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Analytical Methods

Direct technique for monitoring lipid oxidation in water-in-oil emulsions based on micro-calorimetry

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ABSTRACT

An experimental device based on the measurement of the heat flux dissipated during chemical reactions, previously validated for monitoring lipid oxidation in plant oils, was extended to follow lipid oxidation in water-in-oil emulsions. Firstly, validation of the approach was performed by correlating conjugated diene concentrations measured by spectrophotometry and the heat flux dissipated by oxidation reactions and measured directly in water-in-oil emulsions, in isothermal conditions at 60 °C. Secondly, several emulsions based on plant oils differing in their n-3 fatty acid content were compared. The oxidability parameter derived from the enthalpy curves reflected the α-linolenic acid proportion in the oils. On the whole, the micro-calorimetry technique provides a sensitive method to assess lipid oxidation in water-in-oil emulsions without requiring any phase extraction.

1. Introduction

Emulsions are metastable systems made out of two immiscible fluids, e.g. an oil phase and an aqueous phase. In processed foods, lipids are most often involved in emulsified formulations, either as the dispersed phase (oil-in-water; O/W), like in commercial salad dressings, mayonnaise, sauces, or as the continuous phase (water-in-oil; W/O), like in margarines. Whatever the emulsion type, the presence of polyunsaturated fatty acids prone to oxidation restricts the potential use of these products in the food market. Indeed, lipid oxidation can cause food damage because of undesirable aroma, texture, shelf life and color modifications (Frankel, 1991; Fritsh, 1994; Jacobsen, 1999), in addition to nutritional degradation (Halliwell & Gutteridge, 1990; Kubow, 1992). Many qualitative and quantitative methods, standardized or not, have been developed to assay lipid oxidation (Shahidi & Zhong, 2005). The simplest are based on chemical tests and may lead to normalized values (for example, peroxide value for primary oxidation products, para-anisidine value for secondary oxidation products). These methods have major drawbacks as they require organic solvents and they are time consuming. In the case of multicomponent food products such as emulsions, characterization of lipid oxidation requires prior extraction of the oil phase. Since this preliminary step may involve oxidative conditions (like mixing steps), antioxidant species are often added at the beginning of the extraction process. Moreover, some oxidized lipid derivatives are surface-active or water-soluble and will not therefore be extracted by nonpolar solvents (Coupland & McClements, 1996). Thus, direct methods avoiding any extraction step are highly sought after to characterize lipid oxidation (Frankel, 2012; Shahidi & Zhong, 2005). Only few direct methods are available, such as headspace gas chromatography to measure secondary volatile products (e.g. aldehydes) and conductivity allowing the determination of the oil stability index (OSI) by measuring the formation of volatile organic compounds (Frankel, 2012; Shahidi & Zhong, 2005). Beside these physicochemical methods, sensory

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analysis is also used to determine the extent of oxidation in food products (Frankel, 2012; Malcolmson, 2005). In this context, the development of novel methods to characterize lipid oxidation would be of great interest, especially regarding primary oxidation products.

We recently developed a micro-calorimeter device capable of measuring continuously the heat flux dissipated during oxidation reactions under isothermal conditions. We showed that the conjugated diene concentration (primary oxidation compounds) measured in neat plant oils by spectrophotometry and the heat flux dissipated by oxidation reactions were highly correlated (Dridi, Sommier et al., 2016). Because measurements can be operated with a resolution of a few μW, the sensitivity level of this micro-calorimeter is suitable to assess lipid oxidation. Thus, the aim of the present study was to extend the use of this apparatus to water-in-oil emulsions. For this purpose, emulsions based on virgin plant oils varying in their n-3 polyunsaturated fatty acid content were formulated and analyzed under isothermal conditions at 60 °C. The heat fluxes emanating from the oxidative reactions were measured. To validate the method, the concentration of conjugated hydroperoxide dienes was measured by spectrophotometry after oil extraction for correlation with the enthalpy signal.

2. Experimental part

2.1. Materials

Four different virgin plant oils were used, namely rapeseed oil (Vigean, Clion-sur-Indre, France), linseed oil (Bioplanète, Bram, France), camelina oil (Bioplanète, Bram, France) and olive oil (Elbarka, Ksar Said, Tunisia). The oils mainly differed in their α-linolenic acid content: 0.7 wt% in olive oil, 8.7 wt% in rapeseed oil, 35.1 wt% in camelina oil, and 57.3 wt% for linseed oil (Dridi, Sommier et al., 2016). Upon receipt, the initial lipid oxidation status of the oils was quantified by the peroxide value according to the standardized method AFNOR T60-220. All peroxide values were lower than 4 (Dridi, Sommier et al., 2016). Hexane (purity 96%) and sodium chloride were purchased from Sigma Aldrich (Steinheim, Germany). Lipohilic surfactants, i.e. polyglycerol polyricinoleate (PGPR) and distilled mono-glycerides (DMG) were from Palsgraad (Juelsminde, Denmark).

2.2. Emulsion preparation and structural characterization

W/O emulsions were prepared as already described in (Dridi, Essafi, Gargouri, Leal-Calderon, & Cansell, 2016). Briefly, the continuous phase was a mixture of 96 wt% plant oil, 2 wt% PGPR, and 2 wt% DMG. The aqueous phase contained sodium chloride at 0.1 M, to inhibit destabilization phenomena like Oswald ripening (Kabalnov, 2001) and coalescence (Aronson & Petko, 1993). W/O emulsions were prepared by gradually dispersing the aqueous phase into the oil phase, up to a fraction of 80 g per 100 g, by means of a homogenizer (RZR 2102 control Z Heidolph, Schwabach, Germany). Emulsification is provoked by the viscous stress applied to the external phase which is transmitted to the droplets. These latter adopt a thread-like shape and are fragmented into smaller droplets (Mabille et al., 2000). In order to produce break-up at relatively low shear rates (laminar regime), a sufficiently large average viscosity is required. This was achieved by adopting a high-dispersed phase content and a large PGPR/MDG concentration (12 wt% each). After the emulsification step, the emulsions were diluted with oil in order to set the final droplet fraction at 40 wt % as well as the PGPR/MDG concentration at 2 wt% each. The average aqueous droplet size was about 1 μm as confirmed by particle size measurements based on optical microscopy (Leica DM2500P microscope equipped with an oil immersion × 100 objective, Zeiss, Germany) and static light scattering (Mastersizer 2000, Malvern Instruments). For each plant oil, the emulsion was prepared at least in triplicate.

2.3. Lipid oxidation measurements

Lipid oxidation in W/O emulsions was monitored by micro-calorimetry by measuring the reaction heat in the isothermal mode at 60 °C. The micro-calorimeter device was described in (Dridi, Sommier et al., 2016). Briefly, it is composed of four rooms, one for the reference cell, three others for the samples. The quantitative heat flux from the oxidative reactions was deduced from the voltage given by thermopiles and measured, in parallel, using a digital multimeter model Agilent 34410 A with $6^{1/2}$ digit resolution. Sample cells were made with copper. This metal was adopted due to its very high thermal conductivity (401 $W \cdot m^{-1} \cdot K^{-1}$) despite the fact it can act as a catalyst for oxidation (Andersson & Lingnert, 1998; Osborn-Barnes & Akoh, 2003). Instrumental control and data acquisition were operated with a computer using Labview software, version 9.0. Sample cells had a diameter of 2.3 cm and were open to atmosphere. The empty cells located in a thermostatically controlled chamber were first heated at 60 °C. The W/O emulsion (about 0.5 g) was initially introduced in a syringe and preheated for a few minutes, at 60 °C. The injection into the cell that was performed through a hole specially designed in the chamber wall to minimize heat loses. The residence time of W/O emulsions in the calorimetric cells was always shorter than 8 h, in order to avoid any significant variation of mass due to water evaporation. Because the heat capacity of water is higher than that of oil (4.2 $J \cdot g^{-1} \cdot K^{-1}$ and 2 $J \cdot g^{-1} \cdot K^{-1}$, respectively), it took longer for the thermal signal to stabilize in emulsions than in neat oil. As a matter of fact, the presence of water droplets dispersed in the oil phase prevented the accurate measurement of the heat flow during the 2 first hours. For this reason, the heat signal was integrated only after 2 h for the enthalpy calculation. For each formulation, at least three independent heat flux measurements were performed. Enthalpy values are means ± Standard Deviation (SD).

For incubation times of 4, 5, 6 and 8 h, the cells containing the W/O sample were removed from the micro-calorimeter, for primary oxidation product analysis. Oil was extracted from the O/W emulsions by centrifugation (30 min, 14,000 rpm, minispin plus spinner, Brumath, France). The extracted oil phase was analyzed by the conjugated diene hydroperoxide products produced in the early stages of the lipid oxidation process (Schaich, 2005). Their concentration was deduced from optical density (OD) measurements at 233 nm, as described in (Dridi, Sommier et al., 2016), using a Hitachi (U-2810) double beam spectrophotometer and a 1 cm thick quartz cell. Pre-weighted oil was dissolved in hexane to a final lipid concentration that ensured an absorbance measurement in the spectrophotometer linear range, so that OD was simply proportional to the conjugated diene concentration according to the Beer-Lambert's law. In order to take into account the dilution factor imposed by the measuring conditions, the OD measured at 233 nm was normalized by the mass of lipids (mg) per unit volume of solution (mL) (Dridi, Sommier et al., 2016). For each sample of extracted oil, at least three independent measurements were performed. Values are means ± SD.

3. Results and discussion

In this work, different plant oils were used to evaluate the effect of the polyunsaturated fatty acid content on the oxidability of W/O emulsions. The emulsion formulations based on 0.1 M NaCl aqueous phase and a mixture of PGPR and DMG in the oil phase were