

Effects of Commercial Processing on Levels of Antioxidants in Oats (*Avena sativa* L.)

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The effects of various commercial hydrothermal processes (steaming, autoclaving, and drum drying) on levels of selected oat antioxidants were investigated. Steaming and flaking of dehulled oat groats resulted in moderate losses of tocotrienols, caffeic acid, and the avenanthramide Bp (*N*-(4'-hydroxy)-(E)-cinnamoyl-5-hydroxy-anthranilic acid), while ferulic acid and vanillin increased. The tocopherols and the avenanthramides Bc (*N*-(3',4'-dihydroxy-(E)-cinnamoyl-5-hydroxy-anthranilic acid) and Bf (*N*-(4'-hydroxy-3'-methoxy)-(E)-cinnamoyl-5-hydroxy-anthranilic acid) were not affected by steaming. Autoclaving of grains (including the hulls) caused increased levels of all tocopherols and tocotrienols analyzed except β -tocotrienol, which was not affected. Vanillin and ferulic and *p*-coumaric acids also increased, whereas the avenanthramides decreased, and caffeic acid was almost completely eliminated. Drum drying of steamed rolled oats resulted in an almost complete loss of tocopherols and tocotrienols, as well as a large decrease in total cinnamic acids and avenanthramides. The same process applied to wholemeal made from groats from autoclaved grains resulted in less pronounced losses, especially for the avenanthramides which were not significantly affected.

KEYWORDS: *Avena sativa*; oat; antioxidants; tocopherols; tocotrienols; *p*-coumaric acid; ferulic acid; caffeic acid; vanillin; avenanthramides; hydrothermal processing

INTRODUCTION

Antioxidants contribute toward protecting foods from rancidity and may help to preserve their color, flavor, and texture (1). Antioxidants can also provide health benefits as generation of free radicals *in vivo* is associated with membrane damage, aging, heart disease, and cancer in humans (2). Consumer concern regarding chemical food additives has prompted research to focus on antioxidants from natural sources, and a long-term goal for the food industry is to produce food products with maximum conservation of endogenous antioxidants, both for food stabilization and for nutritional purposes.

Oat grains are rich in unsaturated lipids (3) and also in lipolytic enzymes (e.g., lipase and lipoxygenase) making the lipids vulnerable to oxidation (4). Lipoxygenase catalyzes not only the oxidation of polyunsaturated fatty acids but also the co-oxidation of lipid-soluble vitamins, such as carotenoids and vitamin E (5, 6). For food purposes, oats are subjected to hydrothermal processes in order to inactivate enzymes and thereby prevent rancidity during storage of the final oat products. Hydrothermal processes are also used to facilitate flaking of the groats, to develop the characteristic "oat taste", and to kill bacteria. Some milled oat products are also pre-cooked in order to provide products with a shorter cooking time.

The main commercially manufactured food products of oats are rolled oats, wholemeal, sifted flour, and bran, and these are used as ingredients in hot and cold breakfast cereals, infant foods, bread, cookies, and snacks.

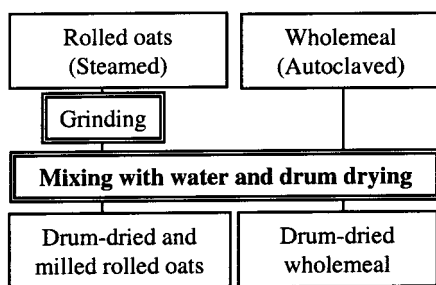
Commercial manufacturing of oat products also provides low-value fractions such as hulls and polish waste. Hulls are always removed prior to food production, and polish waste is a fraction provided when the groats are polished in order to prevent condensation of water within the trichomes and thus inhibit fungal growth during storage. The low-value fractions may be potential sources of natural antioxidants if concentrations of active compounds are reasonably high.

Early work showed that oat flour has a good antioxidant capacity (7), and several compounds in oat grains are known to have antioxidant activity. Examples of such compounds are the E-vitamins (tocopherols and tocotrienols), phenolic acids in free and esterified forms, and avenanthramides (8). Commercial processes may affect the various antioxidants present in the oat grain differently. Breakdown of cell structures may increase the bio-availability of antioxidant compounds, but their degradation may also take place. The tocopherols are reported to be sensitive to heat and to undergo degradation during processing, especially in the presence of water (9, 10). Avenanthramides have been reported to be rather stable to heat treatment under certain conditions (11, 12).

The objectives of the present study were to investigate the effect of three commercially used hydrothermal processes

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Scheme 3. Preparation of Drum-Dried Samples



during a separate production day, and they are referred to later in the paper as Batch 2. The rolled oats were milled with a Retsch ZM 100 mill using a 0.5-mm sieve. A slurry of appropriate thickness to be used for drum drying was obtained by adding 13 and 20 kg of water to 5 kg of milled rolled oats and the wholemeal, respectively. Drum drying in a pilot-plant drum (using three applicator rolls and a steam pressure of 8 bar) started 45 min after water addition, and drum-dried products were collected as five replicates (500 g) at intervals of 10 min.

Chemicals and Reagents. Tocopherols (α - and β -tocopherol) and D,L-tocotrienols (α - and β -tocotrienols) were purchased as isomer kits from Merck (Darmstadt, Germany). Synthetic avenanthramides (*N*-(3',4'-dihydroxy-(*E*)-cinnamoyl-5-hydroxy-anthranilic acid (Bc), *N*-(4'-hydroxy-3'-methoxy)-(*E*)-cinnamoyl-5-hydroxy-anthranilic acid (Bf), and *N*-(4'-hydroxy)-(*E*)-cinnamoyl-5-hydroxy-anthranilic acid (Bp)) were provided by Dr. Sunnerheim (Department of Chemistry, Uppsala University, Sweden). Caffeic acid, *p*-coumaric acid, ferulic acid, and vanillin (all of a purity grade $\geq 97\%$) were purchased from Sigma. All solvents were of analytical grade and were used without further purification.

Extractions. The collected samples were kept at $-20\text{ }^{\circ}\text{C}$ prior to extractions. Grains, groats, hulls, and rolled oats were ground in a Retch mill using a 0.5-mm sieve prior to extraction, and all samples were extracted as duplicates.

Triplicates of the ground samples (5 g) were extracted twice with intermittent centrifugation using 35 mL of methanol on the same day that grinding was performed. Extractions were performed at room temperature under vertical rotation (20 rpm, 20 min). Supernatants were pooled and dried under vacuum at $40\text{ }^{\circ}\text{C}$. The residuals were dissolved in methanol (1 mL) and stored at $-20\text{ }^{\circ}\text{C}$. Aliquots of these extracts were used for high-performance liquid chromatography (HPLC) analysis of tocopherols and tocotrienols (normal-phase HPLC), and cinnamic acids and avenanthramides (reversed-phase HPLC), as described below.

Chemical Analyses. Tocopherols and Tocotrienols. After evaporation of the methanol, hexane was added, and the samples were centrifuged and stored at $-20\text{ }^{\circ}\text{C}$ prior to analysis. HPLC analysis of tocopherols and tocotrienols was performed on a silica column (Genesis silica, 5 μm , 4 μm 250 \times 4.6 mm, Jones Chromatography) using a mobile phase of hexane/1,4-dioxane (96:4 v/v) at a flow rate of 1.5 mL/min (13). Peaks were detected by fluorescence using an excitation wavelength of 294 nm and an emission wavelength of 326 nm. Identification and quantification were made against external standards, and the coefficient of variation was $<10\%$.

Avenanthramides and Cinnamic Acids. HPLC was performed directly on the methanol extracts on a HP series 1100 instrument (Hewlett-Packard, Waldbronn, Germany) equipped with a diode-array detector using a HP ODS Hypersil column (5 μm , 125 \times 4 mm). A combination of 0.01 M phosphate buffer (A) (pH 2.8, 5% acetonitrile) and acetonitrile (B) was used as a mobile phase (0–40% B in A for 60 min with a flow rate of 1 mL/min) (12). The UV-absorbing substances were detected at 340 nm. Peaks were identified by comparison of their retention times and UV-spectra with those of standards. Quantification was made by external standard calibration and levels were expressed as mg kg^{-1} DM. The coefficient of variation was $<10\%$.

Statistical Analysis. Tukey's pairwise comparison ($\alpha = 0.05$) was conducted using the software Minitab release 11.12 (Minitab Inc., State College, PA).

Table 1. Levels of the Antioxidants Analyzed in Two Batches of Rolled Oats and Wholemeal^a

	batch 1		batch 2 ^b	
	rolled oats (<i>n</i> = 5)	wholemeal (<i>n</i> = 4)	rolled oats (<i>n</i> = 1)	wholemeal (<i>n</i> = 1)
α -tocopherol	5.49 \pm 0.4	5.79 \pm 1.9	4.22	0.15
β -tocopherol	0.37 \pm 0.0	13.82 \pm 1.0	2.29	12.44
α -tocotrienol	10.62 \pm 0.6	16.11 \pm 1.3	9.78	0.49
β -tocotrienol	1.31 \pm 0.1	2.26 \pm 0.3	1.63	1.33
caffeic acid	1.48 \pm 0.1	n.d.	n.d.	n.d.
ferulic acid	2.18 \pm 0.0	1.43 \pm 0.1	3.17	2.81
<i>p</i> -coumaric acid	0.54 \pm 0.0	5.59 \pm 0.6	0.72	8.92
vanillin	1.24 \pm 0.0	9.10 \pm 0.7	3.46	11.40
Bc	3.11 \pm 1.0	2.06 \pm 0.1	11.01	4.09
Bf	2.19 \pm 0.7	1.82 \pm 0.1	6.77	2.17
Bp	1.89 \pm 0.6	1.75 \pm 0.1	6.61	2.16

^a All values are given as mg kg^{-1} DM, and as mean \pm SD. n.d. = not detected.

^b Used for drum drying.

RESULTS

There were no significant differences in levels of the selected antioxidants found in groats according to grain size (data not shown). The results in the present study should therefore not depend on the proportions of groats of different sizes.

Effects of Steaming and Flaking. Preparation of rolled oats was accompanied by a moderate decrease in levels of α - and β -tocotrienols but no change in levels of α - and β -tocopherols (**Figure 2A**). The main decrease in the tocotrienols took place after the first steaming of intact groats, even though statistically significant ($p < 0.05$) differences were found only between raw groats and rolled oats. Caffeic acid decreased, while ferulic acid increased, and *p*-coumaric acid and vanillin were unaffected, during the first steaming (**Figure 2B**). A further increase in ferulic acid was found during steam-flaking, when an increase in vanillin was also found. The avenanthramides Bf and Bc were not significantly affected during preparation of rolled oats, while the level of Bp was 45% lower in rolled oats compared to that in raw groats (**Figure 2C**). The main decrease in Bp took place with the first steaming. The changes in levels of β -tocotrienol, caffeic acid, ferulic acid, vanillin, and Bp were significant ($p < 0.05$), but it should be noted that the levels of these compounds were fairly low, and the absolute changes were not very large. Storage of the groats between the first steaming and the steam-flaking did not influence levels of any of the analyzed antioxidants.

Two batches of rolled oats were analyzed because flakes used for drum drying (batch 2) were manufactured during a separate day. Comparisons of levels of the analyzed antioxidants in the two batches of flakes showed some moderate differences. Levels of β -tocopherol, vanillin, and all three avenanthramides analyzed were higher in rolled oats from batch 2, but caffeic acid was not detected in this batch (**Table 1**). These differences may be due to different origins of the raw oats, as it has been found that cultivation conditions may influence levels of the analyzed antioxidants in oat grains (14).

Preparation of rolled oats provides two waste fractions: raw hulls and steamed polish waste. As the polish waste corresponded to only approximately 0.3% of the groat weight, the direct comparison between raw groats and steamed and polished groats which was made above is justified. The raw hulls had low levels of E-vitamins and no detectable levels of caffeic acid (**Table 2**). Compared to raw groats, they also had lower levels of avenanthramides but several times higher levels of *p*-coumaric acid and vanillin (**Figure 2; Table 2**). Similar results have

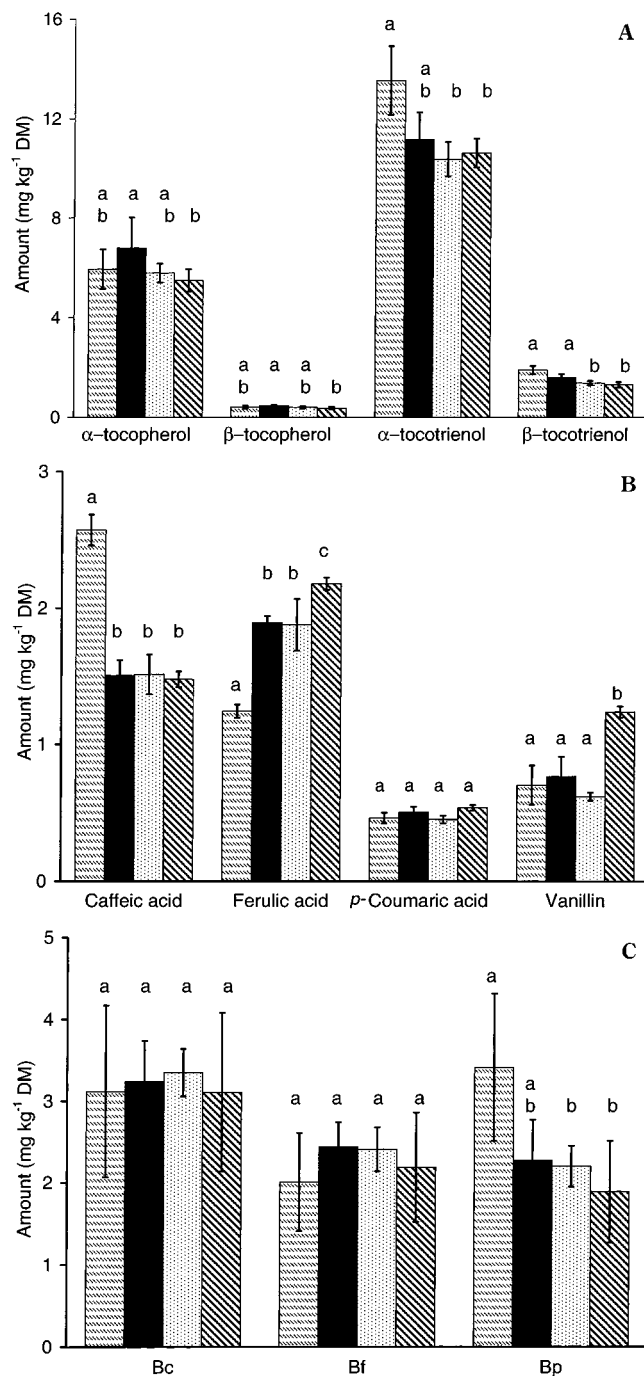


Figure 2. Levels of tocopherols and tocotrienols (A), cinnamic acids and vanillin (B), and avenanthramides (C) in raw groats (open box with open right-angled dots), steamed and polished groats (■), stored groats (open box with small random dots), and rolled oats (open box with solid right-angled dots). The bars show the means of five replicates analyzed as duplicates. The spread is given as \pm SD. Bars with the same superscript within a respective group are not significantly different ($p < 0.05$).

previously been found for hulls separated by hand (15, 16). The most remarkable result found from analysis of the polish waste was the extremely high levels of α -tocopherol in this fraction (Table 2). Compared to raw hulls, polish waste also had higher levels of all other E-vitamins, caffeic acid, ferulic acid, and avenanthramides. Comparison between polish waste and steamed groats showed that polish waste had higher levels of all antioxidants analyzed except tocotrienols and avenanthramides (Figure 2; Table 2).

Table 2. Levels of the Antioxidants Analyzed in Low-Value Fractions of Oats^a

	raw hulls (<i>n</i> = 15)	autoclaved hulls (<i>n</i> = 2)	polish waste (<i>n</i> = 5)
α -tocopherol	1.1 \pm 0.3	3.1 \pm 0.1	29.6 \pm 3.3
β -tocopherol	0.1 \pm 0.0	12.9 \pm 1.6	2.3 \pm 0.3
α -tocotrienol	0.2 \pm 0.1	2.3 \pm 0.3	5.1 \pm 0.5
β -tocotrienol	0.1 \pm 0.0	0.4 \pm 0.1	0.4 \pm 0.1
caffeic acid	n.d.	n.d.	3.8 \pm 0.4
ferulic acid	1.5 \pm 0.4	6.6 \pm 0.5	3.5 \pm 0.5
<i>p</i> -coumaric acid	6.7 \pm 1.5	53.2 \pm 1.2	3.7 \pm 0.4
vanillin	4.6 \pm 0.9	23.2 \pm 0.7	2.1 \pm 0.4
Bc	1.2 \pm 0.9	1.3 \pm 0.4	2.6 \pm 0.6
Bf	1.1 \pm 0.6	1.7 \pm 0.2	2.6 \pm 0.8
Bp	2.4 \pm 1.3	2.1 \pm 0.2	3.4 \pm 1.3

^a All values are given as mg kg^{-1} DM, and as mean \pm SD. n.d. = not detected.

Effects of Autoclaving. Autoclaving of grains, including the hulls, resulted in increased levels of α - and β -tocopherol and α -tocotrienol, whereas the increase in β -tocopherol was tremendous (4350%) (Figure 3A). Drying contributed to a further increase in β -tocopherol and α -tocotrienol in particular, even if this increase was not statistically significant. Compared to steaming of groats, autoclaving of grains also had a much larger impact on levels of all cinnamic acids and vanillin (Figures 2 and 3). Caffeic acid decreased to nondetectable levels, whereas the levels of ferulic acid, *p*-coumaric acid, and vanillin were greatly increased (222%, 1137%, and 1044%, respectively) (Figure 3B). The subsequent drying caused a significant decrease in vanillin and also small nonsignificant decreases in ferulic and *p*-coumaric acid. The avenanthramides Bc and Bp decreased both during autoclaving and drying, whereas Bf was unaffected by autoclaving but decreased during drying (Figure 3C).

After autoclaving, the hulls are separated from the groats and wholemeal is manufactured from the autoclaved groats. Similarly to rolled oats, two batches of wholemeal were analyzed and levels of α -tocopherol and α -tocotrienol in wholemeal from batch 2 were found to be very low (Table 1). As mentioned earlier, differences in levels of antioxidants may depend on different origins of the raw oats. However, different handling, processing, and storage conditions could also possibly contribute to such differences, and the very low levels of α -tocopherol and α -tocotrienol suggest prior oxidative degradation. Levels of the other antioxidants analyzed were within the same range for both batches of wholemeal.

One way to compare differences in effects of autoclaving with those of steaming is to compare rolled oats and wholemeal. However, the respective differences found between the two batches of rolled oats and wholemeal make such a comparison difficult. The finding that wholemeal had higher levels of β -tocopherol and also higher concentrations of *p*-coumaric acid and vanillin than rolled oats was, though, valid for both batches (Table 1).

Autoclaved hulls, a low-value fraction obtained during preparation of wholemeal, had the highest levels of β -tocopherol, *p*-coumaric acid, and vanillin of the three waste fractions studied (Table 2). Compared to raw hulls, they also had higher levels of all E-vitamins as well as of ferulic acid, while levels of avenanthramides did not differ.

Effects of Drum Drying. Drum drying was performed on milled rolled oats and wholemeal from batch 2. This process largely decreased levels of most tocopherols and tocotrienols even though the initial levels of α -tocopherol and α -tocotrienol

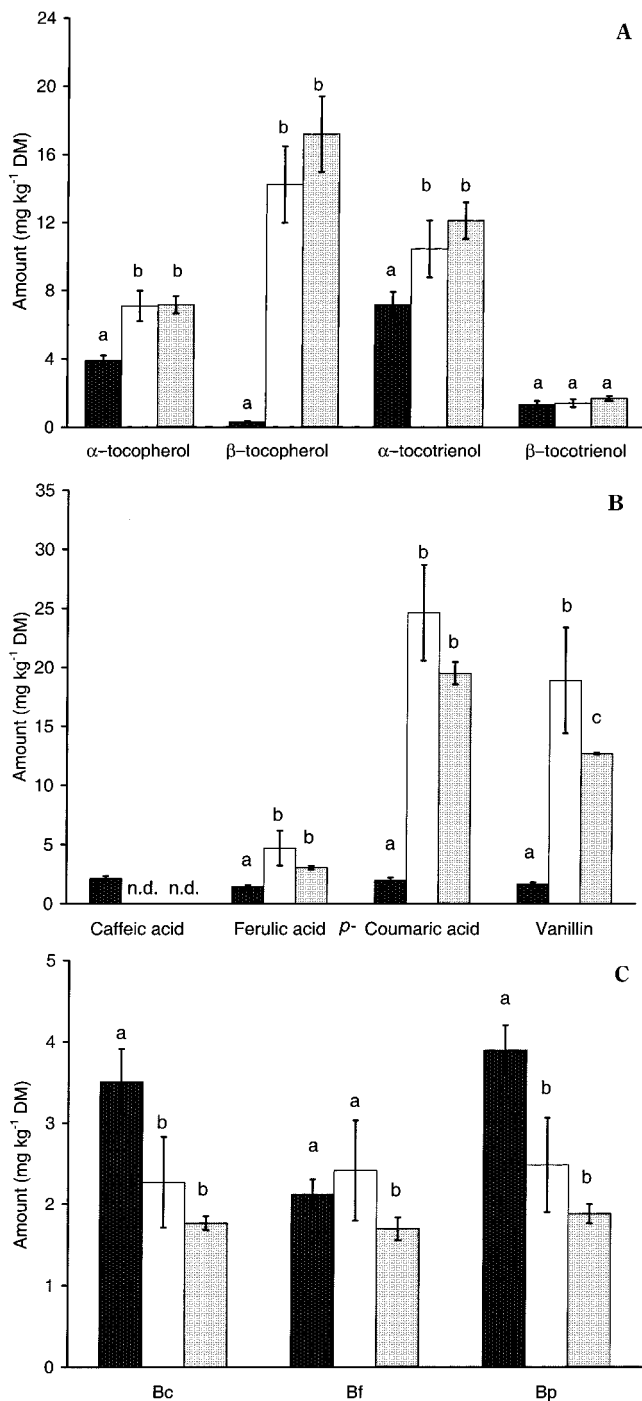


Figure 3. Levels of tocopherols and tocotrienols (A), cinnamic acids and vanillin (B), and avenanthramides (C) in rolled oats and whole groat meal before (open box with hyphen) and after (■) drum-drying. The bars show the means of two or four replicates (see Scheme 2) analyzed as duplicates. The spread is given as \pm SD. Bars with the same superscript within a respective group are not significantly different ($p < 0.05$).

were very low in the wholemeal (Figure 4A). Drum drying also caused decreased levels of ferulic acid, *p*-coumaric acid, and vanillin (Figure 4B). Caffeic acid was detected neither before nor after drum drying. Drum drying of milled rolled oats was also accompanied by a decrease in all three avenanthramides, but the same process did not affect levels in the wholemeal (Figure 4C). Final levels of avenanthramides in the two drum-dried products were, however, approximately the same.

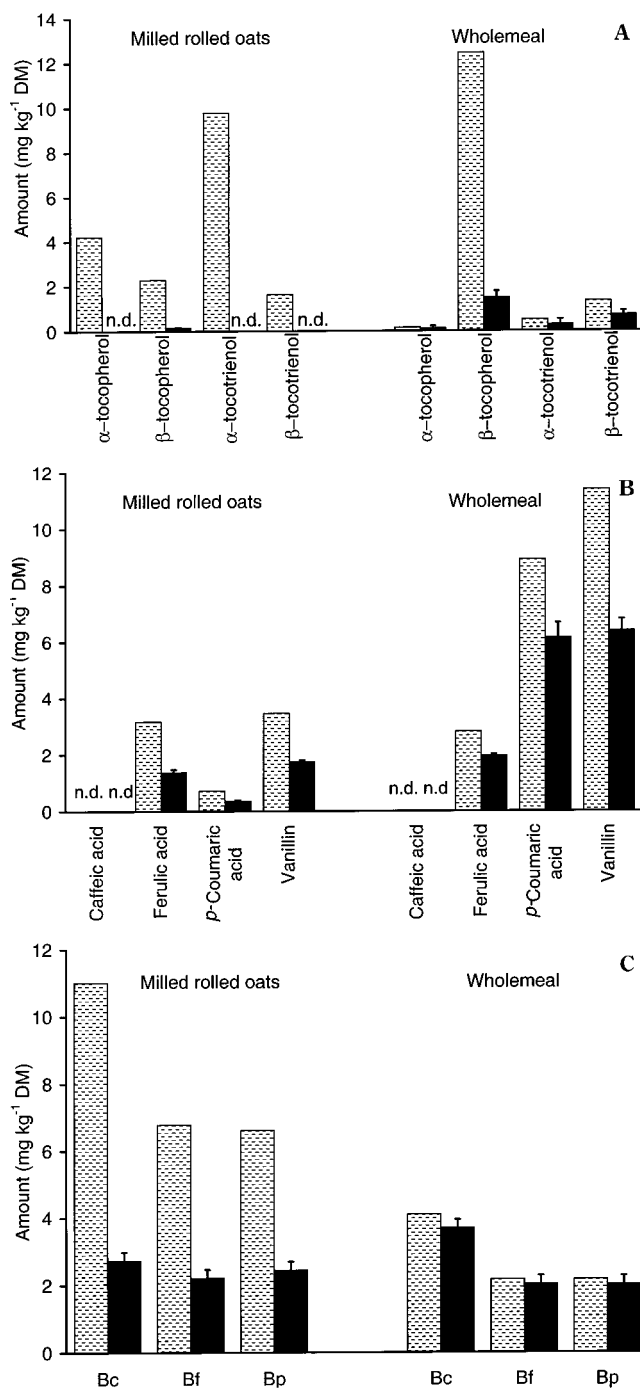


Figure 4. Levels of tocopherols and tocotrienols (A), cinnamic acids and vanillin (B), and avenanthramides (C) in rolled oats and whole groat meal before (open box with hyphen) and after (■) drum-drying. The bars show levels in a single sample taken before drum drying and the mean of levels in five replicates after drum drying. Each replicate was analyzed as duplicates. The spread is given as \pm SD. n.d. = not detected.

DISCUSSION

That the different tocopherols and tocotrienols were differently affected by steaming may be explained by their different locations in the groat. Oat tocopherols are known to be present in higher concentrations in the germ than in the endosperm, where the tocotrienols are the predominant E-vitamins (10). Furthermore, α -tocotrienol has also been found in the bran at higher concentrations than in the starchy endosperm. That tocopherols were well retained during steaming may indicate a

chemical or physical protection by germ tissue. Other explanations could be that α -tocotrienol, as a bran component, was removed by steam-distillation or by oxidation due to lipoxygenase activity. Currently, we cannot explain why E-vitamins, especially β -tocopherol, increased during autoclaving. However, two novel tocotrienols were recently isolated from stabilized and heated rice bran (17). Thus, the increased levels found in the present study may be due to liberation of some bound forms of the known E-vitamins and/or some unidentified compounds coeluting with the known peaks in the HPLC-chromatograms.

The decrease in tocopherol and tocotrienol levels caused by drum drying may be due to hydrolytic and/or enzymatic oxidation of free fatty acids during the process. Although the materials used for preparation of rolled oats and wholemeal were previously steamed and autoclaved, respectively, to inactivate the lipolytic enzymes, some enzyme activity might still remain (4). The lipoxygenase-catalyzed oxidation in dry flours is relatively slow, but when an excess of water is added prior to drum drying, the reaction may take place in seconds (5). Furthermore, drum drying also caused a small decrease in percentage concentrations of linoleic and linolenic acid and a small increase in percentage concentrations of oleic acid and saturated fatty acids (data not shown), which supports the assumption that some oxidation took place during this process.

Large amounts of *p*-coumaric acid and ferulic acid, but not caffeic acid, are known to be bound to the cell walls of cereal grains (18). Increases in *p*-coumaric acid and ferulic acid during hydrothermal processes may therefore be explained by a release of these compounds by hydrolysis. The large increase in *p*-coumaric acid during autoclaving of grains is probably mainly due to release from the hulls because this cinnamic acid is part of Klason lignin, which amounts to about 22% of the oat hull but only 1.4% of the groats (19). The higher levels in wholemeal compared to rolled oats also indicate that compounds released in hulls are transferred to the groats during processing. Dimberg et al (11) also found increased levels of *p*-coumaric acid in groats dehulled after heat treatment but not in groats which were heat-treated without hulls. Caffeic acid is labile to heat in solutions at physiological pH, whereas *p*-coumaric and ferulic acids are more stable (11, 12). This may explain the preferential loss of caffeic acid in steamed and autoclaved samples. Vanillin may be produced by decomposition of ferulic acid (20). The decrease in all cinnamic acids and vanillin caused by drum drying may be due to oxidation, as it was indicated that some oxidation took place during this process.

It is not known whether some avenanthramides are present in bound forms in oat grains, even though Peterson mentioned in a recent review that he found increased levels after alkaline hydrolysis (8). However, as no net increase was found for any of the three avenanthramides analyzed during steaming or autoclaving, it might be suggested that they are either mainly present in the free form or in forms not easily hydrolyzed, contrary to ferulic and *p*-coumaric acids.

That the avenanthramide Bp was found to be more sensitive to steaming than were Bc and Bf is in agreement with a previous study (11). However, this cannot be explained based on their chemical structures and is in contradiction with results from recent stability tests, where pure Bp and Bf were found to be heat stable and Bc was found to be unstable at physiological pH (12). The lack of decrease in the avenanthramides during drum drying of wholemeal might be because their levels have already decreased during autoclaving. However, the observation that levels of avenanthramides seem to decrease only to a certain level cannot be explained.

Table 3. Summary of Changes in Levels of Selected Antioxidants during Processing of Oat Samples^a

	steaming and flaking (groats)	autoclaving (grains)	drum drying (milled rolled oats)	drum drying (whole meal)
α -tocopherol	—	↑	↓	↓
β -tocopherol	—	↑	↓	↓
α -tocotrienol	↓	↑	↓	↓
β -tocotrienol	↓	—	↓	↓
caffeic acid	↓	↓	n.d.	n.d.
ferulic acid	↑	↑	↓	↓
<i>p</i> -coumaric acid	—	↑	↓	↓
vanillin	↑	↑	↓	↓
Bc	—	↓	↓	—
Bf	—	↓	↓	—
Bp	↓	↓	↓	—

^a n.d. = not detected in the starting material. ↑ = increase, — = no change, ↓ = decrease.

Table 3 summarizes the effects of the three hydrothermal processes investigated on levels of the selected antioxidants. The different processes apply different temperatures, moisture, and pressure to the products. The fact that the selected antioxidants are differently affected by processing may be due to their different distribution in the grain and susceptibility to oxidation because of different chemical structure. Whether they are present bound to carbohydrates or other components or not is probably also of importance.

As levels of most of the endogenous antioxidants analyzed were well retained, moderately decreased, or even increased during steaming, this process seems well suitable for production of oat-based foods of high stability and nutritional value. The increase in levels of many antioxidants during autoclaving and the higher concentrations found in wholemeal compared to rolled oats may result in higher stability and nutritional value of wholemeal compared to rolled oats. However, the lack of intact structures in wholemeal may be a disadvantage during storage. Among the waste fractions analyzed, the polish waste (rich in E-vitamins, caffeic acid, and avenanthramides) may be an antioxidant source of interest.

During further processing of rolled oats and wholemeal for use as ingredients in breakfast cereals, cookies, bread, and porridges, further changes in levels of antioxidants may occur. This was exemplified here by a decrease in many antioxidants during drum drying, but a previous study showed increased levels of free avenanthramides during preparation of certain oat-based products (bread, fresh pasta, muffins, and macaroni) (12). This shows that it is of great importance to analyze the actual product, and not the raw material, to know the levels of antioxidants in the food product. For future optimization of commercial processing of oats in order to conserve the endogenous antioxidants, further work focusing on the mechanism(s) responsible for the changes observed in the present study is needed.

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