

Stability of Oat Avenanthramides

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ABSTRACT

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The three main oat avenanthramides, N-(4'-hydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bp**), N-(4'-hydroxy-3'-methoxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bf**), and N-(3',4'-dihydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bc**), and their corresponding cinnamic acids, *p*-coumaric (**P**), ferulic (**F**), and caffeic (**C**), were investigated for stability to pH, temperature, and UV-light treatment. The retention of the avenanthramides after processing of oat-based food products was also analyzed.

The avenanthramide **Bc** and the cinnamic acid **C** were sensitive to alkali and neutral conditions, especially in combination with heat treatment, whereas the other compounds studied were more stable. The cinnamic acids but not the avenanthramides were isomerized when irradiated with UV-light. The avenanthramides were restored after processing of oat-based products.

Autoxidation of polyunsaturated fatty acids lowers the keeping qualities and nutritional value of foods. However, by protecting against rancidity and by conserving color, flavor, and texture, antioxidants can considerably increase the shelf life of foods. Antioxidants are currently receiving increasing interest as to health benefits because autoxidation is also associated with membrane damage, aging, heart disease, and cancer in living organisms (Halliwell 1994, 1999). Today, many food processing technologies use synthetic antioxidants to stabilize foods because they are effective and less expensive than natural antioxidants. However, consumer concern regarding chemical food additives has prompted researchers to focus on antioxidants from natural sources. Perhaps a long-term goal for the food industry should be to produce food products with maximum conservation of endogenous antioxidants, both for food stabilization and for nutritional purposes.

Oat grains have a good edible oil quality because of a high content of mono- and diunsaturated fatty acids (Zhou et al 1999). Unfortunately, oats are also rich in lipolytic enzymes, making the lipids vulnerable to oxidation (Ekstrand et al 1993). Therefore, for food purposes, oats are heat-processed to enhance stability. Oats also contain several antioxidants such as tocopherols, tocotrienols, various hydroxycinnamic acids, and esters (Daniels et al 1963; Daniels and Martin 1967, 1968; Peterson 1995; Xing and White 1997; Emmons and Peterson 1999; Emmons et al 1999; Handelman et al 1999) and amides (Collins and Mullin 1988; Collins 1989; Dimberg et al 1993, 1996). Those antioxidants may also be destroyed during the heat process. The tocopherols are reportedly sensitive to heat, especially in water, and undergo degradation during processing (Wennermark 1993; Peterson 1995).

Among cereals, only oats contain avenanthramides, a group of phenolic compounds that are positively correlated to the fresh taste of oats and negatively correlated to rancid odor and flavor of oat porridge (Molteberg et al 1996). Thus, the avenanthramides may contribute to the oxidative stability of processed oats.

The aim of this study was to examine the stability of oat avenanthramides toward heat treatment, various pH levels, and UV-light irradiation. The three main avenanthramides studied were N-(4'-hydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bp**), N-(4'-hydroxy-3'-methoxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bf**), and N-(3',4'-dihydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bc**), and their corresponding cinnamic acids, *p*-coumaric (**P**), ferulic (**F**), and caffeic (**C**) (Fig. 1). The retention of avenanthra-

mides after food processing was studied by analyzing different oat-based products for **Bp**, **Bf**, and **Bc**.

MATERIALS AND METHODS

Chemicals

p-Coumaric acid, ferulic acid, and caffeic acid, all with a purity grade of at least 97%, were purchased from Sigma-Aldrich. The avenanthramides (**Bp**, **Bf**, and **Bc**) were synthesized and had an apparent purity of ≈97% (determined by nuclear magnetic resonance). All solvents used were obtained from local suppliers and were of analytical grade.

Synthesis of the Avenanthramides

Bp and **Bf** were synthesized by reacting 5-hydroxyanthranilic acid with protected *p*-coumaric and ferulic acid chloride, respectively. The phenolic groups in the cinnamic acids were protected as acetyl derivatives by reaction with acetic anhydride. The deprotection was performed with aqueous ammonia. The procedure is described by Collins (1989). **Bc** was synthesized from 5-hydroxyanthranilic acid, acetic anhydride, and protocatechuic aldehyde (Mayama et al 1981; Bratt 2000). Nuclear magnetic resonance (¹H NMR) data for the synthetic avenanthramides were similar to those in oats described by Collins (1989).

Oat-Based Products

Five products based on wheat flour, but with the inclusion of oats, were selected for this study. The raw materials for the breads and muffins were delivered as mixtures by Kungsörnen AB, Sweden. Before the baking process was performed, yeast and water were added to the bread mixtures, and water was added to the muffin mixture according to ordinary recipes. The muffin mixture contained baking soda as a leavening agent. The macaroni and fresh pasta were also delivered by Kungsörnen AB. A yeast bread (Oat Health) was fermented twice (30 and 40 min at room temperature) before baking in an oven at 210°C for 20 min. Oat inclusions (% dm) were oat bran (4%), oat bran concentrate (OBC) (5%), rolled oats (4%), and oat flour (6%). A yeast-fermented tea cake bread included OBC (5%) and rolled oats (6%). The bread was fermented twice (30 and 40 min at room temperature) and baked in an oven at 220°C for 8 min. Muffins were baked in an oven at 220°C for 8 min and included OBC (4%) and oat bran (9%). Macaroni was boiled in water for 9 min and included (OBC 9%). Fresh pasta was boiled for 3 min and included OBC (7%).

Analytical Determinations

HPLC was performed on a Hewlett Packard Series 1100 instrument equipped with a diode-array detector and a reversed-phase C-18 column (HP ODS Hypersil, 5 μm, 125 × 4 mm²). Mobile phases consisted of solvent A (0.01M phosphate buffer, pH 2.8, and acetonitrile, 95:5, v/v) and solvent B (acetonitrile). The samples

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P	R = H	<i>p</i> -coumaric acid	Bp	R = H	N-(4'-hydroxy)-(<i>E</i>)-cinnamoyl-5-hydroxyanthranilic acid
F	R = OCH ₃	ferulic acid	Bf	R = OCH ₃	N-(4'-hydroxy-3'-methoxy)-(<i>E</i>)-cinnamoyl-5-hydroxyanthranilic acid
C	R = OH	caffeic acid	Bc	R = OH	N-(3',4'-dihydroxy)-(<i>E</i>)-cinnamoyl-5-hydroxyanthranilic acid

Fig. 1. Compounds used in this study.

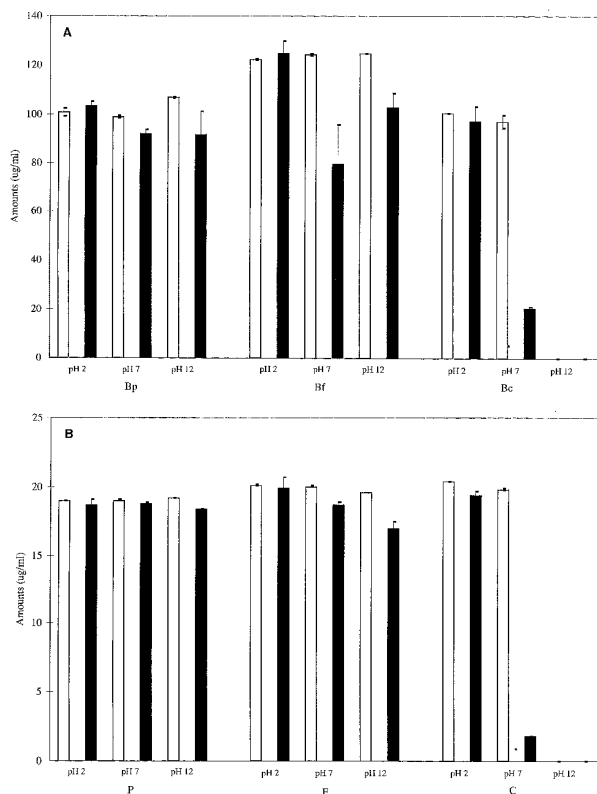


Fig. 2. Effect of pH and temperature on concentrations of avenanthramides (**A**) and cinnamic acids (**B**) after 3 hr of incubation; □ = room temperature, ■ = after heat treatments. Mean of duplicate samples. Error bars ± standard deviation values.

were run with a linear gradient over 60 min from 0 to 40% B in A. The column temperature was 30°C and the flow rate was 1 mL/min. The components were detected at 340 ± 2 nm. The peak areas were manually integrated with software (ChemStation ver. 05.01). Quantification was performed using the synthetic or authentic compounds as external standards. ¹H-NMR spectra were recorded (Varian VXR or a Varian Unity) at 400 MHz with CD₃OD as solvent.

Stability Tests

For sample preparation, duplicates of **P**, **F**, **C** (in methanol at 0.40 mg/mL) and **Bp**, **Bf**, **Bc** (in methanol at 2.0 mg/mL) were all diluted 20 times with 0.01M sodium phosphate buffer at pH 2, 7, or 12 (pH-temperature study) or with methanol (UV study). Samples dissolved in methanol and incubated at room temperature were used as reference.

For the pH-temperature study, samples (1 mL) in screw-capped glass tubes were incubated for 3 hr at room temperature or in a water bath (95–98°C). Thereafter, the samples were cooled in an

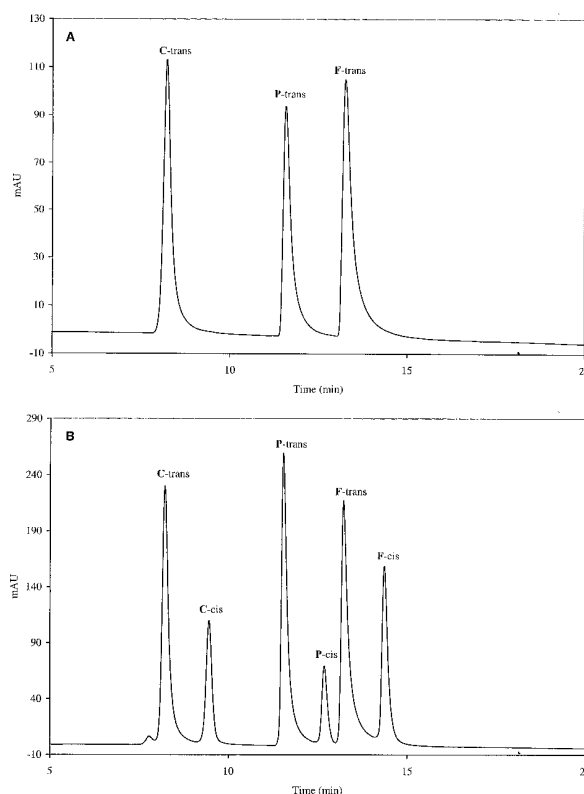


Fig. 3. HPLC results of *p*-coumaric (**P**), ferulic (**F**), and caffeic (**C**) acids before (**A**) and after daylight treatment (**B**) for 5 hr.

ice bath and stored at –20°C before HPLC analyses. The experiment at room temperature was repeated with a 24-hr incubation time. In addition, two samples of **C** were dissolved in 0.01M NaOH (aq) and 0.01M sodium phosphate buffer (pH 12), respectively, and were repeatedly analyzed for 4 hr. The two alkaline solutions were acidified with concentrated HCl and reanalyzed. The pH level of the samples was checked with a pH meter or pH indicator paper (1–10).

For the UV studies samples were incubated in quartz cuvetts under UV-light at 254 nm (Mineralight, multiband UV-254/366 nm, UVP, Upland, CA) for 18 hr. The controls were wrapped in foil and incubated in darkness for the same time period. Controls and samples were analyzed with HPLC and ¹H NMR. In addition, the samples were incubated in CD₃OD in quartz cuvetts in daylight for 5 hr. HPLC and ¹H NMR were performed before and after treatment.

Oat-Based Products

Triplicates of freeze-dried and milled samples (10 g of each) were extracted three times with 35 mL of methanol. The extracts were evaporated to dryness and the residues suspended in 1.0 mL of methanol and centrifuged. The supernatants were subjected to

TABLE I
Nuclear Magnetic Resonance Data of Olefinic Protons of Cinnamic Acids and Avenanthramides After Daylight Exposure^a

Acids ^b	<i>trans</i> H7/H7'	<i>trans</i> H8/H8'	<i>cis</i> H7/H7'	<i>cis</i> H8/H8'
P	7.36, d, $J_{7,8} = 15.8$	6.33, d, $J_{7,8} = 15.8$	6.26, d, $J_{7,8} = 12.5$	5.87, d, $J_{7,8} = 12.7$
F	7.41, d, $J_{7,8} = 15.7$	6.33, d, $J_{7,8} = 15.8$	6.31, d, $J_{7,8} = 12.0$	5.88, d, $J_{7,8} = 12.8$
C	7.32, d, $J_{7,8} = 15.9$	6.27, d, $J_{7,8} = 15.9$	6.20, d, $J_{7,8} = 13.0$	5.84, d, $J_{7,8} = 12.7$
Bp	7.54, d, $J_{7,8'} = 15.8$	6.54, d, $J_{7,8'} = 15.7$
Bf	7.52, d, $J_{7,8'} = 15.5$	6.56, d, $J_{7,8'} = 15.9$
Bc	7.47, d, $J_{7,8'} = 15.6$	6.47, d, $J_{7,8'} = 15.6$

^a Chemical shifts in ppm and coupling constants in Hz. Compounds dissolved in CD₃OD.

^b **P** = *p*-coumaric; **F** = ferulic; and **C** = caffeic; **Bp** = N-(4'-hydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid; **Bf** = N-(4'-hydroxy-3'-methoxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid; **Bc** = N-(3',4'-dihydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid.

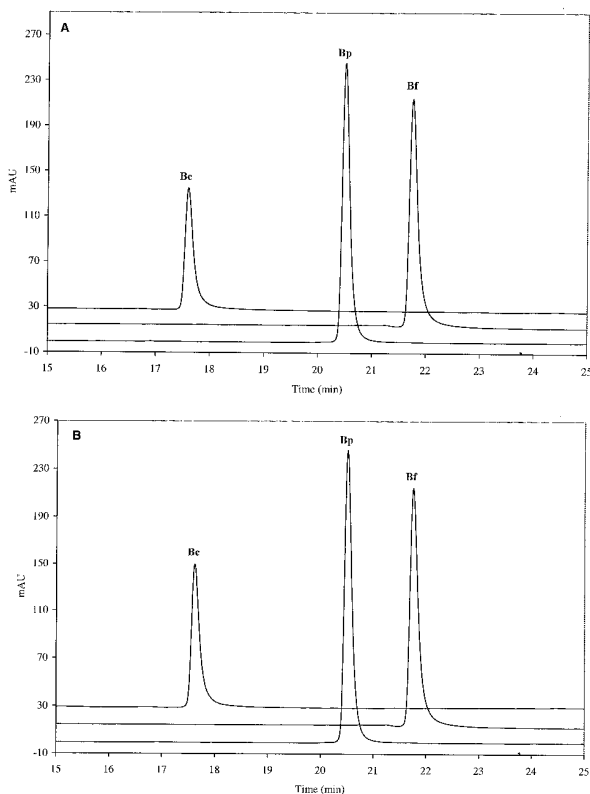


Fig. 4. HPLC results of N-(4'-hydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bp**), N-(4'-hydroxy-3'-methoxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bf**), and N-(3',4'-dihydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bc**) before (**A**) and after daylight treatment (**B**) for 5 hr.

HPLC analysis. The bread mixtures, before yeast and water addition, the muffin mixture before water addition, and the uncooked pastas were used as controls. The analysis are reported on a dry matter (dm) basis; dm content was determined by oven drying at 105°C for 5 hr. The oat ingredients (oat bran, OBC, rolled oats, and oat flour) also were analyzed.

RESULTS AND DISCUSSION

pH-Temperature

Bp and **Bf** and their corresponding cinnamic acids, **P** and **F**, did not seem to be sensitive to any change in pH within 3 hr (Fig. 2), remaining the same as for the controls. This was also true after 24 hr of incubation (data not shown). **Bc** and **C**, however, diminished completely in alkaline solution. **C** diminished 62% in 25 min and 79% within 1 hr when incubated in alkaline phosphate buffer or in aqueous sodium hydroxide. Acidification did not restore **C**, which is in accordance with the results of Friedman and Jürgens (2000).

The instability of **Bc** and **C** was even more pronounced when the pH-treated samples were heat treated. They were almost completely decomposed at pH 7 (Fig. 2) and, as mentioned above,

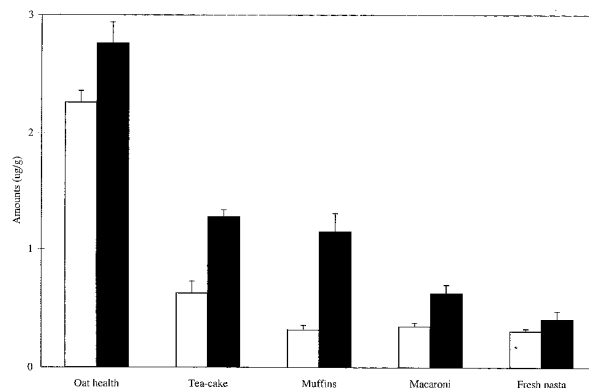


Fig. 5. Concentrations of avenanthramides N-(4'-hydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bp**), N-(4'-hydroxy-3'-methoxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bf**), and N-(3',4'-dihydroxy)-(E)-cinnamoyl-5-hydroxyanthranilic acid (**Bc**) before (□) and after processing (■) of oat-based products. Mean of triplicate samples. Error bars \pm standard deviation values.

total decomposition was observed at pH 12 even without heat treatment. In addition, **Bf** and **F** were degraded to a minor extent at pH 7 and 12 in the heat-treated samples, but **Bp** and **P** were unaffected. According to Collins (1989), avenanthramides are slowly decomposed above pH 10. Our results showed that the stability differs with the structure; **Bc** was more sensitive to base than **Bf**, which in turn was more sensitive than **Bp**.

P, **F**, and **C** are all susceptible to heat, but the temperature used in the present study was generally well below the decomposition temperatures reported for those acids. Ferulic acid starts to decompose at 200°C (Fiddler et al 1967; Pyysalo et al 1977; Stadler et al 1996). However, Pyysalo et al (1977) found that heating at 100°C with pH 4 resulted in decarboxylation of **P** and **F** and that the decarboxylation rate of **C** was lower than these two acids. They noticed that a lower pH resulted in lower stability. However, in our study a lower pH resulted in higher stability. Thus, the breakdown of **C** in alkali was probably not due to a decarboxylation reaction. Moreover, **Bc** also disappeared in base and lacks the corresponding free carboxylic acid group. It probably was not due to a simple acid-base reaction because acidification did not restore **C**. Collins (1989) found that the decomposition of the avenanthramides in alkali was due to hydrolysis of the amide bonds, but in the comparatively mild conditions of our experiment, none of the corresponding anthranilic or cinnamic acids could be detected. Hence, the disappearance could not be explained by hydrolysis. Rather, it might be due to radical reactions involving two vicinal hydroxyl groups in the phenyl rings because the catecholic compounds (**Bc** and **C**) were more sensitive than the noncatecholic compounds. Free radicals are formed during oxidation of caffeic acid in alkaline solution (Atherton and Willder 1993). Furthermore, the decomposition sensitivity parallels the radical scavenging activity measured with diphenylpicrylhydrazyl radical; **Bc** and **C** had higher scavenging activity than **Bf** and **F**, respectively, whereas **Bp** and **P** were almost inactive (Bratt 2000).

UV

It is a well-known phenomenon that cinnamic acids undergo *cis-trans*-isomerization when irradiated with UV light. When light-treated, avenanthramides also isomerize to $\approx 5\text{--}10\%$ (Collins and Mullin 1988; Collins 1989). In the present study, **P**, **F**, and **C** were, as expected, partly transformed from *E* to *Z*-isomers (20–40%) when irradiated at 254 nm for 18 hr (data not shown) or daylight for 5 hr (Fig. 3). The isomerization was studied with HPLC and ^1H NMR. For each of the three cinnamic acids, only one compound could be detected before irradiation; whereas, after irradiation, one additional compound appeared. The coupling constants for the olefinic protons of the original compounds were 16 Hz, showing *trans* configuration, whereas the new compounds showed *cis* configuration with coupling constants of 12–13 Hz (Table I). The avenanthramides **Bp**, **Bf**, and **Bc** showed no tendency to isomerize during any of the above-mentioned treatments. HPLC chromatograms (Fig. 4) and NMR and UV spectra for the avenanthramides were almost identical before and after the irradiations, and no NMR signals corresponding to *cis* double bonds could be observed; therefore, we concluded that no isomerization had occurred. In addition, no *Z*-avenanthramides were formed synthetically, although no precautions were taken to protect the compounds against light during the synthetic work.

The contradictory results obtained, compared with those of Collins and Mullin (1988) and Collins (1989), might be due to different experimental conditions.

Oat-Based Products

In all five oat-based products tested, avenanthramides **Bp**, **Bf**, and **Bc** seemed to be stable. Actually, except for fresh pasta, the concentrations of free avenanthramides increased during processing (Fig. 5). Concentrations of the avenanthramides in unprocessed products were in accordance with the concentrations calculated from the amounts in oat ingredients. The increase of avenanthramides might be explained by a *de novo* synthesis, a release of bound forms, an increasing extractability after processing, or a combination of these factors. Further studies to elucidate the mechanisms are in progress.

CONCLUSIONS

This study showed that the avenanthramides were somewhat stable to pH, temperature, and UV-light treatment. Alkali treatment and neutral conditions in combination with heat were the most destructive conditions, especially for **Bc**. The two cinnamic acids (**P** and **F**) were fairly stable to changes in pH and temperature, whereas **C** showed sensitivity similar to that of **Bc**. All three *trans*-cinnamic acids were partly converted to the corresponding *cis*-isomer after UV irradiation, whereas the avenanthramides were not.

Oat grains contain various antioxidants that protect the lipids from oxidation. The avenanthramides seemed to be stable against different treatments and passed normal food-processing conditions; therefore, they are certainly important for the storage stability of various oat products and may have value as an antioxidant source in nutrition.

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