

# Capabilities of Oat Extracts in Inhibiting Cholesterol and Long Chain Fatty Acid Oxidation During Heating<sup>1</sup>

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## ABSTRACT

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The antioxidant activities of oat groat extracts using different solvents were investigated. For acetone, hexane, and methanol extracts, the total phenolic compound contents were 12.83, 8.56, and 26.17  $\mu\text{g}$  of catechin equivalent/g, respectively. Their corresponding free radical scavenging activities measured using the DPPH (2,2'-diphenyl-1-picrylhydrazyl) method were 0.68, 0.35, and 0.79  $\mu\text{mol}$  of Trolox equivalent/g, respectively. All oat extracts presented significantly greater capability in preventing cholesterol oxidation than the control (no extract added) during heating at the three levels of addition (1, 5, and 10 mg). The capability of the hexane or acetone extract in preventing cholesterol oxidation was not

as effective as the methanol extract. In the docosahexaenoic acid (DHA, C22:6) oxidation test, all oat extracts at the level of 5 and 10 mg had greater inhibition capability on DHA oxidation than the control (DHA only). No significant inhibition was found for the hexane and acetone extract at the level of 1 mg, except for the methanol extract. The capabilities of oat extracts to inhibit oxidation of cholesterol and DHA from high to low was methanol, acetone, and hexane extract, which agreed with their free radical scavenging activities and total phenolic compound contents.

Oat is a cereal that possesses health benefits for humans such as preventing cardiovascular disease and certain cancers. Although the soluble fiber content of oat, especially  $\beta$ -glucan, is generally believed to be primarily responsible for its effects on cardiovascular disease, undesirable lipid oxidation reactions in the body also contribute to these disease conditions (Handelman et al 1999; Gray et al 2002). Cholesterol oxidation could contribute to the development of a progressive thickening of the artery wall due to the accumulation of cholesterol oxidation products in low-density lipoprotein (LDL) particles after they are oxidized. Lipid oxidation reactions in the cell membrane also result in mutation of cell duplication processes and damage to the cell membrane that could result in various types of cancer (Jadhav et al 1996). Recent studies have suggested that some health-promotion capabilities of oats are due to antioxidants as well as  $\beta$ -glucan gum (White and Armstrong 1986; Peterson and Qureshi 1993; Wood et al 2000). Oat is a good source of various antioxidants. Similar to other cereal grains, oat contains relatively high levels of tocopherols, tocotrienols, and phytosterols (Peterson 2001). It also contains many different types of phenolic antioxidant compounds such as avenanthramides, *p*-hydroxybenzoic acid, and vanillic acid (Shahidi and Naczk 1995; Emmons et al 1999; Frankel and Meyer 2000).

Because oat is a good source of a variety of antioxidants, extracts with highly concentrated antioxidants from oat could be used as a natural preservative in preventing food oxidation during cooking and storage, especially for foods rich in unsaturated long-chain fatty acids and cholesterol. The oxidation products generated from long-chain fatty acids are directly responsible for off-flavor of foods that cause deterioration of food quality. Various meat, egg, and dairy products, especially butter, butter oil, cheese, etc., have been reported to produce various toxic cholesterol oxidation products during heat processing. Intake of food containing high levels of the cholesterol oxidation products such as 7-ketocholesterol will eventually cause coronary heart diseases (Kumar and Singhal 1991; Morel and Lin 1996). The oxidation of cholesterol and long chain fatty acids may be prevented by adding an extract of oat antioxidants to those foods; however, little infor-

mation is available on the protection effect of oat extracts against oxidation reactions in food.

The polarities of the antioxidants in oat are different, which may produce different extraction yields when solvents of different polarity are used. Small molecular weight phenolic compounds are readily extracted by methanol but tocopherols, tocotrienols, and most phytosterols are more soluble in hexane, a more nonpolar solvent. Fewer phenolics are extracted by hexane due to their polar nature. Because of the existence of different types of antioxidants in the extracts obtained by different extraction solvents, the extracts may have variable capabilities to prevent fatty acid or cholesterol oxidation. Most previous studies of oat antioxidant activity were evaluated using a methanol extract and a free radical quenching method (Horrocks and Yeo 1999). In this study, the capabilities of oat extracts from different solvent extractions in preventing cholesterol and long chain fatty acid during heating were investigated. The DPPH (2,2'-diphenyl-1-picrylhydrazyl) method, as a measure of free radical quenching, was also used to measure the antioxidant activities of those different extracts for comparison with previous studies. The total phenolic compound contents in the extracts were determined. This information would be helpful in the development and utilization of oat products as a food antioxidant or as an antioxidant nutritional supplement.

## MATERIALS AND METHODS

### Chemicals and Materials

Oat groat without hulls (*Avena sativa* L.) was a gift from the Bell Institute of Health and Nutrition of General Mills Company (Minneapolis, MN). It was stored at 4°C before use. DPPH, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu reagent, heptadecanoic acid (C17:0), docosahexaenoic acid (DHA), and cholesterol were purchased from Sigma-Aldrich (St. Louis, MO).  $\text{BCl}_3$ -methanol and 2,2-dimethoxypropane were purchased from Supelco (Bellefonte, PA). Methanol, acetone, and hexane were HPLC-grade and purchased from Fisher Scientific (Springfield, NJ).

### Preparation of Oat Extracts

Oat groat was ground for 1 min at medium speed in a kitchen blender. The particles of the ground oat flour were able to pass through a 1-mm sieve. Oat flour (200 g) was weighed into a 1,000-mL glass beaker and extracted using 400 mL of acetone, hexane, or methanol. The mixtures were incubated at 60°C in a water bath and stirred gently for 20 min. After incubation, the solvent layer was separated from solid residue by centrifuging the

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mixture at  $2,000 \times g$  for 10 min using a Hermel Z383 K table top centrifuge (National Labnet Company, Woodbridge, NJ). The clear supernatant was transferred to a clean round-bottom flask. Then the solid residue was extracted with another 400 mL of the same solvent (acetone, hexane, or methanol). The separated acetone, hexane, or methanol extracts were combined and dried using a vacuum evaporator at  $50^\circ\text{C}$  for 4 hr. The dried extracts were weighed to obtain the extract yields. The data were expressed as a percentage of fresh weight. The water content of the ground oat flour was  $11.77 \pm 0.04\%$  ( $n = 4$ ).

### Total Phenolic Compound Content

The total phenolic content of oat extract was determined using Folin-Ciocalteu reagent (Velioglu et al 1998). Folin-Ciocalteu reagent was diluted 10 $\times$  with deionized water. Dried oat extract (0.02 g) was redissolved in 1 mL of methanol; 0.1 mL of this oat extract solution was mixed with 0.75 mL of diluted Folin-Ciocalteu reagent. The reaction solution was left at room temperature for 5 min. Then 0.75 mL of sodium bicarbonate solution (60 g/L) was added. The mixture was incubated at room temperature for 90 min and filtered through a 0.45- $\mu\text{m}$  syringe filter (Pall Corporation, Ann Arbor, MI). The absorbance of the solution was determined at 750 nm using a UV-visible spectrophotometer (SpectraMax Plus<sup>384</sup>, Molecular Devices, Sunnyvale, CA). Catechin was used as a standard to prepare a standard curve. The total phenolic compound content of oat extract was calculated and expressed as  $\mu\text{g}$  of catechin equivalent/g of fresh weight of sample.

### Antioxidant Activity from DPPH Radical Scavenging Method

The oat extract solution for the DPPH test was prepared by redissolving 0.20 g of each dried extract (acetone, hexane, or methanol) in 10 mL of methanol. The concentration of DPPH solution was 0.025 g in 1,000 mL of methanol. DPPH solution (2 mL) was mixed with 40, 80, and 120  $\mu\text{L}$  of the oat extract/methanol solution and transferred to a cuvette. The reaction solution was monitored at 515 nm for 30 min at room temperature using the spectrophotometer. The inhibition percentage of the absorbance of DPPH solution was calculated using the equation

$$\text{Inhibition\%} = (\text{Abs}_{t=0 \text{ min}} - \text{Abs}_{t=30 \text{ min}}) / \text{Abs}_{t=0 \text{ min}} \times 100$$

where  $\text{Abs}_{t=0 \text{ min}}$  was absorbance of DPPH at 0 hr and  $\text{Abs}_{t=30 \text{ min}}$  was the absorbance of DPPH after 30 min of incubation.

Inhibition percentage of the absorbance of DPPH was plotted against each quantity of oat extract solution to obtain a regression line. Trolox (0.5 mM) was dissolved in methanol and used as a standard to convert the inhibition capability of oat extract solution to the Trolox equivalent antioxidant activity (TEAC). The ratio between the slopes of the regression lines of oat extraction solution and the Trolox solution was defined as the Trolox equivalent antioxidant activity (TEAC).

### Cholesterol Oxidation During Heating

Cholesterol solution (1 mL) (0.1 mg/mL in hexane) was placed in test tubes (13  $\times$  100 mm). For each oat extract treatment, 50, 250, and 500  $\mu\text{L}$  of the prepared oat extract solution (0.02 g in 1 mL of methanol), which is equivalent to 1, 5, and 10 mg of oat extract, was added to the tubes containing cholesterol and vortexed for 30 sec. A tube without added oat extract solution was used as the control. The solvent in the test tubes was evaporated using a vacuum centrifuge evaporator (CentriVap Mobile system, Labconco, Kansas City, MO). After evaporation, the tubes were immersed in a  $175^\circ\text{C}$  sand bath for 20 min. After the tube was cooled down, 1 mL of methanol was added to the tube and vortexed for 30 sec. The methanol solution was transferred to an HPLC vial for analysis.

An HPLC system was used to determine the cholesterol concentration. It included a Waters 2690 system, a 960 PDA detector (Waters Corporation, Milford, MA), and a 25 cm  $\times$  4.6 mm

diameter and 5- $\mu\text{m}$  C18 Discovery column (Supelco, Bellefonte, PA). The mobile phase was acetone-to-methanol (10:90) at a flow rate of 0.8 mL/min. The injection volume of sample was 100  $\mu\text{L}$ . The HPLC was controlled using Waters Millennium chromatography software. The wavelength for quantifying cholesterol was 215 nm. The percentage of cholesterol that remained in each tube was obtained by comparing its final concentration to its original concentration.

### Determination of DHA Oxidation During Heating

DHA (1 mL) (0.1 mg/mL of hexane) was added to each test tube (13  $\times$  100 mm). Each oat extract was prepared by redissolving 0.2 g of the extract in 10 mL of its extraction solvent. For each oat extract treatment group, 50, 250, and 500  $\mu\text{L}$  of the prepared oat extract solution was added to the DHA tube and vortexed for 30 sec. A test tube containing DHA without added oat extract solution was used as a control. The solvent in the test tubes was evaporated using the vacuum centrifuge evaporator. After evaporation, the tubes were immersed in a  $150^\circ\text{C}$  sand bath for 15 min. Heptadecanoic acid (C17:0) (0.1 mg/mL), as an internal standard for the DHA analysis, was added to each tube after the test tube was cooled down.

After adding 2 mL of  $\text{BCl}_3$ -methanol and 1 mL of 2,2'-dimethoxypropane, all test tubes were capped and incubated in a  $60^\circ\text{C}$  water bath for 10 min to perform the derivatization of fatty acid esters. Hexane (1 mL) and water (1 mL) were added to the tubes and vortexed for 30 sec. The upper hexane layer was transferred to another tube, dried with anhydrous sodium sulfate, and transferred to a GC vial.

A gas chromatograph (Hewlett Packard 5890, Agilent Technologies, Palo Alto, CA) with an FID detector was used to determine DHA concentration. Helium was used as a carrier gas with a column flow rate of 1.2 mL/min. The injection volume was 5  $\mu\text{L}$  and the split ratio was 1:100. The injector and detector temperatures were 250 and  $270^\circ\text{C}$ , respectively. The oven temperature program was set to hold at  $50^\circ\text{C}$  for 3 min and then increased at  $4.0^\circ\text{C}/\text{min}$  to  $250^\circ\text{C}$ . The column was a Supelco SP2380 (30m  $\times$  0.25mm) (Bellefonte, PA). The concentrations of DHA were calculated using the C17:0 internal standard as a reference. The percentage of DHA that remained in each tube was obtained by comparing its final concentration to its original concentration.

### Statistical Analysis

Extraction of oat using each solvent, for each of the DPPH, DHA, and cholesterol oxidation tests were all performed in quadruplicate. The average values and standard deviations were calculated and the data were analyzed by one-way ANOVA with multiple comparisons by Fisher's least significant difference to determine significant difference at  $P < 0.05$  (Zar 1996).

## RESULTS AND DISCUSSION

### Extraction Yield, Total Phenolic Compound Content, and Free Radical Quenching Capability of Oat Extracts

The yields of oat extracts were significantly different with extraction yield from high to low when hexane, acetone, and methanol were used as a solvent (Table I). These yields are slightly lower than a previous study, which reported the yield of oat extract varied from 5.5 to 6.7% using a high-pressure solvent method (Moreau et al 2003). The solvent polarity from high to low is methanol, acetone, and hexane, and it is likely that the chemical compositions of the three extracts could be different. Hexane may extract more lipid (nonpolar triglycerides and phytoosterols) than acetone and methanol extracts (Moreau et al 2003). The acetone extract may contain more polar lipids such as phospholipids than the methanol and hexane extracts. Methanol, which is a relatively more polar solvent, could extract more polar

phenolic compounds and lipids from oat. Thus, total phenolic compound content of oat extract appeared to be directly related to the relative polarity of the extracting solvents (Table I). Methanol extract of oat had two and three times higher total phenolic compound content than acetone and hexane extract, respectively, although its total extraction yield was the lowest among the three solvents. Several studies suggested that the total phenolics content may have positive correlation with antioxidant activity (Velioglu et al 1998; Emmons et al 1999).

The results of free radical quenching capabilities (TEAC) of the three oat extracts measured by the DPPH method are shown in Table I. The order of the free radical quenching capabilities from high to low was methanol, acetone, and hexane extracts. The DPPH test has been widely used for evaluating antioxidant activity of oat methanol extract. Antioxidant activities of methanol extracts from seven oat cultivars were determined using the DPPH test and the higher activity was positively correlated with the concentration of phenolic acids in the extracts (Bryngelsson et al 2002). Also, the correlation between the antioxidant activity measured using the DPPH test and total phenolic compound content in an 80% ethanol extract of pearling fraction has been evaluated (Peterson et al 2001). In this study, the antioxidant activity of methanol extract was two times higher than that of hexane extract. This suggests that most antioxidants in oat are more polar phenolic compounds and likely more extractable in methanol than in hexane. Even though the hexane extract would have contained lipophilic phytochemicals and tocopherols, antioxidant activity was not as high as the more hydrophilic antioxidants in methanol extract. This difference could also be due to a dilution effect brought about by greater extraction of neutral lipid material. However, Adom and Liu (2005) found that the hydrophilic antioxidant activity in oat contributed over 98% of the total antioxidant activity. Acetone may also extract polar phenolic compounds. Also, it has been reported that acetone can react with anthocyanin (Lu and Foo 2001), so it is quite possible that acetone reacted with the phenolics in oat extract and formed products with reduced antioxidant activity. That may be one reason why acetone extract had lower antioxidant activity than methanol extract.

### Capabilities of Oat Extracts in Preventing Cholesterol Oxidation During Heating

Figure 1 shows the percentages of cholesterol that remained after being heated with 1, 5, and 10 mg of oat extracts. In the control sample, only 12.3% of cholesterol remained after 15 min of heating. All three added amounts of extract demonstrated significant capability to prevent cholesterol oxidation. For methanol and acetone extract, the cholesterol that remained was >75 and 55%, respectively, regardless of the amount of oat extract. The lowest antioxidant capability was for 1 mg of hexane extract (32.4%). The activities of the oat extracts for preventing cholesterol oxidation from high to low were methanol, acetone, and hexane extract, which was similar to the order obtained with the DPPH test.

The capability of a methanol extract of oat in preventing human LDL cholesterol oxidation was evaluated using 2,2'-azobis(2-amidinopropane) HCl (AAPH) and Cu<sup>2+</sup> metal ion as accelerating catalyst (Handelman et al 1999). In the LDL cholesterol oxidation accelerated by AAPH, the capability of inhibiting cholesterol oxidation was increased with the increased dose of oat methanol

extract (Handelman et al 1999). For the oxidation initiated by Cu<sup>2+</sup> metal ion, the inhibition ability of oat extract was observed only when using a high dose of oat methanol extract (Handelman et al 1999). Gray et al (2002) also found that 50% ethanol or isopropanol extract of oat can protect LDL particles from oxidation. These results were similar to our results, although we used heat to accelerate cholesterol oxidation. The phenolic compounds in oat extract may greatly contribute to the inhibition of cholesterol oxidation (Peterson 2001). Another study evaluating the capability of extracted grain lipids in preventing cholesterol oxidation during heating also suggested that the inhibition capability is correlated with the concentration of phenolic compounds in the extract (Xu et al 2005). Thus, the compounds in oat methanol extract could play an important role in preventing cholesterol oxidation.

### Capabilities of Oat Extracts in Preventing DHA Oxidation During Heating

The capabilities of oat extracts in preventing DHA oxidation during heating are shown in Fig. 2. In the control group, DHA was completely degraded to an undetectable level, while a signi-

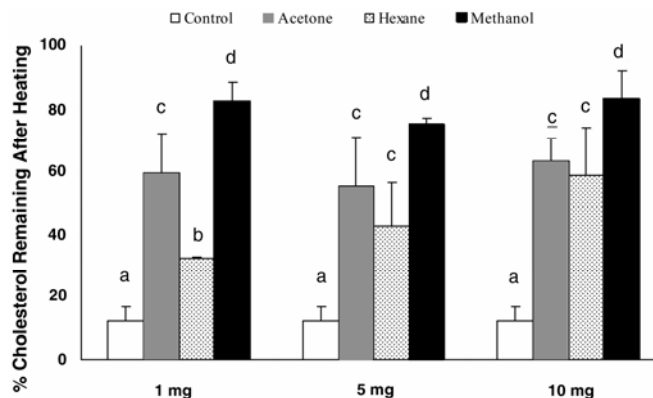


Fig. 1. Cholesterol (%) retained after heating with different concentrations of oat extracts. Bars topped with the same letter are not significantly different ( $P < 0.05$ ).

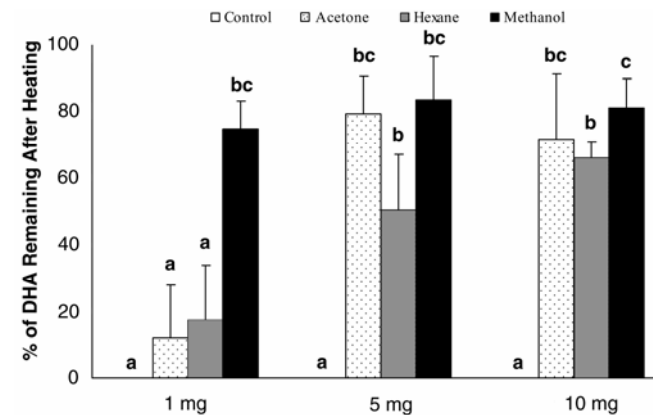


Fig. 2. DHA (%) retained after heating with different concentrations of oat extracts. Bars topped with the same letter are not significantly different ( $P < 0.05$ ).

TABLE I  
Yields, Total Phenolic Compound Contents, and DPPH Scavenging Ability (expressed as TEAC) of Oat Extracts Using Different Solvents

Solvent	Acetone	Hexane	Methanol
Yield (%)	4.10 ± 0.22b	4.94 ± 0.14c	3.43 ± 0.16a
Total phenolics content (µg of catechin equivalent /g)	12.83 ± 3.49b	8.56 ± 2.34b	26.17 ± 5.87a
TEAC (µmol of Trolox equivalent/g)	0.68 ± 0.07b	0.35 ± 0.01c	0.79 ± 0.02a

<sup>a</sup> Values followed by the same letter in the same row are not significantly different ( $P < 0.05$ ). Data expressed on a fresh weight basis.

ficant capability of preventing DHA oxidation was found for all three extracts at the 5- and 10-mg level. For methanol and acetone extract at the two higher concentrations, >80 and 70% of DHA, respectively, remained after heating. The capability of the hexane extract was lower among the three extracts with ≈50% of DHA retained at the 5-mg extract level. The order of antioxidant capability in the DHA test was similar to that of the DPPH and cholesterol tests. However, only the methanol extract was able to prevent DHA oxidation at the 1-mg level.

The inhibition ability of oat extract in autooxidation of methyl linoleate has been studied, and oat methanol extract showed lower antioxidant activity than that of fruits, vegetables, and herbs (Kahkonen et al 1999). A problem in the aforementioned study was that the oat methanol extract contained a residual level of linoleate that may have interfered with the quantification of changes in the added methyl linoleate. As DHA was not detectable in the oat fatty acid analysis of this study, it was selected to monitor oxidation to avoid any interference between the extract and the added DHA. The methanol extract of oat demonstrated greater capability in inhibiting the DHA oxidation than other solvent extracts in our study, which agreed with several studies that used oat methanol extract to stabilize vegetable oils (Duve and White 1991; Tian and White 1994). Those studies found that the oat extract at a level of 0.05–0.10% (w/w) significantly improved the stability of the oil at a frying temperature of 180°C and reduced peroxide values during 26 days of storage at 60°C. Although the phenolic compounds in oat methanol extract contribute significantly to its antioxidant activity, directly adding those known pure phenolic compounds to the oil were less effective in lowering the peroxide value than the oat extract (Xing and White 1997). The reason could be that there were some unidentified antioxidants in the oat methanol extract that contributed to the total antioxidant activity. In this study, in contrast to the inhibition of cholesterol oxidation during heating, the inhibition of DHA oxidation for acetone and hexane extracts was not significant at the lowest amount of 1 mg compared with control.

## CONCLUSIONS

The oat methanol extract showed the greatest capability of preventing cholesterol and DHA oxidation during heating. In other words, the oat methanol extract could significantly reduce cholesterol decomposition and DHA degradation in foods during cooking. That could prevent the production of harmful and toxic cholesterol oxidation products and reduce dietary risk factors for heart disease and cancer formation. Also, it could decrease the degradation of fatty acids that may cause deterioration of food flavor and taste. Therefore, oat methanol extract could be used to maintain the stability of cholesterol and fatty acids in foods during cooking or storage.

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