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Antioxidant properties of hard winter wheat extracts

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Abstract

Extracts from three winter wheat varieties ('Trego', 'Akron' and 'Platte') were evaluated and compared to α-tocopherol for their inhibitory effects on lipid peroxidation in fish oils by measuring the oil stability index (OSI). Free radical scavenging capacities and chelating potencies were also measured to better understand the potential mechanism(s) of their effects on lipid peroxidation. Trego extracts showed the greatest capacity to suppress lipid peroxidation in fish oils. The OSI time of the oil sample containing 600 ppm Trego extract was 2.85 h beyond the control sample containing no antioxidant, which is 3.1 times longer than the OSI time of oil containing 300 ppm tocopherol. Dose effects were observed for Trego extract, but not for Akron or Platte extracts. Furthermore, the higher level of Platte extract corresponded to a shorter OSI time. All three wheat extracts directly reacted with and quenched DPPH radicals and showed chelating activity. Akron extract had the greatest radical scavenging and chelating activities. Neither radical scavenging nor chelating activities of the wheat extracts can explain the relative activities of these extracts on lipid peroxidation in fish oils under the experimental conditions. The results of this study indicate possibility of to developing natural food antioxidants from selected wheat varieties, including Trego hard white winter wheat.

Keywords: Wheat; Antioxidant; Lipid peroxidation; Radical scavenging; Fe^{2+}-chelating; Fish oil

1. Introduction

Lipid peroxidation leads to rapid development of rancid and stale flavours and is considered as a primary mechanism of quality deterioration in lipidic foods and oils (Güntensperger, Hammerli-Meier, & Escher, 1998; Krings, El-Saharty, El-Zeany, Pabel, & Berger, 2000). Antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisols (BHA), are added in food during processing to suppress lipid peroxidation and consequently to improve food quality and stability. Recently, interest in natural antioxidants has increased because of concerns about the long-term safety and negative consumer perception of synthetic antioxidants.

Some natural antioxidants, including vitamin E, soy protein isolates, cherry tissue and rosemary extracts, were reported to be effective against lipid oxidation in meat products (Güntensperger et al., 1998; Yu, Scalin, Wilson, & Schmidt, 2002a), several food systems (Offord, Guillot, Aeschbach, Loliger, & Pfeifer, 1997), bulk oils and oil-in-water emulsions (Frankel, Huang, Prior, & Aeschbach, 1996b). These natural antioxidants may also protect DNA, protein, and membrane lipids from oxidative damage in biological systems and provide additional health benefits for disease prevention and health promotion (Halliwell, 1996). New natural antioxidants are in high demand since antioxidants with suitable physicochemical properties are required for individual food or oil systems, and because the antioxidative capacity of a particular antioxidant could be significantly influenced by the type of system tested, oil substrates, analytical methods, and antioxidant levels (Frankel et al., 1996b).

Antioxidant activities were detected in wheat and wheat-based food products (Onyeneho & Hettiarachchy, 1992; Zielinski & Kozlowska, 2000). Wheat (winter cultivar Almari and spring cultivar Henika) extracts suppressed radical-induced liposome lipid peroxidation and showed radical cation scavenging activity (Zielinski & Kozlowska, 2000). In another study,

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extracts from Durum wheat (*Triticum durum*) inhibited oil oxidation using an active oxygen method (Onyeneho & Hettiarachchy, 1992). In our previous study, extracts prepared from three hard winter wheat varieties (*Triticum aestivum*) were shown to directly react with and quench free radicals, as determined by spectrophotometric and electron spin resonance (ESR) spectrometry methods. However, these wheat extracts have not been evaluated for their effects on lipid oxidation.

In this study, extracts from three hard winter wheat varieties were examined and compared to tocopherol for their inhibitory effects on lipid peroxidation in fish oils. Free radical quenching capacity and chelating potency of each wheat extract were also investigated for better understanding of their actions in inhibition of lipid oxidation. Results from this study demonstrate the potential to develop natural antioxidant preparations from selected hard winter wheat and provided information for wheat breeders and producers to promote the development and production of value-added wheat varieties.

2. Materials and methods

2.1. Materials

Grain samples of three winter wheat varieties ('Akron', 'Trego', and 'Platte') adapted for production in Colorado, were used for this study. The variety Akron is a hard red winter wheat, while Trego and Platte are both hard white winter wheat varieties. Samples were obtained at harvest from breeding trials conducted at a single dryland testing location in eastern Colorado. This field location was considered to be representative of typical wheat production conditions in eastern Colorado. Grain samples were cleaned using seed cleaners to remove all non-grain debris present following harvest. 2,2-Diphenyl-1-picryhydrazyl radical (DPPH·) and disodium ethylenediaminetetracetate (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Extraction of antioxidants

Wheat grain of each variety was ground and extracted for 3 h with ethanol under nitrogen, using a Soxhlet extractor. The ethanol extracts were concentrated to a final volume of 250 ml using a rotary evaporator and kept in the dark under nitrogen until further analyses. In order to prepare dimethyl sulfoxide (DMSO) solution, ethanol was removed under vacuum from a certain volume of the ethanol solution, and the solid residue was quantitatively re-dissolved in DMSO. The resulting DMSO solution was kept under nitrogen in the dark until further analysis.

2.3. Inhibitory effect on lipid peroxidation

Wheat extracts were evaluated for their potential to inhibit lipid peroxidation in additive-free fish oil using the oil stability index (OSI) method (Chen & Ho, 1997). The OSI was determined using the Rancimat instrument (Model 679, Metrohm Ltd., Switzerland). Briefly, a known volume of antioxidant solution (wheat extracts or tocopherol) was mixed into fish oil. The solvent was removed at 40 °C under vacuum. Six ml of each resulting oil was transferred into the reaction tube for the OSI measurement. The oxidation was carried out at 80 °C, and the airflow rate was 7 l per hour. The same volume of the solvent was added to and evaporated from the control sample in replacement of wheat extract. The tests were conducted using two levels of each wheat extract.

2.4. Free radical scavenging activity

Total free radical scavenging capacity of extracts from three wheat varieties were estimated according to the previously reported procedure using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH·) (Yu, 2001). Briefly, freshly-made DPPH· solution was mixed into wheat extracts to start the radical-antioxidant reaction. The final concentration was 100 μM for DPPH·. The absorbance at 517 nm was measured against a blank of pure ethanol at certain time points and used to estimate the remaining radical levels according to a standard curve. The concentration of antioxidant required to scavenge 50% DPPH (ED_{50}) of each wheat extract was obtained by plotting the percent DPPH· remaining at steady state of the reaction against the corresponding antioxidant level (Yu, 2001).

2.5. Chelating activity

Fe^{2+} chelating activity was measured by 2,2′-bipyridyl competition assay (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000). The reaction mixture contained 0.25 ml of 1 mM $FeSO_4$ solution, 0.25 ml of antioxidant solution, 1 ml of Tris–HCl buffer (pH 7.4), 1 ml of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), 0.4 ml of 10% hydroxylamine-HCl and 2.5 ml of ethanol. The final volume was made up to 5 ml with pure water. The absorbance at 522 nm was determined and used to evaluate Fe^{2+} chelating activity using disodium ethylenediaminetetracetate (EDTA) as a standard.

2.6. Statistical analysis

All tests were conducted in triplicate. Data were reported as mean ± S.D. Analysis of variance and least significant difference tests were conducted to identify differences among means. Statistical significance was declared at $P < 0.05$.

3. Results and discussion

Lipid peroxidation is a critical problem affecting food quality and stability. Two factors promote the development of novel natural antioxidants for food applications. The first factor is consumer preference of natural antioxidants due to the reported carcinogenic activity of synthetic antioxidants, including BHT and BHA (Onyeneho & Hettiarachchy, 1992). The other factor is related to the so-called "polar paradox" (Frankel, Huang, Aeschbach, & Prior, 1996a), which describes the observation that polar antioxidants are more effective in nonpolar lipids, while nonpolar antioxidants are more active in polar lipid emulsions (Frankel et al., 1996a). The polarity of an antioxidative compound is determined by its chemical structure and the environmental conditions, such as pH and interactions with other components in food systems. Therefore, new natural antioxidants are needed for suppressing lipid peroxidation in individual food or oil products that have different polarities and contain multiple components.

The oil stability index (OSI) represents a measure of the resistance of lipids to oxidation, with OSI duration being positively associated with oil or food stability. The Rancimat is an instrument that automates the OSI measurements, and has been widely used to evaluate the oxidative stability of lipidic foods and the antioxidative potency of antioxidants (Chen & Ho, 1997). The extracts from all three hard winter wheat varieties showed inhibitory effects against lipid peroxidation in fish oils (Fig. 1). However, the wheat extracts differed in their relative abilities to delay the lipid oxidation. Extracts from the variety Trego had greatest activity to suppress fish oil oxidation, while extracts from the variety Platte showed the lowest protection against lipid peroxidation (Fig. 1a–c). 600 ppm Trego extracts resulted in an OSI time of 2.85 h beyond the control containing no antioxidants. This inhibition is about 3.1 times stronger than that provided by 300 ppm α-tocopherol (Fig. 1a). In a previous study, rosemary extract, a well-known commercial natural antioxidant, resulted in 63.9 and 81.3% inhibition of lipid peroxidation in corn oil at 250 and 500 ppm levels, respectively, while 100 ppm tocopherol had 68.0% inhibition under the same experimental conditions (Frankel et al., 1996a). These data suggested that Trego extracts may have potential to be developed as food antioxidant for commercial applications. In addition, higher levels of Trego extracts corresponded to an increased OSI time although there was no linear relationship between the

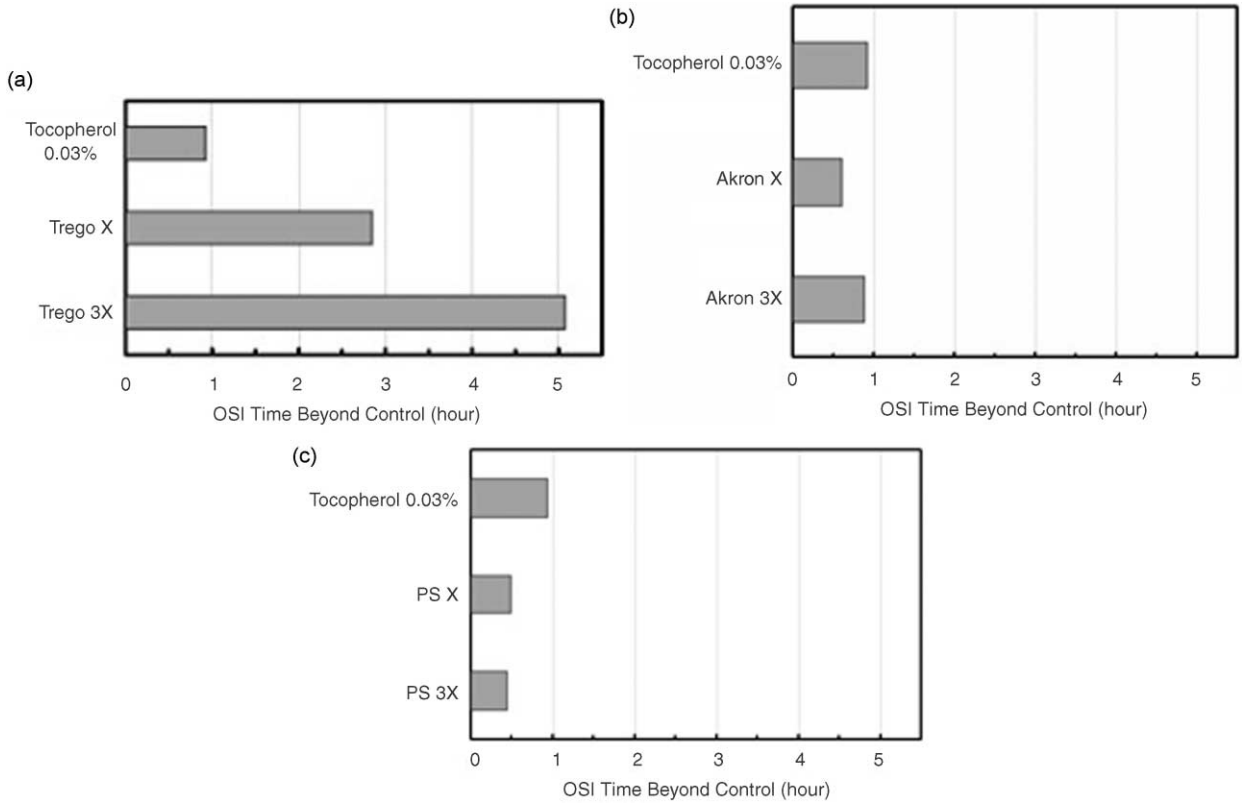

Fig. 1. Inhibitory effects of wheat extracts against lipid peroxidation in fish oils. Trego **X** = Trego extracts 0.06%, Trego 3**X** = Trego extracts 0.20%; Akron **X** = Akron extracts 0.09%, Akron 3**X** = Akron extracts 0.27%; Platte = Platte extracts 0.07%, Platte 3 = Platte extracts 0.21%; Tocopherol 0.03% represents the oil samples containing 0.03% α-tocopherol. All antioxidant concentrations are weight based. The OSI for the control sample containing no antioxidant was 2.5 h under the same experimental conditions. All tests were conducted in triplicate and the means are used.

concentration of Trego extract and the OSI time under the experimental conditions.

In contrast to Trego, extracts from Akron and Platte resulted in shorter OSI times than 300 ppm of α-copherol at the tested levels of 900 and 700 ppm, respectively (Fig. 1b and c). Increasing the levels of either Akron or Platte extracts did not significantly further delay the lipid oxidation in fish oils under the experimental conditions. Furthermore, the higher level of Platte extract resulted in decreased OSI duration, suggesting that Platte extract may act as a pro-oxidant instead of antioxidant at a higher concentration. These data suggested that wheat extracts from different varieties differed in their capacities to inhibit lipid oxidation in multicomponent food systems, and not all varieties of wheat are suitable for developing food antioxidants.

The free radical chain reaction is widely accepted as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to terminate the peroxidation chain reaction and improve the quality and stability of food products. The stable DPPH radical has been used to evaluate antioxidants for their radical quenching capacities (Bran-Williams, Cuvelier, & Berset, 1995; Chen & Ho, 1997). To better understand their antioxidant mechanism(s), each wheat extract was evaluated for radical scavenging activities against DPPH·. The greatest ED_{50} value of 7.10 mg/ml was detected in Trego extracts, followed by Platte extracts (0.95 mg/ml) and Akron extract (0.60 mg/ml; Fig. 2). The lower the ED_{50} value, the greater the radical scavenging activity. The order of the radical scavenging activity against DPPH radical is Akron extracts > Platte extracts > Trego extracts, under the experimental conditions. Trego extracts had the greatest ability to inhibit lipid peroxidation in fish oil but showed the lowest ability to directly react with and quench radical DPPH. These data suggest that it might be more critical, in delaying the lipid peroxidation, to suppress the initiation of the radical chain reaction, than to terminate the radical chain reaction by quenching or removing the radicals generated during propagation of the radical chain reaction.

It has been recognized that the initiation of free radical lipid peroxidation must be catalyzed (Nawar, 1996). Transition metals have been proposed to catalyze the formation of the first few radicals to start the propagation of radical chain reaction in lipid peroxidation. Chelating agents may inhibit lipid oxidation by stabilizing transition metals. To better understand the mechanism of the inhibitory effects of wheat extracts on lipid oxidation in fish oils, chelating activity of each wheat extract was evaluated against Fe^{+2} and expressed as EDTA equivalents. The three wheat extracts differed in their chelating capacities (Fig. 3). The greatest chelating capacity was detected in Akron extracts (5.44 mg/g extracts), but not Trego (5.41 mg/g extracts). The

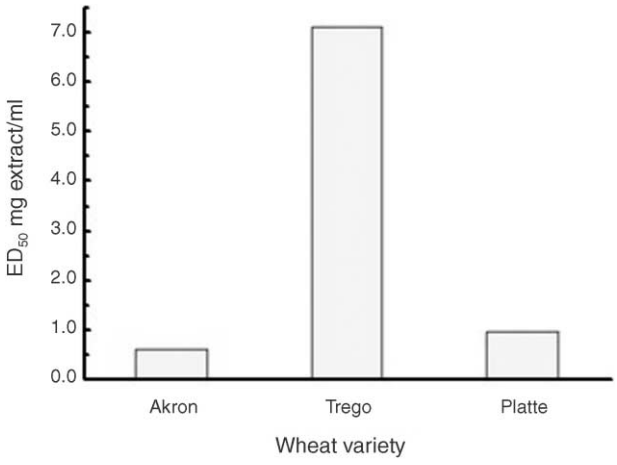

Fig. 2. Radical DPPH scavenging activity. Akron, Trego and Platte represent hard winter wheat varieties 'Akron', 'Trego' and 'Platte'.

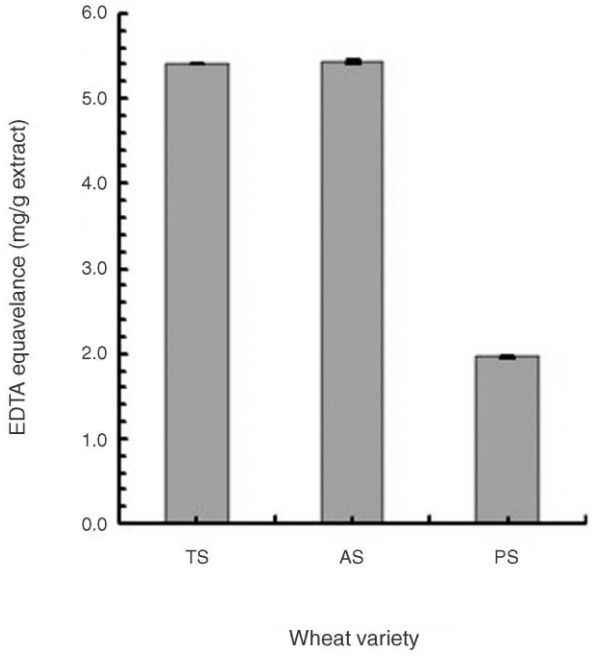

Fig. 3. Chelating capacity of wheat extracts. Akron, Trego and Platte represent hard winter wheat varieties 'Akron', 'Trego' and 'Platte'. Vertical bars represent the standard deviation ($n = 3$).

chelating activities of the wheat extracts also cannot provide a reasonable explanation for their effects on lipid oxidation in fish oils.

The phenolic compounds may contribute to both chelating activities and free radical scavenging capacities of the wheat extracts, while other chemical components may also present and account for the total antioxidant activities of the extracts (Yu, Haley, Perret, Harris, Wilson, & Qian, 2002b). These compounds may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron

transfer, radical addition, or radical recombination. Further composition analysis is necessary to better understand the relationships between chemical structures/composition and antioxidant properties.

In summary, extracts from three hard winter wheat varieties, including Trego, Akron and Platte, were evaluated for their potential to inhibit lipid peroxidation in fish oils. Trego extracts had the greatest capacity to suppress the lipid oxidation in fish oils and the capacity was comparable with tocopherol. These data demonstrate the possibility of developing wheat extracts from selected varieties, such as Trego, for applications as food antioxidants. In addition, the free radical scavenging capacity and chelating activities of the three wheat extracts were examined to better understand the mechanism(s) of their inhibitory effects against lipid peroxidation. The radical scavenging capacity and chelating activity could not explain the greatest inhibitory effect of Trego extract on lipid peroxidation under the experimental conditions.

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