JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Evaluation of Antioxidant Capacity of Cereal Brans

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Several oat brans (crunchy oat bran, oat bran alone, and oat breakfast cereal) and wheat brans (wheat bran alone, wheat bran powder, wheat bran with malt flavor, bran breakfast cereal, tablet of bran, and tablet of bran with cellulose) used as dietary fiber supplements by consumers were evaluated as alternative antioxidant sources (i) in the normal human consumer, preventing disease and promoting health, and (ii) in food processing, preserving oxidative alterations. Products containing wheat bran exhibited higher peroxyl radical scavenging effectiveness than those with oat bran. Wheat bran powder was the best hydroxyl radical ($OH^{\bullet}$) scavenger. In terms of hydrogen peroxide (H_2O_2) scavenging, wheat bran alone was the most effective, while crunchy oat bran, oat bran alone, and oat breakfast cereal did not scavenge H_2O_2. The shelf life of fats (obtained by the Rancimat method for butter) increased most in the presence of crunchy oat bran. When the antioxidant activity during 28 days of storage was measured by the linoleic acid assay, all of the oat and wheat bran samples analyzed showed very good antioxidant activities. The Trolox equivalent antioxidant capacity (TEAC) assay was used to provide a ranking order of antioxidant activity. The wheat bran results for TEAC (6 min), in decreasing order, were wheat bran powder > wheat bran with malt flavor ≥ wheat bran alone ≥ bran breakfast cereal > tablet of bran > tablet of bran with cellulose. The products made with oat bran showed lower TEAC values. In general, avenanthramide showed a higher antioxidant level than each of the following typical cereal components: ferulic acid, gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, vanillic acid, vanillin, and phytic acid.

KEYWORDS: Antioxidant; wheat; oat; bran

INTRODUCTION

The U.S. Department of Agriculture in its Food Guide Pyramid suggests at least three servings a day of whole grains (*1*), to reach a daily level of consumption of 20 to 30 g of fiber. Most developing countries rely on cereals as their major food source, from which more than half of the calories consumed for energy are obtained (*2*). Cereals are high in nutritional structures such as starch, protein, and polar lipids; low in saturated fat (*3*); and relatively high in ω-3 fatty acids such as linolenic acid ($C_{18:3}$), linoleic acid ($C_{18:2}$, ω-6) (*4*), and α-tocopherols and α-tocotrienols (*5*). Furthermore, whole grains are rich in bioactive compounds, insoluble fiber (mainly cellulose, hemicelluloses, and lignin) and soluble fiber, pectin, guar gum, β-glucan, xylan, and arabinoxylan. Wheat bran contains more insoluble fiber while oat brans are excellent sources of soluble fiber (*6*, *7*).

Grains are a major source of phytoestrogens such as lignans (*3*, *8*, *9*), phytic acid, tannins (*3*), sterols (β-sitosterol and avenasterols) (*5*), phenolic compounds and flavonoids (*10*, *11*), and ferulic, caffeic, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, gentisic, sinapic, isoferulic, chlorogenic, vanillic, *p*-hydroxy-phenylacetic, and syringic acids, as well as vanillin. These last compounds occur in the grain primarily in the bound form as conjugates with sugars, fatty acids, or protein (*12*, *13*); as esters; and as diols (*3*). In addition, oats contain N-cinnamoylanthranilate alkaloids, avenanthramides (*11*), apigenin, luteolin, and tricin, while wheat seeds contain lutein (*14*).

There is considerable epidemiological evidence demonstrating the protective role of diets high in whole grains as regards chronic diseases. Soluble fiber, particularly pectin and guar gum, has been reported to decrease coronary heart disease risk (*15*). β-glucan attenuates postprandial elevations in both glucose and

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10.1021/jf049621s CCC: $27.50
Published on Web 06/25/2004

Table 1. Summary of Information on Oat and Wheat Brans, Their Ingredients, or Active Material and Data on Industrial Processing

commercial samples	ingredients	processing/step
crunchy oat bran	oat bran (99.9%)	mixed
	mixed tocopherols (100 ppm)	extrusioned through steam
		dried high temperature (120–130 °C approx. 20′)
		packed
oat bran alone	oat bran (100%)	mixed through steam
		extrusioned
		packed
oat breakfast cereal	whole oat flour (50.4%)	mixed through steam
	wheat flour (36.6%)	extrusioned (2 atm, 150 °C)
	brown sugar (4%)	packed
	malt flour (2%)	
	salt (1.4%)	
wheat bran alone	wheat bran (100%)	packed
wheat bran powder	wheat bran powder (100%)	packed
wheat bran with malt flavor	wheat bran (94.8%)	mixed
	malt flavoring (10^3 ppm)	extrusioned through steam
	fruit aroma E-6046 (10^3 ppm)	dried high temperature (120–130 °C approx. 20′)
		packed
bran breakfast cereal	wheat bran (87%)	mixed through steam
	brown sugar (4%)	extrusioned (2 atm, 150 °C)
	malt flour (2%)	packed
	salt (1.4%)	
tablet of bran	wheat bran powder (64%)	mixed
	lactose (26%)	granulated through steam
	wheat germ (10%)	dried (60–65 °C approx. 8 h)
	calcium phosphate, dibasic (10^3 ppm)	compression
tablet of bran with cellulose	wheat bran powder (67%)	mixed
	cellulose (30%)	compression
	malt flavoring (10^3 ppm)	
	silicon dioxide (10^3 ppm)	

insulin, and possibly triglycerides, especially in diabetics (*16*); insoluble fiber improves colonic functioning (*7*).

Phytoestrogens are recognized for their health-promoting properties inhibiting platelet aggregation (*11*); their antiallergic, anthelminthic, hepatoprotective, antihormonal, antiviral, antimicrobial, and antiinflammatory actions; and their effect on certain cancers (*6*). These phytochemicals, in their role as antioxidants, may also play an important part in human health by scavenging reactive oxygen and nitrogen species and modulating several enzyme systems, such as lipoxygenases (high in oats) (*10*), presumably by chelation of prooxidant metal ions (*5*) such as magnesium and zinc (*17*); alternatively, they may transfer one electron to the radical (*14*).

o-Diphenols exhibit a strong antioxidant activity as compared with less sterically hindered phenolic acids, such as tyrosol. In addition, phenolic acids that are hydroxy derivatives of cinnamic acid are more actively antioxidant than hydroxy derivatives of benzoic acid. Avenanthramides display a higher antioxidant activity than tocopherols or phenolic compounds (*5*).

Antioxidants are concentrated in bran fractions, although the endosperm has significant activity. It is not easy to determine how much of the total bound or insoluble antioxidants is measured by a given assay; furthermore, Maillard reaction products, which also have antioxidant activity, are formed during the processing of breakfast cereals (*6*).

The level of antioxidant activity in blood increases after the consumption of foods high in antioxidants. If the bran of whole grains is essentially intact when it is consumed, the cell contents will be much less available for absorption. Whole grain antioxidants can act as free radical scavengers through the entire digestive tract and in colon tissues (*6*).

New products with high fiber contents have been formulated by the food industry (*18*); in addition, cereal components have been added to food and beverage products in order to preserve and conserve flavor, color, and texture (*10*).

Although several studies on the antioxidant activity of cereal bran have been conducted, the data are still insufficient. Antioxidants are often tested alone, while several structures may be present in foods, allowing antioxidant or prooxidant interactions. For this reason, health and safety demands point to the need for further evaluation.

Our aim was to evaluate some cereal brans (used as dietary fiber supplements by consumers), antioxidant alternatives for use as sources, (i) in the normal human consumer, preventing disease and promoting health, and (ii) in food processing, preserving oxidative alterations.

MATERIALS AND METHODS

Materials. All chemicals were of chromatographic grade quality and were purchased from Sigma Chemical Co. (Poole, Dorset). Oat brans (crunchy oat bran, oat bran alone, and oat breakfast cereal) and wheat brans (wheat bran alone, wheat bran powder, wheat bran with malt flavor, bran breakfast cereal, tablet of bran, and tablet of bran with cellulose) used as dietary fiber supplements (20−50% fiber content according to the labels) and ingredients or active materials were also obtained from the manufacturer (Casa Santiveri, S. A., Barcelona, Spain) (see **Table 1**).

The widely used food antioxidants, BHA (E-320), BHT (E-321), and PG (E-310) [at the permitted concentration of 100 μg/g (*19*)] and Trolox (water soluble analogue of vitamin E at 0.5 mM) were used as antioxidant standards, and some compounds (avenanthramide, ferulic acid, gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, vanillic acid, vanillin, and phytic acid at 0.5 mM) described in the composition of the cereals were used as active standards.

Samples Preparation. Forty grams of ground sample (crunchy oat bran, oat bran alone, oat breakfast cereal, wheat bran alone, wheat bran powder, wheat bran with malt flavor, and bran breakfast cereal) or 10

ground tablets (tablet of bran and tablet of bran with cellulose) (recommended daily dose on the label) or their ingredients/active materials (according **Table 1**) was extracted with water (200 mL, similar to cup size used by consumers) while being continuously stirred, using a Teflon-coated magnetic stir bar and stir plate for 30 min. This "soluble" fraction is probably of greatest interest, as it is the most easily released from the food matrix into the digestive tract (*20*). The sample was centrifuged at 3200*g* for 5 min. The resulting supernatant was collected, frozen, and used for antioxidant analysis (*21*). Each extract was analyzed in quintuplicate, and five separate extracts were taken from each bran sample.

Peroxidation of Phospholipid Liposomes. The ability of samples to inhibit lipid peroxidation at pH 7.4 was tested using ox brain phospholipid liposomes, essentially as described in Murcia and Martínez-Tomé (*22*). The experiments were conducted in a physiological saline buffer (3.4 mM $Na_2HPO_4-NaH_2PO_4$ 0.15 M NaCl), pH 7.4. In a final volume of 1 mL, the assay mixtures were made up with PBS, 0.5 mg/mL phospholipid liposomes, 100 μM $FeCl_3$, 100 μL of samples (or 100 μL of food common antioxidants dissolved in water), and 100 μM ascorbate (added last to start the reaction). Because BHT is not fully soluble in aqueous solution and its emulsion is not homogeneous, deionized water with a conductivity of not more than 4 μs/cm was used to dissolve it. The incubations were at 37 °C for 60 min, at the end of which 1 mL each of 1% (wt/v) TBA and 2.8% (wt/v) trichloroacetic acid was added to each mixture. The solutions were heated in a water bath at 80 °C for 20 min to develop the MDA−TBA adduct [$(TBA)_2$−MDA)]. The $(TBA)_2$−MDA chromogen was extracted into 2 mL of butan-1-ol, and the peroxidation extent was measured in the organic layer as absorbance at 532 nm.

Hydroxyl Radical Scavenging. In a final volume of 1.2 mL, the reaction mixtures contained the following reagents: 10 mM KH_2PO_4−KOH buffer (pH 7.4), 2.8 mM H_2O_2, 2.8 mM deoxyribose (where used), 50 μM $FeCl_3$ premixed with 100 μM EDTA before addition to the reaction mixture, and 100 μL of the tested samples (or 100 μL of food common antioxidants dissolved in water). Ascorbate (100 μM), where used, was added to start the reaction. The tubes were incubated at 37 °C for 1 h. The products of the hydroxyl radical ($OH^{\bullet}$) attack upon deoxyribose were measured as described in Murcia et al. (*23*).

Scavenging of Hydrogen Peroxide. The samples (100 μL) (or 100 μL of food common antioxidants dissolved in water) to be tested with H_2O_2 were incubated with 0.84 mM H_2O_2 for 10 min at 25 °C. Aliquots of these compounds were then taken and assayed for remaining H_2O_2 by using the peroxidase system (*24*). The remaining H_2O_2 was measured as the formation of a chromophore recorded at 436 nm in reaction mixtures containing, in a final volume of 1 mL, 0.150 M KH_2PO_4−KOH buffer, pH 7.4, 50 μL of guaiacol solution (made by adding 100 μL of pure guaiacol to 100 mL of water), and 10 μL of Sigma type IV horseradish peroxidase (5 mg/mL in the same phosphate buffer). N-Acetyl-L-cysteine (NAC) was used as a positive control of hydrogen peroxide scavenging.

Rancimat Test for Oxidative Stability. Sample preparation for the Rancimat test consisted of macerating 25 g of refined olive oil or butter with 5 g of sample (crunchy oat bran, oat bran alone, oat breakfast cereal, wheat bran alone, wheat bran powder, wheat bran with malt flavor, or bran breakfast cereal) or two tablets (tablet of bran or tablet of bran with cellulose) or 100 mg/g common food antioxidants for 3 h at room temperature before analysis.

The Rancimat method (Metrohm model 743, Herisan, Switzerland) determines the IP by measuring the increase in the volatile acidic byproducts released from the oxidizing oil at 120 °C. The concentration of the degradation products, which is transferred into distilled water, is assessed by measuring the conductivity. Longer IPs suggest a stronger activity of the added antioxidants. The relative activity of the antioxidants is expressed by the PF, which is calculated by dividing the IP of oil with the addition of antioxidants by the IP of the control (olive oil alone or butter alone) (*25*).

This technique has been questioned by some authors (*26*), but in agreement with Martínez-Tomé et al. (*24*) and Murcia et al. (*27*), we decided to apply it in this work because it is a commonly used procedure in the food industry and governmental analytical laboratories.

Determination of Antioxidant Activity in Linoleic Acid System. To a solution of 10 mL of linoleic acid (11.7 g/L in 99.8% ethanol) and 10 mL of phosphate buffer (200 mM, pH 7.0), 5 mL of the analyzed sample (or 5 mL of the common food antioxidants dissolved in water) was added. The total volume was adjusted to 25 mL with deionized water. This solution mixture was incubated at 40 °C, and the degree of oxidation was measured. For this, 10 mL of ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%), 0.2 mL of sample (solution mixture), and 0.2 mL of ferrous chloride solution (20 mM in 3.5% HCl) were stirred for 3 min. The absorption values of the mixtures measured at 500 nm were taken as the peroxide content. The inhibition percentage of linoleic acid peroxidation, 100 − [(Abs increase of sample/Abs increase of control) × 100] was calculated to express antioxidant activity (*28*).

Measurement of Total Antioxidant Activity by TEAC Assay. The $ABTS^{\bullet-}$ radical solution was generated from the following reagents: 2.5 mM ABAP and 20 mM $ABTS^{2-}$ stock solution in phosphate buffer solution (containing 100 mM phosphate and 150 mM NaCl, pH 7.4). These were incubated at 60 °C for 12 min, protected from light, and stored at room temperature. The absorbance at 734 nm was measured to check $ABTS^{\bullet-}$ formation (the results must be between 0.35 and 0.45) (*29*).

The antioxidant activity of the samples analyzed (40 μL mixed with 1960 μL of the radical solution) was measured at 734 nm for 6 min. The decrease in absorption at 734 nm observed 6 min after the addition of each compound was used to calculate the TEAC.

A calibration curve was prepared with different concentrations of Trolox (standard solution used to evaluate equivalent antioxidant capacity). By measuring the increase in absorption during 6 min (standard range of 0−10 μM), the absorbance values were corrected for the solvent [samples giving absortion > standard (at 10 μM) were diluted to reduce the measurement within the appropriate part of the Trolox standard curve].

$$\Delta Abs_{Trolox} = Abs_{t=6\ min\ Trolox} - Abs_{t=6\ min\ solvent}$$

The regression coefficient (rc) was calculated from the calibration curve.

$$\Delta Abs_{Trolox} = rc \times [Trolox]$$

To establish the TEAC of commercial antioxidants or analyzed samples, the increase in absorption was measured in the same way. The TEAC was calculated as follows:

$$TEAC_{sample} = \Delta Abs_{sample}/rc$$

The TEAC represents the concentration of a Trolox solution that has the same antioxidant capacity as the analyzed sample.

Statistical Analysis. All experiments were carried out in quintuplicate. The results were analyzed using the Statistical Package for Social Sciences Windows 11.0 and the analysis of variance (ANOVA) procedure.

RESULTS AND DISCUSSION

Inhibition of Phospholipid Peroxidation. This assay examines whether a substance inhibits the peroxidation of artificial lipid systems, such as brain phospholipid liposomes incubated with $FeCl_3$ and ascorbic acid, by scavenging peroxyl radicals (*22*).

Table 2 shows the inhibition of lipid peroxidation in the presence of oat and wheat brans as compared with the activity of the standards (typical cereal compounds and common food additives). Wheat bran powder was the most effective scavenger of peroxyl radical followed, in this order, by wheat bran alone, bran breakfast cereal, wheat bran with malt flavor, oat bran alone, tablet of bran, tablet of bran with cellulose, oat breakfast cereal, and crunchy oat bran.

The products made with wheat bran (wheat bran alone, bran breakfast cereal, and wheat bran with malt flavor) exhibited a

Table 2. Inhibition of Peroxidation in the Lipid System Using Ox Brain Phospholipids by Oat and Wheat Brans as Compared with the Activities of Standards (Typical Cereal Compounds and Common Food Additives)

added to reaction mixtures	% inhibition[a]
oat bran	
crunchy oat bran	47.0 ± 1
oat bran alone	57.8 ± 2
oat breakfast cereal	48.5 ± 1
wheat bran	
wheat bran alone	68.4 ± 1
wheat bran powder	78.5 ± 2
wheat bran with malt flavor	67.3 ± 2
bran breakfast cereal	67.5 ± 1
tablet of bran	51.8 ± 2
tablet of bran with cellulose	50.2 ± 2
standards	
avenanthramide	57.0 ± 1
ferulic acid	27.6 ± 1
gentisic acid	26.5 ± 2
p-hydroxybenzoic acid	10.4 ± 1
protocatechuic acid	15.9 ± 1
syringic acid	24.8 ± 2
vanillic acid	21.9 ± 1
vanillin	17.3 ± 1
phytic acid	34.3 ± 1
BHA	71.4 ± 2
BHT	22.3 ± 1
Trolox	70.6 ± 2
PG	52.5 ± 2

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$).

higher antioxidant activity than those made with oat bran (oat bran alone, oat breakfast cereal, and crunchy oat bran). However, when wheat products were subjected to industrial processing to elaborate tablets (**Table 1**), the inhibition percentages decreased significantly ($p < 0.05$) from those observed for the other wheat samples, being 51.8% for tablet of bran and 50.2% for tablet of bran with cellulose, because they contained a lower amount of the basic ingredient (wheat bran) and some other additional ingredients (see **Table 1**) that showed lower antioxidant activities. This provides some idea of how much the different ingredients affected the antioxidant activity when they are analyzed separately (**Table 7**). Some of these additional ingredients produce a dilution effect on the antioxidant capacity of the final preparation. Tablets of bran are made with wheat bran powder, wheat germ, lactose, and calcium phosphate, the first two components having a very high antioxidant capacity, while a tablet of bran with cellulose contains wheat bran powder, malt flavoring, silicon dioxide, and cellulose.

With regards to the oat samples, oat bran alone showed a better antioxidant activity than the other oat products (oat breakfast cereal and crunchy oat bran), which had been subjected to industrial processing at high temperatures (more than 120 °C) (see **Table 1**) thus decreasing the lipid peroxidation inhibition percentages.

The inhibition of lipid peroxidation by all of the oat and wheat products was significantly ($p < 0.05$) higher than that exhibited by the standards, except BHA and Trolox. PG showed a similar antioxidant activity to the tablets. The inhibition obtained with the standards, except avenanthramide (57% inhibition), was lower than 50% in all cases and in the following decreasing order: phytic acid, ferulic acid, gentisic acid, syringic acid, BHT, vanillic acid, vanillin, protocatehuic acid, and *p*-hydroxybenzoic acid.

The antioxidant activity of wheat bran extract was comparable with that of BHA, although the nonheated wheat bran solution prevented lipid peroxidation slightly more strongly than a BHA solution (*2*).

In an earlier study, Yu et al. (*30*) attributed the potential of wheat extracts as food antioxidants to the phenolic compound that they contain, since these suppress lipid peroxidation by both chelating and free radical scavenging. As regards oat bran, Kähkönen et al. (*13*) observed an 80% inhibition of conjugate diene hydroperoxide formation.

Table 3. Deoxyribose Damage by OH• Radical in the Presence of Oat and Wheat Brans as Compared with the Activities of Standards (Typical Cereal Compounds and Common Food Additives)

	damage to deoxyribose[a]		
added to reaction mixtures	for RM + DR	% inhibition	without ASC[b]
none (control)	1.015 ± 0.03		0.226
oat bran			
crunchy oat bran	0.624 ± 0.01	38.5	0.217
oat bran alone	0.595 ± 0.01	41.3	0.164
oat breakfast cereal	0.223 ± 0.02	78.0	0.084
wheat bran			
wheat bran alone	0.211 ± 0.04	79.2	0.032
wheat bran powder	0.036 ± 0.05	96.4	0.028
wheat bran with malt flavor	0.306 ± 0.02	69.8	0.238
bran breakfast cereal	0.229 ± 0.02	77.3	0.136
tablet of bran	0.288 ± 0.03	71.6	0.101
tablet of bran with cellulose	0.474 ± 0.04	53.2	0.115
standards			
avenanthramide	0.516 ± 0.02	49.2	0.072
ferulic acid	0.796 ± 0.02	21.5	0.051
gentisic acid	1.022 ± 0.01		0.234
p-hydroxybenzoic acid	0.789 ± 0.02	22.3	0.078
protocatechuic acid	0.748 ± 0.04	26.3	0.147
syringic acid	0.674 ± 0.03	33.6	0.058
vanillic acid	0.782 ± 0.03	23.0	0.054
vanillin	0.948 ± 0.02	6.6	0.286
phytic acid	0.077 ± 0.04	88.1	0.049
BHA	0.757 ± 0.01	25.4	0.176
BHT	0.924 ± 0.02	8.9	0.491
PG	1.016 ± 0.02		0.707
Trolox	0.693 ± 0.04	31.6	0.081

[a] RM, reaction mixtures; DR, deoxyribose; ASC, ascorbate. Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] When deoxyribose was omitted, the values ranged from 0.001 to 0.006 absorbance units.

Assessment of the Antioxidant Action of Wheat and Oat Bran by the Deoxyribose Assay. Hydroxyl radicals can be generated under physiological conditions. The deoxyribose assay is used to detect possible scavengers of OH• radicals, which are formed by a mixture of ascorbate and $FeCl_3$−EDTA (*23*).

Table 3 shows the results expressed as the inhibition percentage of the deoxyribose damage caused by OH• in the presence of oat and wheat brans as compared with standards. Wheat bran powder is the best OH• scavenger, followed by wheat bran alone = oat breakfast cereal = bran breakfast cereal > tablet of bran = wheat bran with malt flavor > tablet of bran with cellulose > oat bran alone ≥ crunchy oat bran, the last two showing less than 50% inhibition and without significant differences between them ($p < 0.05$).

In this assay, thermal processing was efficient in the case of oat breakfast cereal and tablet of bran due to the presence of brown sugar and lactose, respectively (**Table 1**), which contribute to enhancing the antioxidant activity of the final preparation (**Table 7**).

The Maillard process reaction involves the formation of a Schiff's base, which is rearranged into enediol structure reductones by the sugar or oxidized lipids and the amino acid free

Table 4. Scavenging of Hydrogen Peroxide by Oat and Wheat Brans as Compared with the Activities of Standards (Typical Cereal Compounds and Common Food Additives) by Using the Peroxidase-Based Assay

added to reaction mixtures	% inhibition[a]
oat bran	
crunchy oat bran	
oat bran alone	
oat breakfast cereal	
wheat bran	
wheat bran alone	84.3 ± 1
wheat bran powder	59.8 ± 2
wheat bran with malt flavor	28.5 ± 1
bran breakfast cereal	6.4 ± 2
tablet of bran	40.5 ± 1
tablet of bran with cellulose	57.1 ± 1
standards	
avenanthramide	36.1 ± 1
ferulic acid	
gentisic acid	33.3 ± 2
p-hydroxybenzoic acid	14.4 ± 2
protocatechuic acid	33.3 ± 1
syringic acid	12.0 ± 2
vanillic acid	15.4 ± 1
vanillin	18.1 ± 1
phytic acid	14.4 ± 1
BHA	
BHT	
Trolox	32.9 ± 2
PG	27.9 ± 1
NAC[b]	87.8 ± 1

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Used as positive control.

Table 5. Effect of Oat and Wheat Brans as Compared with the Activities of Standards (Typical Cereal Compounds and Common Food Additives) on the Oxidative Stability of Butter Expressed as a Factor of Protection Tested by the Rancimat Method[a]

added to reaction mixtures	PF[b,c]
oat bran	
crunchy oat bran	2.11 ± 0.1
oat bran alone	1.47 ± 0.1
oat breakfast cereal	1.93 ± 0.2
wheat bran	
wheat bran alone	1.35 ± 0.1
wheat bran powder	1.23 ± 0.1
wheat bran with malt flavor	1.84 ± 0.2
bran breakfast cereal	1.91 ± 0.2
tablet of bran	1.30 ± 0.1
tablet of bran with cellulose	1.50 ± 0.2
standards	
avenanthramide	0.38 ± 0.2
ferulic acid	0.99 ± 0.2
gentisic acid	0.47 ± 0.1
p-hydroxybenzoic acid	1.10 ± 0.2
protocatechuic acid	0.96 ± 0.1
syringic acid	1.01 ± 0.1
vanillic acid	0.61 ± 0.1
vanillin	1.02 ± 0.2
phytic acid	0.68 ± 0.2
BHA	2.40 ± 0.2
BHT	1.40 ± 0.1
PG	6.48 ± 0.1
Trolox	7.17 ± 0.2

[a] Rancimat tested at 120 °C. [b] Statistical differences were analyzed by ANOVA ($p < 0.05$). [c] PF = IP (butter + samples)/IP (butter alone).

amino group. These compounds generated during thermal processing were found to be highly antioxidant (*31*).

The oat and wheat brans tested also exhibited very good antioxidant activities even when ascorbate was omitted because they are able to scavenge any $OH^{\bullet}$ generated, although the level $OH^{\bullet}$ generated was lower, thus protecting deoxyribose sugar.

Among the standards analyzed, phytic acid showed the strongest antioxidant activity with significant differences ($p < 0.05$) from the other standards even when ascorbate was omitted. Similarly, avenanthramide, syringic acid, Trolox, protocatechuic acid, BHA, vanillic acid, *p*-hydroxybenzoic acid, and ferulic acid exhibited their capacity as $OH^{\bullet}$ scavengers, although with inhibition percentages lower than 50% inhibition. When ascorbate was omitted, absorbance levels were lower than the control sample.

However, vanillin and BHT produced low inhibition percentages and, when ascorbate was omitted, the level of pink chromogen exceeded that of the control. These compounds do not directly scavenge $OH^{\bullet}$ radicals, but they react with ascorbate, decreasing $OH^{\bullet}$ generation. Finally, gentisic acid and PG were prooxidants in this assay, in accordance with the results of Murcia et al. (*27*) in the latter case. The components of dietary fibers, such as cellulose, hemicellulose, pectins, other polysaccharides, and lignin, may form insoluble complexes with mineral elements and thus reduce the generation of some free radicals (*17*).

Hydrogen Peroxide Scavenging. Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes. When the samples scavenge the hydrogen peroxide, there is a decrease in the absorption spectrum, as assessed by the peroxidase test (*23*).

Table 4 shows the effect on hydrogen peroxide of the oat and wheat brans as compared with the effect of the standards. Wheat bran alone was the most effective H_2O_2 scavenger, showing a similar capacity to NAC, which was used as a positive control (*23*). The rest of the analyzed fiber products scavenged H_2O_2 in the following decreasing order: wheat bran powder = tablet of bran with cellulose > tablet of bran > wheat bran with malt flavor > bran breakfast cereal. However, crunchy oat bran, oat bran alone, and oat breakfast cereal did not scavenge H_2O_2. Furthermore, the bran and oat breakfast cereals analyzed contained added ingredients, such as whole oat flour, wheat flour, brown sugar, malt flour, and salt (**Table 1**), which, in this assay, did not scavenge H_2O_2 (**Table 7**). Also, the thermal industrial processing to which the samples were subjected further decreased the antioxidant capacity of those active ingredients.

Wheat products, except bran breakfast cereal, provided better results than PG. Bran breakfast cereal markedly decreased the activity as a consequence of the prooxidant activity that its ingredients/active materials showed (**Table 7**). The standards analyzed, except avenanthramide, gentisic acid, proteocatechuic acid, Trolox, and PG, gave inhibition percentages lower than 20%. According to Murcia et al. (*27*), BHA and BHT react inefficiently with H_2O_2. There are no more data in the literature on the scavenging ability of this reactive oxygen species.

Rancimat Results. The Rancimat test is an accelerated oxidation test, which is used for the determination of the shelf life of fats and oils (*32*). Because different kinds of substances can accelerate and/or inhibit the formation of hydroperoxides, it is possible to evaluate the protection that a given ingredient affords toward a food (rich in oils or fats) elaborated in given conditions of heating.

The PF obtained by the Rancimat method for butter in the presence of oat and wheat brans and standards is shown in **Table 5**. Crunchy oat bran had the strongest stabilizing effect ($p < 0.05$) as compared with the rest of the fiber products, followed by oat breakfast cereal, bran breakfast cereal, and wheat bran

with malt flavor. These last three do not show significant differences between them and protect the butter better than the rest of the commercial products analyzed, which also delay the time of onset of the propagation phase of the radical chain reaction in the following decreasing order ($p < 0.05$): tablet of bran with cellulose = oat bran alone ≥ wheat bran alone ≥ tablet of bran ≥ wheat bran powder. This suitability is very high as revealed by the data shown in **Table 7** for sample constituent ingredients or active materials.

All of the oat and wheat brans studied produced higher PFs than the standards described as typical cereal compounds. When avenanthramide, gentisic acid, vanillic acid, and phytic acid were added to the butter, the time required for the formation of a sufficient concentration of initiating radicals was reduced to a PF lower than 1, thus qualifying them as prooxidants. However, among the common food additives, Trolox provided the greatest protection, followed by other common food additives such as PG, BHA, and BHT ($p < 0.05$).

Wheat showed slightly higher antioxidant properties than oat in sunflower oil at 110 °C (*32*). In a review, Peterson (*11*) claimed that oat extracts at 0.05−0.10% significantly improved the stability of the oil at frying temperature (180 °C) and were superior to the standard antioxidants BHT and TBHQ. Probably due to the presence of the active components of oat, the ethylene group of Δ^5-avenasterol isomerizes to produce an allylic free radical, interrupting the oxidation chain (*33*). Our results on refined oil (data not shown) were also very similar to those reported by Lehner et al. (*32*), the PF of wheat and oat samples being slightly greater than 1.

Linoleic Acid System Assay. This method, which is used to determine the antioxidant activity of the samples during storage at unfavorable temperatures (40 °C), measures the inhibition of linoleic acid autoxidation. All of the oat and wheat bran samples analyzed showed very good antioxidant activity after 28 days of storage, with inhibition percentages higher than 95% (data not shown).

Figure 1 shows the absorbance values obtained for each final product during the autoxidation of linoleic acid for all of their ingredients during 28 days of storage. By this means, it is possible to test the ingredient/active material alone or in conjunction with other ingredients to assess the antioxidant activity of the final product. The high antioxidant activity of crunchy oat bran is due to oat bran while oat breakfast cereal exhibits a high antioxidant activity due to two additional ingredients, wheat flour and whole oat flour.

In this assay, both wheat bran with malt flavor and bran breakfast cereal exhibited a high percentage of inhibition of linoleic acid autoxidation during the 28 days of storage because of the wheat bran that they contained. However, in the case of tablet of bran with cellulose and tablet of bran, the good antioxidant activity is a consequence of the inhibitory effect of wheat bran powder. The last product also includes wheat germ, which is a very active ingredient (**Figure 1**).

Note the time−course curves for the standards, from which we select (i) a curve for very good antioxidant substances, such as PG (avenanthramide, phytic acid, BHT, and BHA exhibited similar activities to PG); (ii) a curve for medium activity compounds such as ferulic acid (syringic acid showed a similar behavior); and, finally, (iii) a third curve for the group including standards with absorbance values similar to the control sample, such as gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, and vanillin, which did not show a protecting activity in this assay (**Figure 1**).

Table 6. Scavenging of ABTS Radical Anions by Oat and Wheat Brans as Compared with the Activities of Standards (Typical Cereal Compounds and Common Food Additives)[a]

added to reaction mixtures	TEAC[b]	TEAC[c]
	oat bran	
crunchy oat bran	3.56 ± 0.01	9.90 ± 0.01
oat bran alone	3.35 ± 0.02	8.29 ± 0.01
oat breakfast cereal	3.07 ± 0.01	10.53 ± 0.02
	wheat bran	
wheat bran alone	10.10 ± 0.01	17.38 ± 0.02
wheat bran powder	15.77 ± 0.02	15.10 ± 0.01
wheat bran with malt flavor	10.41 ± 0.01	18.03 ± 0.01
bran breakfast cereal	10.03 ± 0.02	17.38 ± 0.01
tablet of bran	4.68 ± 0.02	18.08 ± 0.02
tablet of bran with cellulose	3.31 ± 0.01	8.41 ± 0.02
	standards	
avenanthramide	14.20 ± 0.01	14.30 ± 0.01
ferulic acid	14.78 ± 0.01	>19 ± 0.01
gentisic acid	15.09 ± 0.02	>19 ± 0.02
p-hydroxybenzoic acid		1.03 ± 0.01
protocatechuic acid	14.01 ± 0.02	>19 ± 0.02
syringic acid	13.54 ± 0.01	>19 ± 0.01
vanillic acid	0.18 ± 0.02	15.04 ± 0.01
vanillin		16.95 ± 0.02
phytic acid	3.94 ± 0.02	14.80 ± 0.01
BHA	0.44 ± 0.01	1.41 ± 0.01
BHT	0.26 ± 0.01	0.72 ± 0.02
PG	17.20 ± 0.02	17.44 ± 0.01

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] TEAC is the micromolar concentration of a Trolox solution showing the antioxidant capacity equivalent to the dilution of the substance under investigation at 6 min. [c] TEAC is the micromolar concentration of a Trolox solution showing the antioxidant capacity equivalent to the dilution of the substance under investigation at 24 h.

Cereal compounds are a potential source of hydrocolloids that can act as physical antioxidants. It is clear that the absorption of linoleic acid to insoluble material and its protection against oxygenation are correlated. The physical protection of linoleic acid may represent the primary mechanism. When oat was fractionated, the inhibitory properties were further concentrated into the fiber and, especially, the soluble fiber fractions. Oat fiber, but not oat flour, was found to be able to retard the oxidation of a heated barley flour−water suspension (*4*).

Individual phenolic acids, such as caffeic acid and vanillic acid, are positively associated with an increased antioxidant activity in linoleic acid oxidation systems (*12*). Nevertheless, although Velioglu et al. (*34*) observed 64.9% inhibition for wheat germ and Lehtinen and Laakso (*4*) observed around 50% for an oat suspension, Peterson (*11*) cited in a review that only a moderate antioxidative activity was measured for oat grain, bran, or flakes at 72 h. Lehtinen and Laakso (*4*) described the high antioxidant activity of an aqueous extraction of oat fiber in this assay although it was pH-dependent.

TEAC Assay. A TEAC value can be assigned to all compounds able to scavenge the $ABTS^{\bullet-}$ by comparing their scavenging capacities to that of Trolox. Quantitative evaluation of the antioxidant capacity using TEAC can be used to provide a ranking order of antioxidants (*27*).

Table 6 shows the TEAC of the different products elaborated with oat or wheat brans as compared with the activity of standards. The wheat bran results for TEAC (6 min) are, in decreasing order, wheat bran powder > wheat bran with malt flavor ≥ wheat bran alone ≥ bran breakfast cereal > tablet of bran > tablet of bran with cellulose.

The tablet of bran exhibited a better TEAC value than the tablet of bran with cellulose due to the ingredients or active materials used. Both samples contained wheat bran powder and different additional ingredients. The tablet of bran includes a

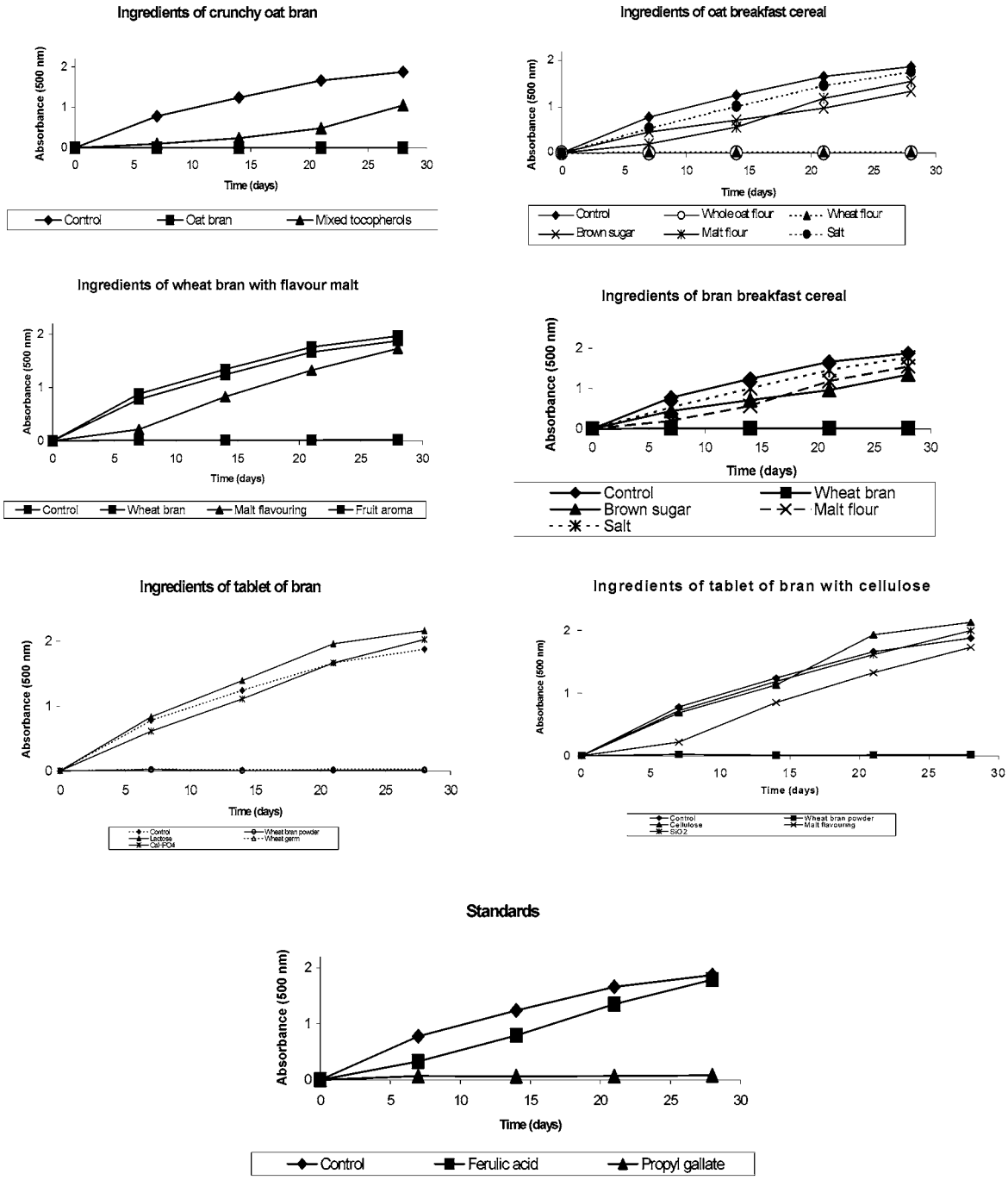

Figure 1. Evolution of the absorbance at 500 nm for the oxidation of linoleic acid in the presence of ingredients corresponding to cereal brans as compared with the activity of standards (typical cereal compounds and common food additives) during 28 days of storage.

wheat germ ingredient (with high antioxidant activity) while the tablet of bran with cellulose is elaborated using as an ingredient 30% cellulose (a compound that does not act as an antioxidant in this assay), resulting in a dilution effect of the antioxidant activity of the final preparation (**Tables 1** and **7**).

Products made with oat bran showed lower TEAC values (3.56 and 3.07), any difference between them being a consequence of the ingredients/active materials used. Crunchy oat bran increased the TEAC because it contains tocopherols, while oat breakfast cereal decreased the value due to the lower antioxidant activity of its ingredients, whole oat flour, wheat flour, brown sugar, malt flour, and salt (**Tables 1** and **7**).

The typical cereal standards exhibited antioxidant activities, in the following decreasing order: gentisic acid = ferulic acid > avenanthramide = proteocatechuic acid > syringic acid > phytic acid > vanillic acid. However, *p*-hydroxybenzoic acid and vanillin did not scavenge $ABTS^{\bullet-}$. Of the common food additives analyzed, PG exhibited the best TEAC. BHA and BHT showed a lower TEAC than the oat and wheat bran samples analyzed.

TEAC (24 h) values were higher for all of the samples. It has been established that TEAC values may change with the measuring times used (*35*). Some of the samples such as wheat bran powder, wheat bran with malt flavor, wheat bran alone, bran breakfast cereal, gentisic acid, ferulic acid, proteocatechuic acid, syringic acid, avenanthramide, and PG exert immediate fast radical trapping, and the reaction is completed within 10 s, while others have a slower onset of radical trapping (6 min) but a rise toward high TEAC (24 h) values, such as tablet of

Table 7. Antioxidant Activities of the Ingredients (Analyzed at the Amount Present in the Final Products) of Cereal Brans Evaluated in the Different Assays[a]

ingredients[b]	lipid peroxidation (%)[c]	deoxyribose assay (%)[c]	peroxidase assay (%)[c]	rancimat test PF[d]	TEAC[e] (6 min)	TEAC[e] (24 h)
oat bran	64.1 ± 1	56.2 ± 1		1.50 ± 0.1	3.01 ± 0.01	9.23 ± 0.02
mixed tocopherols	58.1 ± 1	3.2 ± 2	24.2 ± 1	0.42 ± 0.1	1.68 ± 0.02	3.04 ± 0.01
whole oat flour	40.1 ± 1	18.4 ± 1		0.78 ± 0.2	1.50 ± 0.02	4.43 ± 0.02
wheat flour	28.0 ± 2	28.5 ± 2		0.38 ± 0.2	0.76 ± 0.01	3.24 ± 0.01
brown sugar	10.8 ± 2	43.5 ± 1		0.55 ± 0.1	0.42 ± 0.01	1.12 ± 0.02
malt flour	27.6 ± 1	2.3 ± 2		0.39 ± 0.1	1.84 ± 0.02	5.43 ± 0.01
salt	5.4 ± 2			0.94 ± 0.2		0.09 ± 0.01
wheat bran	60.9 ± 1	71.9 ± 1	60.4 ± 2	1.28 ± 0.1	11.10 ± 0.02	15.11 ± 0.01
wheat bran powder	59.6 ± 2	89.2 ± 2	66.1 ± 1	1.45 ± 0.2	15.04 ± 0.01	14.80 ± 0.01
wheat bran powder (64%)	47.1 ± 2	37.7 ± 1	60.7 ± 1	1.06 ± 0.1	3.50 ± 0.01	10.00 ± 0.01
malt flavoring	19.5 ± 2	14.5 ± 1	24.9 ± 1	1.43 ± 0.1	0.40 ± 0.01	1.45 ± 0.01
fruit aroma E-6046		15.0 ± 2		0.68 ± 0.2		3.89 ± 0.02
lactose	12.2 ± 1	56.0 ± 1	32.5 ± 1	1.03 ± 0.1	0.07 ± 0.01	0.34 ± 0.01
wheat germ	34.2 ± 2	14.6 ± 2	46.8 ± 2	0.56 ± 0.2	1.38 ± 0.01	3.91 ± 0.02
calcium phosphate, dibasic	23.9 ± 1			1.16 ± 0.1	0.12 ± 0.02	0.23 ± 0.02
cellulose	11.9 ± 1	13.0 ± 1	34.7 ± 2	1.64 ± 0.2	0.07 ± 0.01	0.05 ± 0.01
silicon dioxide	17.2 ± 2			1.15 ± 0.1		0.09 ± 0.01

[a] Statistical differences were analyzed by ANOVA ($p < 0.05$). [b] Without industrial process. [c] Percentage of inhibition. [d] PF = IP (butter + samples)/IP (butter). [e] TEAC is the micromolar concentration of a Trolox solution showing the antioxidant capacity equivalent to that of the dilution of the substance under investigation.

bran, oat breakfast cereal, crunchy oat bran, tablet of bran with cellulose, oat bran alone, vanillin, vanillic acid and phytic acid.

The tablet of bran showed a very good TEAC (24 h) because of the presence of active ingredients such as wheat bran powder and wheat germ (**Tables 1** and **7**), while the oat samples included tocopherols, whole oat flour, and wheat flour as ingredients. Finally, BHA, BHT, and *p*-hydroxybenzoic acid yielded low TEAC (24 h) values with time. In this assay, the products containing wheat bran exhibited better TEAC values than those with oat bran.

The antioxidant activity in this assay (TEAC) is partially dependent on the number of free phenolic hydroxyls and is also affected by the type of linkage between monomer structures, partially due to the more hydrophobic nature of the dimers, which lead to a higher concentration in the lipid phase (*36*).

The existence of slow and fast acting antioxidants is therefore evident. Assay times of 10 min and several hours revealed that the activity increased with increasing total phenolic content. This may simply reflect reactivity preferences toward radical types by specific phenolics. This phenomenon is well-established and generally explains why different free radical trapping assays do not necessarily yield the same findings (*20*). Peterson (*11*) also observed 10% lower activity for oat with respect to wheat cereals.

Although some authors have suggested that antioxidant activities of cereal extracts are very low (*13*), several groups have pointed to the good antioxidant capacity of wheat and, to a lesser extent, oat (*37*), as is the case in the present study.

Several reports associate aleurone (*10*), germ, endosperm (*11*), testa, and pericarp (*13*) with antioxidant capacity. Whatever the case, Miller et al. (*37*) estimated that bran products had a higher activity, but it is necessary to bear in mind that these often contain residual starch, which has a diluting effect. Furthermore, intervarietal differences in geographical location, seasonal effects, processing, and methodology are significant variables (*11*).

The greatest antioxidant activities are localized in the outermost layers of the grain. Operations such as drum drying, blanching, roasting, steaming, or extrusion, all routine in grain processing for human consumption (*38*), inactivate lipases and retard peroxidative degradation (rancidity), although these processed samples had significantly lower levels of Maillard products (*39*). Industrial processing can also inactivate 20−50% of phenolic compounds (*10*) at 65 °C (*2*), producing losses of tocotrienols, tocopherols (*40*), or other antioxidants by disintegration of the membranous structures (*41*). However, some avenanthramides and lignans do not change during processing (*9*). Moreover, such processing appears to make the β-glucan more biologically active (*7*).

The reactions of different samples in in vitro assays also differ. Each antioxidant method measures the inhibition of oxidation due to specific chemical reactions and reactivities of the individual components of the mixture (*11*).

The extraction efficacy is an important variable for any comparison of activity among products. Grain antioxidants are difficult to extract since solubility ranges from water soluble to lipid soluble and many are covalently bound to the cell wall material, a cellular structure that inhibits extraction (*32*). Because of this, the total antioxidant activity is due to a complex mixture of several antioxidant materials and, possibly, pro-oxidant compounds (*12*).

In conclusion, the antioxidant activity is a fundamental property important for life (*34*). From a nutritional point of view, it is preferable not to remove bioactive compounds from food materials rather than isolating and then incorporating them into the food (*18*). Our studies demonstrated clearly that cereal brans are a rich source of antioxidant activity. Our research also shows that the antioxidant activity of cereals withstands storage for 28 days (see linoleic test results) and thermal processing (see Rancimat test), meaning that cereal brans could be used to protect food during processing. As a consequence, instead of eating refined grain products (*6*), a very convenient way of significantly increasing the average daily fiber and antioxidant intake of our diet could be by introducing modest changes in our eating habits by adding whole grain cereals or bran to our meals.

ABBREVIATIONS USED

ABAP, 2,2′-azobis(2-amidinopropane)HCl; $ABTS^{2-}$, 2,2′-azinobis(3-ethylbenz-thiazoline-6-sulfonate); $ABTS^{\bullet-}$, 2,2′-azinobis(3-ethylbenz-thiazoline-6-sulfonate) radical anions; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; IP, induction period;

LOO•, peroxyl radical; MDA, malondialdehyde; $O_2^{\bullet -}$, superoxide anion radical; OH•, hydroxyl radical; PBS, phosphate-buffered saline; PF, protection factor; PG, propyl gallate; TBA, thiobarbituric acid; TBHQ, *tert*-butylhydroquinone; TEAC, Trolox equivalent antioxidant capacity.

ACKNOWLEDGMENT

We thank Professor Atsushi Ishihara from Kyoto University (Japan) for his collaboration with avenanthramide.

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Received for review March 8, 2004. Revised manuscript received May 11, 2004. Accepted May 17, 2004. This work was supported by an University of Murcia project (Ref. UMU-5142).

JF049621S