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Decoloration kinetics of chlorophylls and carotenoids in virgin olive oil by autoxidation

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ABSTRACT

Kinetic models are capable of predicting shelf life in keeping with the different variables that can affect the degradation of the food item. In this work, virgin olive oils (VOOs) extracted from olive fruits at three ripening stages with high, medium and low pigment contents respectively, were thermodegraded to characterize the kinetic and thermodynamic parameters for the oxidation of two pigment fractions: a green fraction (chlorophylls) and a yellow fraction (carotenoids). A first-order kinetic mechanism was appropriate for describing the decoloration processes under non-oxygen thermal auto-oxidation. A marked effect of temperature has been pointed out, with the carotenoids (CARs) being the most affected by heat. The kinetic constants for the CAR degradation were about 3.6 times higher than the respective constants for chlorophylls (CHLs) that showed a more stable structure to decoloration by heat. As well, higher activation energy of CHLs (16.03 ± 0.26 kcal·mol^{-1}) as compared to CARs (15.45 ± 0.17 kcal·mol^{-1}) implies that a smaller temperature change is needed to increase the kinetic constant of CHLs.

Neither isokinetic ratio nor compensation existed between the three VOO matrixes and further, for each pigment fraction (CHLs or CARs) all kinetic constants were explained by a single Arrhenius line. Consequently, the differences between the oily matrixes did not significantly affect the decoloration mechanisms, and moreover, the kinetic parameters obtained as temperature functions according to the Arrhenius model, can be used to develop a prediction mathematical model for decoloration of CHL and CAR pigment fractions in VOO over time and depending on temperature.

1. Introduction

Each technological process for obtaining and/or storage of vegetable foods is associated with a specific transformation of their carotenoid and chlorophyll pigments. This fact makes these functional constituents appropriate as quality indicators for final product quality and also demonstrates their potential applicability as a tool for process traceability.

Virgin olive oil (VOO) is known for its high levels of monounsaturated fatty acids that help maintain normal blood cholesterol levels (Commission Regulation (EU), 2012). It is also a good source of phytochemicals including polyphenolic compounds, squalene, alpha-tocopherol, and carotenoids and chlorophylls which have health benefits that include reduction of risk factor of coronary heart disease, prevention of several varieties of cancers, modification of immune and inflammatory responses and antioxidant activity (García-González, Aparicio-Ruiz, & Aparicio, 2008; Lercker & Caramia, 2010). A nutrition claim for olive oil polyphenols have been recently authorized (EFSA, 2011; Commission Regulation (EU), 2012).

Chlorophyll and carotenoid pigments are highly appreciated as functional components both for its coloring properties and its health benefits for the human consumption. Carotenoids, besides their participation in yellow coloring of fruits, vegetables and oils, are bioactive compounds which have provitamin A function (β-carotene and β-cryptoxanthin), and antioxidant activity, and prevent age-related macular degeneration and cataract formation (lutein) (Seddon et al., 1994). Also, it has been demonstrated, in both in vitro and in vivo animal model assays, that the chlorophyll compounds, in addition to its function as green coloring, exhibit a series of biological properties, such as antioxidant and antimutagenic activities, modulation of xenobiotic enzyme activity, and induction of apoptotic events in cancer cell lines, all consistent with the prevention of degenerative diseases (Ferruzzi & Blakeslee, 2007).

The importance of the biological properties of chlorophylls and carotenoids together with the potential of those compounds in the determination of quality and authenticity of a VOO leads to the importance of tracking the degradation of those compounds during the storage or heat treatment in order to know the loss of biological properties of VOO and possible conditions of the olive oil before marketing. Carotenoids and chlorophylls are widely affected by heat treatment, with the former undergoing reactions of *trans–cis* isomerization and rearrangements of

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5,6-epoxide groups to 5,8-furanoxide groups in vegetable foods thermally processed (Mínguez-Mosquera & Jarén-Galán, 1999; Pérez-Gávez, Jarén-Galán, & Mínguez-Mosquera, 2000; Shi & Le Maguer, 2000; Sanchez, Carmona, Ordoudi, Tsimidou, & Alonso, 2008; Zhao, Kim, Pan, & Chung, 2014), and the latter undergoing decarbomethoxilation and allomerization in C-13^2 of the isocyclic ring of the chlorophylls (Mínguez-Mosquera, Gandul-Rojas, Gallardo-Guerrero, Roca, & Jarén-Galán, 2007). Under a powerful process, both pigment fractions undergo auto-oxidation with the destruction of chromophore groups (Aman, Schieber, & Carle, 2005; Schwartz & Lorenzo, 1990). All these reactions can modify the functional properties of these compounds and/or alter their bioavailability.

Lutein and β-carotene are the major carotenoids in virgin olive oil (VOO) but it is also known that other xantophylls such as neoxanthin, violaxanthin, anteraxanthin and β-crytoxanthin are present. Pheophytin *a and b* are the major chlorophyll pigments in VOO followed by chlorophyll *a and b*, OH-pheophytin *a and b* and lactone-pheophytin *a and b* (Gandul-Rojas & Mínguez-Mosquera, 1996).

Kinetic models are capable of predicting shelf life in keeping with the different variables that can affect the degradation of the food item. Numerous experimental works describe VOO degradation, but until recently the kinetic performance in oxidation parameters (Farhoosh & Hoseini-Yazdi, 2014; Mancebo-Campos, Fregapane, & Salvador, 2008) and individual pigment thermodegradation products have not been reported (Aparicio-Ruiz & Gandul-Rojas, 2012; Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2010; Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2011).

This research work is aimed at the kinetic study and characterization of the thermodynamic parameters governing the thermal degradation reactions of two pigment fractions: green fraction (chlorophylls) and yellow fraction (carotenoids) in VOO, to advance in the knowledge of the thermal stability of these pigment fractions in an oily matrix, and for the first time to establish mathematical models enabling the prediction of the behavior of its decoloration reactions by autoxidation versus thermal variables governing critic points in storage and/or processing of this food e.g. soft deodorization or cooking/frying.

2. Materials and methods

2.1. Chemicals and standards

Tetrabutylammonium acetate and ammonium acetate were supplied by Fluka (Zwijndrecht, The Netherlands). HPLC reagent grade solvents were purchased from Teknokroma (Barcelona, Spain), and analytical grade solvents were supplied by Panreac (Barcelona, Spain). For the preparation, isolation, and purification of chlorophyll pigments, analytical grade reagents were used (Panreac). The deionized water used was obtained from a Milli-Q 50 system (Millipore Corp., Bedford, MA). Standards of chlorophyll *a*/*b* (chl *a*/*b*) were supplied by Sigma-Aldrich Co. Standards of pheophytin *a*/*b* (phy *a*/*b*) and pyropheophytin *a*/*b* (pyphy *a*/*b*) were provided by Wako Chemicals Gmbh (Neuss, Germany). The C-13 epimer of phy *a*/*b* was prepared by treatment with chloroform according to the method of Watanabe et al. (1984). 13^2-OH-phy *a*/*b* was obtained by selenium dioxide oxidation of phy *a*/*b* at reflux heating for 4 h in pyridine solution under argon (Hynninen, 1991). 15^1-OH-lactone-phy *a*/*b* was obtained from phy *a*/*b* by alkaline oxidation in aqueous media according to the method of Mínguez-Mosquera and Gandul-Rojas (1995).

Reference samples of lutein, β-carotene, neoxanthin, violaxanthin and antheraxanthin were obtained from a pigment extract of fresh spinach saponified with 3.5 M KOH in methanol and isolated by TLC on silica gel GF254 (0.7 mm thickness) on 20 × 20 cm plates using petroleum ether (65–95 °C)/acetone/diethylamine (10:4:1, v/v/v) (Mínguez-Mosquera, Gandul-Rojas, & Gallardo-Guerrero, 1992). Luteoxanthin, auroxanthin, neochrome, and mutatoxanthin were obtained by acidification with 1 M HCl in ethanol (Khachik, Beecher, & Whittaker, 1986). β-Cryptoxanthin was obtained from red peppers (Mínguez-Mosquera & Hornero-Méndez, 1993). All standards were purified by TLC using different eluents (Mínguez-Mosquera et al., 2007).

2.2. Samples

The study of thermal degradation of pigments was carried out with virgin olive oils obtained by physical extraction into a two-phase system (Di Giovanchino, 2013) and supplied by a single industrial mill (Cooperativa Sor Ángela de la Cruz, Estepa, Seville) to avoid any effect of pedoclimatic and agricultural parameters and the industrial variables of the extraction systems in the comparative studies. In order to have three lots of oil with a differing pigment content, the starting material used was a mixture of two oil variety olives – Hojiblanca and Manzanilla – picked in three different months: November (sample N), December (sample D), and January (sample J). The proportion of fruits between varieties was 20/80, 80/20 and 100/0 respectively. The dates of picking correspond to high, medium, and low pigment levels (referring to the green color) and correlated inversely with the degree of fruit ripening according to the method of Walalí-Loudiyi, Chimitah, Loussert, Mahhou, and Boulouha (1984).

2.3. Heat treatment

Preliminary assays, with a commercial sample of VOO, enabled an approximate determination of the degree of conversion for the main reactions to be studied (Aparicio-Ruiz & Gandul-Rojas, 2012; Aparicio-Ruiz et al., 2010, 2011) and established a range of times for an appropriate sampling at each temperature. The total time of each experiment changed depending on the assay temperature: 42 h (120 °C), 64 h (100 °C), 370 h (80 °C) and 744 h (60 °C). At least 128 aliquots (32 for each of the four assay temperatures) were separated from each oil lot (samples N, D, and J). These aliquots were put into glass tubes that were sealed in the absence of air and placed in thermostatted ovens at the temperatures fixed for each experiment. These four temperatures were used to determine the kinetic and thermodynamic parameters (reaction order, reaction rate, and activation energies).

For each oil lot, two samples were analyzed for each time/temperature pair. The samples were removed from the thermostatted ovens at fixed time intervals, depending on each experiment, to obtain a total of at least 16 duplicate samples. The samples were cooled rapidly in an ice bath and then kept at −20 °C until analysis of the pigments.

2.4. Extraction and analysis of chlorophyll and carotenoid pigments

All procedures were performed under green lighting to avoid any photooxidation reactions. Pigment extraction was performed by liquid-phase distribution. This method was developed for virgin olive oil by Mínguez-Mosquera, Gandul-Rojas, Garrido-Fernández, and Gallardo Guerrero (1990). The technique is based on the selective separation of components between N,N-dimethylformamide (DMF) and hexane. The oil sample (10–15 g) was dissolved directly in 150 mL of DMF and treated with five 50 mL successive portions of hexane in a decanting funnel. The hexane phase carried over lipids and carotene fraction while the DMF phase retained chlorophyll pigments and xanthophylls. This system yielded a concentrated pigment solution that was oil free and could be adequately analyzed by chromatographic techniques.

HPLC analysis of chlorophyll pigments was performed according to a modification of the method of Mínguez-Mosquera et al. (1992), as is described by Roca, Gallardo-Guerrero, Mínguez-Mosquera, and Gandul-Rojas (2010). A reverse phased column (20 cm × 0.46 cm) packed with 3 μm C18 Spherisorb ODS2 (Teknokroma, Barcelona, Spain) and an elution gradient with the solvents (A) water/ion-pair reagent/methanol (1:1:8, v/v/v) and (B) acetone/methanol (1:1 v/v), at a flow rate of 1.25 mL/min were used. The ion-pair reagent was 0.05 M tetrabutylammonium acetate and 1 M ammonium acetate in