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Performance of structured lipids incorporating selected phenolic and ascorbic acids ☆

Eliza Gruczynska [a], Roman Przybylski [a], Felix Aladedunye [a,b,*]

[a] Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada
[b] Max Rubner-Institut (MRI), Federal Research Institute for Nutrition and Food, Department for Safety and Quality of Cereals, Working Group for Lipid Research, Schützenberg 12, D-32756 Detmold, Germany

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ABSTRACT

Conditions applied during frying require antioxidant which is stable at these conditions and provides protection for frying oil and fried food. Novel structured lipids containing nutraceuticals and antioxidants were formed by enzymatic transesterification, exploring canola oil and naturally occurring antioxidants such as ascorbic and selected phenolic acids as substrates. Lipozyme RM IM lipase from *Rhizomucor miehei* was used as biocatalyst. Frying performance and oxidative stability of the final transesterification products were evaluated. The novel lipids showed significantly improved frying performance compared to canola oil. Oxidative stability assessment of the structured lipids showed significant improvement in resistance to oxidative deterioration compared to original canola oil. Interestingly, the presence of ascorbic acid in an acylglycerol structure protected α-tocopherol against thermal degradation, which was not observed for the phenolic acids. Developed structured lipids containing nutraceuticals and antioxidants may directly affect nutritional properties of lipids also offering nutraceutical ingredients for food formulation.

1. Introduction

Recently there has been great interest in naturally occurring antioxidants with a high reducing ability, such as ascorbic and phenolic acids. However, the hydrophilic character of these compounds reduces their applicability and effectiveness in stabilizing fats and oils, particularly during frying (Stamatis, Sereti, & Kolisis, 1999). Therefore, the enzymatic incorporation of ascorbic and/or phenolic acids into triacylglycerol structure, offering structured lipids with solubility in oil, became a field of great interest. There are several published papers dealing with lipase-catalysed synthesis of phenolic lipids. For instance, dihydrocaffeic acid was incorporated into triacylglycerol structure of fish liver oil (Sabally, Karboune, St-Louis, & Kermasha, 2007) and trilinolein and trilinolenin (Sabally, Karboune, St-Louis, & Kermasha, 2006). Safari, Karboune, St-Louis, and Kermasha (2006) converted selected phenolic acids into phenolic lipids by transesterification with triolein. Karboune, Safari, Lue, Yeboah, and Kermasha (2005) studied lipase-catalysed biosynthesis of cinnamoylated lipids at different molar ratios of cinnamic acid to triolein. Xin et al. (2009) carried out their studies of ethyl ferulate and triolein with the intention to prepare feruloyl oleins in a solvent-free medium. However, the reports on structured lipids incorporating phenolic compounds focused on their synthesis with sole aim at antiradical scavenging activity, neglecting frying performance and oxidative stability of developed structured lipids.

As far as ascorbic acid is concerned, the reports on its enzymatic modification give attentions principally to production of free fatty acids ascorbyl derivatives and their utilisation as additives for the stabilization of fats and oils (Torres, Kunamneni, Ballesteros, & Plou, 2008). For example, the enzymatic acylation of ascorbic acid with palmitic acid in ethyl methyl ketone, pyridine, dioxane, tetrahydrofuran, or 2-methyl-2-butanol has been investigated (Enomoto, Miyamori, Sakimae, & Numazawa, 1990; Sakashita, Miyamoto, & Sakimae; 1992; Humeau, Girardin, Coulon, & Miclo, 1998; Humeau, Girardin, Rovel, & Miclo, 1998). Alkyl or vinyl esters have been also successfully employed as acyl donors in the enzymatic syntheses of ascorbyl derivatives by lipases, especially that from *Candida antarctica* (Song & Wei, 2002).

However, to the best of our knowledge, there is no scientific data on enzymatic incorporation of ascorbic acid into triacylglycerol

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* Corresponding author at: Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada. Tel.: +1 403 894 5543.
E-mail address: felix.aladedunye@alumni.uleth.ca (F. Aladedunye).

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structure, and consequently assessing frying performance and oxidative stability of lipids containing incorporated ascorbic and other phenolic acids. Thus, the aim of this study was implementation of ascorbic and selected phenolic acids into canola oil triacylglycerols structure and assessment of frying performance and oxidative stability of developed structured, hybridized acylglycerols.

2. Materials and methods

2.1. Materials

Commercially refined, bleached and deodorized regular canola oil (CAN) was donated by Richardson Oilseed Processing (Lethbridge, Canada). Its parameters were as follows: tocopherols content = 317 μg/g of α-tocopherol, and 353 μg/g of γ-tocopherol, free fatty acids (FFA) at 0.05%, mono- and diacylglycerols at 0.5%, peroxide value (PV) 2.9 meq/kg. Ascorbic, gallic, ferulic, caffeic and p-hydroxyphenylacetic acids were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Lipozyme RM IM was purchased from Sigma–Aldrich Chemical Co. (St Louis, MO, USA). All chemicals and solvents used in this study were of analytical grade. Standards of triacylglycerols were purchased from Larodan Fine Chemicals AB (Malmo, Sweden). Tocopherols standards were obtained from Calbiochem-Novabiochem (San Diego, CA, USA). Standards of fatty acid methyl esters were purchased from Nu-Chek-Prep Inc. (Elysian, MN, USA).

2.2. Transesterification

Vials containing mixtures of canola oil with ascorbic or other tested phenolic acids in organic solvents were flushed with nitrogen and placed in a hybridization oven (Tek Star, Greensboro, NC, USA). The transesterification reactions were performed under the following optimised conditions: substrate molar ratio 5:1 (CAN:ascorbic acid, mol/mol) and 8:1 (CAN:phenolic acids, mol/mol), temperature 50 °C for ascorbic acid and 60 °C for phenolic acids, reaction time 24 h and 7 days, respectively. The transesterification reactions were performed in acetone and ethyl methyl ketone correspondingly, for ascorbic and other phenolic acids. After thermal equilibration at desired temperature, catalyst (Lipozyme RM IM lipase) was added at 30 mg/mL and 45 mg/mL for ascorbic and phenolic acids, respectively. The transesterification was performed with continuous shaking and reaction was monitored by HPLC as previously reported (Sabally et al., 2006; Safari et al., 2006). Control experiments without enzymes were carried out in parallel.

2.3. Isolation and purification of transesterified fats

The transesterification reaction was stopped by removing the enzyme through filtration and the solvents were evaporated under vacuum using rotary evaporator (BÜCHI Labortechnik AG, Flawil, Switzerland) at 40 °C. In order to remove unreacted acids, the transesterified fats were repeatedly washed with methanol until no significant amounts of acids were detected in the methanol wash, monitoring by HPLC.

The HPLC analysis was carried out using a Finnigan Surveyor HPLC system (Thermo Electron Corp., Waltham, MA, USA). The sample was separated at ambient temperature on a reversed phase C18 column (4 μm; 300 × 3.9 mm; Novapak, Waters, MA) using a mobile phase consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at a flow rate of 1 mL/min using the following gradient: 100% A (0 min); 95% A (5 min); 25% A (40 min); 100% B (45 min); 100% B (50 min); 100% A (55 min). Injection volume was 10 μL and the analytes were detected at 254 and 280 nm using a Finnigan Surveyor photodiode-array (PDA) detector.

2.4. Frying fast test

Hybrid acylglycerols were tested for frying performance utilising a fast frying test according to Aladedunye & Przybylski, 2011 with some modifications. Briefly, 12 g of the transesterification products were weighed into a glass beaker (Pyrex, USA; outer diameter 35 mm, capacity 30 mL). Octagonal stir bars (9.5 × 25 mm, Fischer Scientific, USA) were placed into the vessel, increasing the final surface to volume ratio to 0.42. Then vessel was heated at 180 ± 5 °C for 10 min and 1.2 g of formulated starch (a mixture of potato starch, glucose and silica gel at 4:1:1, wt/wt/wt) added. Heating was maintained for 2 h with intermittent stirring at 400 rpm for 20 min. About 0.5 mL of oil samples were withdrawn after 30th, 70th, 100th and 120th min of heating. Selected times reflects frying time at standard conditions for 1, 3, 5 and 7 days based on the amount of polar components formed. Frying performance of fats was assessed by measurement of total polar components, composition of polymeric materials, fatty acid composition, and residual tocopherols determinations.

2.5. Schaal Oven test

The hybrid acylglycerols were tested for oxidative stability using Schaal Oven protocol. The samples were stored at 65 °C in vials in the dark for 5 days and examined at 24 h intervals. Oxidative stability of fats was assessed by peroxide value and residual tocopherols.

2.6. Tocopherols analysis

Tocopherols were analysed according to AOCS Official Method Ce 8-89 (Firestone, 2009). Briefly, oil samples (50 mg) were weighed directly into autosampler vials and dissolved in 1.0 mL hexane. Analyses were performed on a Finnigan Surveyor HPLC (Thermo Electron Corp., Waltham, MA, USA) with a Finnigan Surveyor Autosampler Plus and Finnigan Surveyor FL Plus fluorescence detector set for excitation at 292 nm and emission 324 nm. The column was a normal phase Diol column (5 μm; 250 × 4.6 mm; Monochrom, Varian, CA). Of each sample, 10 μL was injected. Mobile phase consisted of 7% methyl-*tert*-butyl ether in hexane with a flow rate of 0.6 mL/min. The amounts of tocopherols were quantified using external calibration with each isomer calibrated individually.

2.7. Size exclusion chromatography

The composition of polar components was assessed using high performance size exclusion chromatography (HPSEC) according to ISO Method 16931 (2007). Separation was performed on a Finnigan Surveyor liquid chromatograph (Thermo Electron Corporation, Waltham, MA). Components were separated on three size exclusion Phenogel columns connected in series (500 A, 100 A and 50 A; 5 μm, 300 × 4.6 mm; Phenomenex, Torrance, CA) kept at 30 °C. Tetrahydrofuran (THF) was used as a mobile phase at flow rate of 0.3 mL/min. Sample of 10 μL was injected, and eluting components detected with evaporative light scattering detector (Sedex 75; Sedere, Alfortville, France), operated at 30 °C with purified air at pressure of 2.5 bar.

2.8. Total polar compounds (TPC)

TPC were determined by gravimetric method following AOAC Method 982.27 (1990) with Schulte modification (Schulte, 2004).