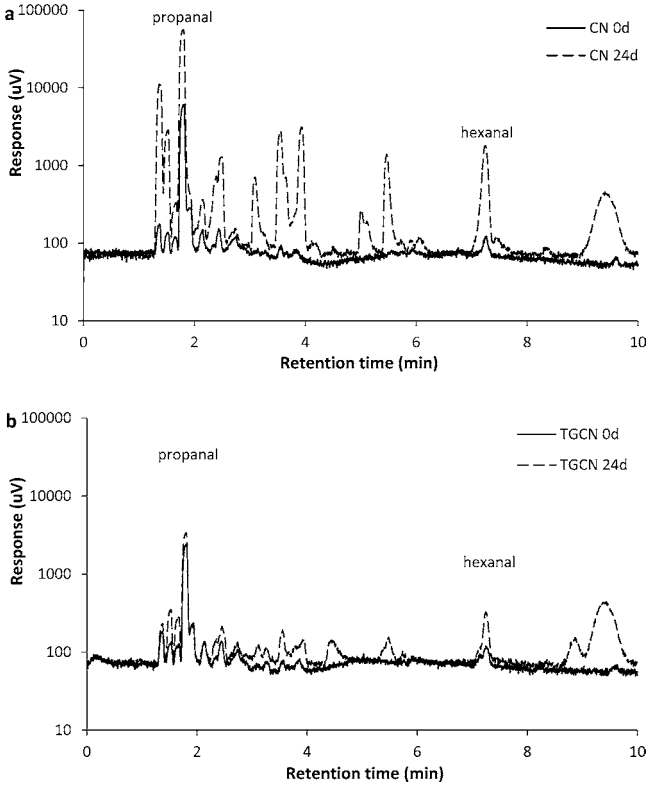

Figure 5. Headspace analysis. The secondary products of lipid oxidation were followed by head space gas chromatography. The whole chromatograms of fresh emulsions prepared with non-cross-linked and cross-linked sodium caseinate and the emulsions after 24 days of storage were presented by plotting the detector response against retention time.

products. The GC analysis was conducted at 0, 6, 12, 18, 24, and 30 days of storage. For fresh CN emulsions, only one sound peak of propanal was detected with peak area 2.8×10^4; the peak area for hexanal was nearly zero. During the following 18 days of storage, a gradual growth was found for the peak area of propanal (1.2×10^5) and hexanal (3900) and no significant change was found for the overall shape of the chromatogram (data not shown). At day 24, the peak area of propanal and hexanal sharply increased to 2.7×10^5 and 1.1×10^4 and a number of other unidentified products were detected (Figure 5a). The production of volatiles in the TGCN emulsion was effectively inhibited. The peak area of propanal and hexanal increased from 1.1×10^4 to 1.6×10^4 and from 0 to 1300, respectively, after 24 days of storage (Figure 5b). The result obtained from the emulsion samples after 30 days of storage showed the same trend: apparent increase for each peak for the CN emulsion and slight increase for the TGCN one (data not shown).

The rate of oxidation was followed by measuring the oxygen consumption in the emulsions with increasing duration of storage (Figure 6). Oxygen consumption is a direct measurement of oxygen transfer from the continuous aqueous phase to the dispersed oil phase and therefore also directly linked with the formation of fatty acid hydroperoxides in the oxygen-consuming step of the lipid oxidation reaction. The emulsions were stored under conditions where large air space and constant stirring allowed quick and sufficient oxygen supply to the aqueous phase so that the oxygen concentration in the emulsion samples for the following oxygen consumption measurement can start from the same saturated level. The oxygen consumption measurement is based on the luminescence quenching by oxygen from the aqueous phase. The measurement is performed by minimizing the air space in the measurement vial. Thus, as oxygen is transferred to the oil phase, its amount in the aqueous phase is simultaneously decreasing, which can be measured as reduced rate of luminescence quenching. The same trend as shown in the peroxide value measurement results was found indicating that the lipid oxidation in the TGCN emulsion was obviously retarded. For the CN emulsion, the lipid oxidation went through an induction phase for the first 10 days of storage. The rate of oxygen consumption increased from 0.34 to 1.01 mmol/(L·day). After this phase, the rate sharply increased to 68.7 mmol/(L·day) for the CN emulsion stored for 30 days, which means the oxygen in the oil phase was rapidly consumed by high concentration of intermediate products of oxidation. As comparison, the oxygen consumption rate in TGCN emulsion was much slower, although a slight increase was observed with time. The rate of oxygen consumption increased from 0.41 to 3.54 mmol/(L·day) after 30 days of storage.

DISCUSSION

The results of oxygen consumption, peroxide value measurement, and headspace analysis showed good correlation with each other. Under the storage conditions in this work, the lipid oxidation was probably in the initiation stage during the first 10 days, as the rate of oxygen consumption in the aqueous phase and the production of peroxides were found to be at a low level (Figures 4and 6). Then the CN emulsion went through the propagation stage of oxidation as seen from the acceleration of peroxide production and a much higher oxygen consumption rate for the next 5−10 days. Similar increase was observed in the oxygen consumption rate of TGCN emulsion after 20 days of storage. Only a small amount of volatiles was produced during this period, since most of the droplets had not reached the termination stage of oxidation yet. Therefore, no significant change in the GC chromatograms was detected in the CN and TGCN emulsions during the first 24 days (Figure 5). Many secondary products of oxidation became detectable by GC only after 24 days of storage. Meanwhile the rate of oxygen consumption and peroxide production were still increasing. This may be explained by the variation of the oxidative stage of oil droplets; some of them may have been oxidized more quickly than the others.

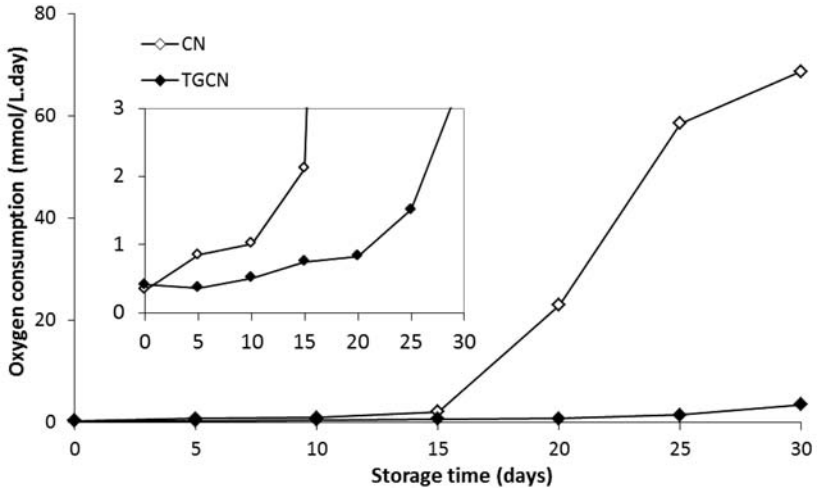

Figure 6. Oxygen consumption measurement. The oxygen consumption in emulsions stored for 0, 5, 10, 15, 20, 25, and 30 days was monitored using a single-channel oxygen meter. The consumed oxygen was plotted against time, and the rate of oxygen consumption was calculated as the slope of the linear part of each plot. The unit is presented in millimole of oxygen consumed in 1 L of emulsions per day.

Besides the above-described properties, particle size was monitored and obtained to be the same for both emulsions during the first 10 days of storage (Figure 3). The particle size increased later to less extent of the TGCN emulsion, indicating that the cross-linking had improved the physical stability of the emulsion. On the other hand smaller particles mean that TGCN emulsion had larger surface area than CN emulsion, which in turn mean larger oxygen flux and higher probability of the oxidation products to interact with the interface.[1]

The primary hypothesis of this work was that by increasing the interactions between the protein molecules that are responsible for forming the interfacial layer, diffusion of oxygen across the layer could be hindered. Overall the initiation stage of oxidation was 10 and 20 days for CN and TGCN emulsions, respectively. Thus, the oil in TGCN emulsion oxidized much more slowly than in CN emulsion. But the rate of oxygen consumption during the initiation period was roughly the same for both emulsions, 0.5−1 mmol/(L·day), considering the small variation in the oxidation rate during the initiation stage. A steady state Fickian diffusion model could be applied for the interface to help the interpretation of the obtained results. The steady state means that the oxygen flux does not depend on time, but it depends on the diffusion coefficient of oxygen within the interface, on the thickness of the layer, and on the concentration gradient across the interface. It is also necessary to assume that oxygen is consumed once it is transported into the oil phase, and thus, the oxygen concentration is zero in the oil phase. This means that oxygen transport across the interface is the rate-controlling step of oxidation.[3,21] Furthermore, as Fickian diffusion is applied, the thickness of the interface has to be considerably larger than the dimensions of the oxygen molecule, which is a reasonable assumption as casein forms multilayers of approximately 10 nm in thickness.[22] Since the particle sizes of the emulsions were almost the same for the first 10 days and later did not much differ from each other, it can be assumed that the effective surface area of the oil droplets was the same during the initiation period of oxidation. Thus, the data can be evaluated based on steady state Fickian parameters. Because the oxygen consumption rates were similar for both emulsions with the same surface area, the retarded oxidation of the oil in TGCN emulsion cannot be explained by the change in diffusion coefficient or increase in the thickness of the interface or decrease of the oxygen gradient across the interface. Apart from oxygen transfer, the cross-linked interfacial layer might inhibit the formation of lipid radicals by limiting the interaction between transition metals in the aqueous phase and the oil phase. Therefore, less hydroperoxides were formed in the TGCN emulsion, supported by the peroxide value measurement (Figure 4), and as a result, the lipid oxidation in the cross-linked emulsion was retarded. The peroxide value measurement is not measuring the rate of hydroperoxide formation but instead the difference between their formation and decomposition rates.[23] Contact with transition metals could also have a role in decomposition of hydroperoxides. This contribution was not supported by the results of the present study, as no accumulation hydroperoxides in the oil phase could be observed for the cross-linked emulsion. A possible explanation for the same oxygen fluxes but delayed oxidation in the case of TGCN emulsion could be due to the different interplay between the reaction products and the interface. The cross-linked sodium caseinate could better protect the interface against adsorption of surface-active compounds produced during the oxidation than CN emulsion and thereby could limit the transfer of these oxidation intermediates into the aqueous phase. Previous study of the displacement of polymerized interfacial protein by surfactant could indirectly provide evidence for the inhibition effect of the cross-linked proteins on the transfer of transition metals and oxidation intermediates across the interface.[24]

A comparable study reported by Kellerby et al. had shown that increasing the cohesiveness of the adsorbed sodium caseinate at the O/W interface did not improve the oxidative stability of emulsions. The transglutaminase cross-linked interfacial sodium caseinate did not influence the diffusion of small pro-oxidant molecules to the core of oil droplets.[2] With the lipids that are both very susceptible to oxidation, the major differences between these two studies were the time point where cross-linking was applied and the storage methods of emulsions. In Kellerby's study, the emulsions were stored at high temperature (55 °C) with limited air space and no agitation. Compared with the present study, where the aqueous phase of the emulsion was kept saturated with oxygen, the conditions were very different and probably responsible for the different results. Also the interfacial protein layers formed may

be structurally different as in the work by Kellerby et al.; interfacial modification was used in comparison to the bulk modification used in the present study. In Kellerby's study, the modification after the formation of emulsions was shown to occur between the adsorbed interfacial proteins rather than between the adsorbed proteins and proteins in the aqueous phase. Therefore, the cross-linking reaction resulted in an increased cohesiveness. In our study, sodium caseinate was cross-linked prior to the emulsification process. For this type of cross-linking, we have previously shown that dilatational elastic modulus of the air−water interface was little affected compared with interfacial modification in the same system.[25] When the protein polymers had adsorbed onto the droplet surface, an interfacial layer with stronger resistance against the penetration of pro-oxidants and against the adsorption of oxidation products compared to that formed with the uncross-linked proteins may form.

This study has shown the potential use of transglutaminase to improve the oxidative stability of protein stabilized emulsions. A remarkable increase in oxidative stability of flaxseed oil emulsions was found when enzymatic cross-linking of emulsifying protein was performed. The increased stability was directly evidenced with delayed propagation stage based on reduced formation of fatty acid hydroperoxides and volatiles and a longer period of low rate oxygen consumption. The exact mechanism of increased stability is yet to be clarified, but at present the most likely explanation is the improved stability of TGCN interface in the presence of oxidized oil. Cross-linking may also have changed the ratio of adsorbed versus nonadsorbed protein, which could increase the thickness of the interfacial protein layer and thus also the amount of antioxidative functional groups of protein localized at the oil−water interface. In future work, it is very interesting and necessary to find out the actual reason for the phenomena reported in this study. It would also be interesting to modify the interfacial proteins in a way that both the electrostatic and steric repulsion against pro-oxidants can be strengthened at the same time. An ideal modified protein could form a thick interfacial membrane with extensive positive charge. Making a protein conjugate with cationic polysaccharide by Maillard reaction could be one possible scenario. Besides, Ma et al. reported modification methods to modify the surface charge of milk protein emulsifiers to positive or negative values at one certain pH. This would provide a good model for studying the effect of interfacial charge on the oxidative stability of emulsions.[26,27]

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Notes

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ABBREVIATIONS USED

GC, gas chromatography; GIDH, glutamate dehydrogenase; M_w, molecular weight; NIDH, nicotinamide dinucleotide; O/W, oil in water; PAGE, polyacrylamide gel electrophoresis; pI, iso-electric point; POV, peroxide value; SDS, sodium dodecyl sulfate

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