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Research Note

Lipid Oxidation of Sodium Caseinate-stabilized Emulsion-gels Prepared Using Microbial Transglutaminase

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Abstract Development of lipid oxidation in casein-stabilized emulsion-gels prepared using microbial transglutaminase was investigated. The oxidative stability of emulsion-gels was assessed based on measurement of the amount of lipid hydroperoxide, hexanal, and 1-octen-3-ol. Profiles of lipid hydroperoxide showed that formation of lipid hydroperoxide was reduced in emulsion-gels, compared with emulsions, regardless of the storage temperature. Quantitative analysis of hexanal and 1-octen-3-ol showed no significant ($p>0.05$) difference between oxidative stabilities of emulsion-gels and emulsions. The storage temperature did not affect formation patterns of hydroperoxide, hexanal, and 1-octen-3-ol. Transglutaminase can be a useful tool for retardation of lipid oxidation based on formation of emulsion-gels.

Keywords: emulsion-gel, lipid oxidation, microbial transglutaminase, sodium caseinate, sunflower oil

Introduction

The susceptibility of lipids to oxidation is a major cause of quality deterioration in many types of natural and processed foods (1). Except for cheeses, lipid oxidation is undesirable in most foods due to quality deterioration that includes changes in texture, shelf life, appearance, and nutritional profiles, and development of off-flavors and potentially toxic reaction products (1,2). Polyunsaturated lipids have health-promoting biological activities, but incorporation into many food products has been restricted because of susceptibility to lipid oxidation (3).

In real food systems, many natural foods and most processed foods exist in either a partially or completely emulsified state. An emulsion consists of 2 immiscible liquids, generally oil and water, with one being dispersed in the other in the form of small spherical droplets (4). Owing to oil, emulsions are vulnerable to lipid oxidation. In water-in-oil (W/O) emulsions, lipid oxidation can occur at a rate similar to rates in bulk oils because the surface of the lipid phase is exposed directly to air (5). However, in oil-in-water (O/W) emulsions, molecules involved in lipid oxidation can partition into the lipid core of an emulsion droplet, the emulsion droplet interfacial membrane, and the continuous phase, resulting in oxidation rates and mechanisms that differ from bulk oils (1,6,7). Emulsion-gels, developed for changes and/or improvements in textural and rheological properties of O/W emulsions, are not free from lipid oxidation due to the reasons described above. In addition to a capacity for improving textural and rheological properties, potential applications as controlled-release carriers for lipid-soluble bioactive components have encouraged an interest in the food industry in emulsion-gels (8). Protein emulsion-gels can be successfully formed based on thermal and non-thermal (acidification, addition of divalent salts, and enzymatic cross-linking) treatments (8,9). Thermal treatments are not suitable for formation of emulsion-gels as carriers for heat-labile bioactive components (10). Among non-thermal treatments for formation of protein emulsion-gels, enzymatic cross-linking shows unique textural and rheological properties and also has potential for use with controlled-release carriers for heat-labile/lipid-soluble bioactive components (8,10). Microbial transglutaminase (mTGase) is an enzyme used for generation of protein emulsion-gels. mTGase (EC 2.3.2.13) is an acyl transferase that catalyzes transfer of γ-carboxamide groups in proteins and peptides to primary amines. When the ε-amino group of peptide-bound lysine acts as an acceptor in the reaction, an ε-(γ-glutaminyl) lysine cross-link is formed. Thus, mTGase can form both intra and intermolecular covalent bonds between glutaminyl and lysine residues of proteins and peptides (11). The objective of this study was, therefore, to investigate development of lipid oxidation in emulsion-gels converted from sodium caseinate-stabilized O/W emulsions using enzymatic cross-linking with mTGase.

Materials and Methods

Materials Ca^{2+}-independent transglutaminase derived from *Streptoverticilium* was supplied by Ajinomoto (ACTIVA TG-S; Ajinomoto Co., Inc., Tokyo, Japan). According to information from the supplier, the enzyme concentration in the commercial preparation accounted for 1% of the total material, the remainder being 79.5% lactose, 10.0% sodium phosphate, 9.0% dextrin, and 0.5% L-sodium ascorbate. Before use, the enzyme was purified to remove salts and other compounds. A 4 g sample of the commercial enzyme product was suspended in 20 mL of a 0.2 M Tris-HCl buffer (pH 7.0) at 4°C for 4 h. After passage through a 5 μm membrane filter (Millipore Corporation, Billerica, MA, USA), the solution was subjected to centrifugal concentration with a 10 kDa polyethersulfone membrane (Vivaspin 6; Sartorius AG, Göttingen, Germany). Then, the concentration of the enzyme was determined following the Bradford method (12) using a Bio-Rad Protein Assay (Bio-Rad, Hercules, CA, USA). This enzyme solution was used without storage.

Sodium caseinate (Alanate 180®), obtained from the New Zealand Dairy Board (Wellington, New Zealand), consisted of 91.3% protein, 4.0% moisture, 3.5% ash, 1.1% fat, and 0.1% lactose and was used without purification. Reagent-grade sunflower oil, purchased from Sigma-Aldrich (St. Louis, MO, USA), was composed of 61.53% linoleic acid, 27.52% oleic acid, 5.99% palmitic acid, 3.81% stearic acid, 0.38% linolenic acid, 0.21% arachidic acid, 0.18% eicosenoic acid, and 0.07% myristic acid based on manufacturer supplied information. All other reagents were of at least analytical grade.

Emulsion-gel preparation Sodium caseinate was used due to excellent emulsification properties (13) and as a substrate for mTGase (14). An aqueous solution of 8% (w/v) sodium caseinate with 0.2% sodium azide was prepared with gentle stirring at room temperature for 17 h to ensure complete dissolution, after which the final pH of the protein solution was approximately 6.8. O/W emulsions were prepared using 45% (v/v) sunflower oil and 55% (v/v) protein solutions using a sonicator (VCX-400; Sonics & Materials, Newtown, CT, USA). Identical energy input conditions were used for preparation of emulsions with maintenance of the same geometry of the sample container, the solution volume, the position of the probe, and the time of emulsification. To ensure that the temperature of freshly prepared emulsions was below 30°C, samples were stored in a container previously stored on ice. Sodium caseinate-stabilized emulsions were prepared, and mTGase was added to emulsions to achieve a final concentration of 0.05%. Incubation at 37°C for 6 h was required for emulsion-gel formation.

Measurement of lipid oxidation For evaluation of primary product formation of lipid oxidation, emulsions (2 g) were placed in capped glass test tubes. For headspace analysis, emulsion samples (1 g) were placed into 10 mL headspace glass vials (Agilent Technologies, Santa Clara, CA, USA) and sealed with poly(tetrafluoroethylene)/butyl rubber septa and aluminum caps (Agilent Technologies) using a crimper. All emulsions were incubated at 4, 20, and 37°C in the dark for up to 7 days.

Lipid hydroperoxide concentrations were determined using a method adapted from Nuchi *et al.* (15). Emulsions (0.2 mg) were transferred to 1.5 mL of a 3:1 (v/v) mixture of isooctane/2-propanol, vortexed for 30 s, and centrifuged at 2,000×*g* for 2 min. An organic phase (30 μL) was added to 2.97 mL of a 2:1 (v/v) mixture of methanol/butanol, followed by addition of 15 μL of 3.94 M ammonium thiocyanate and 15 μL of a ferrous iron solution prepared based on mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$. The solution was vortexed, and 20 min after iron addition, the absorbance was measured at 510 nm (V-530 UV/VIS spectrophotometer; Jasco, Tokyo, Japan). The concentration of lipid hydroperoxides was calculated using a cumene hydroperoxide standard curve.

The volatile compounds hexanal and 1-octen-3-ol were used as markers of secondary product of lipid oxidation and analyzed using GC of volatile compounds sampled in the headspace of vials equilibrated at 50°C with a solid-phase microextraction (SPME) fiber coated with polydimethylsiloxane (10 mm length, 100 μm film thickness) (Supelco, Bellefonte, PA, USA) exposed in the headspace for 5 min at 50°C. The fiber was then transferred to the injection port of a gas chromatograph (3900 GC; Varian, Palo Alto, CA, USA) equipped with a single taper splitless liner (Varian) at 260°C. The purge was kept off during the first 5 min of desorption to ensure complete injection of volatiles into the column. Volatiles were separated using an HP-1 fused silica capillary column (30 m length, 0.32 mm internal diameter, 1 μm film thickness; Agilent Technologies). The temperature program was holding for 5 min at 35°C, followed by an increase at a rate of 10°C/min to 220°C, and holding for 5 min isothermally. Eluted compounds were detected using a flame ionization detector set at 250°C and hydrogen and air flows were set at 23 and 230 mL/min, respectively.

Volatile compounds were identified based on comparison of retention times with authentic reference standards. Concentrations were determined based on peak areas using a standard curve constructed with authentic hexanal, and results were expressed in ng of the volatile compound desorbed from the fiber. Fibers used throughout this study were tested daily based on checking peak areas obtained after 5 min of fiber exposure in the headspace of a test solution containing known amounts of the 4 volatile compounds and equilibrated at 50°C.

Mean values and standard deviations were obtained from triplicate experiments. Duncan's multiple range test was performed for mean comparisons (p<0.05) using IBM SPSS statistics version 21.0 (IBM, Armonk, NY, USA).

Results and Discussion

No significant (p>0.05) changes in hydroperoxide concentrations

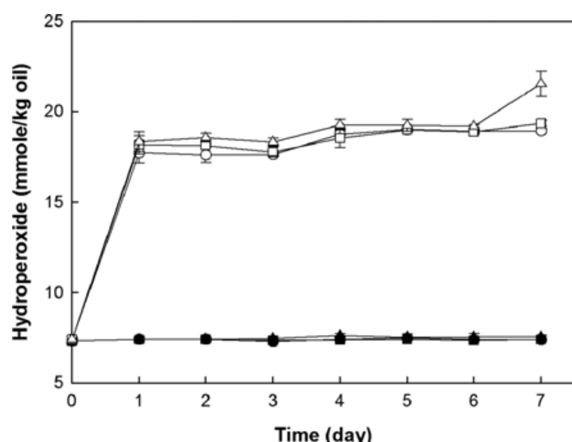

Fig. 1. Formation of lipid hydroperoxide in emulsions and emulsion-gels stabilized using sodium caseinate at 4, 20, and 37°C in darkness. ●, 4°C; ■, 20°C; ▲, 37°C. Filled symbols=emulsion-gel; open symbols= emulsion.

after 1 day were observed for emulsion systems. The amount of hydroperoxide was approximately 18 mmol/kg of oil after 1 day, regardless of the storage temperature (Fig. 1). Hydroperoxide formation increased rapidly for the first day and remained fairly constant during storage up to 7 days. In emulsion-gel systems, the level of formation of lipid hydroperoxide was lower than for emulsion systems. The concentration of hydroperoxide in the plateau region was 7 mmol/kg of oil, indicating that formation of lipid hydroperoxide in emulsion-gels was retarded, compared with emulsion systems. Presumably, the physical barrier produced by mTGase at the surface of O/W emulsion droplets suppressed formation of hydroperoxides based on retardation of oxygen movement toward emulsified oil and prevention of lipids from reacting with oxygen. The protein network barrier of the emulsion-gel was tighter and more viscous than the emulsion membrane. Additionally, the physical barrier produced by mTGase may have prevented movement of transition metals to emulsified oil that would have accelerated lipid oxidation (16). The physical barrier produced by mTGase in emulsion-gels was more effective than the interfacial layer of the emulsion for oxidative stability. Prevention of penetration and diffusion of oxygen is a reason for lower hydroperoxide concentrations in emulsion-gels.

A lipid radical formed between unsaturated oils and oxygen reacts rapidly with oxygen to form a lipid peroxide that extracts a hydrogen atom to form a hydroperoxide. Lipid peroxides are relatively stable but decompose into aldehydes, ketones, alcohols, and hydrocarbons (17). Hexanal and 1-octen-3-ol are the major compounds produced from sunflower oil and are often used as indices of lipid oxidation in oils and fatty materials containing high levels of linoleic acid (18). In addition, hexanal and 1-octen-3-ol are involved in development of off-flavors during lipid oxidation (19).

No significant (p>0.05) increase occurred in peak areas of hexanal and 1-octen-3-ol over 5 min of adsorption time. Concentrations of each volatile compound detected in the headspace of emulsions and

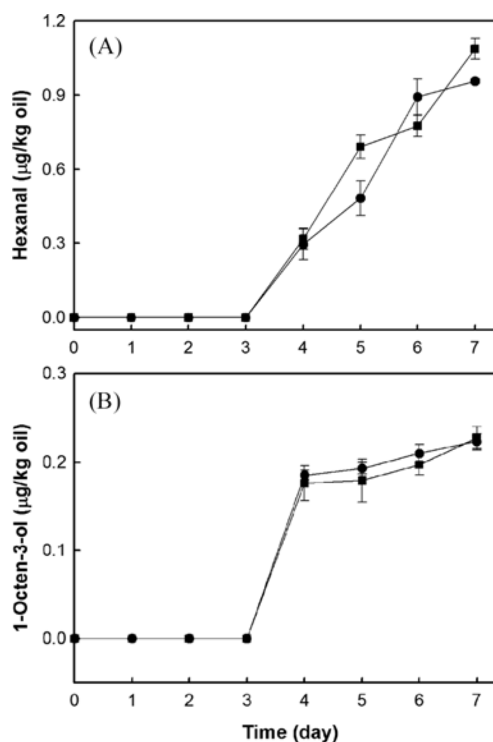

Fig. 2. Development of hexanal (A) and 1-octen-3-ol (B) in the headspace of emulsions and emulsion-gels stabilized using sodium caseinate during aging at 37°C in darkness. ●, emulsion-gel; ■, emulsion.

emulsion-gels were plotted against time (Fig. 2). Shapes of curves differed for each volatile compound, highlighting the complexity of lipid oxidation kinetics and production of volatile secondary products of oxidation. Regardless of the storage temperature, shapes of the curves were similar. The amount of hexanal (Fig. 2A) desorbed from the SPME fiber was constant during the first 3 days. However, a significant (p<0.05) difference was observed in the amount of hexanal between 3 and 7 days. A continued increase in the amount of hexanal in emulsion and emulsion-gel headspaces indicated that further lipid oxidation occurred during 7 days of storage. Based on the hexanal profile, no significant (p>0.05) difference was detected in the oxidative stabilities between emulsion and emulsion-gels.

Quantitative analysis of 1-octen-3-ol showed patterns dissimilar to the hydroperoxide profile (Fig. 2B). The amount of 1-octen-3-ol was low for the first 3 days, but the concentration of 1-octen-3-ol in emulsion and emulsion-gel headspaces increased significantly (p<0.05) between days 3 and 4.

Hydroperoxides must first be present before decomposition into secondary products, such as hexanal and 1-octen-3-ol. However, concentrations of hexanal and 1-octen-3-ol and rates of production were similar between emulsion systems and emulsion-gel systems, indicating that the protein network introduced by mTGase in the emulsion-gel system retarded the initiation step of lipid oxidation in emulsified oil, but could not prevent decomposition of hydroperoxides into secondary products.

Formation of lipid hydroperoxide can be successfully retarded based on introduction of physical barriers in the food matrix. Use of mTGase during processing of emulsion-gel products offers new opportunities for texture improvements and for retardation of lipid oxidation.

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Disclosure The authors declare no conflict of interest.

References

1. McClements DJ, Decker EA. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. J. Food Sci. 65: 1270-1282 (2000)
2. Halliwell B, Murcia MA, Chirico S, Aruoma OI. Free radicals and antioxidants in food and *in vivo*: What they do and how they work. Crit. Rev. Food Sci. 35: 7-20 (1995)
3. Watkins SM, German JB. Unsaturated fatty acids. pp. 513-537. In: Food Lipids: Chemistry, Nutrtion, and Biotechnology, 3rd ed. Akoh CC, Min DB (eds). CRC Press, Boca Raton, FL, USA (2008)
4. McClements DJ. Context and background. pp. 1-26. In: Food Emulsions: Principles, Practice, and Techniques. 2nd ed. McClements DJ. CRC Press, Inc., Boca Raton, FL, USA (2005)
5. Fritsch CW. Lipid oxidation- the other dimensions. Inform 5: 426-436 (1994)
6. Mancuso JR, McClements DJ, Decker EA. The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. J. Agr. Food Chem. 47: 4112-4116 (1999)
7. Frankel EN, Huang S-W, Kanner J, German JB. Interfacial phenomena in the evaluation of antioxidants: Bulk oils vs emulsions. J. Agr. Food Chem. 42: 1054-1059 (1994)
8. Tang C-H, Chen L, Foegeding EA. Mechanical and water-holding properties and microstructures of soy protein isolate emulsion gels induced by $CaCl_2$, glucono-δ-lactone (GDL), and transglutaminase: Influence of thermal treatments before and/or after emulsification. J. Agr. Food Chem. 59: 4071-4077 (2011)
9. Lee HA, Choi SJ, Moon TW. Characteristics of sodium caseinate- and soy protein isolate-stabilized emulsion-gels formed by microbial transglutaminase. J. Food Sci. 71: C352-C357 (2006)
10. Yang M, Liu F, Tang C-H. Properties and microstructure of transglutaminase-set soy protein-stabilized emulsion gels. Food Res. Int. 52: 409-418 (2013)
11. Ando H, Adachi M, Umeda K, Matsuura A, Nonaka M, Uchio R, Tanaka H, Motoki M. Purification and characteristics of a novel transglutaminase derived from microoganisms. Agr. Biol. Chem.Tokyo 53: 2613-2617 (1989)
12. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254 (1976)
13. Dickinson E, Rolfe SE, Dalgleish DG. Competitive adsorption of α_{s1}-casein and β-casein in oil-in-water emulsions. Food Hydrocolloid. 2: 397-405 (1988)
14. Nio N, Motoki M, Takinami K. Gelation mechanism of protein solution by transglutaminase. Agr. Biol. Chem.Tokyo 50: 851-855 (1976)
15. Nuchi CD, McClements DJ, Decker EA. Impact of Tween 20 hydroperoxides and iron on the oxidation of methyl linoleate and salmon oil dispersions. J. Agr. Food Chem. 49: 4912-4916 (2001)
16. Dickinson E, Hong S-T. Surface coverage of β-lactoglobulin at the oil-water interface: Influence of protein heat treatment and various emulsifiers. J. Agr. Food Chem. 42: 1602-1606 (1994)
17. Coupland JN, McClements DJ. Lipid oxidation in food emulsions. Trends Food Sci. Tech. 7: 83-91 (1996)
18. Abdalla AE, Roozen JP. Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. Food Chem. 64: 323-329 (1999)
19. Forss DA. Odor and flavor compounds from lipids. Prog. Chem. Fats Other Lipids 13: 177-258 (1973)