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ORIGINAL PAPER

Antioxidant Activity of Oat Malt Extracts in Accelerated Corn Oil Oxidation

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Abstract Oat cultivar AC Vermont was malted to concentrate antioxidants, milled to fractionate only the endosperm portion and extracted with methanol to isolate the crude antioxidants. The oat malt antioxidant fraction was assessed as a natural antioxidant based upon enhancing the stability of corn oil against oxidation and compared to the synthetic antioxidant butylated hydroxytoluene (BHT). The induction time (time required for the formation of 10 meq hydroperoxide per kilogram corn oil thermally oxidized) was used to measure antioxidant activity of oat antioxidant or BHT. The protection factor achieved by crude oat malt antioxidant extract concentrate at 0.26% (2,600 μg/g) was comparable to BHT (75 μg/g). The antioxidant activity of the oat and barley malt extract concentrates was not significantly different. However, the extract concentrate of oat malt had 44% less color compared to that of barley malt at equal concentrations showing its potential as a natural food antioxidant.

Keywords Oat malt · Antioxidant activity · BHT · Peroxide value · Protection factor · Malt extract color

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Introduction

Antioxidants obtained from a natural source would have commercial value to both the food and personal health care product industries provided that they are concentrated and are effective at inhibiting unwanted oxidation reactions. Concentrating and isolating antioxidants from grains could present a processing opportunity to manufacture natural antioxidants. Malt processing has been found to increase antioxidant activity in cereal grains by several times depending upon malting conditions. Munich-style or melanoidin-rich barley malts are known to exhibit antioxidant properties that are beneficial in stabilizing beer flavor [1]. Malt processing releases intrinsic bound phenolic compounds and creates new antioxidants through the Maillard reaction in barley which results in enhancing antioxidant activity [2–4].

Oats have been recognized as a source of antioxidant compounds. Oat antioxidants include a variety of substances such as quinones of benzoic acid, cinnamic acids, flavones, flavonols, flavanones, anthocyanidins and aminophenols [5]. Malt processing includes steeping and germination phases prior to kilning. Reported studies demonstrated that the short-germination of oat increased total phenolic content three to fourfold [6], and increased total antioxidant activity [6, 7]. More importantly, avenanthramides, a group of phenolic antioxidants intrinsic to oat, also increased in concentration during steeping and germination processes [6–8]. Avenanthramides have demonstrated stable antioxidant activity after steam processing [9, 10]. Thus, avenanthramides may retain antioxidant activity after kilning.

Oat malt processing theoretically could increase the concentration of heat-stable Maillard reaction products and phenolic type antioxidants such as avenanthramides, and

p-coumaric, ferulic, and caffeic acids [10]. The heat-stable avenanthramides are present in the endosperm with the Maillard reaction products and both could be isolated through specific fractionation milling and solvent extraction procedures. The objectives of this study were: (1) to enhance antioxidant activity in oat by malt processing, (2) to fractionate and concentrate antioxidant-rich oat fractions by dry milling and solvent extraction, and (3) to compare the antioxidant activity of the oat fractions to that of butylated hydroxytoluene (BHT).

Materials and Methods

Cereal Grains

Oat cultivar AC Vermont was obtained from the 2002 harvest in New Liskeard, ON, Canada. A sample of commercial Munich-style barley malt known for its high antioxidant activity was obtained from Cargill (Sheboygan, WI, USA) courtesy of Gilbertson and Page (Fergus, ON, Canada) and used for comparison purposes since barley is a common malting cereal crop.

Malt Processing

Oats were steeped in spring water at 13–15 °C for 3 h, air-rested for approximately 12 h followed by a second water steep for 4 h until the kernel moisture content reached approximately 47%. Germination occurred in a controlled environment at 13–15 °C and 95% relative humidity for 6 days duration intermittent with brief 30 min water steeps on each day. During the germination phase, the average kernel moisture content was 43% (±5%). Malt kilning began on the 6th day at which time the acrospire had grown to approximately 90% of the groat length (~12 mm). Kilning was accomplished with a forced air convection laboratory oven fitted with a controlled vent with germinated oats contained in a 250 μm sieve at a depth of 5 cm. The kilning protocol consisted of three stages including: (1) a closed oven with high humidity environment at 40 °C for 12 h, (2) a closed oven with high humidity environment at 65 °C for 2 h, and (3) a vented oven at 90 °C for 14 h. The germinated oats were collected at the conclusion of the 40 °C stage and lyophilized.

Milling and Fractionation of Endosperm

All oat malt and raw oat samples were dehulled with an impact dehuller and milled with an Udy Mill (Fort Collins, CO, USA) fitted with a 1.0 mm screen. The barley malt was not dehulled but milled under the same conditions. The milled samples were fractionated using sieves to recover predominately the endosperm portion of the grain. The milled oat malt and raw oat samples were passed through a 177-μm sieve to remove the bran and trichome material and passed again through a 250-μm sieve to remove the large bran and broken hull material. The fractionated oat malt and raw oat yield was approximately 76% and had moisture content of 5%. The milled barley malt sample was passed through a 250 μm sieve to remove the bran particles and broken hull material. The fractionated barley malt yield was approximately 71% and had moisture content of 5%. Fractioned samples were stored in airtight containers.

Extraction of Antioxidants

Initially, the oat materials were extracted two times with hexane (at a 5:1 solvent to sample ratio) in order to remove oil. The defatted materials were extracted three times with methanol (at a 5:1 solvent to sample ratio) in order to extract the antioxidant compounds. Milled grain samples and solvent mixtures were shaken with a laboratory rotary shaker at 1,200 rpm for 15 min and centrifuged for 20 min at 20,900 g. The supernatant was collected, reduced in a rotavap and the solvent residue evaporated for a few days in a fume hood. The resulting residue was purified by re-dissolving in a few milliliters of methanol, centrifuged for 20 min at 20,900 g, and evaporated for a few days in a fume hood. The resulting residue is called the crude antioxidants, which had the consistency of thick honey.

Antioxidant Assay

The antioxidant activity was assayed by quantitatively determining the peroxide value according to the approved method Cd 8–53 [11]. Observations were determined for each sampling interval in triplicate and all experimental treatments were performed in duplicate. The oat malt antioxidants, barley malt antioxidants, and BHT solutions were added directly to separate 125 mL Erlenmeyer glass beakers (previously washed in chromic acid), containing stripped corn oil (antioxidant-free corn oil from Fisher Scientific), and maintained at 125 °C in a laboratory oven. Aliquot samples of oxidizing corn oil were taken at specific time intervals and analyzed for lipid hydroperoxides. In the case of untreated or control corn oil, an average of 1.8 meq/kg lipid hydroperoxide concentration was obtained (average of 20 determinations). The thermal oxidation process was terminated whenever the lipid hydroperoxide concentration exceeded 100 meq/kg.

Determination of Induction Time and Protection Factor

The induction time was determined by the intersection of two linear regressions constructed from a plot of experi-

mental time and lipid hydroperoxide concentration for each antioxidant treatment in the corn oil (i.e., the two linear regressions constructed from the horizontal and vertical portions of the oxidation curve). A hydroperoxide concentration at 10 mequiv/kg was estimated from the consistent intersection of these linear regressions of the oxidation curve. This concentration depicts the inflection point of the oxidation curve at which the induction time was calculated. Consequently the induction time can be defined as the time required for the formation of 10 mequiv/kg. The protection factor is the quotient of induction time for each antioxidant treatment and the control (no antioxidant treatment).

Measurement of Malt Color

Malt color was determined by dissolving crude antioxidant from oat and barley malt in methanol and measured spectrophotometrically according to the approved method [12]. The color value is presented in the unit degrees.

Statistical Analysis

A profile of the oxidation curve was constructed by plotting the elapsed time and the corresponding lipid hydroperoxide concentration as determined from the average of two replicate trials per antioxidant treatment. A linear regression was applied to the initiation phase of each oxidation curve in order to determine the slope using statistical software (GraphPad, San Diego, CA, USA). The relationship between induction time and the antioxidant concentration was determined by curve regression analysis using the same statistical software. Least significant difference analysis was applied to the induction time and initiation phase slopes using the pairwise *t*-test ($P < 0.05$).

Results and Discussion

Antioxidant Activity of Oat Malt Extracts

The antioxidant activity demonstrated by BHT and crude antioxidants extracted from oat malt are illustrated in Fig. 1. A comparison of induction times demonstrated no significant differences between the control treatment and the 1,500 μg/g crude barley malt, 1,500 μg/g crude oat malt, and 500 μg/g crude oat malt antioxidant treatments (Table 1). These antioxidant concentrations achieved minimal antioxidant protection. The treatments containing crude antioxidants from oat and barley malt at the 2,500 μg/g level provided some antioxidant protection which was significantly higher than the control and 40 μg/g BHT treatments. No significant differences were observed

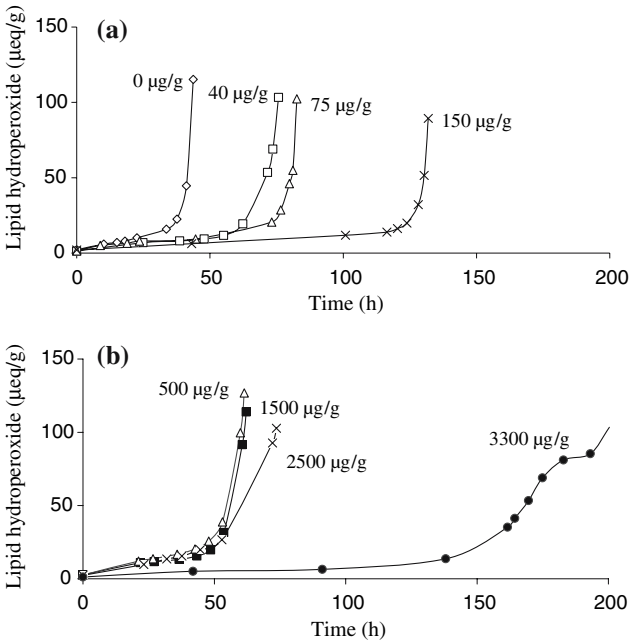

Fig. 1 Oxidation curves of stripped corn oil heated at 125 °C and spiked with BHA and antioxidant extract concentrates from oat malt. **a** BHT at 0 μg/g (*open diamonds*), 40 μg/g (*open squares*), 70 μg/g (*open triangles*), 150 μg/g (*times*). **b** Crude oat malt antioxidant at 500 μg/g (*filled squares*), 1,500 μg/g (*open triangles*), 2,500 μg/g (*times*), 3,300 μg/g (*open circles*)

between crude oat malt and barley malt antioxidant treatments at 2,500 μg/g. The 3,300 μg/g crude oat malt antioxidant treatment had significantly longer induction times than both the 70 and 150 μg/g BHT treatments indicating better antioxidant activity. The induction time of the corn

Table 1 Induction time and initiation slope for various concentrations of BHT and crude antioxidant

Antioxidant treatment (μg/g)	Induction time (h)	Initiation slope
BHT		
0	21.3a	0.4042g
40	35.0b	0.2380f
70	51.6d	0.1446e
150	74.9e	0.1099cd
Oat malt		
500	18.5a	0.1243de
1,500	22.9a	0.1094cd
2,500	41.7c	0.0559ab
3,300	93.5f	0.0246a
Barley malt		
1,500	24.7a	0.0932c
2,500	42.2c	0.0549ab

Means within a column followed by the same letter are not significantly different at $P > 0.05$

oil treated with the 3,300 μg/g crude oat malt extract was 1.2 and 1.8fold higher compared with the corn oil treated with 150 and 70 μg/g BHT, respectively.

A comparison of significant differences for the initiation slopes was less discriminating compared to induction time (Table 1). In other words, treatments that did not demonstrate any significant difference in the initiation slope did have a significant difference in the induction time. The initiation slopes were not significantly different between 150 μg/g BHT and 1,500 μg/g crude oat malt extract; however, large significant differences existed in the induction times. For example, the 150 μg/g BHT and 1,500 μg/g crude oat malt antioxidant treatments had induction times of 74.9 and 22.9 h, respectively; however, the initiation slopes were not significantly different at 0.1099 and 0.1094, respectively. The significant differences between the induction times and initiation slopes suggested that a more gradual deterioration of corn oil occurred during the initiation phase with the crude oat malt antioxidant treatment compared to the BHT.

The discordant relationship that appeared to exist between the induction time and initiation slope for the BHT and crude antioxidant treatments was explored further. The relationship between antioxidant concentration and induction time is illustrated in Fig. 2. In the case of BHT, a linear relationship existed between concentration and induction time with an adjusted determination coefficient (r^2) of 0.9764. This agrees with previous findings [13]. However, a second-order quadratic regression explains the relationship between the concentration of crude oat malt antioxidant and induction time with an adjusted determination coefficient (r^2) of 0.9588. Comparing these differences in antioxidant behavior suggests that the actual antioxidant mechanism may differ between the BHT and crude antioxidant treatments. The crude oat malt antioxidant appears to have greater antioxidant effectiveness than BHT as seen at concentration 0.33% or 3,300 μg/g of crude oat malt antioxidants. A mathematical comparison of the induction time for each antioxidant system indicates that approximately 2,600 μg/g crude oat malt antioxidants yielded the same antioxidant protection as 75 μg/g BHT.

Reported studies demonstrated that the short-germination of oats increased the total phenolic content three to fourfold [6] and increased total antioxidant activity [6, 7]. Quantifying specific phenolic concentrations in the raw, germinated, and malted oat cultivar AC Vermont was outside the scope of this investigation. The current study shows that total antioxidant activity of crude oat malt antioxidant was comparable to that of BHT and it could replace it as a natural antioxidant. In contrast, a germinated oat sample was found to have lower total antioxidant activity compared to oat malt, e.g., induction time of 38 versus 42 h at 2,500 μg/g. It is possible that oat cultivar

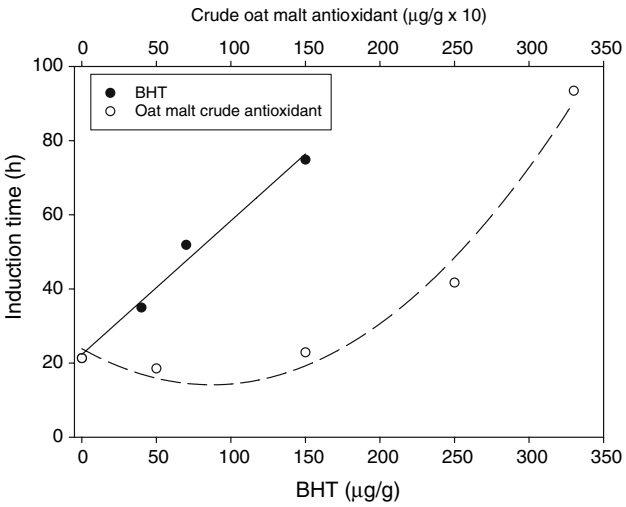

Fig. 2 Relationship between induction time and concentrations of BHA and oat malt antioxidants

AC Vermont may have a predisposition to lower total phenolic levels upon germination or that the some or all of the antioxidant-active phenolics may have been enzymatically degraded at the conclusion of the 6 days germination period. Alternatively, the antioxidant-active phenolics may have exhibited weak antioxidant activity without possible synergistic activity with the Maillard reaction products upon the total antioxidant activity.

Likewise, a comparison between the antioxidant activity of crude oat malt and barley malt antioxidants investigated the connection between malt processing and antioxidant properties of two different cereals. There were no significant differences in the induction time or initiation slopes between the crude oat malt and barley malt antioxidant treatments at both the 1,500 and 2,500 μg/g levels. Thus, the antioxidant properties appear similar among malted oat cultivar AC Vermont and Munich malt made from two row malting barley cultivars.

Crude Antioxidant Color and Protection Factor

Previous studies indicated a direct relationship between barley malt extract color and antioxidant activity [14]. The trend of increasing malt extract color and increasing protection factor was observed in this study. As shown in Fig. 3, increasing the concentration of the specific extracts resulted in a darker extract color test medium, which corresponded to better antioxidant protection. However, comparisons between the same level of the oat and barley extracts gave the same protection factor even though the crude oat malt extract had less color. The degrees of color in the 1,500 μg/g crude oat malt and barley malt extracts were 0.519 and 0.935, respectively. At 2,500 μg/g crude oat malt and barley malt extracts, the degrees of color were

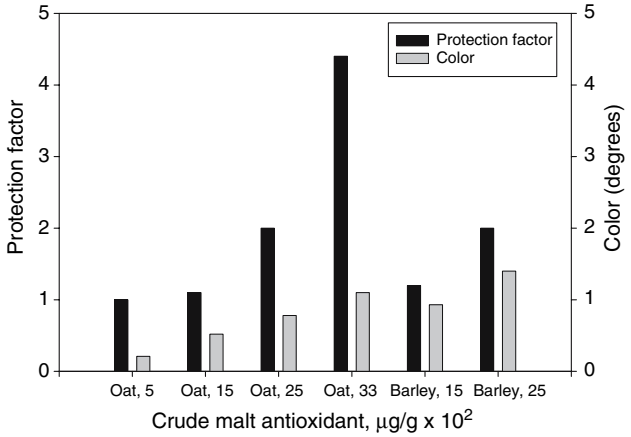

Fig. 3 Color and protection factor of oat and barley malt antioxidants

0.784 and 1.41, respectively. The color of crude oat malt extract is approximately 44% less than the crude barley malt extract but had similar antioxidant activity. Avenanthramides are colorless and reported as heat-stable phenolics [9, 10] that could be present in the mixture of crude oat malt extract. Avenanthramides are absent in the crude barley malt extract for genetic reasons. As a result, the presence of avenanthramides in the crude oat malt extract could be responsible for the greater protection factor and less color compared to the crude barley malt extract at the same antioxidant concentration. There would also be possible synergistic antioxidant effects between Maillard reaction products and avenanthramides in the crude oat malt extract. When these crude malt extracts were added to corn oil in the oxidation experiments, the oils became slightly darker than the control oil particularly for barley malt extract. However, no color measurements were carried out due to the small amount of oil sample. This may suggest that crude malt extracts would require refinement if they would be used as antioxidants in some applications such as oils and beverages.

The regulated usage limit of BHT in edible fats and oils is 75 µg/g [15]. The crude oat malt antioxidant extract at approximately 2,600 µg/g (<1% usage rate) achieved the same antioxidant protection as 75 µg/g. The improved antioxidant effectiveness of the crude oat malt antioxidant extract, as indicated by the decreased slope during the initiation phase, is apparent compared to BHT. The lower color in the crude oat malt extract compared to the crude barley malt extract is advantageous in achieving broad application in consumer products.

Promoting the attractiveness of oat malt antioxidants for use by industry requires clarifying the link between oat genotype and heat-stable phenolic concentration in the germinated oat and determining the optimum germination conditions. In addition, the heat stability of oat phenolics before and after the kilning process needs clarification, as well as identifying the antioxidative substances in the oat malt fraction.

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