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Oxidative stabilization of ultra-high omega-3 concentrates as ethyl esters or triacylglycerols

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A comparative study of the oxidative stability of "ultra-high" ω3 concentrates (80%) from fish oils as triacylglycerols (TAG) or ethyl esters (EE), as well as their stabilization by supercritical extracts of rosemary (SER), α-tocopherol or their mixture, by Rancimat method and throughout storage time, was carried out. No significant differences were found between EE and TAG on oxidative stability when measured by Rancimat conditions in the range of 50—90 °C. However, storage experiments revealed a poor stability of the EE concentrate form, measured by the monitorization of peroxide and p-anisidine values. Concerning the stabilization by antioxidants, there was a lack of antioxidant effect of evaluated compounds when Rancimat assays were performed at 70 °C, whereas an antioxidant effect began to evidence at 60 °C, and a clearest antioxidant protection was measured at 50 °C for the mixture of the SER plus α-tocopherol in both EE and TAG forms. This selected binary mixture efficiently stabilized the ω3-TAG concentrate throughout 50 days of storage conditions, whereas the stabilization of ω3-EE concentrate was worse. Therefore, the combination of SER and α-tocopherol seemed to be a good antioxidant mixture for the efficient stabilization of extremely labile ultra-high ω3 concentrates, especially in the form of ω3 TAG oils.

1. Introduction

General consumption of fish is quite low to reach the minimal recommended intake level of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are ω3 fatty acids well known for their beneficial role as antithrombotic, anti-inflammatory and hypolipidemic fatty acids (Kamal-Eldin & Yanishlieva, 2002; WHO, 2005). A popular way to increase long ω3-polyunsaturated fatty acids (PUFA) intake is by available fish oils supplements. These sources can be found both as purified fish oil and as concentrates in the form of triacylglycerols (TAGs), free fatty acids and ethyl esters (EEs). The range of concentration of total ω3 fatty acids in these commercial products is quite variable, as values between 20%and 60% can be found. Moreover, although less frequent, levels of 70% and up to 80% can be also found for "ultra-high" ω3 concentrates (Drusch, Grob, & Schwarz, 2008; Ganga et al., 1998).

Despite the high nutritional value of these products, it is well known that the high susceptibility to oxidation of polyunsaturated oils is the main cause of their deterioration and loss of quality (De Leonardis, Pizzella, & Macciola, 2008; Yanislieva & Marinova, 2001). Therefore, the general production and handling of ω3-PUFA concentrates, fish oils or ω3 oils-enriched food products, is unavoidably linked to the use of antioxidants. Most studies concerning the oxidative stabilization of ω3-PUFA have been focused on the commercial fish oils or concentrates that contain relative low or medium levels of these fatty acids; whereas the data concerning the oxidation, oxidative stability or oxidative stabilization of "ultra-high" ω3 oils is limited, and can be considered a challenge, since these products contain the highest level of the most susceptible fatty acids.

The number of antioxidants that have been tested for oxidative stabilization of ω3-PUFA oils is in general quite large and are the subject of many studies. The most classical antioxidant in ω3 oil concentrates are tocopherols (Kulas & Ackman, 2001; Yanislieva & Marinova, 2001). However, a decrease in the antioxidative efficiency of tocopherols is frequently found with increasing degree of unsaturation (Witting, 1969; Yanislieva & Marinova, 2001). The combination of tocopherols with other compounds, as the ternary or binary antioxidant system of α- and γ-tocopherol, ascorbic acid or lecithin has shown notable success in fish oil stabilization (Bandarra, Campos, Batista, Nunes, & Empis, 1999; Halmilton, Kalu, McNeill, Padley, & Pierce, 1998; Nishina, 1991). Other natural compounds, especially those from plant sources with antioxidant properties, have been tested for fish oil and fish oil ω3 concentrates. This is because natural antioxidants are preferred, given the evidences of undesirable side effects reported for

Abbreviations: AA, antioxidant; AAI, activity antioxidant activity index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EE, ethyl esters; IT, induction time; p-AnV, p-anisidine value; PV, peroxide value; PUFA, polyunsaturated fatty acids; TAG, triacylglycerols; SER, supercritical extracts of rosemary; TOTOX, total oxidation value.

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synthetic compounds (Ito, Fukushima, & Tsuda, 1985). Furthermore, many natural antioxidants have shown the additional advantage as bioactive compounds, such as antioxidant, antimicrobial, antiinflammatory or antitumorigenic (De Leonardis et al., 2008). Therefore, the addition of natural antioxidants to oils is interesting from the technological point of view in order to control the lipid oxidation, but this approach would give an added value to such oils due to the presence of bioactive antioxidants. Quercetin, (Ganga et al., 1998), chlorogenic acid, caffeic acid (De Leonardis et al., 2008), catechins (Sullivan, Mayr, Shaw, Murphy, & Kerry, 2005) or oregano extracts (Bhale, Xu, Prinyawiwatkul, King, & Godber, 2007) are examples of natural bioactive antioxidants tested for fish oil and fish oil ω3 concentrates. A current popular example of bioactive natural antioxidants are rosemary extracts and their major phenolic compounds, carnosic acid, carnosol or rosmarinic acid, both solely or in combination with other antioxidants, which have shown antioxidant properties in diverse oils, included fish oils (Bhale et al., 2007; Drusch et al., 2008; Sullivan et al., 2005; Wada & Fang, 1992). Concerning the production of rosemary extracts, utilization of supercritical fluid technology, considered as a “green” technology, is reaching a great interest, in comparison to more conventional method of extraction based on utilization of organic solvents.

The aim of the present work was the comparative study of the oxidative stability of ultra-high ω3 concentrates from fish oils as TAGs or EEs, as well as the evaluation of the antioxidant activity of supercritical extracts of rosemary (SER), α-tocopherol or their mixture for the stabilization of such oils. Accelerated conditions of oxidation were used for these aims by the Rancimat method, which was performed at diverse temperatures, in order to evaluate the dependence on temperature of the oil stability and the antioxidant activity of compounds. Finally, the efficiency of the compound that showed the best antioxidant activity under accelerated conditions of Rancimat was confirmed under normal storage conditions of the oils, by the monitorization of the oxidative indices (peroxide value, p-anisidine value) along time of storage.

2. Materials and methods

2.1. Samples

The ω3 concentrate fish oil as EE 80% DHA/10% EPA (ω3-EE) was purchased from Chemport (Jeollanam-do, Korea). The ω3-concentrate fish oil as TAG 60% DHA/20% EPA (ω3-TAG) was obtained by enzymatic interesterification of the commercial ω3-EE oil. For the studies of stabilization, α-tocopherol, SER or their mixture (50:50) were added to samples at 500 mg/kg.

SER were obtained as follows. Rosemary (*Rosmarinus officinalis L.*) leaves were ground in a cooled mill and sieved to 200–600 μm. Then, supercritical fluid extraction of the sample was accomplished using a pilot-plant extractor (Thar Technology, Pittsburgh, PA, USA, model SF2000) comprising a 2 L cylinder extraction cell, a two-step decompression system (separators cells S1 and S2) and a CO_2 recirculation device. Extraction was carried out for 5 h at 30 MPa and 313 K (Garcia-Risco et al., 2011). In order to remove the essential oil present in the plant matrix and further concentrate the rosemary antioxidant components, fractionation of the extract was carried out during the first hour of extraction. Then, two different extracts were collected in S1 and S2, being SER the fraction obtained in S1.

2.2. Oxidative stability under Rancimat test

Samples (3 g) were subjected to accelerated oxidative conditions by a Metrohm Rancimat model 743 (Herisau, Switzerland) at airflow rate of 20 L/h and diverse temperatures (50–90 °C). The conductivity measuring cells contained 70 mL of distilled water. The induction time (IT) was automatically determined as the inflection point of the generated plot of conductivity (μS/cm) of the water versus time (h). Analyses were performed in triplicate.

The samples in presence and absence of antioxidants were oxidized under Rancimat conditions as previously described in the range of 50–70 °C. The index of antioxidant activity (AAI) was estimated according to Nwosu, Boy, and Sheldon (1997) as:

$$\mathrm{AAI} = \mathrm{IT\ with\ antioxidant} / \mathrm{IT\ without\ antioxidant}$$

2.3. Oxidative indices at storage experiments

Two samples of 15 g from each form of ω3 concentrate (ω3-EE and ω3-TAG) containing the selected antioxidant were stored in screw-capped glass tubes of 15 mL at room temperature under dark. Samples were purged with nitrogen prior to storage and each time that samples were measured. Control samples of ω3-EE and ω3-TAG without addition of antioxidant were also stored in duplicate and handled under the same conditions.

Oxidative indices were monitored throughout storage until significant differences between the control and the treated samples were observed. Peroxide value (PV) and p-anisidine value (p-AnV) were measured by the photometric Oxitester method (model “FoodLab Fat”, CDR S.r.L., Ginestra Fiorentina) (Kamvissis, Barbounis, Megoulas, & Koupparis, 2008). The required PV and p-AnV reagents were provided by CDR S.r.l. Measurements of PV and p-AnV were done in duplicate.

The total oxidation value (TOTOX) was calculated according to Shahidi and Wanasundara (2002) as:

$$\mathrm{TOTOX} = (2 \times \mathrm{PV}) + \mathrm{p{-}AnV}$$

Finally, the antioxidant activity (AA) with respect to the control samples was calculated similarly to DeJong and Lanari (2009) as:

$$\%\mathrm{AA} = 100 \times (\Delta\mathrm{TOTOX}_C - \Delta\mathrm{TOTOX}_T) / \Delta\mathrm{TOTOX}_C$$

where $\Delta\mathrm{TOTOX}_C$ was the increment of TOTOX value of the control sample (without added antioxidants) throughout the time of storage and $\Delta\mathrm{TOTOX}_T$ was the increment of TOTOX value of the treated samples throughout the time of storage.

2.4. Statistical analysis

Statistical analyses were performed by means of the general linear model procedure of the SPSS 17.0 statistical package (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA). Differences were considered significant at $p \leq 0.05$. Post-hoc Tukey's tests were performed in the specific analysis of antioxidant compounds, in order to establish significant differences between the antioxidant treatments.

3. Results and discussion

3.1. Oxidative stability of ultra-high ω3 concentrates under Rancimat test

In general, lipid oxidation seems to go through different steps or reaction pathways depending on low or high temperature conditions (Tan, Che Man, Selamat, & Yusoff, 2002). One reason that seems to explain this phenomenon is related to the effect of oil temperature on the solubility of oxygen, which decreases with temperature (Robertson, 2000). Therefore, the prediction of kinetic rate constants at low temperatures based on accelerated tests performed at high temperatures shows some limitations, which has been pointed out as one of the problems of the Rancimat methodology (Farhoosh, Niazmand, Rezaei, & Sarabi, 2008). Hence, depending on the type of oil, the predictions may vary from acceptable estimation, to overestimation or underestimation (Farhoosh, 2007). Consequently, determination of fish-oil IT at 60 °C under Rancimat conditions have been suggested, because the kinetic of lipid oxidation at this temperature seems to be similar to