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Lipid Oxidation in Corn Oil-in-Water Emulsions Stabilized by Casein, Whey Protein Isolate, and Soy Protein Isolate

MIN HU, D. JULIAN MCCLEMENTS, AND ERIC A. DECKER*

Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

Proteins can be used to produce cationic oil-in-water emulsion droplets at pH 3.0 that have high oxidative stability. This research investigated differences in the physical properties and oxidative stability of corn oil-in-water emulsions stabilized by casein, whey protein isolate (WPI), or soy protein isolate (SPI) at pH 3.0. Emulsions were prepared with 5% corn oil and 0.2−1.5% protein. Physically stable, monomodal emulsions were prepared with 1.5% casein, 1.0 or 1.5% SPI, and ≥0.5% WPI. The oxidative stability of the different protein-stabilized emulsions was in the order of casein > WPI > SPI as determined by monitoring both lipid hydroperoxide and headspace hexanal formation. The degree of positive charge on the protein-stabilized emulsion droplets was not the only factor involved in the inhibition of lipid oxidation because the charge of the emulsion droplets (WPI > casein ≥ SPI) did not parallel oxidative stability. Other potential reasons for differences in oxidative stability of the protein-stabilized emulsions include differences in interfacial film thickness, protein chelating properties, and differences in free radical scavenging amino acids. This research shows that differences can be seen in the oxidative stability of protein-stabilized emulsions; however, further research is needed to determine the mechanisms for these differences.

KEYWORDS: Casein; whey protein; soy protein; emulsions; lipid oxidation; antioxidant

INTRODUCTION

Proteins are commonly used in food products to facilitate the formation and improve the stability of oil-in-water emulsions (*1*−*4*). During homogenization, proteins are capable of rapidly absorbing to the surface of oil droplets, where they lower interfacial tension and inhibit droplet coalescence by forming protective membranes around the droplets (*2*, *3*). Proteins also stabilize oil-in-water emulsions by imparting an electrical charge to the emulsion droplet at pH values above or below the p*I* of the proteins. This positive or negative electrical charge causes repulsive forces that inhibit droplet coalescence and flocculation, thus further stabilizing the emulsion system (*1*−*4*).

Although a great deal of research has been focused on the physical stability and interfacial properties of protein-stabilized oil-in-water emulsions, very little research has focused on the oxidative stability of these emulsions. When iron was used to promote the oxidation of menhaden oil-in-water emulsions stabilized by whey protein isolate (WPI), oxidation rates were found to be lower at pH values below the p*I* of WPI, where the emulsion droplets were cationic (*5*). In the absence of added iron, salmon oil-in-water emulsions stabilized with WPI were again found to be more oxidatively stable at pH values below the p*I* (*6*). The ability of cationic protein-stabilized emulsion to decrease lipid oxidation has been postulated to be due to the electrostatic repulsion of transition metals away from the lipid droplets (*5*). However, in a comparison of the lipid oxidation rates in salmon oil-in-water emulsions stabilized by WPI, sweet whey, β-lactoglobulin, and α-lactalbumin at pH 3, oxidative stability was in the order β-lactoglobulin ≥ sweet whey > WPI > α-lactalbumin, whereas the positive charge of the emulsion droplets was in the order β-lactoglobulin ≥ α-lactalbumin > WPI > sweet whey (*6*). These results suggest that the droplet charge is not the only factor responsible for differences in the oxidative stability of protein-stabilized oil-in-water emulsions.

Sodium caseinate is commonly used as an emulsifier in the food industry (*1*). It is composed of a mixture of four principal proteins: α_{s1}-, α_{s2}-, β-, and κ-caseins (*2*). The monomeric forms of the casein proteins have relatively low molecular weights (15000−26000 Da), but in nature casein exists as a heterogeneous multisubunit protein complex known as a micelle. β-Casein and α_{s1}-casein, which together make up more than three-fourths of the total protein, are mainly responsible for sodium caseinate's excellent emulsifying properties (*3*). Compared with many other food proteins, such as WPI, the caseins are particularly disordered and substantially hydrophobic, which assists their rapid absorption during emulsification leading to the rapid establishment of a thick sterically stabilizing layer that protects newly formed droplets against flocculation and coalescence (*3*). Soy protein isolate (SPI) and its individual proteins have also been reported to form physically stable oil-in-water emulsions. The major proteins from soybeans are 7S and 11S with molecular weights of approximately 200,000 and 350,000

* Address correspondence to this author at the Department of Food Science, Chenoweth Lab, University of Massachusetts Amherst, MA 01003 [telephone (413) 545-1026; fax (413) 545-1262; e-mail edecker@foodsci.umass.edu].

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Da, respectively. These proteins have heterogeneous quaternary structures with some glycolation and no phosphorylation.

Although research has been conducted on the utilization of whey proteins to produce cationic, oxidatively stable oil-in-water emulsions at low pH, little is known about whether emulsions stabilized with other proteins would impact oxidative reactions in a similar manner. Therefore, the objective of this study was to utilize corn oil-in-water emulsions stabilized by casein (CAS), WPI, and SPI to better understand differences in the oxidative stability of protein-stabilized emulsions.

MATERIALS AND METHODS

Materials. Corn oil was purchased from a local grocery. Bovine casein (sodium salt, >99% protein), imidazole, sodium acetate, ferrous sulfate, cumene hydroperoxide, and hexanal were purchased from Sigma Chemical Co. (St. Louis, MO). WPI was obtained from Davisco Food International Inc. (Eden Praire, MN), and SPI (Supro 545) was from Protein Technologies International (St. Louis, MO). Proteins were used without further purification. The protein content of WPI was 97.6%. The major protein components of WPI were 55−61% β-lactoglobulin, 19−22% α-lactalbumin, and 6−8% bovine serum albumin. The protein content of SPI was ≥90.0%, ≤6.0% moisture, and ≤1.5% fats. All protein concentrations and composition data cited were obtained from the respective manufacturers. All other reagents were of analytical grade or purer.

Methods. *Preparation and Characterization of Emulsions.* An oil-in-water emulsion was prepared using 5.0 wt % corn oil and 95% acetate−imidazole buffer (5 mM each, pH 3.0) containing 0.2−1.5% WPI or SPI (*6*). Sodium caseinate (0.2−1.5%) was difficult to dissolve at pH 3.0; therefore, it was dissolved at pH 7.0, and then the pH was slowly adjusted to pH 3.0. Oil-in-water emulsions were made by blending the lipid and aqueous phases for 2 min using a hand-held homogenizer (M 133/1281-Biospec Products, Inc., Bartlesville, OK). The coarse emulsion was then homogenized four times at 5000 psi through a high-pressure value, two-stage APV Lab 1000 homogenizer (Albertslund, Denmark). The particle size distribution ($d_{3,2}$) of the emulsions was measured using a Coulter LS 230 laser light scattering instrument (Coulter Corp., Miami, FL). Droplet size distributions were measured at 1, 48, and 96 h after homogenization to monitor emulsion stability. Emulsion droplet charge (zeta-potential, ζ) was measured by directly injecting diluted (1:1000, sample: acetate−imidazole buffer, pH 3.0) oil-in-water emulsions into the measurement chamber of a ZEM 5003 Zetamaster (Malvern Instruments, Worcester, U.K.). The ζ-potential measurements are reported as the average of two separate injections, with five readings made per injection.

Measurement of Lipid Oxidation. To monitor lipid hydroperoxide formation during storage, emulsions (5 mL) were placed in lightly sealed screw-cap test tubes and allowed to oxidize at 37 °C in the dark. Lipid hydroperoxides were measured by mixing 0.3 mL of emulsion with 1.5 mL of isooctane/2-propanol (3:1, v/v) by vortexing (10 s, 3 times) and isolation of the organic solvent phase by centrifugation at 1000*g* for 2 min. The organic solvent phase (200 μL) was added to 2.8 mL of methanol/1-butanol (2:1, v/v), followed by 15 μL of 3.97 M ammonium thiocyanate and 15 μL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). The absorbance of the solution was measured at 510 nm, 20 min after the addition of the iron (*7*). Hydroperoxide concentrations were determined using a standard curve made from cumene hydroperoxide.

For headspace analysis, emulsion samples (1 mL) were placed into 10 mL headspace vials and sealed with poly(tetrafluoroethylene) butyl rubber septa. Headspace hexanal was determined using a Shimadzu 17A gas chromatograph equipped with a Hewlett-Packard 19395A headspace sampler (*8*). The headspace conditions were as follows: sample temperature, 55 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1.0 min. The aldehydes were separated isothermally at 65 °C on an HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 μm film thickness). The splitless injector temperature was 180 °C, and the eluted compounds were detected with a flame ionization detector at 250 °C. Concentrations were determined from peak areas using a standard curve made from authentic hexanal.

Statistical Analysis. Assays were measured in triplicate. Statistical analysis was performed using the Student *t* test (*9*).

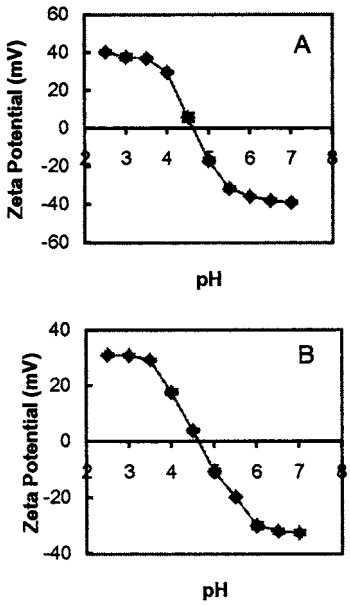

Figure 1. Droplet ζ-potential of 5% corn oil-in-water emulsion stabilized by casein (A) or soy protein isolate (B) as a function of pH. Data points represent means ($n = 5$).

RESULTS

Physical Characterization of Corn Oil-in-Water Emulsions Stabilized by WPI, CAS, and SPI. The p*I* of proteins used to stabilize oil-in-water emulsions is a very important factor in oxidative stability because pH values below the p*I* of the proteins will produce cationic droplets that can repel iron and other prooxidative metals and thus inhibit lipid oxidation. The p*I* of CAS-, WPI-, and SPI-stabilized emulsion droplets was determined by measuring the electrical charge of droplets (ζ-potential) as a function of pH. The p*I* values of WPI-, CAS-, and SPI-stabilized emulsion droplets were found to be 4.8 (*6*), 4.7 (**Figure 1A**) and 4.6 (**Figure 1B**), respectively. CAS, WPI, and SPI solubilized in water have p*I* values of 4.6, 5.1, and 4.5 (*10*−*12*). At pH 3.0, the surface charges of the oil-in-water emulsions stabilized with 0.5% CAS, WPI, and SPI were 29.9 ± 0.6, 55.9 ± 0.4, and 29.4 ± 0.5 mV, respectively. Increasing protein concentration had no effect on the ζ-potential of the emulsion droplets for any of the proteins tested (data not shown), which indicated that the droplet surfaces were saturated with protein even at the lowest protein concentration used.

The particle size and physical stability of emulsions made with CAS, WPI, and SPI were needed before comparisons of oxidative stability could be made. Protein concentrations are known to influence emulsion droplet size, surface protein concentration, and storage stability (*13*−*15*). Thus, four different concentrations of CAS, WPI, and SPI (0.2, 0.5, 1.0, and 1.5%) were tested, and the particle sizes of these emulsions are shown in **Table 1**. The initial mean particle diameters of WPI-, SPI-, and CAS-stabilized corn oil-in-water emulsions ranged from 0.25 to 0.29 μm, from 0.30 to 0.80 μm, and from 0.30 to 0.48 μm, respectively. Initial particle sizes decreased with increasing protein concentration for both SPI and CAS over the entire range of protein concentrations tested. Initial droplet diameters for SPI-stabilized emulsions decreased dramatically from 0.80 to 0.38 μm when protein concentrations were increased from 0.2

Table 1. Mean Particle Size (Micrometers) of Corn Oil Emulsions Stabilized by Different Concentrations of Whey Protein Isolate (WPI), Soy Protein Isolate (SPI), and Casein during Different Storage Times at 20 °C

storage time (h)	0.2%	0.5%	1.0%	1.5%
		WPI		
1	0.29 ± 0.17(m)[a]	0.27 ± 0.16(m)	0.25 ± 0.15(m)	0.26 ± 0.15(m)
48	0.30 ± 0.19(m)	0.27 ± 0.16(m)	0.25 ± 0.16(m)	0.23 ± 0.14(m)
96	0.35 ± 0.31(b)	0.28 ± 0.17(m)	0.25 ± 0.15(m)	0.22 ± 0.14(m)
		SPI		
1	0.80 ± 0.64(b)	0.38 ± 0.26(b)	0.33 ± 0.18(m)	0.30 ± 0.16(m)
48	1.02 ± 0.87(b)	0.36 ± 0.21(b)	0.33 ± 0.19(m)	0.30 ± 0.16(m)
96	1.07 ± 1.02(b)	0.37 ± 0.26(b)	0.33 ± 0.17(m)	0.29 ± 0.16(m)
		Casein		
1	0.48 ± 0.37(b)	0.47 ± 0.36(b)	0.42 ± 0.36(b)	0.30 ± 0.16(m)
48	0.47 ± 0.36(b)	0.46 ± 0.39(b)	0.41 ± 0.35(b)	0.30 ± 0.17(m)
96	0.40 ± 0.24(b)	0.49 ± 0.37(b)	0.40 ± 0.29(b)	0.29 ± 0.17(m)

[a] m, monomodal distribution; b, bimodal distribution.

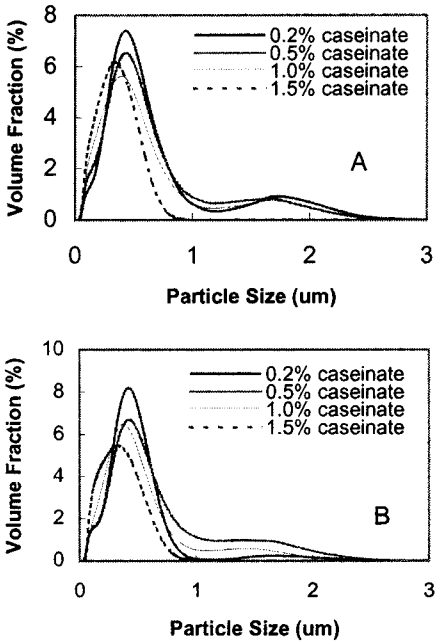

Figure 2. Particle size distributions of casein (0.2–1.5%)-stabilized oil-in-water emulsions after 1 (A) and 96 (B) h of incubation.

to 0.5%. This large decrease in particle diameter suggests that the reduction of particle size during homogenization was limited by protein concentration in the 0.2% SPI emulsion. The initial particle diameter (day 0) for WPI-stabilized emulsions also decreased with increasing protein concentration from 0.2 to 1.0% with no additional change in size at 1.5% WPI. In addition to a decrease in particle size, the droplet distribution patterns for the different emulsions also varied with increasing proteins concentrations. WPI-stabilized emulsion exhibited monomodal droplet distributions (e.g., only a single peak was observed on the particle sizer over the entire protein concentration tested at 0 days of storage). SPI had bimodal emulsion droplet size distributions at 0.2 and 0.5% protein, whereas CAS-stabilized emulsions were bimodal from 0.2 to 1.0% protein with 1.5% protein producing monomodal droplet distributions. Examples of the particle size distribution for CAS-stabilized emulsions after 1 and 96 h of storage are shown in **Figure 2**.

The particle size distributions and characteristics of the emulsions changed during the storage of the emulsions (**Table 1**). Emulsions prepared with 0.2% WPI had an increase in mean

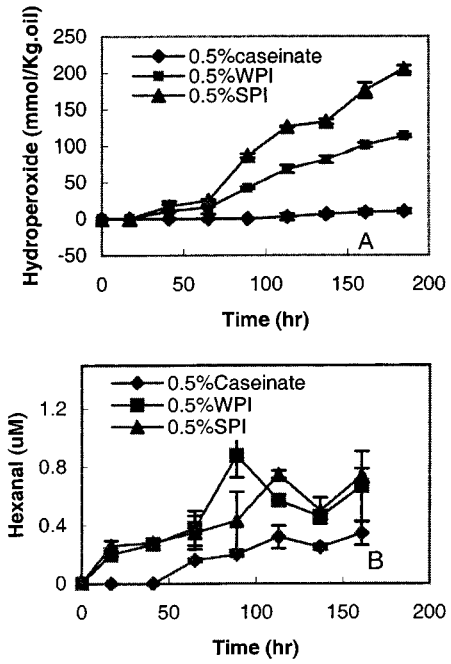

Figure 3. Formation of lipid hydroperoxides (A) and headspace hexanal (B) in 5% corn oil emulsions stabilized by 0.5% whey protein isolate, soy protein isolate, or casein at pH 3 and 37 °C. Data points represent means ($n = 3$) ± standard deviations.

particle size from 0.29 to 0.35 μm with the droplet size profile changing from monomodal to bimodal after 96 h of storage. The droplet size distribution of emulsions stabilized with 0.2% SPI also increased during storage. The droplet size of emulsions prepared with WPI or SPI at concentrations of 0.5−1.5% did not increase over the 96 h of storage; however, emulsions made with 0.5% SPI had a bimodal distribution during the entire storage period. Large increases in particle size distributions for CAS-stabilized emulsions were not observed at any protein concentrations. Protein concentrations of 0.5 and 1.5% were used for oxidative stability studies because these emulsions exhibited good physical stability for all of the proteins tested.

Comparison of Differences in Oxidative Stability of Emulsions Stabilized by CAS, WPI, and SPI. The oxidative stability of the oil-in-water emulsion stabilized with WPI was greatest when the pH was lower than the p*I* of the absorbed WPI, thus producing cationic emulsion droplets that can repel prooxidative metals (*5*, *6*). Because production of cationic emulsion droplets could be used as a strategy for the production of oxidatively stable emulsions, differences in lipid oxidation rates between CAS-, WPI-, and SPI-stabilized corn oil-in-water emulsions were compared at pH 3. These emulsions were incubated at 37 °C to accelerate oxidation rates, thus making it easier to determine differences in the oxidative stability of the samples.

Figure 3 shows the formation of lipid hydroperoxide and headspace hexanal in corn oil-in-water emulsions stabilized with 0.5% CAS, WPI, and SPI. The oxidative stability of the protein-stabilized emulsion was in the order CAS > WPI > SPI as determined by both lipid hydroperoxide and headspace hexanal. For emulsions made with CAS, lipid hydroperoxide concentrations were basically unchanged after 185 h of incubation. Lipid hydroperoxides were not observed in the WPI- and SPI-stabilized emulsions after 24 h of oxidation; hydroperoxide concentrations increased to 113.2 and 205.5 mmol/kg of oil, respectively, after 185 h of storage. Hexanal formation in the emulsions stabilized with 0.5% protein exhibited similar trends,

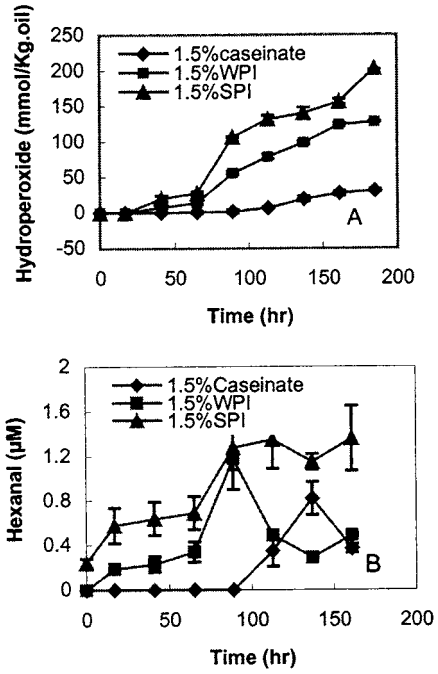

Figure 4. Formation of lipid hydroperoxide (A) and headspace hexanal (B) in 5% corn oil emulsions stabilized by 1.5% whey protein isolate, soy protein isolate, or casein at pH 3 and 37 °C. Data points represent means ($n = 3$) ± standard deviations.

with CAS-stabilized emulsions having no detectable headspace hexanal for up to 48 h of storage, whereas hexanal was detected in the WPI- and SPI-stabilized emulsions after 24 h of storage. Emulsions made with 1.5% WPI and SPI had similar lipid hydroperoxide formation rates and slightly higher hexanal formation rates compared to emulsions made with 0.5% protein (**Figure 4**). In emulsions made with 1.5% CAS, formation of hydroperoxides was again very low, whereas the increase in protein resulted in an increase in the lag phase of hexanal formation from 48 h with 0.5% protein to 96 h with 1.5% protein.

DISCUSSION

Transition metals, and in particular iron, are major prooxidants in oil-in-water emulsions due to their ability to decompose lipid hydroperoxides into free radicals (*16*). Factors that could influence lipid oxidation rates in oil-in-water emulsions include particle size, which influences surface area, emulsion droplet charge, which can cause either attraction or repulsion of transition metals, thickness of the emulsifier layer at the interfacial region of the emulsion droplet that can impact interactions between lipids and aqueous phase prooxidants, and chemical components of the proteins that can scavenge free radicals (e.g., cysteine and tyrosine) or chelate prooxidant metals (phosphoserine) (*16*).

The particle size of emulsion droplets could impact lipid oxidation rates because smaller particle sizes result in larger surface area and thus greater possibility for lipid−aqueous phase prooxidant interactions. Lethuat and co-workers (*17*) found that increasing surface area in bovine serum albumin-stabilized oil-in-water emulsions increased lipid oxidation as measured by oxygen consumption and formation of conjugated dienes. In our system particle size and thus surface area may have been a factor in the oxidative stability of the 0.5% CAS-stabilized emulsions because the particle size of these droplets was slightly larger and the formation of lipid hydroperoxides and headspace hexanal was slower than in SPI- and WPI-stabilized emulsions (**Table 1** and **Figure 3**). The SPI-stabilized emulsions had larger particles sizes (**Table 1**) and thus lower surface area than the WPI-stabilized emulsions, whereas formation of lipid hydroperoxides was similar (**Figure 3A**) and formation of headspace hexanal was greater (**Figure 3B**). The fact that SPI-stabilized emulsion droplets had lower surface area but similar or lower oxidative stability than WPI-stabilized emulsions suggests that surface area was not the major factor in differences in oxidative stability. In the emulsions stabilized with 1.5% protein, emulsion droplet sizes were similar (0.26−0.30 μm) and were in the order WPI < SPI = CAS (**Table 1**). The oxidative stability of these emulsions was in the order CAS > WPI ≥ SPI (**Figure 4**). Lack of correlation between particle size and oxidation rates in the 1.5% protein-stabilized emulsion suggests that surface area was also not a major factor in the observed differences in oxidative stability of the emulsions made with the different proteins.

Another factor that could influence lipid oxidation rates in protein-stabilized emulsions is the droplet charge, with a higher cationic charge density potentially being able to more effectively repel aqueous phase prooxidant metals. However, the fact that the ζ-potential of the emulsion droplets stabilized by WPI was almost twice as high as those of the CAS- and SPI-stabilized emulsion droplets whereas the oxidative stability of the WPI-stabilized emulsions was intermediate among the three proteins suggests that the magnitude of the positive charge of the emulsion droplet charge did not have a major impact on lipid oxidation rates. A similar pattern was observed in emulsions stabilized with WPI, β-lactoglobulin, and α-lactoalbumin, with the degree of cationic surface charge not having a large impact on lipid oxidation rates (*6*).

The oxidative stability of oil-in-water emulsions is also influenced by the size of the emulsifier, which can affect the thickness of the interfacial layer of the emulsion droplet with larger hydrophilic headgroups decreasing oxidation rates (*18*). Casein can form a thick interfacial layer around dispersed oil droplets of up to 10 nm compared to 1−2 nm for whey proteins (*18*). The thickness of the interfacial layer of SPI-stabilized emulsion droplets has not been reported. The ability of casein to form a thick layer around the emulsion droplets could help to explain why the casein-stabilized emulsions had the greatest oxidative stability.

An additional factor that could be involved in differences in the oxidative stabilities of the different emulsions is differences in amino acid composition among the proteins. The free sulfhydryl group of cysteine can inhibit lipid oxidation. Casein was the only protein of the three tested that does not contain free cysteine, yet it produced the most oxidatively stable emulsion. In addition, previous work has shown that blocking free sulfhydryls in WPI with *N*-ethylmaleimide (NEM) prior to the formation of emulsions did not alter oxidation rates, suggesting that free sulfhydryls at the emulsion interface do not inhibit lipid oxidation rates (*6*). These observations suggest that free sulfhydryls were not a major determinant in differences in oxidative stability among the emulsions.

Other amino acids and in particular tyrosine, phenylalanine, tryptophan, proline, methionine, lysine, and histidine have also been reported to be antioxidative. **Table 2** shows the concentrations of these amino acids in the proteins used in these experiments. Casein, which produced the most oxidatively stable emulsions, had the highest concentrations of tyrosine, methionine, and proline and also had high concentrations of phenylalanine (similar to SPI). SPI, which produced the least oxidatively

Table 2. Concentrations of Potentially Antioxidative Amino Acids (Grams per 100 g of Protein) in Casein (CAS), Whey Protein Isolate (WPI), and Soy Protein Isolate (SPI)[a]

amino acid	CAS	WPI	SPI
histidine	2.7	2.3	2.6
methionine	2.6	2.2	1.4
phenylalanine	5.1	3.7	5.2
proline	10.0	5.1	4.2
tryptophan	1.1	2.8	1.3
tyrosine	5.2	1.8	3.8

[a] Amino acid concentrations are cited from data obtained from the respective manufacturers.

stable emulsions, had lower concentrations of lysine, methionione, and tryptophan than WPI. Unfortunately, no clear relationship can be made between amino acid composition and oxidative stability of the emulsions made with the different proteins. In addition, it is unclear how these amino acids are physically oriented (e.g., toward the lipid or water), a factor that could dramatically influence their reactivity. Therefore, detailed analysis of the kinetic oxidation of these antioxidative amino acids is needed to better understand their role in the oxidative stability of protein-stabilized emulsions.

Another mechanism that could affect oxidation rates is prooxidant metal chelation. Upon the production of protein-stabilized emulsions the protein equilibrates between the emulsion droplet interface and the continuous phase with continuous phase protein concentrations increasing with increasing protein concentrations at a constant lipid level (*19*). If the continuous phase proteins are able to chelate metals, they can remove metals away from the lipid droplet and inhibit lipid oxidation. Continuous phase whey proteins are capable of removing metals away from emulsion droplets at pH 7.0 when the proteins are anionic (*20*). This effect would not be expected at pH 3.0 when the proteins are cationic. One exception would be casein, which contains phosphoseryl groups that remain anionic and can chelate iron at low pH (*21*).

CONCLUSIONS

Physically stable corn oil-in-water emulsions at pH 3 can be made using casein, SPI, and WPI, respectively. At pH 3.0, the oxidative stability of casein-stabilized corn oil-in-water emulsions was greater than of emulsions made with WPI and SPI. These data indicate that casein is an excellent candidate to produce oil-in-water emulsions that have both high physical and oxidative stability. The exact reasons why casein-stabilized oil-in-water emulsions have greater oxidative stability are not known, although casein's ability to produce thick layers on the emulsion droplet interface and its unique chelating properties are potential candidates. Further research is needed to evaluate its antioxidative mechanisms to provide information that could be used to maximize the oxidative stability of food emulsions.

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