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Stability of encapsulated olive oil in the presence of caffeic acid

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ABSTRACT

The aim of this study was to investigate the influence of microencapsulation and addition of the phenolic antioxidant caffeic acid (CA) on the storage stability of olive oil. Olive oil in the absence or presence of 300 ppm CA was encapsulated in 1.5% w/w sodium alginate shells. Encapsulated oil (with/without added CA) and unencapsulated oil were stored at 20 or 37 °C for 30 days and then subjected to stability and quality evaluation based on peroxide value (PV), *p*-anisidine value (*p*-AV), Totox value, free fatty acid (FFA), total extractable phenolic content (TEPC), and fatty acid composition. The CA addition increased the stability and TPC of the final oil product. Oxidation changes were generally slower in the encapsulated oil samples. Both encapsulation and addition of CA preserved unsaturated fatty acids (UFAs) including C18:1 (omega-9 FA), C18:2 (omega-6 FA) and C18:3 (omega-3 FA). We conclude that the current oil encapsulation method using alginate microspheres could be a feasible approach to increasing olive oil stability. The addition of CA to olive oil not only provides additional protection to the oil, but also improves the nutritional values of the final oil product in terms of elevated TEPC and desired UFAs.

1. Introduction

Olive oil from olive fruit (*Olea europaea* L.; *Oleaceae*) is of dietetic importance, containing essential vitamins, monoenoic and dienoic fatty acids, and other natural nutrients (Kiritsakis & Markakis, 1987; Nawar, 1985). Benefits of increased intake of olive oil include its rapid digestibility, together with its reported anti-ulcer, anti-aging, stress and plasma cholesterol lowering properties, as well as therapy potentials for type 1 & 2 diabetes and skin care (Assmann et al., 1997; Christakis, Fordyce, & Kurtz, 1980; Owen et al., 2000; Ozyilkan, Colak, Akcali, & Basturk, 2005; Shah, Adams-Huet, Grundy, & Garg, 2004; Strychar et al., 2003; Visioli & Galli, 1998). Olive oil contains triglycerides composed mainly of oleic acid (monounsaturated fatty acid, MUFA) and a small amount of saturated fatty acid resulting in its poor storage stability (O'Brien, 2004). Oxidation results in the loss of nutrients and flavour, conversion of unsaturated to saturated fatty acid, and development of deleterious products such as reactive oxygen species. Protection from lipid oxidation is a critical factor in oil quality. Fortification of oils with antioxidants and encapsulation of oils have been adopted as approaches to address this issue (Anwar, Bhanger, & Kazi, 2000; Heinzelmann & Franke, 1999).

Recently, natural antioxidants have increased in consumer popularity compared to synthetic ones such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), because of the perceived health risks of synthetic antioxidants such as carcinogenesis (Prior, 2004). Plant phenolic antioxidants possess diverse health-promoting properties, including antioxidant activity and protection against diseases such as cardiovascular disease and some forms of cancer (Arts & Hollman, 2005; Bravo, 1998; Kampa et al., 2004; Mateos, Goya, & Bravo, 2006). Caffeic acid (CA), a potent antioxidant among the hydroxycinnamic acids, is widespread in the plant kingdom (Gülçin, 2006; Murkovic, 2003; Rice-Evans, Miller, & Paganga, 1996). The use of CA may provide dual benefits through inhibiting lipid oxidation since it is a free radical scavenger, while also increasing the nutritional values of the final product (Gülçin, 2006; Marinova & Yanishlieva, 1992, 1994; Pino, Campos, Lopez-Alarcon, Aspee, & Lissi, 2006).

Microencapsulation is an approach for stabilizing active substances through enclosing liquid droplets or small particles of a sensitive substance (called the "core") within a physical barrier, either a continuous coating film or a solid wall material (called the "shell") (Dziezak, 1988). Biopolymer materials such as gelatin, plant gums, modified celluloses or starch, dextrin or whey proteins can be used for the shell (Ashady, 1993; Kanawija, Pathania, & Singh, 1992; Krasaekoopt, Bhandari, & Deeth, 2003; Ouyang

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et al., 2004; Sun & Griffths, 2000; Young, Sarda, & Rosenberg, 1993). Sodium alginate is an inexpensive, nontoxic co-polymer extracted from brown seaweed or kelp, containing two monomeric units D-mannuronic acid and L-guluronic acid. Sodium alginate is capable of forming rigid gels by the action of calcium ion or multivalent cations. Alginate gel microspheres have been conventionally prepared *via* extrusion by dropping an alginate solution through a needle into a $CaCl_2$ solution (external gelation) (Lencki, Neufeld, & Spinney, 1989).

While the microencapsulation techniques alone may not be a completely satisfactory approach to protecting oil from oxidation, addition of a phenolic antioxidant may offer an additional preservative effect. This study compares the oil stability achieved by using microencapsulation singly and in combination with CA addition. The PV, *p*-AV, total FFAs, fatty acid composition and TEPC were analysed over the 30-day storages period at 20 °C (a normal ambient temperature for home storage) and 37 °C (a typical tropical temperature) to evaluate the oil quality and nutritional value.

2. Materials and methods

2.1. Chemicals and materials

Extra virgin olive oil (*Olea europaea*) was obtained from Ti Kouka Estate, New Zealand, and stored in the dark at 4 °C until used. Sodium alginate (food grade) was obtained from Danisco, Auckland, New Zealand.

Active carbon (activated charcoal), caffeic acid, Folin–Ciocalteu's phenol reagent (2 N), gallic acid, *p*-anisidine, tridecanoic acid C13:0 and a 37-component mix of fatty acid methyl esters (FAMEs) (Supelco, 1 g/ml in CH_2Cl_2) were from Sigma–Aldrich Chemie, Steinheim, Germany. Absolute ethanol and sodium carbonate were from Merck, Darmstadt, Germany. Glacial acetic acid, potassium iodide, chloroform, concentrated HCl (36%), iso-octane, methanol, *n*-hexane (95%), potassium dichromate, sodium thiosulphate, and sulphuric acid were from Ajax Finechem Pty Ltd., Sydney, Australia. Ammonium chloride was from May & Baker, Dagenham, England. Starch and sodium sulphite were from BDH laboratory chemicals Ltd, Poole, England. Phenolphthalein indicator and sodium hydroxide were from Scharlau, Scharlau Chemie, Barcelona, Spain. All reagents were of analytical grade.

2.2. Preparation of encapsulated olive oil with/without caffeic acid for storage trials

Caffeic acid was added at a level of 300 ppm to olive oil (in 250-ml Duran Schott bottles wrapped with aluminium foil). The headspace of the Schott bottles was flushed with nitrogen gas. This process was carried out under low intensity light conditions and away from natural sunlight to prevent oil deterioration. In parallel to the encapsulation, the unencapsulated oil was stored in glass tubes next to the Inotech IE-50R Encapsulator (Inotech Encapsulation AG, Dottikon, Switzerland). These glass tubes were selected to have the same measurements and transparency as the core syringe of the encapsulator, in order to ensure the same light and heat exposures between the unencapsulated oil and the oil being encapsulated.

Aqueous alginate solution (1.5%) was prepared by dissolving Na-alginate in water, homogenised using a Silverson high shear Mixer (10,000 rpm for 5 min, L5T, Silverson Machines Ltd., Chesham, Bucks, England), and stored overnight at 4 ± 0.5 °C. Olive oil in the absence or presence of CA (300 ppm) was encapsulated in alginate shell at a level of 50 mg oil/g alginate solution using the Inotech IE-50R Encapsulator and collected in glass vessels. During encapsulation, the nozzle of the encapsulator extruded the core fluid (olive oil) and shell fluid (alginate solution) simultaneously into an outer (shell) and inner (core) structure. The diameter orifice of the nozzle allowed the core–shell stream to exit under laminar flow conditions and break into regular-sized microdroplets under a vibration frequency 1706 Hz. A 3% calcium chloride solution was used to induce cross-linking of the alginate *via* an ion-induced polymerisation mechanism. The obtained encapsulated beads along with the cross-linking solution were transferred to a clean container and placed on an orbital shaker for hardening. After a 10-min hardening, the beads were spread on an 11-μm nylon mesh, backed by an absorbent paper towel and drained until no further moisture was found on the paper towel. The resultant beads were further dried under nitrogen gas (oxygen free – BOC Gases New Zealand Limited, Auckland, New Zealand) and placed in glass bottles (wrapped in aluminium foil) at 10 °C. Microsphere morphology was evaluated *via* visual observation and by optical microscopy, using a Nikon Eclipse E600 microscope (Nikon Corporation, Chiyoda-ku, Tokyo, Japan) equipped with a Nikon Coolpix 995 3.34 mega pixel camera (40×, Nikon corporation, Chiyoda-ku, Tokyo, Japan).

Duplicate unencapsulated and encapsulated oils were stored in the same types of sealed glass containers (with the same headspace: volume ratio) for 30 days at 20 °C (in a temperature-controlled food laboratory) or 37 °C (in a food-grade incubator oven, MIR 162, Sanyo Electric Co., Ltd., Japan). Two subsamples of control or treated oil at Day 0, 7, 14 and/or 30 were obtained and subjected to chemical analysis after an oil extraction process. After subsampling, the glass bottles were flushed with nitrogen gas and capped until the next measurement.

2.3. Extraction of olive oil from the encapsulation beads

The encapsulated beads (5 g) were put into an extraction thimble that contained methanol (100 ml). The obtained mixture was homogenised using an Ultra-Turrax homogenizer (24,000 rpm for 2.5 min, IKA Werke, Janke and Kunkel, Staufen, Germany). Hexane (100 ml) was added, and the mixture was shaken vigorously to facilitate the transfer of oil into the hexane layer. The hexane layer was then separated *via* a separation funnel and collected. The extraction steps were repeated three times. The obtained hexane layers were combined and evaporated using a Labconco RapidVap® (40 °C, 10 kPa for 50 min, 60% speed; Model 79100-01, Labconco Corporation, Kansas City, MO) under nitrogen gas, to a final volume of 5 ml. The mixture was then dried in a Labconco CentriVap® Centrifugal Concentrator (Model 78100-01; Labconco CentriVap®, Kansas, MO, USA) at 40 °C for 4 h under vacuum.

2.4. Peroxide value (PV) determination

The PVs of all the oil samples were determined using the AOCS Official Method (1998a) and expressed as peroxide milliequivalent per kg oil. Oil (0.5 g) was dissolved in acetic acid-chloroform solution (3 ml, 3:2 v/v). After saturated potassium iodide KI solution (50 μl) was added, the mixture was left to stand for 1 min with occasional shaking. An aliquot (3 ml) of Milli-Q water was added. The mixture was titrated with 0.01 N standardised sodium thiosulphate solution until the yellow iodine colour just disappeared. Starch indicator solution (0.2 ml, 1 g/100 ml) was added. The titration continued until the blue colour derived from the iodine just disappeared. A blank sample as reagent control was set up and carried through all the steps.

2.5. p-Anisidine value (p-AV) determination

The *p*-AVs of the oil samples were determined using the AOCS Official Method (1998b). Oil (0.5 g) was dissolved in iso-octane (12.5 ml). The iso-octane solvent was used as the reference (blank)