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Impact of laccase on the colour stability of structured oil-in-water emulsions

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ABSTRACT

The optical properties of food emulsions play a key role in determining their perceived quality because they are the first sensory cue that many consumers receive. The purpose of the current study was to investigate the impact of a cross-linking enzyme (laccase) on the appearance of structured oil-in-water emulsions containing a lipophilic model colorant (Nile red). A layer-by-layer electrostatic deposition approach was used to prepare oil-in-water emulsions stabilized by interfacial protein-pectin complexes under acidic conditions (pH 3.5, 10 mM citrate buffer). Laccase (an oxidoreductase) was then added to the system, since this enzyme is often used to covalently cross-link interfacial biopolymer layers. The optical properties of the emulsions were monitored during storage using spectral reflectance to determine the L^*a^*b values, while the physical properties were monitored by measuring changes in droplet surface charge and particle size distribution. No changes in the size or charge of the droplets were observed during storage, indicating that the emulsions had good physical stability. In the absence of laccase, the emulsions were stable to colour fading, but in the presence of laccase rapid colour changes occurred (red to blue to white). These results have important implications for the formation of structured food emulsions containing certain types of food dyes.

1. Introduction

Structured food colloids are being developed for a variety of applications within the food industry because of the enhanced functional attributes, including encapsulation of bioactive components, modulation of rheological properties, control of food digestibility, and alterations in optical properties (McClements, 2012; Zeeb, Fischer, & Weiss, 2014; Zeeb, Stenger, Hinrichs, & Weiss, 2016). The appearance of food colloids is of critical importance because it is the first impression that most consumers experience of food or beverage products (Chantrapornchai, Clydesdale, & McClements, 2001; McClements, 2002). The colour attributes of food products have been associated with various quality parameters, including perceptions of flavour, safety, or nutritional properties (Sigurdson, Tang, & Giusti, 2017). The utilization of synthetic colour additives in foods is still popular, but consumer demand for natural colorants has steadily increased due to health, ethical, and ecological reasons. However, the replacement of synthetic colorants is challenging since many natural alternatives are highly sensitive to environmental matrix conditions such as pH, proteins, metal ions, oxygen, enzymes, and light (Sigurdson et al., 2017).

Previous studies have shown that the optical properties of emulsions are determined by their interaction with light waves (Chantrapornchai et al., 2001; McClements, 2002). In general, two main factors affect emulsion appearance: (i) light scattering by droplets (which depends on their concentration, size, and refractive index) and (ii) light absorption by chromophores (which depends on their concentration and absorbance spectra). For example, the spectral reflectance of *n*-hexadecane oil-in-water emulsions increased with increasing droplet concentration (from 0 to 20%), but decreased with increasing droplet diameter (from 0.26 to 26 μm), which was attributed to changes in the light scattering efficiency of the droplets (Chantrapornchai, Clydesdale, & McClements, 1999a). The lightness and colour of emulsions has been reported to depend on dye type and concentration, which was attributed to changes in light absorbance by the chromophores (Chantrapornchai, Clydesdale, & McClements, 1999b).

In general, emulsions are thermodynamically unstable systems that are prone to destabilization through mechanisms such as coalescence, flocculation, creaming, or Ostwald ripening (Dickinson, 2010; McClements, 2004; Zeeb, Gibis, Fischer, & Weiss, 2012b; Zeeb, McClements, & Weiss, 2017). The resultant changes in local droplet concentration and particle size brought about by these instability

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mechanisms would be expected to lead to changes in the optical properties of emulsions. Indeed, studies have reported that the spectral reflectance and colour coordinates of emulsions may be altered by droplet flocculation or Ostwald ripening (Chantrapornchai et al., 2001; Weiss & McClements, 2001). Moreover, chemically-driven alterations in the emulsion composition or structure, such as those resulting from oxidation, hydrolysis, or Maillard reactions might also have a major impact on the appearance of emulsions. For example, polyphenol oxidases (such as tyrosinase and laccase) are typically involved in the browning reactions of fruits and vegetables, which leads to unacceptable pigmentation in the final product (Claus & Decker, 2006; Octavio, Ma, Ricardo, & Francisco, 2006). Laccase has also been shown to promote colour bleaching of lutein-loaded emulsions, which was attributed to its ability to promote oxidation of the carotenoid (Beicht, Zeeb, Gibis, Fischer, & Weiss, 2013). Consequently, food scientists and manufacturers are greatly interested in understanding the physical and chemical factors that impact the appearance of food emulsions to improve their ability to control, maintain, and protect their colour properties throughout processing, storage, transport, and utilization.

The objective of the present study was to gain further insights into the impact of polyphenol oxidases on the colour stability of structured oil-in-water emulsions. Initially, emulsions containing a lipophilic model colorant (Nile red) were prepared using a well-established interfacial engineering technique based on electrostatic deposition of oppositely charged biopolymers on the droplet surfaces. Laccase (from *Trametes versicolor*) was then added to the emulsions, because this enzyme has previously been shown to be suitable to cross-link interfacial biopolymer complexes, and thereby improve emulsion stability against environmental stresses (Littoz & McClements, 2008; Zeeb, Gibis, Fischer, & Weiss, 2012a). The impact of adding the enzyme on the physical and optical properties of the emulsions was then assessed during storage under various environmental conditions.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI 895) was obtained from Fonterra (Auckland, New Zealand). As stated by the manufacturer, the composition of the WPI used was 93.9% protein (69.2% β-lactoglobulin, 14.2% α-lactalbumin, 3.3% bovine serum albumin, 2.1% immunoglobulin G, 1.6% glycomacropeptide, 1.2% proteose peptone 5), 4.7% moisture, 0.3% fat, 0.4% carbohydrates, and 1.5% minerals. Sugar beet pectin (Betapec RU 301, Batch-No. 1093135) was donated by Herbstreith & Fox (Neuenbürg, Germany). This pectin has been determined to have a degree of esterification of 55%, a galacturonic acid content of 65%, and a ferulic acid content of 0.75 ± 0.02% (Zeeb et al., 2012a). Laccase (Batch-No. 0001437590, from *Trametes versicolor*) and Nile red were purchased from Sigma-Aldrich Co. (Steinheim, Germany). The laccase obtained was reported to have > 10 units per mg (AU) enzyme. Medium chain triglycerides (MCT, Miglyol 812) were obtained from Cremer Oleo GmbH & Co. KG (Witten, Germany). Citric acid monohydrate (purity = 99.5%), tri-sodium citrate dihydrate (purity ≥ 99%), analytical grade hydrochloride acid (HCl, ≥ 32%, p.a., Batch-No. P074.4) and sodium hydroxide (NaOH, purity ≥ 99%, Batch-No. 6771.5) were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Distilled water was used for the preparation of all samples. In addition, emulsion samples are expressed on a weight basis (% w/w).

2.2. Fabrication of structured emulsions using electrostatic deposition

Oil-in-water emulsions coated by protein-polysaccharide interfacial complexes were prepared based on a well-established interfacial engineering approach (Guzey & McClements, 2006; Zeeb, Fischer, & Weiss, 2011; Zeeb et al., 2012a, 2012b). Briefly, emulsions

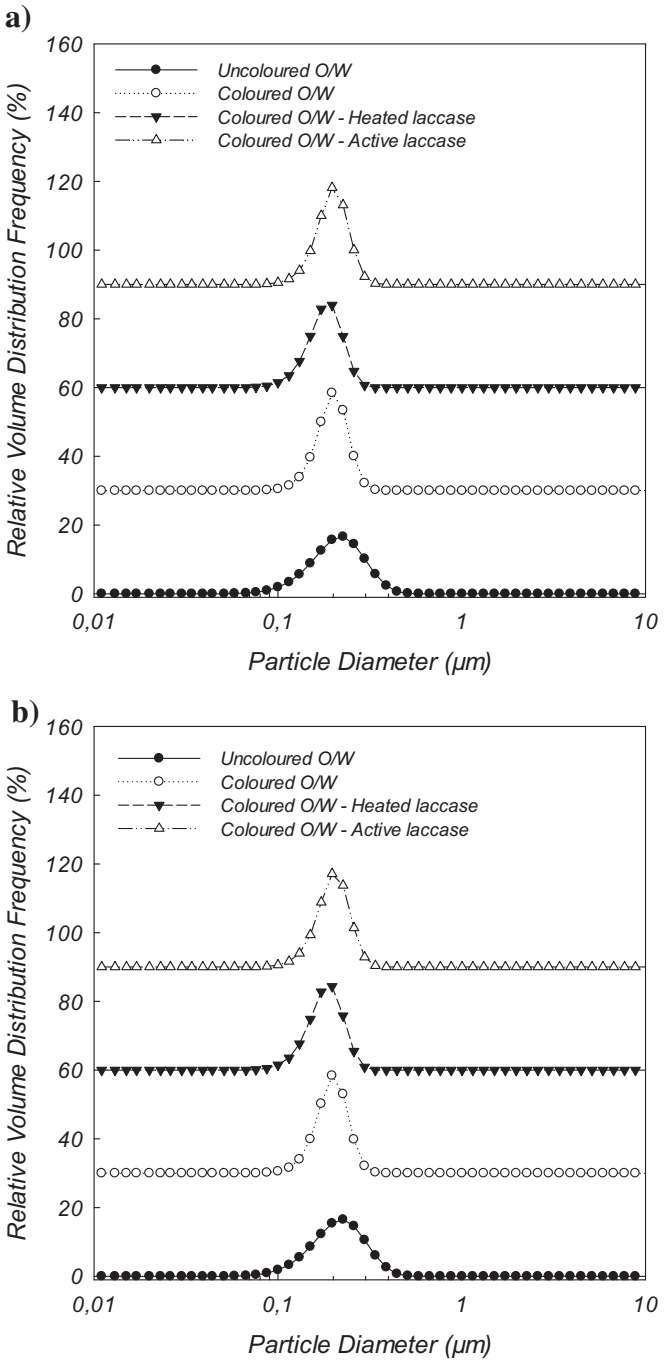

Fig. 1. Time-dependent particle size distribution of structured oil-in-water (o/w) emulsions (1% MCT, 10 mM citrate buffer, pH 3.5): (A) day 1 and (B) day 7.

were prepared by homogenizing 10% (w/w) oil phase (0.0006% Nile red in MCT) and 90% (w/w) aqueous phase (1% WPI in 10 mM citrate buffer, pH 3.5). Initially, a coarse emulsion was formed by blending the oil and aqueous phases in a high shear blender (Silent Crusher M, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 20,000 rpm for 2 min. The coarse emulsion was then passed through a microfluidizer (M110-EH-30, Microfluidics International Corporation, Newton, MA) five times at 1000 bar and then diluted to a final oil droplet concentration of 1% using an aqueous beet pectin solution (0.2%, pH 3.5, 10 mM citrate buffer). The oil phase used (MCT) was selected because it is colourless and therefore does not contribute to the colour of the emulsions. An *uncoloured emulsion* was prepared using the same conditions but in the absence of Nile red so as to act as a control.