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Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals

A. Baublis, E.A. Decker*, F.M. Clydesdale

Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA

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Abstract

Aqueous extracts from high bran, whole grain and refined wheat cereals are capable of inhibiting iron/ascorbic acid induced phosphatidylcholine liposome oxidation. High bran and whole grain cereal extracts contained the highest antioxidant activity and were able to inhibit both iron and peroxyl radical-promoted oxidation. Antioxidants in aqueous extracts from the high bran cereal were found in both high and low molecular weight fractions. Aqueous extracts from cereal homogenates that were subjected to simulated gastrointestinal pH treatments exhibited greater antioxidant activity than the untreated aqueous extracts. These data indicate that wheat-based cereals contain antioxidants whose activity is enhanced by gastrointestinal conditions suggesting that they could be important dietary antioxidants.

1. Introduction

There exists a healthy balance between oxidants (such as free radicals) and antioxidants (such as vitamins E and C and protective enzymes) in vivo (McCord, 1994). Unfortunately, situations occur where antioxidant defense systems that scavenge and minimize the formation and reactivity of prooxidants are not 100% effective. Therefore, oxidative damage does occur in vivo and has been linked to chronic diseases, such as cancer and atherosclerosis (Ames, 1989). To minimize potential oxidative damage in vivo, antioxidants from various dietary sources have been studied extensively. Accumulating evidence has shown that dietary intake of phytochemicals, including carotenoids, α-tocopherol, ascorbic acid and the flavonoids have been linked to the maintenance of health and protection from disease (Block & Langseth, 1994; Hertog, Hollman & Katan, 1992).

The ingredients found in wheat-based breakfast cereals have been shown to contain a number of components which are capable of inhibiting lipid oxidation including phytate, fiber and phenolics. Phytic acid inhibits lipid oxidation through its ability to chelate and inactivate prooxidant metals (Graf & Eaton, 1990). At molar ratios of 0.25 phytate-to-iron and above, the iron-promoted generation of OH· is almost completely blocked by phytate. Polysaccharides including xanthan (Shimada et al., 1997) and gum arabic (Anon., 1993) have been reported to display antioxidant activity through unknown mechanisms. In addition, fibers from cereal flours have been reported to inhibit lipoxygenase-catalyzed oxidation of linoleic acid emulsons (Lehtinen & Laakso, 1997). The proposed mechanism for the antioxidative effect of the cereal flour suspensions in the aqueous systems is due to physical interaction between the lipid material and fiber, with soluble fiber showing the greatest inhibitory activity. Wheat also contains phenolic compounds such as ferulic acid which are known antioxidants (Graf, 1992) and phenolics from ethanol extracts of wheat bran can inhibit oxidation of bulk oils (Onyeneho & Hettiarachchy, 1992).

Wheat contains several antioxidants that could be important in the diet. However, the majority of phytate, phenolics, and dietary fiber are in the bran layer of whole grains (Thompson, 1994) suggesting that the antioxidant potential of wheat-containing foods would be dependent on wheat or wheat fractions (e.g. bran, whole grain or refined) used in the food product. Therefore, the objective of this research was to evaluate the antioxidant potential of ready-to-eat breakfast cereals manufactured with different sources of wheat. The research evaluated the antioxidant potential of aqueous

* Corresponding author. Tel.: +1-413-545-1026; fax: +1-413-545-1266.

E-mail address: edecker@foodsci.umass.edu (E.A. Decker)

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extracts of the breakfast cereals because numerous other water-soluble antioxidants, such as those found in tea and fruits, have been found to have positive health benefits (Decker, 1995).

2. Materials and methods

2.1. Materials

L-α-Phosphatidylcholine (type IV-S from soybean), Folin and Ciocalteu's phenol reagent (2 N), 2-thiobarbituric acid, 1,1,3,3-tetraethoxypropane, and ferric chloride were obtained from Sigma Chemical Company (St. Louis, MO). L-Ascorbic acid was obtained from Fischer Scientific (Pittsburgh, PA). Fiber One™, Wheaties™ and Cream of Wheat™ cereals were the sources of the high bran, whole grain, and refined wheat cereals, respectively. The cereals were purchased from a local supermarket. All other chemicals were of analytical grade or purer.

2.2. Methods

2.2.1. Preparation and characterization of cereal extracts

Cereals were crushed into a powder (8.3 g) using a mortar and pestle and extracted at room temperature with double distilled deionized (dddI) water (125 ml) while being continuously stirred using a teflon coated magnetic stir bar and stir plate for 30 min. Following extraction the samples were centrifuged at 8000×*g* (20°C) for 30 min. The resulting supernatant was collected and used for antioxidant analysis. Simulated gastrointestinal pH conditions were conducted on the cereal homogenates by incubating homogenates at room temperature for 30 min (pH 6.5–7.0), decreasing the pH to 2 and incubating at 37°C for 30 min (to simulate transition into the stomach) and then raising pH to 6 for 30 min at 37°C (to simulate transition into the small intestine) (Platt & Clydesdale, 1984). The pH-adjusted cereal homogenates were centrifuged and the resulting supernatant was collected and used for antioxidant assays.

Low molecular weight compounds in the aqueous extracts were isolated by ultrafiltration using a Spectrum (Spectrum Medical Industries, Houston, TX) 70 ml stir cell unit equipped with a Amicon Diaflo YM3 membrane (Amicon Corporation, Lexington, MA) with a molecular weight cutoff of 3000 daltons. The extract was passed through the membrane using 50 psi of nitrogen gas at room temperature while constantly being stirred. High molecular weight (HMW) compounds were isolated using dialysis tubing with a 3500 dalton molecular weight cutoff. Aqueous extract was dialyzed against 100 volumes of water while constantly being stirred for approximately 8 h. Dialysis was repeated three times. The final volume of the retentate was measured so that the dialyzed aqueous extract could be evaluated at a volume equivalent to the original extract. Phenolics present in the aqueous cereal extracts and ultrafiltration permeates were measured using the Folin–Ciocalteu method according to Eguchi, Curtis and Shetty (1996).

2.2.2. Lipid oxidation studies

Liposomes were prepared from soybean phosphytidylcholine by sonication according to the method of Decker and Hultin (1990). Phospholipid concentration in the liposome solution was estimated by measuring the phosphate content according to the method of Anderson and Davis (1982). Oxidation was performed in a shaking water bath at 37°C for 30 min using an ascorbic acid-ferric chloride redox system as the prooxidant. A typical oxidation reaction contained 30 μM $FeCl_3$, 100 μM ascorbic acid, 2 mg liposomes, various aqueous cereal extracts at the indicated volume and buffer (5 mM K_2HPO_4/0.12 M KCl, pH 7) to make the total volume 5 ml. Reactants were added to the system in the following order: buffer, $FeCl_3$, aqueous cereal extract, liposome and ascorbic acid. Peroxyl radicals were generated with 2,2′-azobis-(2-amidopropane) (AAPH) at 10 mM. AAPH oxidation was conducted under similar conditions as the ascorbic acid–ferric chloride oxidation system with the following changes: 4 mg of phosphatidylcholine was used instead of 2 mg and incubation was carried out for 3.5 h instead of 30 min to increase lipid oxidation.

Assessment of oxidation was achieved by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of McDonald and Hultin (1987) and by using a modification of the lipid peroxide method reported by Shantha and Decker (1994). Lipid peroxides were isolated from 0.08 ml of sample by mixing with 2 ml chloroform–methanol (2:1, v/v) with a vortex mixer for 5 s followed by centrifugation for 5 min at 2000×*g*. The lower organic phase (1.0 ml) was then mixed with 0.63 ml of chloroform-methanol (2:1, v/v), 8 μl ammonium thiocyanate solution and 8 μl of Fe(II). The samples were mixed on a vortex mixer for approximately 2 s and allowed to incubate for 5 min at room temperature. Absorbance was determined at 500 nm against a blank of chloroform–methanol (2:1, v/v). TBARS in cereal extract controls (no liposomes) were determined for all samples to correct for TBARS not resulting from lipid oxidation. Both TBARS and lipid peroxide concentration were determined from standard curves constructed using 1,1,3,3-tetraethoxypropane and cumene hydroperoxide, respectively, and were expressed as μM/mg phospholipid. The data were presented as percent activity which was calculated as:

(TBARS or ROOH in presence of cereal extract/TBARS or ROOH in absence of cereal extract)×100.

2.2.3. Statistics

All data are presented as mean ± standard deviation (SD) and analyzed by paired Student's *t*-test using Statview® 512+ (Brain Power, Inc., Calabasas, CA).

3. Results and discussion

The influence of extracts of foods and the biological tissues from which foods are derived on lipid oxidation represents a balance between the reactivity of both prooxidants and antioxidants. Wheat is known to contain antioxidative compounds such as phenolics and phytic acid (Graf, 1992; Lehtinen & Laakso, 1997; Onyeneho & Hettiarachchy, 1992). However, the impact of extracts from ready-to-eat breakfast cereals on lipid oxidation would differ from cereal grains because they are commonly fortified with iron, a potential prooxidant. In the phophatidylcholine liposome model system used in this study, the breakfast cereal extracts were not observed to promote lipid oxidation in the absence of added prooxidants indicating that they were not strongly prooxidative (data not shown). In addition, these breakfast cereals are fortified primarily with ferric forms of iron which do not promote oxidation in the phosphatidylcholine liposome model used in this study (Decker & Hultin, 1990; Lee & Clydesdale, 1979). Since iron is the primary prooxidant added to ready-to-eat breakfast cereals, it is likely that the ability of the different cereal extracts to impact lipid oxidation is due mainly to differences in antioxidant compositions. However, differences in the reactivity of prooxidants originating from the different breakfast cereals could also be impacting the observed oxidation kinetics.

The ability of various concentrations (8–55% of the assay volume) of aqueous extracts of high bran, whole grain, and refined wheat cereals to inhibit iron-accelerated oxidation of phosphatidylcholine liposomes is shown in Fig. 1. The order of antioxidant activity was high bran (10–55% activity) > whole grain (20–60% activity) > refined wheat (80–100% activity). When inhibition of iron-accelerated lipid oxidation by the different cereal extracts (15% of assay volume) was compared by monitoring both TBARS or lipid peroxides (Fig. 2), similar levels of antioxidant activity were observed. The ability of the cereal extracts to inhibit lipid oxidation accelerated by the nonmetallic peroxyl radical generator, AAPH (Fig. 3), was similar to the antioxidant activity observed in the iron–ascorbate system. The ability of high bran and whole grain extracts to inhibit both metal and nonmetallic catalyzed oxidation suggests that metal chelation was not the major antioxidant mechanism responsible for the observed antioxidant activity.

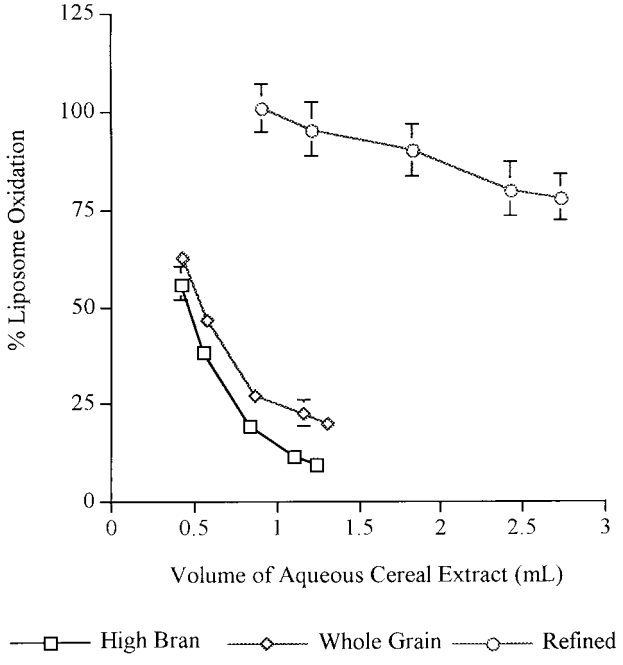

Fig. 1. Phosphatidylcholine liposome oxidation, induced with iron/ascorbic acid, in the presence of high fiber, whole grain, and refined wheat cereal aqueous extracts added at extract concentrations ranging from 8 to 55% of the assay volume. TBARS in the absence of extracts were 1.93 μM TBARS/mg liposome. Error bars represent standard deviations of mean ($n=3$). Some error bars lie within the data point symbol.

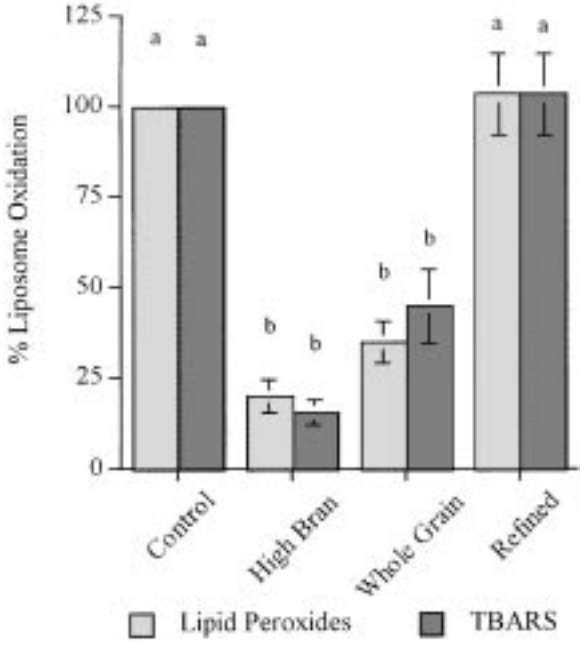

Fig. 2. Phosphatidylcholine liposome oxidation, induced with iron/ascorbic acid, in the presence of high bran, whole grain, and refined wheat cereal aqueous extracts added at 15% of assay volume. TBARS and lipid peroxides in the absence of extracts were 1.66 μM TBARS/mg liposome and 0.143 μM lipid peroxides/mg liposome, respectively. Error bars represent standard deviations of mean ($n=3$). Bars within each cereal treatment sharing the same letters are not statistically different ($P<0.05$).

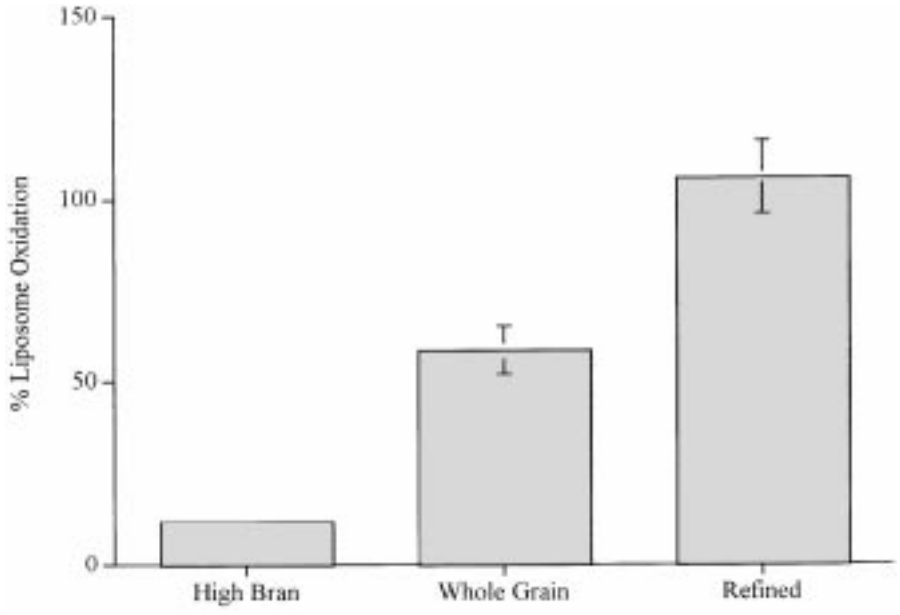

Fig. 3. Phosphatidylcholine liposome oxidation in the presence of high bran, whole grain, and refined wheat cereal aqueous extracts added at 15% of assay volume using 2,2′-azobis-(2-amidopropane) as a peroxy radical generator to promote oxidation. TBARS in the absence of extracts were 0.54 μM TBARS/mg liposome. Error bars represent standard deviations of mean ($n = 3$).

High bran extracts were fractionated into high (HMW) and low (LMW) molecular weight fractions by dialysis and ultrafiltration, respectively. When lipid oxidation was monitored with either lipid peroxides or TBARS the order of antioxidant activity for these fractions was total aqueous extract > HMW fraction > LMW fraction with the LMW fraction showing little to no antioxidant activity at the concentration tested (Fig. 4). Similar results were observed for these different fractions in the whole grain extract (data not shown). Since both lipid peroxides and TBARS gave similar antioxidant activities for both the whole grain and high bran extracts, only TBARS were used for subsequent analysis. In addition, since the refined wheat cereal had little or no antioxidant activity, subsequent studies focused on the high bran and whole grain cereals.

Phenolics are effective antioxidants due to their ability to inactivate free radicals (Decker, 1997; Graf, 1992). Therefore, it was surprising that the LMW fraction of the cereal extracts did not inhibit lipid oxidation since phenolics in wheat such as ferulic acid would have a molecular weight below 3000. When higher concentrations of the LMW fraction were tested, inhibition of iron-accelerated lipid oxidation was observed at LMW concentrations $\geqslant 16$ or 20% of the assay volume for high bran and whole grain cereals, respectively (Fig. 5). The ability of the LMW fractions to inhibit lipid oxidation increased with increasing concentrations.

Acid conditions can increase the extractability of phenolics esterified to carbohydrates from wheat bran (Kroon, Foulds, Ryden, Robertson & Williamson, 1997). Gastrointestinal pH conditions caused a dramatic increase in antioxidant activity for the high bran and whole grain cereal aqueous extracts and their LMW fractions (Fig. 5) suggesting that acid conditions caused alterations in the activity, composition and/or concentration of water-soluble LMW antioxidants. It is

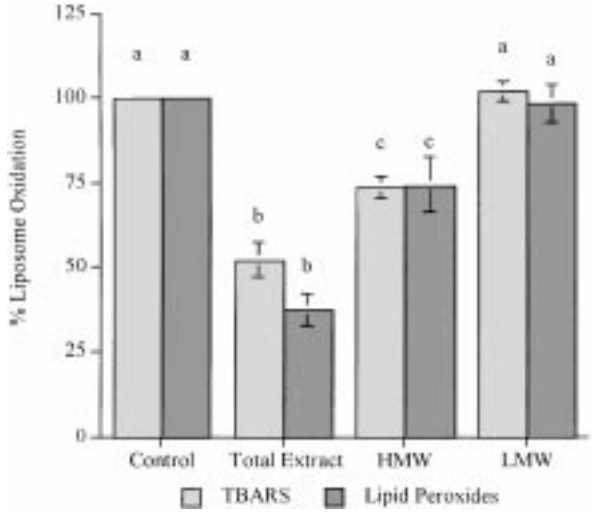

Fig. 4. Phosphatidylcholine liposome oxidation induced by iron/ascorbic acid in the presence of high bran cereal aqueous extract subjected to molecular weight fractionation by dialysis [high molecular weight (HMW) fraction; > 3500 Da], and ultrafiltration [low molecular weight (LMW) fraction; < 3000 Da] added at 15% of assay volume. TBARS and lipid peroxides in the absence of extracts were 1.20 μM TBARS/mg liposome and 0.117 μM lipid peroxides/mg liposome, respectively. Error bars represent standard deviations of mean ($n = 3$). Means not sharing a common letter differ significantly ($P < 0.05$).

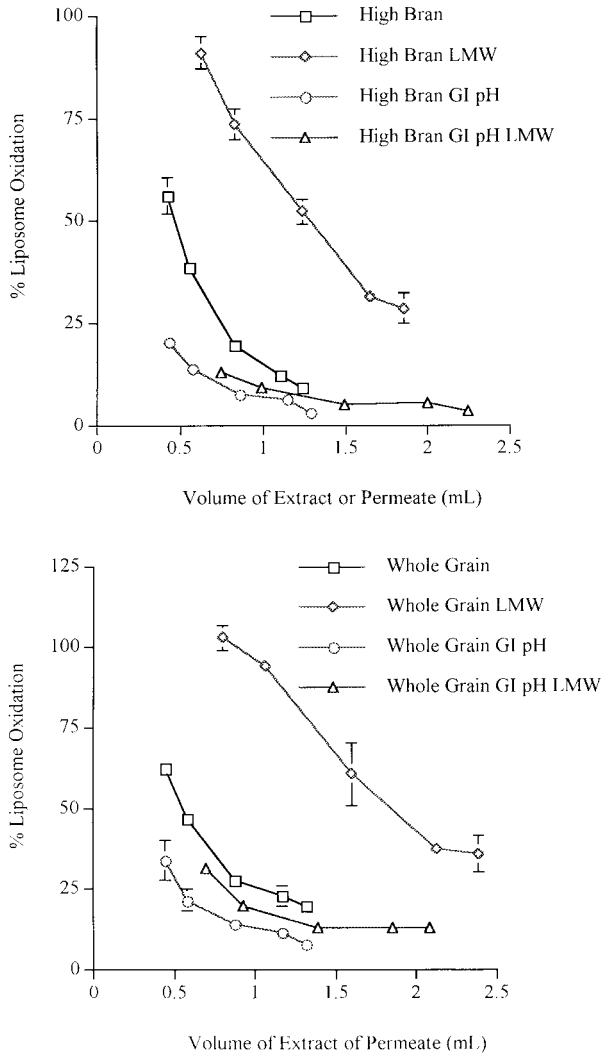

Fig. 5. Phosphatidylcholine liposome oxidation, induced with iron/ascorbic acid, in the presence of high bran and whole grain cereal extracts and their low molecular weight (LMW) fractions which had been subjected to gastrointestinal (GI) pH conditions. TBARS in the absence of extracts were 2.11 μM TBARS/mg liposome. Error bars represent standard deviations of mean ($n=3$). Some error bars lie within the data point symbols.

possible that these acidic conditions could influenced phenolic composition. Phenolic compounds are commonly esterified to sugars or acids. Kroon et al. reported that gastric intestinal treatment of fine wheat bran resulted in the release of 0.41 nmol of free ferulic acid and 6.91 nmol of esterfied ferulic acid (*O*-[5-*O*-(trans-feruloyl)-α-L-arabinofuranosyl]-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose equivalents). Ohta, Yamasaki, Egashira, and Sanada (1994) reported that the antioxidant activity of 5-*O*-feruloyl-L-arabinofuranose and *O*-(5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose were slightly stronger than free ferulic acid in inhibiting oxidation of rat liver microsomes induced by carbon tetra-chloride. Also, Saija et al. (1995) reported that rutin displayed higher antioxidant activity compared to its non-glycosylated counterpart, quercetin, in an iron induced linoleate oxidation system. It is possible that glycosylated phenolics are being released from wheat during the gastrointestinal pH treatment thus altering phenolic composition. If these glycosylated phenolics are more effective antioxidants in the phosphatidylcholine liposomes system used in this study, this could explain why increased antioxidant activity was observed following gastrointestinal pH treatments. Alternatively, increased antioxidant activity could also be due to the release of other LMW antioxidants such as phytate or could be due to decreased reactivity of prooxidants in the cereal extracts.

4. Conclusions

Whole wheat- and wheat bran-based ready-to-eat breakfast cereals contain water-soluble compounds that can inhibit lipid oxidation promoted by both iron and peroxyl radicals. Both high (>3500) and low (<3000) weight fractions can inhibit lipid oxidation indicating that several distinctly different antioxidants exist in aqueous extracts of wheat-based breakfast cereals. Simulated gastrointestinal pH treatment increased antioxidant activity in both the aqueous extracts and the LMW fractions of whole wheat and high bran cereals suggesting that antioxidant activity could be enhanced by the digestive process. This research suggests that wheat-based whole grain or high bran cereals may have beneficial health effects. However, additional research is needed to identify the active antioxidant components and to evaluate their activity in vivo.

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