Extraction and Identification of Antioxidants in Oats

Karen J. Duve and Pamela J. White*

Food Science and Human Nutrition Department, Iowa State University, Ames, IA 50011

Eight separate solvent systems were used with groats and **hulls of several lines** of oats to determine which **system resulted in the most effective, rapid extraction** of antioxidants. **Antioxidant** activity at room temperature was **estimated by using thin-layer chromatography** (TLC) along with a β -carotene spray. The greatest antioxidant **activities were obtained with methanolic antioxidant** extracts **derived from Noble and Ogle oats and hulls. These extracts were added to soybean** oil (SBO) **and their effectiveness was compared with that of butylated** hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and α control (no additives) at 32° C, 60° C and 180° C. A **petroleum ether extract of Noble oats also was tested in** SBO at 180°C. Peroxide values (PV) for oils with added antioxidants during storage at 32^oC and 60^oC showed **that the Ogle oat extract was more effective than the other oat and hull extracts or the control. There were no** significant **differences in effectiveness among the** extracts and the control at 60° C. At 180[°]C, the stability of each oil was determined by **measuring conjugated dienoic** acid **values (CD) and the relative amounts of the unoxidized fatty acid methyl esters (FAME} 18:2, 18:3 and** 18:2/16:0. **All** oils with added oat and **hull extracts had** significantly **lower CD and significantly higher 18:2/16:0 than oils with added** BHT, TBHQ or **the control during** 14 days at frying **temperature. Phenolic and hydroxy-phenolic** antioxidant **compounds** with acids, alcohols, sugars or **glycerides attached were tentatively identified in the oat and hull extracts by using TLC and chromatographic sprays.**

KEY WORDS: Antioxidants, oat extracts, oats.

The addition of antioxidants to fats, oils and foods containing fats and oils is desirable for several reasons. Antioxidants can increase the shelf life of foods by 15-200% (1), allowing food to be transported and stored for long periods (2). The addition of antioxidants may spare essential fatty acids, carotene, vitamin A and biotin from destruction by autoxidation (3}. Autoxidation of polyunsaturated fatty acids not only lowers the nutritional value of foods (4), but also is associated with membrane damage, aging, heart disease and cancer in living organisms (5). In the food industry, synthetic antioxidants are often used because they are effective and less expensive than natural antioxidants. However, consumer concern regarding chemical food additives has prompted researchers to focus on an inexpensive method to extract antioxidants from natural products. Esters of phenolic acids with antioxidant activity have been identified in oats (6). Daniels and Martin (7) reported that alkaline hydrolysis of antioxidant extracts yielded caffeic acid, ferulic acid, glycerol and longchain hydroxy-fatty acid components of oats.

Some sterols present in oats (8) have been shown to retard thermal changes at frying temperature (9). These sterols have a side chain containing an ethylidene group (9). It is thought that free radicals from the heated oils react with the ethylidene group on the sterols that isomerize to produce an allylic free radical, which can interrupt the oxidation chain (7). The addition of sterols may be a natural alternative to the use of antioxidants in frying oils.

The present study describes a simple, inexpensive extraction procedure to remove antioxidants and sterols from oats. The extracts were tested for their ability to reduce oxidation in soybean oil (SBO) at room temperature and accelerated room temperature and to reduce the changes occurring in oil at frying temperature. Extracts containing antioxidants and sterols from oats could be used in food products to prevent oxidation of lipids, which leads to rancidity, or to extend the frying life of an oil.

EXPERIMENTAL PROCEDURES

Extractions. Groats from six lines of oats (Noble, Ogle, D504, B605-1085, Cherokee, CI-9170) and hulls from two lines (Noble, Ogle} were obtained from the Iowa State University Agronomy Department. Diethyl ether and petroleum ether (b.p. range $35-60^{\circ}$ C) were distilled before use All other solvents were used as purchased. The following combinations of solvents were applied to 100.0 g oats or 25.0 g hulls for six days: Diethyl ether (8 changes}; petroleum ether (8 changes}; petroleum ether (3 changes} followed by diethyl ether (8 changes}; petroleum ether (3 changes} followed by chloroform/diethyl ether (2:1) (8 changes}; petroleum ether (3 changes} followed by chloroform/dichloroethane/ether (1:1:2) (8 changes}; petroleum ether (3 changes} followed by methanol (8 changes} methanol (8 changes}; and petroleum ether (3 changes} followed by chloroform/methanol/dichloroethane (1:1:2) (8 changes}.

Solvent changes were collected and rotary-evaporated at 45 $\rm{^{\circ}C}$. They were stored under nitrogen at $-18\rm{^{\circ}C}$ in 10 mL methanol/methylene chloride (1:1) until tested. The concentration of the extracts in solvent was 100 \pm 10 mg/mL.

Thin-layer chromatography (TLC) tests of antioxidants. The procedures of Daniels and Martin (7) and Pratt and Miller (10) were followed for identifying antioxidant activity of the extracts. Thin-layer chromatography (TLC) plates (0.25 mm) precoated with Silica gel G (Fisher Scientific, Itasca, IL) were activated at 100° C for 15 min. They were streaked with 200 μ L of extract and developed in the upper phase of chloroform/ethanol/acetic acid (98:2:2) (7). After development, the plates were sprayed with a solution of 9 mg β -carotene dissolved in 30 mL chloroform, to which two drops of linoleic acid and 60 mL of ethanol were added. The sprayed plates were exposed to daylight for 6 hr. The intensity of the resulting orange color corresponded to the relative antioxidant activity of the extract (11,12}.

TLC tests to identify the chemical composition of extracts. Additional sprays were used to tentatively identify antioxidant compounds in the methanolic oat extracts (12}. Plates (0.25 mm) precoated with Silica gel G were streaked with 200 μ L of methanolic extract from Noble oats, Noble hulls, Ogle oats or Ogle hulls. They were

^{*}To whom correspondence should be addressed at Food Science and Human Nutrition Dept., 3367 Dairy Industry Building, Iowa State University, Ames, IA 50011.

initially developed in ethyl acetate/formic acid/water (10:2:3). Bands were individually scraped from the plates and dissolved in 50 mL methanol. After 30 min, the bands were rotary-evaporated at 45°C and redissolved in 10 mL methanol. Next, 200 μ L of each methanolic band was streaked onto preactivated TLC plates (0.25 mm) and developed in n-butanol/acetic acid/water (4:1:5). The following sprays were used to identify chemical compounds.

Spray 1. A 1% solution of potassium ferricyanide in water and a 1% solution of ferric chloride in water gave a blue color upon reacting with phenolic compounds (13).

Spray 2. Ferric chloride (2 g) dissolved in 100 mL ethanol indicated the presence of trihydroxy-phenolics by turning blue, dihydroxy-phenolics by turning green or other phenolics by turning red or brown (14).

Spray 3. Ammoniacal silver nitrate solution was prepared by mixing 30 mL of ammonium hydroxide and 70 mL water. Silver nitrate (3.4 g) in 100 mL water was added to the solution and sprayed on the chromatograms. After heating for 10 min at 105° C, brown, black and gray streaks were produced as evidence of reducing compounds (15).

Spray 4. Vanillin (2 g) and 1 g p-toluene sulphonic acid were dissolved in 100 mL ethanol, the plates were sprayed and then heated for 10 min at 100° C. Flavonoids reacted to give a red-violet color (16).

Spray 5. Phenolics with free ortho- or para-hydroxy groups gave a brown color upon reacting with a spray made from 5 mL of 0.5% p-nitroaniline in 2N HC1 and 15 mL of 20% sodium acetate solution (17).

Spray 6. Chromatograms sprayed with 20% sodium carbonate solution produced phenolics visible under UV light **(18}.**

Spray Z Iodine was scattered in a TLC tank. Plates were placed in the tank and exposed to the iodine vapor. A brown color indicated the presence of sugar mercaptals, alcohols, acids, glycerides, N-acylamino sugars or polysaccharides (19).

Spray 8. A mixture of 1.8% aniline in ethanol and 1.8% oxalic acid in water was sprayed onto TLC plates. After heating at 100°C for 10 min, hexoses turned green-brown, pentoses turned red and uronic acids turned yellow (20}.

Spray 9. One gram of p-anisidine" HC1 was dissolved in 10 mL methanol and added to 90 mL n-butanol. Sodium hydrosulfite (0.1 g) was added to the mixture, and the chromatograms were sprayed. Yellow colors indicated the presence of ketohexoses; light brown, deoxy sugars and aldohexoses; brown, aldopentoses; green, methyl pentoses; and red, uronic acids (21).

Spray 10. A mixture of 3% anisidine'HC1 in n-butanol was sprayed onto TLC plates, which were then heated for 10 min at 100° C. Identification of the compounds by color was the same as listed under spray 9 (21).

Standards. Compounds that are known antioxidant components of oats (22) were located on TLC plates (0.25 m) with a β -carotene spray to help identify antioxidant components of the oat and hull extracts. The purchased antioxidant standards included: p-hydroxybenzoic acid, salicylic acid, gentisic acid, protocatechuic acid, vanillic acid, syringic acid, p-coumaric acid, m-coumaric acid, o-coumaric acid, caffeic acid, ferulic acid, isoferulic acid and sinapic acid (Aldrich Chemical Company, Milwaukee, WI); and α -tocopherol (Sigma Chemical Company, St. Louis, MO).

Storage tests. Further tests for antioxidant activity were carried out with methanolic extracts of Noble oats, Noble hulls, Ogle oats and Ogle hulls. These extracts were stored in refined, bleached, deodorized (RBD) soybean oil (180 g) (with no additives and no citric acid} that was obtained from a commercial refining operation. Levels of extracts tested at 32 $\rm ^{\circ}C$ and 60 $\rm ^{\circ}C$ included 0.02%, 0.05% and 0.1% by weight for the oat and hull extracts and 0.02% (the legal limit) for the commercial antioxidants butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ). Samples (1-mL aliquots) were taken every 5 days for 80 days at 32° C and every 2 days for 20 days at 60 $^{\circ}$ C. The samples were stored under nitrogen at -18° C until evaluated. Peroxide values were determined by using the Stamm method as modified by Hamm *et al.* (23). The storage tests were run in duplicate, and analyses of all samples were run in duplicate and averaged.

Heating tests. Methanolic extracts of Noble oats, Noble hulls, Ogle oats and Ogle hulls and a petroleum ether extract of Noble oats were tested at frying temperature (180°C) by adding extract levels of 0.05% and 0.1% by weight to 1,000 g RBD soybean oil. Commercial antioxidants (BHT, TBHQ) were tested at their legal limit of 0.02%. Fry Baby® electric deep fryers (Presto Company, Eau Claire, WI, model 05430) were set at 180° C \pm 3°C for 10 hr/day for 14 days. The temperature of each fryer was individually controlled by a rheostat. Oils were sampled (1-mL aliquots) every 2 days and stored under nitrogen at -18° C until analyzed. The AOCS method Ti la-64 (24) was used to determine conjugated dienoic acid (CD) values of the samples. All tests and analyses were run in duplicate and averaged.

Gas chromatography. All treatments of soybean oils from the heating tests were analyzed for fatty acid composition before and after 14 days of frying on a Varian Aerograph Series 3700 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with a hydrogen flame detector and a stainless-steel packed column (10% silar on gas chromQ II, Alltech Associates, Deerfield, IL) of 6 ft \times 0.085 in. Fatty acid methyl esters of 16:0, 18:0, 18:1, 18:2 and 18:3 were determined according to the procedure of Metcalfe *et al.* (25). Soybean oils from each treatment of each replicate were run in duplicate, and the results were averaged.

Data and statistical analyses. For 32°C and 60°C storage, the peroxide values (PV) were plotted against days of heating. Two values from the plot were used to determine differences among treatments. The first was the induction period, or the time from day zero to the beginning of the rapid oxidation period. Figure 1 shows a typical pattern in the rise of PV for several treatments. The induction period for all treatments shown, except TBHQ, was six days. Comparisons among treatments also were made from the slope of the rise in PV after the induction period.

For heating at 180° C, the number of 10-hr heating cycles was plotted against CD values for each treatment. The initial rise of the CD slope, until it leveled off, was determined. Figure 2 shows a typical pattern in the rise of CD for several treatments.

Treatments were compared to determine differences. Values from the slopes of PV and CD, from the induction period of PV, represent averages adjusted for differences among experiments. These are the so-called least square means (LSMEANS) as calculated in the Statistical Analysis System (SAS) software system (26).

RESULTS AND DISCUSSION

Screening of oat lines for antioxidant activity. Thin-layer chromatography coupled with the β -carotene spray was

used to indicate relative antioxidant activity of oat and hull extracts. From this work, it seemed that methanolic extracts of Noble oats and Ogle oats, compared with Cherokee. D504, B605-1085 and CI-9170 oat lines, produced the darkest orange spots and therefore contained the greatest potential for antioxidant activity. These extracts were selected for further study.

FIG. 1. Peroxide values of soybean oils with added methanolic antioxidant extracts from Noble hulls during storage at 60° C.

FIG. 2. Conjugated dienoic acid values of soybean oils with added methanolic and petroleum ether antioxidant extracts from Noble oats and hulls during heating at 180°C.

Identification of compounds in oat extracts. Thin-layer chromatography was used with the sprays described earlier to tentatively identify the types of compounds in methanolic oat and hull extracts (see Table 1). These sprays revealed four major bands with R_f values of 0.80, 0.64, 0.55 and 0.33. All these bands tested positive for antioxidant activity when sprayed with a β -carotene spray.

Sprays 1 and 2 revealed the presence of phenolics in all four bands in Noble oats, Ogle oats, Noble hulls and Ogle hulls. These phenolic compounds were identified further by a positive reaction with p -nitroaniline (spray 5). This spray indicated that phenolics with free ortho- and parahydroxy groups were present. Daniels *et al.* (27) reported that a pink color produced with p-nitroaniline is typical of feruloyl esters, whereas a brown color is typical of caffeoyl esters. They found that oat extracts produced brown bands at 0.80 and 0.30 and a pink band at 0.55 after being sprayed with the p-nitroaniline solution. The TLC results of Noble oats, Ogle oats, Noble hulls and Ogle hulls were similar to those of Daniels *et al.* (27}. Bands at 0.80, 0.64 and 0.33 turned brown, indicating the presence of caffeoyl esters, whereas the band at an R_f value of 0.55 turned pink, suggesting that feruloyl esters may be present in the extracts. There were no positive tests for flavonoids with the vanillin p-toluene sulphonic acid spray (spray 4) or for phenollcs with the sodium carbonate solution (spray 6).

Reducing compounds were identified by the appearance of a brown color after chromatograms were sprayed with an ammoniacal silver nitrate solution (spray 3). Bands with R_f values at 0.80 and 0.64 were identified as containing reducing compounds in extracts of Noble oats, Noble hulls, Ogle oats and Ogle hulls. After being exposed to iodine vapor (spray 7), Noble oat and Ogle oat extracts showed brown bands at 0.80 and 0.64, whereas the Noble hull and Ogle hull extracts revealed three brown bands at 0.80, 0.64 and 0.55. The appearance of these brown bands indicated that one or more of the following compounds were present--sugar mercaptals, alcohols, acids, glycerides, n-acylamino sugars or polysaccharides. The Noble hull band at 0,80 was extremely dark when compared with the other bands, indicating a higher

TABLE 1

Identification of Antioxidant Compounds in Oat Extracts by Using TLC Sprays

Compound identified	Sprav ^a	Color	Bands ^b
Antioxidants	ß-carotene	orange	A.B.C.D
Phenolics		blue	A, B, C, D
Phenolics	2	brown	A, B, C, D
Reducing agents	3	brown	A,B
OH-phenolics	5	brown	A.B.D
OH-phenolics	5	pink	C
Sugars, acids, alcohols, glycerides	7	brown	A.B.Cc
Uronic acids	8	yellow	D
Ketohexoses	9 or 10	vellow	A

 ${}^{a}\mathrm{See}$ section on Experimental Procedures for a description of each spray.

 b The bands correspond to positive identification of compounds at the following R_f values: A, 0.80; B, 0.64; C, 0.55; and D, 0.33.

 c Band C was found only in the hull extracts.

concentration of sugars, alcohols, acids or glycerides in the Noble hulls. The aniline oxalate spray (spray 8) produced a faint yellow band at an R_f of 0.33 for all extracts. This yellow band indicated that uronic acids were present. Yellow bands at 0.80 were produced by *p*-anisidine (sprays 9 and 10), indicating the presence of ketohexoses in all extracts.

Standards of ferulic acid, caffeic acid, p-hydroxy benzoic acid, salicylic acid, p-coumaric acid, m-coumaric acid, o-coumaric acid, isoferulic acid, sinapic acid and tocopherol were all tested by using TLC and identified with a β -carotene spray. Ferulic acid, caffeic acid and the other derivatives of cinnamic and benzoic acids gave similar R_f values at 0.80, owing to their similar structures, whereas to copherol gave an R_f value of 0.65. In the actual oat extracts, these compounds probably are not found as pure substances but are attached to other compounds resulting in derivatives with lower R_f values than the pure substances (22). These antioxidant compounds may account for the positive tests of the bands at R_f 0.55 and 0.33. Further analysis of all bands by gas chromatography or gas chromatography-mass spectrometry is necessary to report any positive identification of compounds in the oat and hull extracts.

Storage at 32°C. Soybean oils with different levels of methanolic antioxidant extracts from Noble oats, Noble hulls, Ogle oats and Ogle hulls and with BHT, TBHQ and a control with no additives were stored at 32° C for 80 days. A summary of the induction periods and slopes of the PV is shown in Table 2. The results are the average of two replicate experiments. The control (no additives) had the highest slope of PV of all oils during storage, indicating that it oxidized the quickest. Statistical analyses indicated no significant differences in induction periods for all treatments except those containing TBHQ $(p<$ 0.0001). Because the concentration of the antioxidants had no significant effect, the results of all treatments containing the same additives were averaged for statistical analyses.

There were no significant differences among the slopes of the control and of the oils containing Noble oat extract, Noble hull extract or Ogle hull extract. The slopes of the

TABLE 2

*Storage at 32°C was for 80 days. Storage at 60°C was for 20 days. Values within a column followed by different superscript letters are significantly different at a minimum of p<0.002.

**Average values of three concentrations: 0.02, 0.05 and 0.10%.

PV of the oils containing Ogle oat extract, BHT and TBHQ, however, were significantly lower (p<0.001) than those of the control and the other treatments. Soybean oil containing TBHQ, BHT or Ogle oat extract had the lowest slopes of PV of all treatments during storage at 32 ~ C; therefore, these treatments were the most effective antioxidants in this experiment, with TBHQ being the best.

Storage at 60^oC. The slopes of PV for sovbean oils with different levels of methanolic antioxidant extracts from Noble oats, Noble hulls, Ogle oats and Ogle hulls stored at 60° C for 20 days also are shown in Table 2. There were no significant differences in the induction periods among the control oil, the oils with added Noble oat extract, Noble hull extract, Ogle oat extract, Ogle hull extract or the BHT-containing oil. The TBHQ-containing oil had a significantly longer induction period at 11.3 days (p< 0.0001) compared with all other treatments at 60° C.

The rises in PV of the control, and of the oils with added oat and hull extracts, were not significantly different from each other. The oil with added BHT had a rise in PV that was significantly lower than the control $(p<0.0005)$, whereas the soybean oil with added TBHQ proved to be the most effective antioxidant, significantly lower than all other treatments (p<0.002).

Preliminary tests were run with petroleum ether extracts of Noble oats in soybean oils at 60°C. It was hypothesized that the antioxidant material may have been removed from the oats with the triglyceride by petroleum ether. However, the results showed no differences in the PV of the control and the oils with added petroleum ether extracts during 20 days of storage; therefore, these data are not shown.

At both 60° C and 32° C, there were no differences among induction periods of any of the treatments with the exception of TBHQ (p<0.0001). There were differences, however, in the slopes of the rise in PV. At 60° C, the treatments fell into three distinct groups. There were no differences among the control and treatments containing Noble oats, Noble hulls, Ogle oats and Ogle hulls. The oil containing BHT differed from the control (p<0.0005) and from the TBHQ-containing oil (p<0.002), but not from the other treatments. The TBHQ treatment differed from all other treatments (p<0.002).

At 32° C there were two distinct groups. There were no significant differences among the control and the treatments containing Noble oats, Noble hulls and Ogle hulls. The treatments: BHT, TBHQ and Ogle oats were not significantly different from each other, but had significantly lower rates of PV accumulation as compared with the oils with added extracts of Noble oats, Noble hulls, Ogle hulls and the control. Overall, TBHQ was the most effective antioxidant at both temperatures because it extended the induction period and lowered the rise in the slope of PV of the soybean oils.

Heating tests. At 180°C, the number of 10-hr heating cycles was plotted against CD values, resulting in a curved line that rose (in all cases) and leveled off (in most cases). The initial rise is due to the rapid formation of CD from polyunsaturated fatty acids (PUFA). This slope levels off as the oil breaks down and less PUFA are available for conversion to CD. The initial slope was determined for each treatment as an indicator of the rate of oxidation of the oil.

The slopes of the CD values of soybean oils with added methanolic antioxidant extracts from Noble oats, Noble hulls, Ogle oats and Ogle hulls and a petroleum ether extract from Noble oats are shown in Table 3. During 14 days of heating, the control had the greatest rise in the slope of the CD values. There were no significant differences in the initial rise in CD values among oils with added methanolic oat and hull extracts; therefore, the slopes were averaged for statistical analysis (average slope $= 0.17$). The oils with added methanolic oat and hull extracts had CD slopes significantly lower than the slopes for the oils with added BHT, TBHQ, 0.05% of petroleum ether extract of Noble oats and the control. The oil containing petroleum ether extract of Noble oats (0.10%) had a CD slope significantly lower than the slopes of the control, the BHT-containing oil and the oil with added TBHQ, but not significantly different from the oils with added methanolic oat and hull extracts. The petroleum ether extract of Noble oats at 0.05% was likely at too low a level to be effective, whereas, at 0.1%, the extract was very effective at protecting the soybean oil from oxidation.

Fatty acid methyl esters. Fatty acid methyl esters were determined for all treatments of the soybean oils before and after 14 days of heating at 180° C (Table 4). The data given are the relative percentages of each fatty acid present in the soybean oil. Also listed is the 18:2/16:0 ratio, which was suggested by Augustin *et al.* (28) as a good way to indicate the heat abuse of an oil.

In this study, the 18:2/16:0 values for the control (7.8) and for the oil with 0.05% added Ogle hull extract (7.7) were the lowest of all treatments, indicating the greatest degree of oil deterioration. The least deteriorated oils, according to this parameter, were those with 0.1% petroleum ether extract (18.5) and 0.1% Ogle oat extract (15.7). These extracts and the treatments [Noble oats (0.1%), Noble oats (0.05%) and Ogle oats (0.05%)] all had 18:2/16:0 values significantly greater than those of the control $(p<0.02)$.

The higher the amount of 18:3 remaining at day 14, the better the antioxidant extract protected the unsaturated fatty acids from thermal and oxidative breakdown. The oil containing 0.1% petroleum ether extract of Noble oats

TABLE3

Slopes of Conjugated Dienoic Acid Values of Soybean Oils with Added Methanolic and Petroleum Ether Extracts from Noble Oats, Noble Hulls, Ogle Oats and Ogle Hulls During Heating at 180°C for 14 Days^{*}

*Values within a column followed by different superscript letters are significantly different at p<0.002.

**Average values of two concentrations: 0.05 and 0.10%.

TABLE 4

Fatty Acid Methyl Esters of RBD Soybean Oils Before and After 14 Days at 180~

*Values within a column followed by different superscript letters are significantly different at p<0.02.

not only had the highest 18:2/16:0 ratio, but also the greatest amount of 18:3 after 14 days at frying temperature. Linolenic acid also was still present at day 14 in soybean oils with added extracts: Ogle oats, 0.1%; Noble oats, 0.05%; Noble oats, 0.1%; Ogle oats, 0.05%; and Noble hulls, 0.1%).

The FAME data showed that the most stable oils (the ones with the greatest amounts of 18:3 and higher 18:2/16:0 values} also were the oils with the lowest CD slopes. The oil with added petroleum ether extract (0.1%) and all oils with added methanolic oat and hull extracts were more stable than the oils with added BHT, TBHQ, petroleum ether extract of Noble oats (0.5%) and the control, according to CD and FAME determinations.

The high-temperature stability of the oils with added methanolic or petroleum ether (0.1%} extracts probably was due to the presence of specific sterols that contain ethylidene side chains. Knights (6) reported that Δ^{5} avenasterol and β -sitosterol were the major sterols present in oats. White and Armstrong (8) examined the hightemperature antioxidative effects of these sterols that were extracted from oats and found that Δ^5 -avenasterol

was the effective agent at reducing changes in the soybean oil during heating.

ACKNOWLEDGMENT

This work was supported by a research grant from the Center for Crops Utilization Research (CCUR), Iowa State University. This is journal paper number J-14184 of the Iowa Agriculture and Home Economics Experiment Station, project 2568.

REFERENCES

- 1. Branen, J., *J. Am. Oil Chem. Soc.* 50:59 (1973}.
- 2. Chang, S.S., B. Ostric-Matijasevic, O.A.L. Hsieh and C. Huang, *J. Food Sci.* 42:1102 (1977}.
- 3. Perkins, E.G., *Food Technol.* 13:508 (1960}.
- 4. Farag, R.S., A.Z.M.A. Badei and G.S.A. E1 Baroty, J. *Am. Oil Chem. Soa* 66:800 (1989}.
- 5. Cosgrove, J.R, D.E Church and W.A. Pryor, *Lipids* 22:299 (1987}.
- 6. Knights, B.A., *Phytochemistry* 4:857 (1965}.
- 7. Daniels, D.G.H., and H.F. Martin, J. *Sci. Food Agric.* 18:589 (1967).
- 8. White, RJ., and L.S. Armstrong, J. *Am. Oil Chem. Soc.* 63:525 (1986}.
- 9. Gordon, M.H., and P. Magos, *Food Chem. 10*:141 (1983).
- 10. Pratt, D.E., and E.E. Miller, *J. Am. Oil Chem. Soa* 61:1064 (1984}.
- 11. Marco, G.J., *Ibid. 45*:594 (1968).
- 12. Taga, M.S., E.E. Miller and D.E. Pratt, *Ibi&* 61:928 (1984}.
- 13. Barton, G.M., R.S. Evans and J.A.F. Gardner, *Nature 170:249* (1952}.
- 14. Reio, L., *J. Chromatogr.* 1:338 (1958}.
- 15. Hollar, N.S., Ph.D. Thesis, Purdue University, West Lafayette, Indiana, 1974.
- 16. Roux, D.G., and H.E. Maihs, *J. Chromatogr.* 4:65 (1953}.
- 17. Swain, T., *Biochem. J.* 53:200 (1953}.
- 18. Swain, T., in *Data for Biochemical Research,* Vol. 1, edited by R.M.C. Dawson, D.C. Elliott, W.H. Elliott and K.M. Jones, Oxford University Press, NY, 1969, pp. 558-562.
- 19. Bailey, R.W., *Ibid.*, pp. 539-544.
- 20. Nicholson, R.L., M.S. Thesis, University of Maine, Orono, 1967.
- 21. Pridham, J.B., *Anal. Chem.* 28:1967 (1956}.
- 22. Collins, F.W., in *Oats: Chemistry and Technology,* American Association of Cereal Chemists, Inc., St. Paul, MN, 1986.
- 23. Hamm, D.L., E.G. Hammond, V. Parvaneh and H.E. Snyder, J. *Am. Oil Chem. Soc.* 42:920 (1965}.
- 24. *Official and Tentative Methods of the American Oil Chemists' Society,* American Oil Chemists' Society, Champaign, IL, 1983.
- 25. Metcalfe, L.D., A.A. Schmitz and J.R. Pelka, *Anal. Chem.* 38:514 {1966}.
- 26. SAS, SAS Institute, Inc, Cary, NC, 1985.
- 27. Daniels, D.G.H., H.G.C. King and H.F. Martin, *J. Sci. Food Agric.* 14:385 (1963}.
- 28. Augustin, M.A., A. Telingai and L.K. Heng, *J. Am. Oil Chem. Soc.* 64:1670 (1987).

[Received September 18, 1990; accepted March 29, 1991]