STUDY OF IRON ENRICHED O/W EMULSIONS STABILIZED BY PROTEINS

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Abstract: *Lipid oxidation leads to the development of off-flavors (rancidity) and potentially toxic compounds. Proteins are generally regarded as safe food ingredients and they are widely used as emulsifiers in food products. Sodium caseinate (NaCas) can prevent lipid oxidation, essentially by chelating metals ions, but other mechanisms such as free-radical scavenging may also be involved. The aim of this paper is to study the oxidative stability of iron enriched o/w emulsions stabilized by proteins such as NaCas is related to availability of metal ions and protein properties.*

Cuvinte cheie: *Lipid oxidation, iron, availability, sodium caseinate, oxidative stability, o/w emulsions*

I. Introduction

Lipid oxidation is a major cause of food quality deterioration. It leads to the development of off-flavors (rancidity) and potentially toxic compounds. The main rules of lipid oxidation in oils are known and recent studies have focused on lipid oxidation in oil-in-water (o/w) emulsions [1]. In many foods, the lipid phase is dispersed as oil droplets in an aqueous matrix.

Oxidation phenomena in emulsions mimic what happens in food products. Lipid oxidation is favored because the droplets present a large contact surface between oxidizable fatty material and the water-soluble chemical compounds, namely, oxygen and ions of transition metals, which participate in the initiation and propagation of oxidation reactions [2,3].

Proteins are generally regarded as safe food ingredients and they are widely used as emulsifiers in food products [*4*]. Many proteins also exert antioxidant activity when added in the aqueous phase of o/w emulsions stabilized by small surfactants such as phospholipids. The antioxidant activity of milk proteins was demonstrated in various studies [*5-6*]. In particular, caseins and casein-derived phosphopeptides inhibited formation of lipid hydroperoxides and hexanal in Brij stabilized corn oil emulsions . The ability of casein phosphorylated groups to chelate pro-oxidant metals ions may explain this inhibition, but free-radical-scavenging activity could also be involved [*7*].

In emulsified systems and in other model systems, some proteins generally considered as antioxidant were pro-oxidant under specific conditions. Numerous examples showed that proteins can behave as pro-oxidant, depending on physicochemical conditions of the systems [*8-10*].

Sodium caseinate (NaCas) is a mixture of disordered proteins of relatively low molecular weights. They can prevent lipid oxidation, essentially by chelating metals ions, but other mechanisms such as free-radical scavenging may also be involved. As their hydrophobic and hydrophilic residues are clustered into large and separate domains, caseins adsorb to the oil-water interfaces through their hydrophobic domain, whereas the hydrophilic domain forms a tail protruding in the aqueous phase (*27*).

The aim of this paper is to study the oxidative stability of iron enriched o/w emulsions stabilized by proteins such as NaCas is related to availability of metal ions and protein properties.

II. Materials and Methods

Materials. Commercial sunflower oil was stripped of tocopherols, mono- and diacylglycerides, and free fatty acids using adsorption chromatography on an alumina column. Powdered NaCas (92.7% purity) : sodium azide (NaN_3, purity ≥99%); hexanal (98%), 1-octen-3-ol (98%), 2-(*E*)-octenal (94%), 1,10-phenantroline monohydrate (99%), hydroquinone (99%), and ammonium iron (II) sulfate hexahydrate [$Fe(NH_4)_2(SO_4)_2 . 6H_2O$] (>99%) were purchased from Aldrich.

Preparation, Characterization, and Storage of Emulsions. The day before emulsion preparation, NaCas solutions (20 g/L; 0.4 g /L NaN_3; pH adjusted to 6.5) were prepared and stirred overnight at +4 ºC to entirely dissolve the proteins without foam formation. The solutions were equilibrated at room temperature just before use and their pH was adjusted to 6.5 by addition of either NaOH or HCl (1 mol /L). O/W emulsions were prepared with 30 vol % sunflower oil and 70 vol % protein solutions. The two phases were premixed for 2 min at 8 000 rpm using a rotor-stator homogenizer.The coarse emulsions were then omogenized for 4 min at 35 bar through a one-stage low-pressure valve homogenizer.

The size distribution of the oil droplets in the emulsions was measured immediately after homogenization with a laser light scattering instrument. It was daily checked to monitor emulsion stability and reported as volume-surface mean diameter ([$d_{3,2}$]; μm). The charge of the emulsion droplets (zeta potential, ζ; mV) was also measured after homogenization.

Aliquots (1,5 mL) of emulsions were distributed in 20-mL headspace vials sealed with Teflon/silicon septa and aluminum crimp caps. The vials were rotated in the dark at 30 ºC, 20 rpm. pH of the samples was measured with a pH-150 MA. with an electrode suitable for measurements in emulsions.

Measurement of Lipid Oxidation. To evaluate the formation of primary products of lipid oxidation in the emulsions, conjugated dienes (CD) were evaluated according to the method described by Lethuaut et al. [2]. Aliquots of emulsions were diluted in 2-propanol to obtain lipid concentrations of 250 mg /L.The solutions were centrifuged for 4 min at 5600 rpm. The absorbance of the supernatants were measured at 233 nm with a UV-vis spectrophotometer (UNICAM, UK). Reference cell contained water in 2-propanol in the same proportions as in samples. Results were expressed in millimoles of conjugated dienes per kilogram of oil (mmol CD kg/ oil) at 233 nm [11].

Four volatile compounds: pentane, hexanal, 1-octen-3-ol, and 2-(*E*)-octenal, were selected as markers of secondary products of lipid oxidation. They were analyzed by gas chromatography (GC) of the volatile compounds sampled in the headspace of the vials equilibrated at 30 ºC, with a solid-phase microextraction (SPME) fiber. All tests have been carried out using the Shimadzu GC system coupled with a single quadrupole mass-spectrometer GCMS-QP2010 Plus equipped with the three-dimensional automated system for the injection of samples AOC-5000 (GCMS-QP2010 PlusxAOC-5000). For the identification, the general library of NIST-5 mass spectra were used. The accuracy of displacement has been verified according to the library of Covatz retention indices (MLRI). The analysis of the experimental data was carried out with the GC/MS Solution software (Shimadzu), which contains the SCAN/SIM options (Fast Automated Scan/SIM Type (FASST); creation of automatic SIM (Scan/SIM) table (COAST).

Iron-Binding Properties of the Proteins. To evaluate the ability of the proteins to bind soluble iron, mixtures of the protein solution and known amounts of ferrous iron were filtrated through cutoff filters and the unbound metal was quantified [12]. Ferrous iron was added to NaCas (1 g/ L, pH adjusted to 6.5) at concentrations ranging from 1.0 to 5.1 mg /L. After one night at room temperature, protein solutions were filtered through disposable cutoff filters to remove proteins and protein-bound iron. The filtrate contains only the free iron.

Free Fe^{2+} reacts with *o*-phenantroline to give a colored complex that is quantified by measurement of solution absorbance at 508 nm. One volume of *o*- phenantroline reagent (2.5 mM phenantroline, 18.2 mM hydroquinone in ethanol/water; 50/50; v/v) was added to one volume of the filtrate. The solution absorbance was read immediately at 508nm against a blank. The concentration of free iron was determined from the calibration curve built with ammonium iron (II) sulfate hexahydrate solutions (1-10 mg/ L). It was then subtracted from the concentration of added iron to obtain the concentration of bound iron. The experiment was also performed with pure water as a control.

III. RESULTS

The freshly prepared emulsions had very similar and monomodal droplet size distributions. For NaCas-stabilized emulsions, [$d^{3,2}$] was 1.32 ± 0.12 μm. The droplet size distributions of the emulsions largely overlap each other and oil-water interface areas ($6/d_{3,2}$]) are very close. Consequently, differences in oxidative stability of the NaCas-stabilized emulsions could not be attributed to differences in droplet size and interfacial surface area [2]. The droplet size distributions of the emulsions remained constant even after 10 days of storage, and no phase separation nor creaming was observed, showing that the emulsions were physically stable

and not destabilized during the rotating agitation at 30 ºC. NaCas-stabilized oil droplets were negatively charged (-81.2 (5.1 mV), as revealed by ζ-potential measurements.

The initial pH of the emulsions was slightly above the pH of the protein solutions (6.5): it was 6.66 ±0.02 for NaCas-stabilized emulsions. During aging, the pH of the NaCas-stabilized emulsions remained stable for the first 8 h, respectively, then decreased rapidly to finally decrease slowly after 24 h aging (Figure 1). pH values of 6.04 ±0.02 were reached after 48 h for the emulsions.

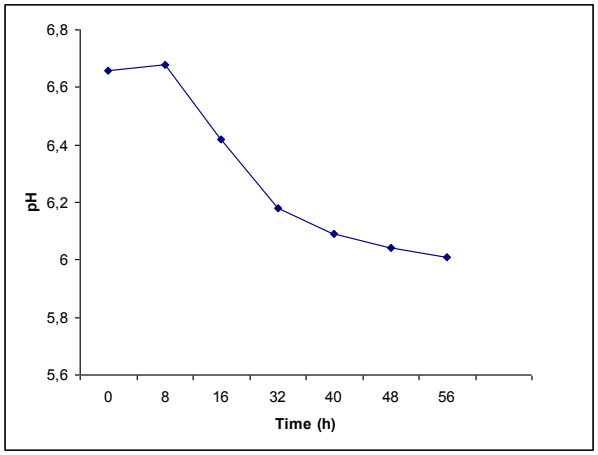

Figure 1. Evolution of pH during aging at 30 ºC of 30% sunflower oil emulsions stabilized by sodium caseinate

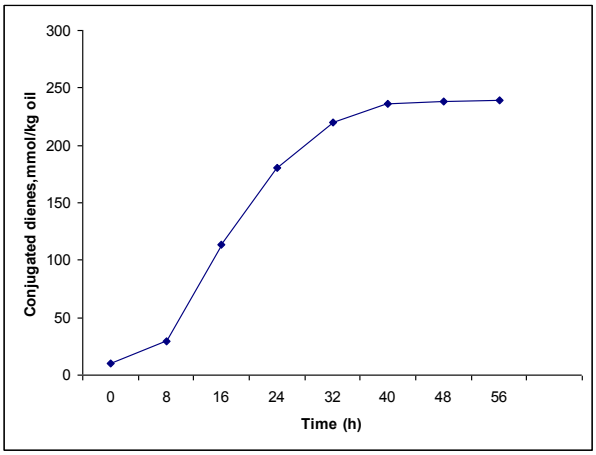

Figure 2. Formation of conjugated dienes during aging of 30% sunflower oil emulsions stabilized by sodium caseinate

The decrease of emulsion pH during aging can be attributed to the formation of short-chain aliphatic acids as secondary products of oxidation [10]. Alteration of basic amino acids of the proteins, such as lysyl or histidyl residues, because of their reaction with primary (free radicals or hydroperoxides) or secondary (aldehydes) products of oxidation may also be involved in this pH decrease [11].

Content of conjugated dienes increased slowly during the first 8 hours of agiing, then increase sharply to a plateau when around 230 -240 mmol ROOH / kg oiil after 24 h. (Figure 2). This level remained constant even for a long time of storage (10 days).

Twenty volatile compounds were identified as secondary products of oxidation in the headspace of the oxidizing emulsions. Pentane, hexanal, 1-octen-3-ol, and 2-(*E*)-octenal were chosen as markers of lipid oxidation. Pentane and hexanal were the major compounds produced and were often used as indexes of lipid oxidation in oils or fatty materials containing high level of linoleic acid (*3*, *6*, *13*, *30-32*). 1-Octen-3-ol, 2-(*E*)-octenal, and hexanal are involved, among others, inthe development of off-flavors during lipid oxidation (*14*, *33*). These four volatile compounds were even detected (from 0.1 to 10 ng) in the headspace of the fresh emulsions equilibrated 15 min at 30 ºC before SPME-GC measurements. Quantities of each volatile compound detected in the headspace of the emulsions were plotted versus time (Figure 3).

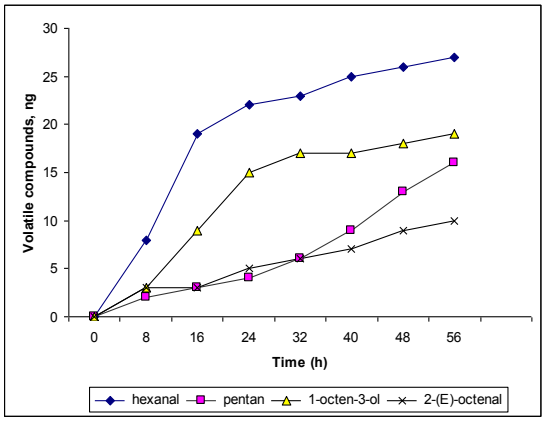

Figure 3. Evolution of volatile compounds: hexanal, 1-octen-3-ol and 2-(E)-octenal in the headspace of 30% sunflower oil emulsions stabilized by NaCas

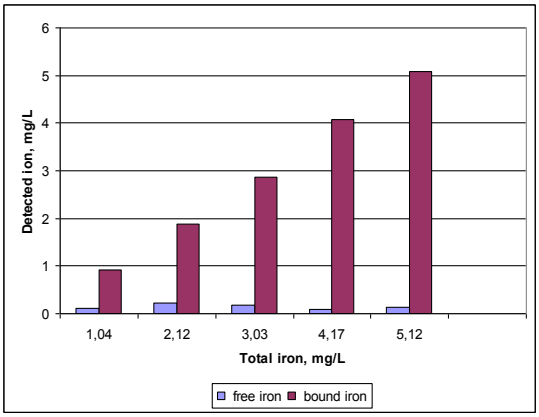

Figure 4. Retention of iron (ferrous sulfate) by pentane, solution of NaCas

The amount of hexanal desorbed from the SPME fiber increased rapidly during the first 24 h and then continued to increase slowly even after 10 days. Pentane quantity increased linearly as a function of time throughout incubation. 2-(*E*)-Octenal amount remained very low during the first 16 h and then increased progressively during aging.The amount of 1-octen-3-ol increased slowly during the first 8 h, then increased sharply until 24 h, and finally remained constant.

When 0.5-5.0 mg/L iron was added to the 1 g /L NaCas solution, nearly all the metal was bound by the proteins (Figure 4). The difference between bound iron and total added iron accounted for experimental losses as shown by values obtained on control. This result is in accordance with previous studies that demonstrated the efficient iron-chelating properties of caseins [10,11]. Indeed, the total iron-binding capacity of caseins was not reached in the present study, but the results show that all the soluble iron that could be present in the emulsions was likely bound by caseins.

Proteins can prevent or promote lipid oxidation by various mechanisms depending on their physicochemical characteristics [12]. In emulsions, lipid oxidation is greatly influenced by electrostatic interactions between ions of transition metals and droplet surface but interfacial film thickness, pH, or the presence of solutes in the aqueous phase may also interfere. A better knowledge of the factors that determine the development of lipid oxidation in the emulsions where proteins constitute the stabilizing interface can lead to an optimized use of proteins as food ingredients. Among these factors, the interactions that can take place between the metal ions and the proteins, either at the interface or in the aqueous phase of the emulsions, and their influence on emulsion chemical stability should be investigated.

These results demonstrate that the oxidative stability of protein-stabilized emulsions both depends on the interactions of proteins with transition metal ions and on the antioxidant properties of the emulsifying proteins. At pH = 6.5, that is above the pI of the proteins, the oxidative stability of NaCas-stabilized emulsions greatly depends on metal availability. The chelating properties of NaCas and electrostatic interactions favored positioning of the metal ions at the interface, a key place to initiate the oxidation reactions. The location of these compounds in emulsions, and more generally in food products, is a critical factor that should be controlled to improve their oxidative stability.

REFERENCES

1. Frankel, E. N. Free radical oxidation. In *Lipid oxidation*; The Oily Press LTD: Dundee, Scotland, 1998; pp 13-22.
2. Lethuaut, L.; Genot, C. Effect of droplet size on lipid oxidation rates of oil-in-water emulsions stabilized by protein. *J. Am. Oil Chem. Soc.* 2002, *79*, 425-430.
3. Dickinson, E. *Les colloides alimentaires*; Dickinson, E., Ed.; Masson: Paris, 1996.
4. Diaz M., Dunn C. M., McClements D. J., Decker, E. A. Use of aseinophosphopeptides as natural antioxidants in oil-in-water emulsions. *J. Agric. Food Chem.* 2003, *51*, 2365-2370.
5. Rival, S. G.; Boeriu, C. G.; Wichers, H. J. Caseins and casein hydrolysates. 2. antioxidative properties and relevance to lipoxygenase inhibition. *J. Agric. Food Chem.* 2001, *49*, 295-302.
6. Tong, L. M.; Sasaki, S.; McClements, D. J.; Decker, E. A. Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *J. Agric. Food Chem.* 2000, *48*, 1473-1478.
7. Jacobsen, C. Sensory impact of lipid oxidation in complex food systems. *Fett/Lipid* 1999, *101*, 484-492.
8. Lu, C. L.; Baker, R. C. Effect of pH and food ingredients on the stability of egg yolk phospholipids and the metal-chelator antioxidant activity of phosvitin. *J. Food Sci.* 1987, *52*, 313-316.
9. Pryor, W. A.; Castle, L. Chemical methods for the detection of lipid hydroperoxydes. In *Oxygen Radicals in Biological Systems*; Packer, L., Ed.; Academic Press: Orlando, FL, 1984; pp 293-295.
10. Mei, L.; McClements, D. J.; Wu, J.; Decker, E. A. Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl. *Food Chem.* 1998, *61*, 307-312.
11. van Ruth, S. M.; Roozen, J. P.; Posthumus, M. A.; Jansen, F. J.H. M. Volatile composition of sunflower oil-in-water emulsions during initial lipid oxidation: influence of pH. *J. Agric. Food Chem.* 1999, *47*, 4365-4369.
12. Guerin-Dubiard, C.; Anton, M.; Dhene-Garcia, A.; Martinet, V.; Brule, G. Hen egg and fish egg phosvitin: composition and iron binding properties. *Eur. Food Res. Technol.* 2002, *214*, 460-464.