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Short communication

Linseed oil stabilisation with pure natural phenolic compounds

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ABSTRACT

Linseed has been used for a very long time in human and animal nutrition. Currently, there is an increasing interest in linseed oil because of its particularly high content in α-linolenic acid (ALA), an omega-3 fatty acid (FA). Unfortunately, ALA turns also the oil extremely sensitive to oxidation. This study aimed at assessing four pure representative phenolic compounds, myricetin (flavonol), (+)-catechin (flavanol), genistein (isoflavone), and caffeic acid (hydroxycinnamic acid) at a concentration of 555 μmol/kg as antioxidants in refined linseed oil (RLO). Their protective effect was assessed by monitoring the hydroperoxide formation, the FA profile and the residual antioxidant concentration in RLO, along its storage at 60 °C according to the Schaal oven test procedure. Caffeic acid, (+)-catechin and myricetin were found to be more efficient than butylated hydroxyanisole (BHA), a synthetic antioxidant. Interestingly, myricetin strongly reduced ALA oxidation. These results confirm that the chemical structure of the phenolic compounds plays a major role in their antioxidant properties.

1. Introduction

Linseed (*Linum usitatissimum*) oil is highly rich in polyunsaturated fatty acids (PUFA) and contains more than 50% of α-linolenic acid (ALA), which is an omega-3 and essential fatty acid (FA). Consumption of omega-3 FA is necessary for many physiological reasons and has been associated with a lower incidence of many types of illnesses among which inflammatory and cardiovascular diseases (Simopoulos, 2002). Unfortunately, when exposed to oxygen, PUFA are rapidly oxidised into hydroperoxides and further degradation products. This oxidation generates radical oxygen species that may cause irreversible damages when reacting with biological molecules such as DNA, proteins or lipids (Choe & Min, 2006).

Improvements in polyunsaturated oil preservation are continuously searched for, and the addition of natural antioxidants is presently being explored to answer the continuous demand of consumers for natural and healthy ingredients or food. Phenolic compounds are ubiquitously found in the plant kingdom and are well-known for their health benefits. Their abilities as antioxidants in oils have already been reported. Caffeic acid (Fig. 1d) was found to be more efficient as antioxidant than butylated hydroxyanisole (BHA) (Fig. 1e) in sunflower oil at 30 °C and 110 °C and in corn oil at 50 °C and 110 °C (De Leonardis, Macciola, & Di Rocco, 2003; Roussis, Tzimas, & Soulti, 2008). Myricetin (Fig. 1b) reduced the loss of linoleic acid and ALA in rapeseed oil heated at 105 °C during 22 h (Chen, Chan, Ho, Fung, & Wang, 1996). It was also a better antioxidant than quercetin, α-tocopherol, (+)-catechin, kaempferol and rutin in methyl linoleate oxidised at 40 °C (Pekkarinen, Heinonen, & Hopia, 1999). (+)-Catechin (Fig. 1a) was found to be a good antioxidant in lard and it was stated that tea extracts had a higher antioxidant activity as their flavonol content increased (Gramza et al., 2006). Genistein (Fig. 1c), the main isoflavone found in soy, is not commonly investigated as antioxidant in food. The protective effects of phenolic compounds in oil are used to be enhanced in the presence of synergistic molecules, such as ascorbyl palmitate (AP, Fig. 1f) (Pokorny, 1999).

In this work, four pure phenolic compounds, myricetin, (+)-catechin, genistein, and caffeic acid, representative of four phenolic classes, namely flavonols, flavanols, isoflavones, and hydroxycinnamic acids, were used as natural antioxidants in linseed oil. This study aimed at a better understanding of the influence of the polyphenol structure on the antioxidant activity, in a highly polyunsaturated oil, using the Schaal oven test to oxidise the oil at 60 °C.

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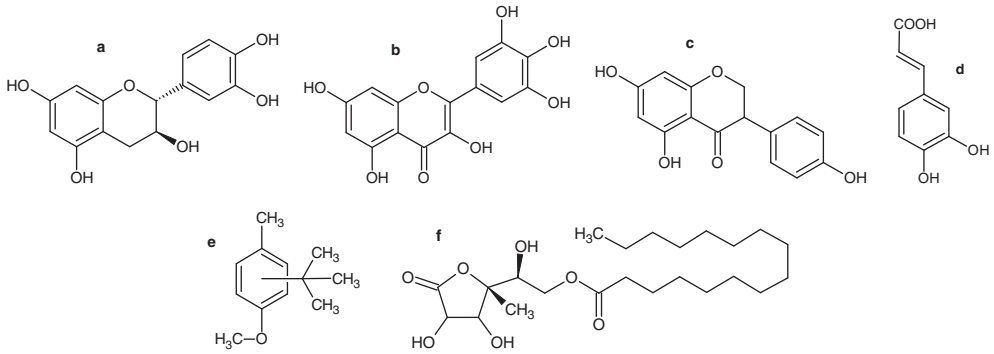

Fig. 1. Chemical structure of (+)-catechin (a), myricetin (b), genistein (c), caffeic acid (d), butylated hydroxyanisole (e), and ascorbyl palmitate (f).

2. Materials and methods

2.1. Materials

Refined linseed oil (RLO) free of synthetic antioxidant was kindly supplied by Vandeputte (Mouscron, Belgium).

2.2. Standards and reagents

Pure myricetin, genistein, and (+)-catechin were obtained from Extrasynthèse (Lyon, France). Pure caffeic acid, BHA, and AP were obtained from Sigma–Aldrich (St. Louis, MO). All other solvents and reagents used were of HPLC/Spectro grade.

2.3. Sample preparation and oxidation conditions

Duplicate samples of RLO (20 g) with added natural antioxidant (555 μmol/kg, corresponding to half the allowable BHA concentration in refined oil defined by the Codex Alimentarius) and AP (241 μmol/kg: suggested concentration for a synergistic antioxidant behaviour with phenolic compounds (Hras, Hadolin, Knez, & Bauman, 2000)) were prepared as follows. First of all, the compounds (phenolic compound with or without ascorbyl palmitate) were precisely weighted in a glass beaker and solubilised in pure ethanol. The quantity of ethanol used was limited so not to exceed 4% of the weight of the final oily solution. Then, RLO was added to the beaker in order to obtain the desired concentration of each compound in RLO. The solution was strongly mixed with a glass rod for 10 min. The mixture obtained was then flushed with nitrogen for 3 min in order to remove the ethanol solvent. The different mixtures were stored at 4 °C in tinted bottles until the start of the experiments. They were then strongly shaken and distributed (20 g) in smaller bottles, which were heated for 0, 6 and 12 days at 60 °C in an oven according to the Schaal oven test. Samples of RLO without antioxidant, with AP, with BHA, and with BHA and AP were also run as controls. Periodically, samples were removed from the oven, flushed with nitrogen, and stored at −20 °C until analysis.

2.4. Peroxide value

Hydroperoxides produced in RLO during the oxidative treatment were measured using the iodometric titration method for peroxide value (PV) determination (AOCS, 1996). The measurement repeatability reached 80%.

2.5. Fatty acid profile

The analytical method used for the FA profile determination of RLO was adapted from Meurens, Baeten, Yan, Mignolet, and Larondelle (2005). The chromatograph used was a Trace GC Thermo Finnigan (Milan, Italy) equipped with a flame ionization detector (225 °C) and an autoinjector Pal GC from CTC Analytics (Zwingen, Switzerland). FA were separated using a Restek Rt-2560 (Bellefonte, PA) column (0.2 μm, 100 m × 0.25 mm ID).

We considered palmitic acid of RLO as an internal standard as it is not oxidised during the thermal treatment. Results were expressed as the percentage of total FA initially identified. The measurement repeatability reached 90%.

2.6. Residual concentration of phenolic compounds

Added phenolic compounds were extracted from one of the two samples of the duplicate thermally treated using the method described by Araújo (1999). Briefly, 2 g of oil were first diluted in 5 ml petroleum ether, then extracted three times with 5 ml of an ethanol:water (80:20) solution. Afterwards, the ethanolic extract was concentrated, flushed with nitrogen, and stored at −20 °C until analysis. The chromatographic analysis of phenolic compounds was performed using a reversed-phase HPLC column on a Waters 2695 Separation module (Waters, Milford, MA) equipped with an autoinjector, a 2996 photodiode array detector and the Empower software. A X-terra RP_{18} (5 μm, 250 × 4.6 mm) column (Waters) was used for phenolic separation at 30 °C. The measurement repeatability reached 85%. The separation methods used were those described by Spiclin, Gasperlin, and Kmetec (2001), in the case of BHA, and by Llorach, Espin, Tomas-Barberan, and Ferreres (2002), in the case of natural phenolic compounds. The maximum absorbance peaks of BHA, (+)-catechin, caffeic acid, myricetin and genistein were 290, 280, 320, 360, and 280 nm, respectively. Each phenolic compound was quantified through a calibration curve by injecting standards at different concentrations. The added phenolic compounds were extracted from the oil at day 0 with yields ranging from 0.6 to 0.8, which were used as corrective factors to determine their real concentration in RLO during the thermal treatment.

2.7. Statistical analysis

The experiment was done in duplicate. The results were thus expressed as the mean of two values for each antioxidant, except in the case of HPLC analysis where only one determination was performed. When applicable, the one-way analysis of variance (ANOVA) was realised with the SAS Enterprise guide software and the distinction between sample means was performed using the Student Newman–Keuls method ($P \leqslant 0.05$).

3. Results and discussion

Fig. 2 presents the PV variation, the fatty acid profile and the residual concentration of phenolic compounds in RLO during the

accelerated oxidative treatment at 60 °C. PVs for all samples in the initial conditions were similar and equivalent to 1.21 ± 0.57 meq O_2/kg. Fig. 2A shows the PV of RLO determined during the oil storage. It reveals that neither AP, nor BHA, with or without AP, significantly reduced the hydroperoxide production. In contradiction to these results, it was already stated that BHA (555 or 1110 μmol/kg) slowed down the peroxidation in unrefined and unbleached linseed oil at room temperature (25 °C) (Rudnik, Szczucinska, Gwardiak, Szulc, & Winiarska, 2000). This discrepancy may be attributed to the presence of naturally occurring antioxidants in unrefined and unbleached oils that could enhance the BHA effectiveness through synergistic interactions. In our hands, AP (241 μmol/kg) did not prevent RLO from peroxidation. This confirms the results of Hras et al. (2000) who showed that AP (241 μmol/kg) did not have a significant impact on sunflower oil stabilisation at 60 °C. By contrast, Zandi and Gordon (1999) found that AP (482 μmol/kg) was capable of delaying rapeseed oil peroxidation at 60 °C. This discrepancy might be explained by the higher AP dose used. Genistein tested with AP did not improve the stability of RLO. Russin, Boye, Pham, and Arcand (2006) tested genistein at a much higher concentration (2000 μmol/kg) in linseed oil stored at 60 °C and observed a slight but significant protective effect, which remained inferior to that of BHA at 1110 μmol/kg. The three other phenolic compounds tested (caffeic acid, (+)-catechin and myricetin), in all cases in combination with AP, significantly inhibited the peroxide formation as compared to the control. Interestingly, myricetin was found to be significantly more efficient than the two other active compounds tested. Moreover, myricetin limited the PV under 10 meq O_2/kg (Codex Alimentarius recommended limit for refined edible oil) at least until day 6, which would be equivalent to a 6 months long storage at room temperature. Then, the PV kept increasing in the myricetin + AP condition but remained significantly below all other conditions tested. Such a high protective effect of myricetin was already observed in less unsaturated edible oils (Pekkarinen et al., 1999; Roussis et al., 2008).

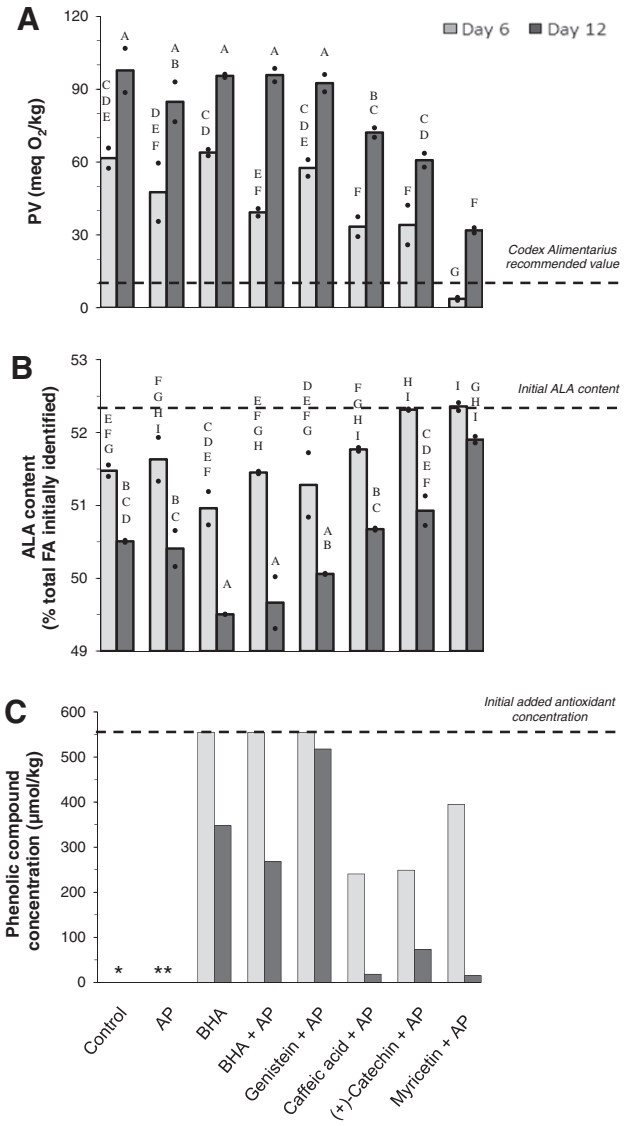

Fig. 2. Peroxide value (PV) (meq O_2/kg) (A), α-linoleic acid (ALA) content (% total FA initially identified) (B), and residual concentration of added antioxidants (μmol/kg) (C) in RLO (refined linseed oil) containing natural phenolic compounds (555 μmol/kg), butylated hydroxyanisole (BHA: 555 μmol/kg) and ascorbyl palmitate (AP: 241 μmol/kg) during an accelerated oxidative treatment at 60 °C (Schaal oven test). Values from which means were calculated are represented by dots (·). Significant differences ($P \leqslant 0.05$) between samples are represented by the absence of common capital letters. *Free of phenolic compounds; **not quantified.

Analysis obtained from three determinations showed that the RLO used in this work contained 69.10 ± 0.08% of PUFA and 52.42 ± 0.04% of ALA. The other main FA were, in the decreasing importance order, oleic (20.16 ± 0.05%), linoleic (16.68 ± 0.04%), palmitic (5.50 ± 0.01%), and stearic (4.03 ± 0.04%) acid.

The unsaturation degree of ALA turns it the most sensitive to oxidation. Although its degradation in the control condition was only about 2% of the total FA initially identified (Fig. 2B), differences in antioxidant protective effects could be easily observed between the conditions tested. Fig. 2B reveals that ALA was gradually degraded throughout the treatment, except in the (+)-catechin + AP and myricetin + AP conditions where no significant loss of ALA could be observed at day 6 in comparison to the initial value. In the case of myricetin + AP, the protection against ALA loss was largely kept until day 12. The other antioxidants tested, both synthetic and natural, did not seem to protect ALA from oxidation. These observations clearly demonstrate that phenolic compounds belonging to different classes of polyphenols do not have the same abilities in polyunsaturated oil protection. This statement may be explained by the chemical structure of each polyphenol. The number of OH groups in myricetin (6) and (+)-catechin (5) are much higher than in caffeic acid (2), and may explain their higher radical-scavenger activity. In the case of myricetin, the unsaturation in the C ring and the oxo function would make it an even more powerful antioxidant. Taken together, our results are thus in agreement with the antioxidant activity assigned to each of these compounds (Rice-Evans, Miller, & Paganga, 1996).

As they exert their antioxidant activity, phenolic compounds progressively disappear because by giving H-atom(s) to radicals they are oxidised and become distinguishable from the initial molecule. Consequently, an assessment of the phenolic content in RLO can be used as a tool to check the antioxidant activity of each phenolic compound.

Fig. 2C presents the concentration of added phenolic compounds in RLO during the thermal oxidation at 60 °C. The initial antioxidant concentration was 555 μmol/kg and is represented by the dotted line.

The fact that BHA did not show protective effects neither in terms of PV nor in terms of ALA content and remained stable during the first 6 days of the thermal treatment suggests that it preferentially reacts with secondary oxidation products that might be produced by hydroperoxide decomposition. The combination of BHA with AP did not improve the BHA stability in the oil. Genistein was extremely stable during the thermal treatment, which confirms its inability to limit RLO oxidation. The other phenolic compounds showed a steady oxidation throughout the treatment and reached a low concentration on day 12. These

results suggest that these compounds progressively react with the radicals formed from the beginning of the thermal treatment and, in turn, limit oxidation reactions. The rapid decrease of (+)-catechin and caffeic acid during the first 6 days would imply that they are less capable than myricetin of reducing the radicals produced by the lipid oxidation.

4. Conclusions

Through the use of simple analytical methods, this work has revealed the high antioxidant properties of (+)-catechin (flavanol), and furthermore of myricetin (flavonol) in the protection of ALA in a RLO matrix. Besides, it underlines the involvement of the structural features of phenolic compounds in their antioxidant activity.

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