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Short communication

Linseed oil presents different patterns of oxidation in real-time and accelerated aging assays

Caroline Douny [a,*], Rina Razanakolona [b], Laurence Ribonnet [b], Jérôme Milet [c], Vincent Baeten [d], Hervé Rogez [e], Marie-Louise Scippo [a], Yvan Larondelle [b]

[a] Department of Food Sciences, Laboratory of Food Analysis, FARAH – Veterinary Public Health, University of Liège, B43bis, Quartier Vallée 2, Avenue de Cureghem 10, 4000 Liège, Belgium
[b] Institut des Sciences de la Vie, Université catholique de Louvain, Croix du Sud, 2/L7.05.08, B-1348 Louvain-la-Neuve, Belgium
[c] Oxylent SA, Chemin du Fundus, 15, B-7822 Ghislenghien, Belgium
[d] Valorisation of Agricultural Products Department, Henseval Building, Walloon Agricultural Research Centre (CRA-W), 24 Chaussée de Namur, B-5030 Gembloux, Belgium
[e] Centre for Agro-Food Valorisation of Amazonian Bioactive Compounds (CVACBA), Universidade Federal do Para, 66.095-780 Belém, PA, Brazil

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ABSTRACT

This study aimed at verifying if the hypothesis that one day at 60 °C is equivalent to one month at 20 °C could be confirmed during linseed oil aging for 6 months at 20 °C and 6 days at 60 °C using the "Schaal oven stability test". Tests were conducted with linseed oil supplemented or not with myricetin or butyl-hydroxytoluene as antioxidants. Oxidation was evaluated with the peroxide and *p*-anisidine values, as well as the content in conjugated dienes and aldehydes. All four indicators of oxidation showed very different kinetic behaviors at 20 and 60 °C. The hypothesis is thus not verified for linseed oil, supplemented or not with antioxidant. In the control oil, the conjugated dienes and the peroxide value observed were respectively of 41.8 ± 0.8 Absorbance Unit (AU)/g oil and 254.3 ± 5.8 meq.O_2/kg oil after 6 months at 20 °C. These values were of 18.2 ± 1.3 AU/g oil and 65.2 ± 20.3 meq.O_2/kg after 6 days at 60 °C.

1. Introduction

Linseed (*Linum usitatissimum*) oil has a high α-linolenic acid (ALA) content and is therefore interesting in nutrition. Indeed, the consumption of n-3 polyunsaturated fatty acids (PUFA) is necessary for various physiological reasons and has been associated with a decrease of the incidence of inflammatory and cardiovascular pathologies (Simopoulos, 2008). Unfortunately, the high PUFA content of linseed oil also contributes to its rapid oxidation (Guillén & Uriarte, 2012). To prevent it, supplementation with antioxidants is required. Synthetic antioxidants commonly used in food include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethoxyquin and ascorbyl palmitate (Reische, Lillard, & Eitenmiller, 2008). These antioxidants show an effective protection of the PUFA (Anwar, Siddiq, Iqbal, & Asi, 2007), but serious doubts concerning their safety oriented research towards the exploration of natural antioxidants (Chirinos, Huaman, Betalleluz-Pallardel, Pedreshi, & Campos, 2011; Martín et al., 2014), such as flavonoids, tocopherols and carotenoids (Pelli & Lyly, 2003). Michotte et al. (2011) showed that myricetin, a flavonol present in many plants, is an effective protector against autoxidation of PUFA in linseed oil. Other studies demonstrated that myricetin can also protect sunflower (Marinova, Toneva, & Yanishlieva, 2008) and rapeseed oils (Chen, Chan, Ho, Fung, & Wang, 1996).

The AOCS Cg 5-97 method, also known as the *Schaal oven stability test*, is an oven storage test used to simulate the real-time aging of oils (Michotte et al., 2011). This test should be conducted with oils as little oxidized as possible, at 60 °C in the dark. This method shows a good correlation with real-time stability studies. In the literature, some authors showed that the oxidation parameters obtained from aging realized at 60 °C and at a temperature close to ambient temperature are linearly linked. Indeed, Abou-Gharbia, Shehata, Youssef, and Shahidi (1996) indicated that when Tehina, a paste obtained with ground sesame seeds, was aged 2 days at 65 °C, the *p*-anisidine (PA) value and peroxide value

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* Corresponding author.
E-mail addresses: cdouny@ulg.ac.be (C. Douny), rina.razanakolona@gmail.com (R. Razanakolona), laurenceribonnet@hotmail.com (L. Ribonnet), Jerome.milet@oxylent.be (J. Milet), V.baeten@cra.wallonie.be (V. Baeten), herverogez@gmail.com (H. Rogez), mlscippo@ulg.ac.be (M.-L. Scippo), yvan.larondelle@uclouvain.be (Y. Larondelle).

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(PV) were similar to the values obtained with Tehina aged for 60 days at 22 °C. Similarly, soybean and cotton oils aged in closed bottles had equivalent «flavor scores» after 4 days at 60 °C and 4 months at 26 °C (Evans, List, Moser, & Cowan, 1973). Those works were reported by Khan and Shahidi (2001) and led to the hypothesis that an aging of 1 day at 65 °C was equivalent to an aging of 1 month at 25 °C. A linear relationship would mean that the results obtained at 60 °C during a short time experiment could be extrapolated to real-time (room temperature) aging of oil. Therefore, the objective of our study was to verify this hypothesis during linseed oil aging, without added antioxidant and with BHT or myricetin. In each aging experiment, primary products of PUFA oxidation were evaluated with the determination of conjugated dienes and PV; secondary products were evaluated with the determination of the PA value and aldehyde concentrations.

2. Materials and methods

2.1. Standards and reagents

1,1,3,3-tetraethoxypropane, 2,4-dinitrophenyl-hydrazine (97%), 2,4-decadienal, BHT (99%), PA were purchased from Sigma-Aldrich (St. Louis, Missouri). Stock solutions of 4-hydroxy-2-hexenal (4-HHE) and 4-hydroxy-2-nonenal (4-HNE) were obtained from Cayman Chemicals (Ann Arbor, Michigan). Myricetin was obtained from Extrasynthese (Genay, France).

Chloroform and water were of Chromanorm quality, while acetic acid 100% was of Normapur Quality, all provided by VWR International (Radnor, Pennsylvania). Ethanol absolute, trichloroacetic acid and hydrochloric acid 12 M were from Merck (Darmstadt, Germany). LC–MS-grade acetonitrile was obtained from Biosolve (Valkenswaard, The Netherlands).

2.2. Sample preparation and oxidation conditions

Refined linseed oil (RLO) free of synthetic antioxidant was kindly supplied by Vandeputte (Mouscron, Belgium). RLO was collected in a 1 L plastic bottle, inerted and stored at 4 °C until use. Samples of RLO (20 g), with added antioxidant and without (controls), were prepared as described by Michotte et al. (2011): BHT and myricetin were solubilized in a limited quantity of ethanol so that it did not exceed 4% of the weight of the final solution. Then, RLO was added in order to obtain the desired concentration of 555 μmol antioxidant/kg oil for each compound. The solution was strongly mixed with a glass rod for 10 min and flushed with nitrogen for 3 min to remove ethanol. The different mixtures were stored at 4 °C until the start of the experiments. The mixtures were then strongly shaken and distributed (20 g) in left open tinted glass bottles (100 ml, diam. 4 cm), which were stored for 6 days at 60 °C (accelerated aging) or 6 months at 20 °C (real-time aging) in two ventilated ovens (ULM800, Memmert, Schwabach, Germany). Both oven temperatures were monitored during the test with temperature probe from VWR International (Radnor, Pennsylvania). For the 60 °C condition, bottles were removed from the oven every 12 h for the two first days, then every 24 h until 6 days. For the 20 °C condition, bottles were removed from the oven every 2 weeks for the two first months, then every month until 6 months. Then, bottles were flushed with nitrogen, closed and stored at −70 °C until random analysis.

2.3. Conjugated dienes

Conjugated dienes were evaluated in 25 mg RLO diluted with 100 mL hexane by measuring the absorbance at 232 nm with a UV–vis Cecil 2041 spectrophotometer (Cecil Instruments Limited, Cambridge, UK) (Dieffenbacher, 1992).

2.4. Peroxide value

The hydroperoxides were measured using the iodometric titration method (AOAC, 1995). Five g of RLO were diluted in 30 mL acetic acid/chloroform solution (3/2, v/v) then 0.5 mL of a saturated potassium iodide solution were added and a titration was realized with a 0.1 N $Na_2S_2O_3$ solution.

2.5. p-anisidine value

The PA value was determined by diluting 0.5 g RLO with 25 mL isooctane and adding PA (0.25% in glacial acetic acid) (American Oil Chemists' Society, 1998). PA value was measured at 350 nm.

2.6. Aldehyde measurement by LC-MS/MS

The aldehyde measurement was performed according to Douny et al. (2015). Briefly, 2 g of oil, added with BHT and methylmalondialdehyde as internal standard were extracted two times with water/ethanol 50/50 (v/v). Dinitrophenylhydrazone derivatives were prepared by adding a 2,4-dinitrophenyl-hydrazine solution (0.05 M in ethanol/HCl 12 M 9:1 (v/v)) and incubating for 2 h at 60 °C.

Separation and detection of aldehydes as derivatives were performed using a Spectra System P4000 HPLC system and a LCQ Deca ion trap mass spectrometer, with an Electrospray source (ThermoQuest Finnigan, San Jose, California).

2.7. Statistical analysis

Statistical Analysis System (SAS Institute, 2000) was used to check the data for normal distribution with a Shapiro–Wilk test and when variables were not normally distributed, a logarithmic transformation was performed. Significant differences between day 0 and other days/months ($p < 0.05$) were tested using the general linear model procedure. The non-parametric test of Kruskal Wallis has been used when normality was not respected despite a logarithmic transformation.

3. Results

The determination of oxidation products obtained from RLO with and without added antioxidants aged at a temperature of 60 °C or 20 °C are presented in Fig. 1. Conjugated dienes (Fig. 1I and II), PV (Fig. 1III and IV) and PA value (Fig. 1V and VI) are expressed as mean ± standard deviation (SD) of three independent experiments, with measurements performed twice on each sample, leading to a total of 6 values per condition. MDA content (Fig. 1VII and VIII) is expressed as the mean concentration ± SD of three independent experiments, with measurements performed once on each sample, leading to a total of 3 values per condition.

3.1. Primary oxidation products

3.1.1. Conjugated dienes

For the dienes, the absorbance value reached 41.8 ± 0.8 AU/g oil after 6 months at 20 °C, for the control, and 28.0 ± 0.6 AU/g oil, for the RLO samples supplemented with BHT, corresponding to an increase of respectively nearly 150% and 75%, compared to the absorbance value measured at day 0 (16.4 ± 0.2 AU/g oil for the control RLO and 15.9 ± 0.6 AU/g oil for RLO supplemented with BHT) (Fig. 1.I). At this temperature, conjugated diene values