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Interfacial protein engineering for spray-dried emulsions – Part II: Oxidative stability

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ABSTRACT

The aim of this work was to investigate how the oxidative stability of encapsulated oil is affected by the humidity response of a Na-caseinate–maltodextrin matrix. Furthermore, the effect of modification of the interfacial Na-caseinate layer through cross-linking was studied. For this purpose, two model spray-dried emulsions containing sunflower oil, maltodextrin, and either non-cross-linked or cross-linked Na-caseinate were stored at different relative humidities (RHs; ~0%, 11%, 33%, 54%, and 75%). Increasing RH improved the oxidative stability of the spray-dried emulsions. This behaviour was mainly linked to the loss of individual powder particles upon caking and collapsing of the matrix at RH 75%. Oxidation of non-encapsulated surface lipids with a proportion of ca. 5% of total lipids was only twofold compared to total lipids. Excess protein on particle surfaces may have delayed oxidation, e.g., by its radical scavenging activity. Under several storage conditions, cross-linking of the protein slightly improved the oxidative stability.

1. Introduction

Lipid oxidation is a major chemical reaction that leads to deterioration of foods containing unsaturated lipids. Oxidation may decrease nutritional value and cause the formation of undesired flavours as well as compounds with possible adverse health effects. Lipid oxidation begins either by free radical-mediated autoxidation, which can be accelerated by factors such as metal ions and heat, or by photo-oxidation in the presence of UV light and sensitisers or enzymes. The primary lipid oxidation products are hydroperoxides, which decompose to alkoxyl and peroxyl radicals that both propagate the chain, increasing the rate of radical turnover, and undergo various recombinations and scissions to form secondary products, e.g., aldehydes, ketones, and alcohols as well as dimers and polymers (Schaich, 2005). Because lipid oxidation strongly limits the shelf life of foods, there is a need to develop structures that can limit contact between unsaturated lipids and oxygen. One approach is to encapsulate the lipids in a carbohydrate and/or protein matrix; this can be accomplished by spray-drying or freeze-drying oil-in-water emulsions. In dried emulsion powders, the stability of lipids is controlled by the properties of the wall material and the oil-matrix interface, the presence of antioxidative and pro-oxidative factors, and the storage conditions (Gharsallaoui et al., 2012; Jimenez, García, & Beristain, 2006; Polavarapu, Oliver, Ajlouni, & Augustin, 2011).

When oil is dispersed, as in spray-dried emulsions, its surface area becomes considerably larger and its stability is expected to be governed by contact and mass transfer with the surroundings, where reactants such as oxygen, radicals, and metal catalysts exist. Under dry conditions, a higher metal catalyst activity (less hydration) and higher hydroperoxide decomposition (less hydrogen bonding to water) may accelerate oxidation. Moisture sorption to dry material may decrease lipid oxidation by quenching free radicals. Labuza's stability map suggests that a minimum oxidation rate is reached at the monolayer water content of the system. Above the monolayer water content, mobilisation of catalysts and oxygen, as well as swelling of the matrix that leads to exposure of more catalytic sites, are thought to accelerate oxidation (Labuza, 1980). Roos and Karel (1991) emphasised the role of moisture-related changes in the physical state of the matrix to explain oxidative stability. Diffusion-controlled chemical reactions are expected to proceed slowly in a glassy state and to be accelerated after uptake of sufficient moisture, leading to a rubbery state. The oxygen transfer rate within starch polymers was shown to be higher at high humidity because the higher water content increased the solubility of oxygen in the matrix and facilitated diffusion across the plasticised matrix (Forssell, Lahtinen, Lahelin, & Myllärinen, 2002).

However, as an equilibrium concept, Labuza's stability map has its limitation for predicting the behaviour of meta-stable systems, such as encapsulated oils and other foods. For example, at low

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moisture content, the morphology of powder particles and the presence of pores and cracks may contribute to oxygen transport, thereby increasing the oxidation rate (Hardas, Danviriyakul, Foley, Nawar, & Chinachoti, 2002; Jimenez et al., 2006). Oxidation rate is increased in a rubbery state when lipids have been excluded from collapsed or crystallised matrix. Oxidation, however, is decreased if, the structural collapse of a rubbery matrix entraps lipids, consequently decreasing porosity and oxygen diffusion (Nelson & Labuza, 1992).

Our previous studies suggested a major difference between protein versus carbohydrate wall materials with respect to their ability to protect oil from oxidation at humid conditions (Partanen, Hakala, Sjövall, Kallio, & Forssell, 2005; Partanen et al., 2008). Proteins are more likely than carbohydrates to interact with lipids, based on their surface active properties, which allow them to serve as emulsifiers that can form an interfacial layer around oil droplets. They have also been shown to quench radicals to decrease lipid oxidation (Schaich & Karel, 1976). Proteins can undergo structural changes, such as polymerisation, cross-linking, or scission through reactions with the primary and secondary products of lipid oxidation (Schaich, 2008). These structural changes can affect the function of proteins in dried emulsions. Park, Murakami, Mori, and Matsumura (2005) showed improved stability of a powder model system by the addition of proteins and peptides. Hence, proteins can have structural as well as chemical functions in dried emulsions. Research on oil microencapsulation has so far focused particularly on the technological aspects and on carbohydrates as wall materials. More research is needed to understand the role of the interfacial protein layer and the possibilities of interfacial engineering in terms of maintaining the stability of oil.

Our main aim in the present study was to investigate in a model system how the oxidative stability of encapsulated polyunsaturated oil is affected by humidity-induced changes in the physical structure of a carbohydrate–protein matrix. As a second aim, the possibility to improve oxidative stability by modification of the interfacial protein layer through enzymatic cross-linking was studied. Therefore, two spray-dried emulsions containing sunflower oil as a simple model for polyunsaturated oil, maltodextrin as wall material, and non-cross-linked or cross-linked Na-caseinate as a protein component, were produced. They were stored at five different relative humidities (RHs) at 22 °C for 29 weeks. The stability of the dried emulsions was studied at given time points by methods that recognise different oxidation states. Fatty acid composition, peroxide value (PV), and α-tocopherol contents were measured separately for surface and total lipids. Hexanal content of the powders was measured by static headspace. For comparison, bulk sunflower oil was stored at the same conditions. The effect of RH on the release of hexanal from spray-dried emulsions was studied in an additional experiment to support the interpretation of the storage tests results. All results were discussed in relation to the physical characterisation (Moisio et al., 2013) of the same spray-dried emulsions.

2. Materials and methods

2.1. Reagents

To obtain selected RHs, phosphorous pentoxide (RH ~0%), lithium chloride (RH 11%), magnesium chloride hexahydrate (RH 33%), magnesium nitrate hexahydrate (RH 54%), and sodium chloride (RH 75%) were purchased from Sigma–Aldrich (Steinheim, Germany). Reagents for fatty acid analysis included sodium hydroxide (Merck, Darmstadt, Germany), boron trifluoride in methanol (Sigma–Aldrich, Steinheim, Germany), sodium sulphate (purity 99%, VWR International, West Chester, PA, USA), C19:0 methyl ester, and a GLC-63 mixture of fatty acid methyl esters (Nu-Check Prep, Elysian, MN, USA). Peroxide values (PV) were determined using iron(III) chloride (Titrisol®; Merck, Darmstadt, Germany), iron(II) chloride tetrahydrate, and ammonium thiocyanate (Riedel-de Haën, Seelze, Germany) as reagents. We purchased α-tocopherol as an isomer kit from Merck (Art. 15496). All solvents used were of HPLC grade: methanol (J.T. Baker, Deventer, The Netherlands), 1-decanol (⩾95%; Fluka Chemie GmbH, Buchs, Germany), 1,4-dioxane (Riedel-de Häen, Seelze, Germany), Milli-Q water (Millipore Corp., Bedford, MA, USA), ethanol (99.5%; ALTIA, Rajamäki, Finland), heptane and 2-propanol (Rathburn Chemicals Ltd., Walkerburn, Scotland).

2.2. Sample materials

The commercially available sunflower oil used in this study was obtained from Bunge Finland Oy (Raisio, Finland). Sodium caseinate from Kaslink Foods (Koria, Finland) contained at minimum 90% protein and at maximum 1.5% fat, 4.0% ash, and 5.5% moisture. Maltodextrin DE 22.2 was purchased from Grain Processing Corporation (Muscatine, IA, USA). Emulsions containing sunflower oil (30% dry matter), either non-cross-linked or cross-linked Na-caseinate (3% dry matter), maltodextrin (67% dry matter), and Milli-Q water, were prepared and spay-dried according to Moisio et al. (2013). Cross-linking of Na-caseinate was performed in 3% protein solution prior to emulsification. Transglutaminase activity was 100 nkat/g of protein and incubation time was 4 h at room temperature. Na-caseinate, sunflower oil and water were pre-homogenised with a Heidolph Diax 900 (Germany) homogeniser and afterwards high-pressure-homogenised with a Microfluidics M-110Y homogeniser. The homogenised emulsion was mixed with a maltodextrin solution and the final mixture was spray dried with a Niro Mobile Minor (Denmark) laboratory spray drier using a rotary atomiser with an inlet air temperature of 180 °C and an outlet temperature of 80 ± 2 °C (Moisio et al., 2013).

2.3. Storage tests

The non-cross-linked spray-dried emulsion (NCL), the cross-linked spray-dried emulsion (CL), and the bulk sunflower oil were stored over a period of 29 weeks at 22 °C, in the dark, in acrylic desiccator cabinets (305 × 305 × 305 mm, Nalgene Nunc™, USA) stabilised at five RHs: ~0%, 11%, 33%, 54%, and 75%. The RHs were obtained with dry phosphorus pentoxide and saturated salt solutions. Stability of the powders was studied at selected time points. Powder samples (3 g) were stored in open petri dishes as 2–3 mm layers for chemical analyses or at 0.50 g in open 20-mL headspace vials (i.d., 22.5 mm) as 2–3 mm layers for analysis of hexanal. Oil samples were stored in similar open headspace vials: 3 g samples for chemical analyses and 0.50 g samples for analysis of hexanal. One powder and one oil sample for chemical analyses and three replicates for hexanal measurements were stabilised for each time point at the respective RHs. Chemical analyses including fatty acid composition, PV, and α-tocopherol content, were conducted separately from triplicate surface and total lipid extracts, whereas hexanal was measured directly from the triplicate samples. In addition, the original quality of the powders and the oil was characterised.

2.4. Extraction of surface and total lipids for chemical analyses

Surface and total lipids were extracted using the methods of Baik et al. (2004) after small modifications. For the extraction of surface lipids, a 0.3 g sample in a tube was washed with 5 mL of heptane by mildly shaking for 15 min. The tube was then centrifuged (3000 rpm for 2 min). The organic phase was separated from