Improved Vitamin E Retention by Using Freshly Milled Whole-Meal Wheat Flour during Drum-Drying

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The retention of α-tocopherol and certain lipid oxidation parameters were followed in freshly milled or stored (6 weeks) whole-meal wheat flour (WMWF) subjected to drum-drying. Drum-drying is a process used in the manufacture of infant products. The two flours were each mixed with whey powder in the proportion 9:2.4, followed by scalding and fermentation, prior to drum-drying. The lipid oxidation parameters that were followed indicated more pronounced lipid oxidation in stored WMWF, on the basis of a 2-fold higher concentration of free fatty acids and a 10-fold higher O_2 uptake compared with those of freshly milled WMWF. It is concluded that lipid oxidation might have occurred enzymatically as well as nonenzymatically by catalysis with iron and copper. The extensive degradation of vitamin E was related to its function as antioxidant during the co-oxidation of lipid peroxidation. However, by using freshly milled WMWF and mild drum-drying conditions, it was possible to retain 42% of the original α-tocopherol from WMWF, which is a marked improvement (4-fold) compared with stored WMWF.

INTRODUCTION

Cereal grains are an important source of vitamin E. According to a recent Finnish study (Piironen et al., 1986), as much as 30% of the recommended dietary allowance of α-tocopherol equivalents originate from cereal products in Finland. As a natural antioxidant, vitamin E prevents oxidation of unsaturated fatty acids by being a free-radical scavenger. Cereal grains from wheat contain 2–3% lipids, of which about 65% consists of polyunsaturated fatty acids (Barnes and Galliard, 1991). Thus, cereals provide both essential, but oxidation-susceptible, polyunsaturated lipids and their natural antioxidant, vitamin E. Nutritional as well as technological reasons require both components to be as well preserved as possible during the manufacture of convenient single or mixed cereal products for human consumption.

Previous studies by Håkansson et al. (1987a), Håkansson and Jägerstad (1990), and Wennermark and Jägerstad (1992) have shown that the retention of vitamin E during processing of cereals could be related to lipid oxidation. Studies using model systems showed that lipid oxidation and vitamin E degradation started as soon as water (cold as well as heated) was added to the flour. The vitamin E losses occurred more slowly in water suspensions of freshly milled wheat flour than in stored flour. Furthermore, drum-drying was shown to destroy almost all vitamin E in white wheat flour as well as whole-meal wheat flour. In another experiment from the same study (Håkansson and Jägerstad, 1990) whole-grain wheat was steam-flaked in an attempt to inactivate the enzymes involved in the lipid oxidation. All vitamin E activity was retained in the steam-flaked wheat, and the lipoxygenase activity was inactivated. However, during the subsequent drum-drying of the steam-flaked wheat, about half of the original vitamin E activity was lost. This could be compared to drum-drying of whole-meal flour without prior steam-flaking, where 90% was lost.

The purpose of the present investigation was to study the α-tocopherol retention during various steps of the drum-drying procedure in relation to lipid oxidation, performed under industrial-like conditions (pilot scale). Of special interest was to compare freshly milled and stored (6 weeks) whole-meal wheat flour (WMWF). A mixture of WMWF (freshly milled or stored) and whey powder was subjected to scalding and fermentation prior to the drum-drying procedure, a common procedure during the manufacture of instant gruel and porridges. The fermentation was performed to degrade the phytic acid present in the whole-meal flour. The scalding was performed either with or without the addition of ascorbic acid or iron oxide as an oxygen-consuming agent. Furthermore, a rapid and simple solid-phase method for the extraction of vitamin E was applied, followed by analysis of α-tocopherol only. α-Tocopherol is the most abundant of the eight natural vitamin E isomers in food and also the most biologically active. In wheat flour approximately 70% of the α-tocopherol equivalents originate from α-tocopherol. Furthermore, previous studies have shown that α-tocopherol is the most susceptible to degradation during processing. However, γ-tocopherol and δ-tocopherol possess higher antioxidant activity than α-tocopherol in vitro.

MATERIALS AND METHODS

Materials. Different batches of freshly milled and stored whole-meal wheat flour (WMWF) from winter wheat were received from a commercial mill (Juvel AB, Sweden). The WMWF was not supplemented with any nutrients or other food additives. The chemical composition of the flours was as follows: water, 10%; ash, 1.4%; fat, 2.5%; protein, 9.5%; carbohydrates by difference, 76.5%. The freshly milled WMWF was stored in a freezer (−50 °C), within 24 h after milling, while the other flour batch was stored in a silo (20 °C) for 6 weeks until processed. The whey powder was obtained from Arla, Sweden, and its chemical composition was as follows: water, 2%; ash, 9%; fat, 1%; protein, 13%; carbohydrates by difference, 74%.

Drum-Drying Procedure. Freshly milled and stored WMWF mixed with whey powder (see Scalding) was drum-dried using a pilot-scale drum (Gouda) at Semper AB, Sweden. The design of the study is shown in Figure 1 along with the sampling scheme for the chemical analysis. To compare mildly and severely processed products, the two batches were drum-dried for 40 min

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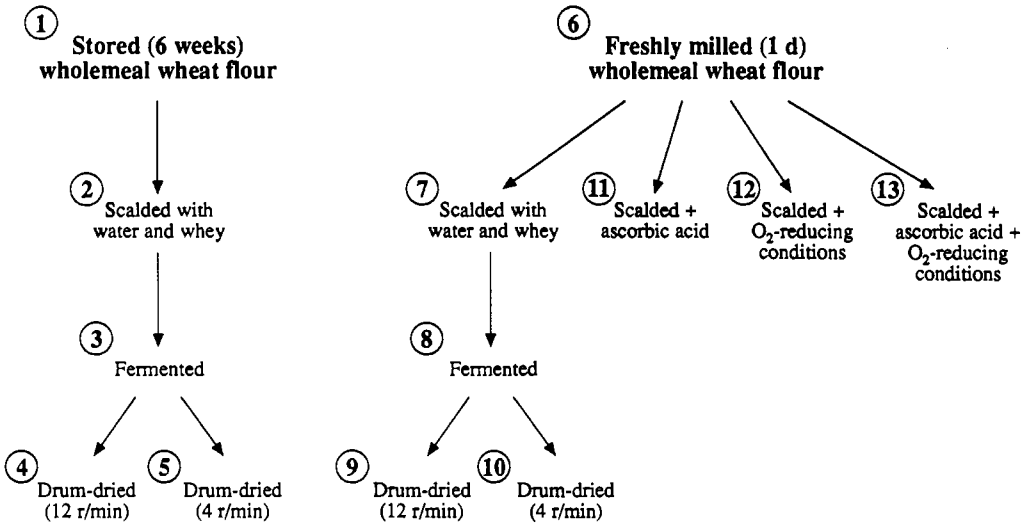

Figure 1. Processing and sampling scheme (sample numbers refer to Table 1).

using a steam pressure of 0.4 MPa and drum speeds of 12 and 4 rpm, respectively. The thin films of material produced on the single rotated drum were milled in a Retch mill with 1-mm mesh and stored frozen (−50 °C) until analysis. Prior to drum-drying, the two flours were subjected to scalding and fermentation to start the gelatinization of starch and to reduce the content of phytic acid.

Scalding. The WMWF (9.0 kg) was added gradually to a mixture of water (22.0 kg) and whey powder (2.4 kg), preheated to 68 °C, during stirring in a steam boiling vessel (Getinge). After 15 min, when all flour had been added, the temperature was decreased to 59 °C for another 25 min. An aliquot of the slurry was lyophilized and stored (−50 °C) until analysis.

Fermentation was performed at 39 °C by adding 6 g of dry yeast (Jästbolaget AB, Sweden) suspended in 200 mL of water to the scalded slurry of WMWF and whey powder during stirring. Prior to yeast addition the slurry was chilled to 39 °C for 10 min. After 60 min of fermentation, the slurry was chilled to room temperature (22 °C) before being drum-dried. An aliquot of the fermented slurry was lyophilized and stored (−50 °C) until analysis.

Alternative Scalding Methods. In separate experiments another three alternative scalding procedures were evaluated with respect to α-tocopherol retention using freshly milled wholemeal wheat flour.

(1) Scalding in the Presence of Ascorbic Acid. The scalding was performed as described above with the exception of the addition of 100 ppm of ascorbic acid, calculated on the basis of the amount of whole-meal wheat flour added to the slurry, before the flour (3.0 kg) was added. The slurry (11.1 kg) was then lyophilized and stored frozen (−50 °C) until analysis.

(2) Scalding under Oxygen-Reducing Conditions. Before scalding, oxygen was reduced by packing the flour in sealed plastic bags (nylon/PVDC/PE) containing bags (40 g/kg) with oxygen-consuming activated powdered iron oxide (FeO; Ageless, Z-type, Mitsubishi Gas Chemical Co. Inc., Tokyo) during storage at -50 °C for 96 h. The scalding procedure took place in a vessel flushed with nitrogen and placed in the steamer as described above.

(3) Scalding in the Presence of Ascorbic Acid under Oxygen-Reducing Conditions. The freshly milled whole-meal wheat flour was treated with ascorbic acid (100 ppm) and oxygen-consuming iron oxide (FeO; Ageless) as described above.

Analysis of Vitamin E. α-Tocopherol was extracted from the wheat samples using disposable cartridge extraction as described by Bourgeois et al. (1985) with some modifications adopted by the National Food Administration in Sweden.

Extraction Procedure. Each sample (6.25 g) was suspended in 75 mL of 95% ethanol, 22.5 mL of KOH (50%), and 0.25 g of ascorbic acid. The volume was adjusted to 125 mL by adding 25 mL of distilled water. After hydrolysis by reflux boiling for 30 min, the samples were cooled to room temperature and diluted with 50% ethanol to 250 mL. An aliquot of 20 mL was taken out and subjected to a dry-packed Extrelut cartridge (20 mL) purchased from Merck AG (Darmstadt, Germany). After 15 min of equilibration, vitamin E was eluted with 60 mL of hexane and collected together with a small amount of BHT. After evaporation to dryness, the samples were dissolved in 3 mL of methanol.

Table 1. Concentrations of α-Tocopherol, Free Fatty Acids (FFA, in Percent of Fat), and Lipase (Units/Gram of Fat) in Stored and Freshly Milled WMWF of Different Fractions Collected during Drum-Drying

no. material/process	α-tocopherol mg/100 g[a]	α-tocopherol %	FFA, %	lipase, units/g
1. **stored milled WMWF**	0.99 ± 0.10	100	37	1300
2. slurry after scalding	0.22 ± 0.01	22	11	<10
3. slurry after fermentation	0.27 ± 0.09	27	11	<10
4. drum-dried flour (12 rpm)	0.10 ± 0.05	10	20	–[b]
5. drum-dried flour (4 rpm)	0.06 ± 0	6	23	–
6. **freshly milled WMWF**	0.95 ± 0.00	100	18	1900
7. slurry after scalding	0.61 ± 0.06	64	2.4	<10
8. slurry after fermentation	0.56 ± 0.06	59	2.9	<10
9. drum-dried flour (12 rpm)	0.40 ± 0.01	42	9.5	–
10. drum-dried flour (4 rpm)	0.27 ± 0.04	28	11	–
11. slurry after scalding + ascorbic acid	0.70 ± 0.04	74	–	–
12. slurry after scalding under reduced O_2	0.69 ± 0.04	73	–	–
13. slurry after scalding of flour treated with ascorbic acid and reduced O_2	0.69 ± 0.04	73	–	–

[a] Mean ± SD of four observations calculated on the wheat fraction only. [b] –, not analyzed.

HPLC Analysis. A Varian Model 5020-B liquid chromatograph, equipped with a 20-μL loop, was used. Detection was achieved with a Shimadzu Model RF-540 spectrofluorometer, and the chromatograms were recorded with a Shimadzu Chromatopac C-R3A integrator. Excitation was set at 295 nm and emission at 327 nm (both slits at 10 nm). The HPLC column, a LiChrospher 100 RP-18 in LiChroChart (5 μm, 4.6 × 250 mm) purchased from Merck AG, was eluted with degassed methanol at a flow rate of 2.0 mL/min. The quantity of α-tocopherol was calculated using a standard of *dl*-α-tocopherol (Merck) dissolved in ethanol (5 μg/mL). The standard was injected after every four samples to correct for changes in the response of the detector. Each sample was analyzed in duplicate and injected twice in the HPLC, resulting in calculation of the mean and standard deviation based on four injections. The concentration of α-tocopherol presented in Table 1 is given for 100 g dry matter of WMWF after correction for the addition of whey powder and/or yeast (assumed to contain negligible amounts of α-tocopherol). The mixture of WMWF and whey powder contained 77.3% WMWF calculated on a dry matter basis. The variation within assay of α-tocopherol concentration in a commercial gruel of WMWF/milk powder from Semper AB was 5.3% (calculated on

10 observations) and the recovery of added α-tocopherol was 92.7% (mean of 4 observations).

Determination of Free Fatty Acids. The free fatty acid analyses were performed by Analycen AB (Lidköping, Sweden) according to the *Laboratory handbook for oils and fat analyses* (1966). The samples were extracted with ether. The oils were dissolved and titrated with 0.1 M NaOH.

Lipase Activity. Hydrolytic activity of flour suspensions was measured spectrofluorometrically according to the method of Heltved (1984). The method is based upon the enzymatic hydrolysis of nonfluorescent 4-methylumbelliferyl heptanoate (MUH) to the highly fluorescent compound 4-methylumbelliferone. The fluorescence measurement of MUH was carried out by suspending the wheat flour (20.0 mg) in 0.2 M Tris-HCl buffer, pH 8.5 (20 mL), during careful mixing. At zero time, 0.1 M MUH in 96% ethanol (25 μL) was added to an aliquot (3 mL) of the flour suspension. The change in fluorescence intensity with time at 20 °C was recorded at excitation and emission wavelengths of 330 and 450 nm, respectively. MUH hydrolysis has been shown to correlate with lipase activity (Heltved, 1984).

Oxygen Consumption. Measuring the O_2 uptake is valuable in checking the state of storage deterioration of milling fractions and is complementary to the lipase assay described above. The oxygen uptake was measured after 200 mg of ground (0.5-mm screen) whole-meal wheat flour was suspended in 4 mL of 0.1 M sodium phosphate buffer, pH 6.0, by using a cell of an O_2 electrode assembly (Rank Bros., Cambridge, U.K.) as described by Galliard (1989).

Iron and Copper. Iron and copper in the whole-meal wheat flours were determined by Semper AB using a Varian AA-575 atomic absorption spectrophotometer. The samples in duplicates were dry-ashed (300–500 °C), washed, and dissolved in concentrated nitric acid and water prior to analysis according to the method description of Semper based on that of Tanner and Barnett (1985).

RESULTS

α-Tocopherol Retention in Stored Whole-Meal Wheat Flour (WMWF). The concentration of α-tocopherol in whole-meal wheat flour and its retention during different processing steps of drum-drying are shown in Table 1. The values are given for the WMWF part only, amounting to 77.3% of the mixture on a dry matter basis. Stored WMWF mixed with whey powder lost as much as 78% of its α-tocopherol content during scalding. The α-tocopherol content was higher after the subsequent fermentation, but the standard deviation of the analyses was also higher. Both scalding and fermentation took place in the presence of excess water (60%) at temperatures between 39 and 68 °C. Another 20% was lost on the drum when the product was mildly (12 rpm) or severely (4 rpm) processed, leaving only 6–10% of the original α-tocopherol in the final product.

α-Tocopherol Retention in Freshly Milled WMWF. Drum-drying of freshly milled WMWF mixed with whey powder retained considerably more of the α-tocopherol than stored WMWF, as shown in Table 1. The normal scalding procedure resulted in a reduction of one-third of the total α-tocopherol. Slightly lower losses (26–27%) of α-tocopherol were obtained if scalding was performed in the presence of ascorbic acid and/or under oxygen-reducing conditions.

The fermentation of freshly milled WMWF mixed with whey powder caused minor losses of α-tocopherol. The reason for using fermentation was to degrade some of the phytic acid. In general, about 80% of the phytic acid was degraded. After drum-drying, the overall retention of α-tocopherol content amounted to 42% in the mildly processed material and 28% after more severe processing conditions.

Lipid Oxidation Parameters. In stored WMWF, 37% of its fat consisted of free fatty acids, while freshly milled WMWF showed half this figure, 18% (Table 1). The concentration of free fatty acids fell dramatically during scalding and fermentation, down to 11% and slightly below 3% for stored and freshly milled WMWF, respectively. After drum-drying, the concentrations of free fatty acids increased again by a factor of 2–4 times depending on the flour and the severity of the process.

Lipase activity was high in both freshly milled and stored WMWF, somewhat higher in freshly milled. After scalding and fermentation, the lipase activity was reduced to nearly zero (Table 1). The oxygen-consuming capacity of WMWF was measured in the fresh and stored WMWF before processing only. The figures were 10 times higher for stored WMWF than freshly milled WMWF (0.5 vs 0.05 μmol of O_2 min^{-1} g^{-1}), indicating a higher degree of oxidation in stored WMWF.

Iron and copper measurements showed initial values of 1.6 and 0.24 mg/100 g dry matter of WMWF, respectively. After drum-drying of stored WMWF under mild and severe conditions, the concentrations of iron increased to 2.7 and 4.1 mg/100 g of dry matter, respectively. The increase in iron was probably due to leakage from the drum. Similarly, the copper concentration increased from 0.24 to 0.38 mg/100 g of dry matter after drum-drying. The increase in copper concentration was equally high for mildly and severely drum-dried WMWF and probably originated from the process water.

DISCUSSION

In accordance with previous studies (Håkansson et al., 1987; Håkansson and Jägerstad, 1990), stored WMWF lost most of its α-tocopherol when drum-dried. About 75% was lost already after scalding, while no further losses occurred during fermentation. Another 15% was lost on the drum, leaving only 6–10% of the original α-tocopherol.

Interestingly, freshly milled WMWF retained 42% of its α-tocopherol when mildly drum-dried and 28% when severely processed. The figure for scalding losses was less than half of that reported for stored WMWF.

The more severely processed WMWF generated higher losses of α-tocopherol. The drum-drying procedure entailed evaporation of the water until a moisture level of either 4.5% or 2% was reached. The difference between mild and severe drum-drying is perhaps not as much the final moisture but rather the speed of the drum. Mild drum-drying is performed with a drum speed of 12 rpm where the product stays about 3 s on the heated drum. Severe drum-drying was performed at lower drum speed (4 rpm), resulting in a 10-s stay on the drum. However, prior to this, the flour slurries are boiled for an average of 10 min (the time depends on processing conditions) on the upper cavity of the drum before being exposed as a thin film on the drum.

The losses of α-tocopherol during pretreatments and subsequent drum-drying were most likely related to lipid oxidations. WMWF has been reported to undergo lipid oxidation during storage (Galliard, 1986). The bran is rich in lipase that hydrolyzes the lipids into free fatty acids. The lipase activity was demonstrated in both freshly milled and stored WMWF. In stored WMWF around one-third of the lipid content consisted of free fatty acids, while freshly milled WMWF showed half this value. For both flours, though, the content of free fatty acids was very high. However, almost equally high levels of free fatty acids were reported in fresh and stored whole-meal flours in a previous study (Håkansson and Jägerstad, 1990). WMWF also contains lipoxygenase, especially in the germ that might oxidize the free fatty acids further into

peroxides and free radicals. This reaction takes place rapidly, especially when water is added to the flour, as was the case for scalding. Consequently, the free fatty acid levels decreased during these steps. During the subsequent drum-drying, the concentration of free fatty acids was increased in spite of no lipase activity. This may be due to a change in extractability of the lipids, or it may be the processing initiated other than enzymatically induced mechanisms for splitting of triglycerides or other compounds consisting of fatty acid.

The stored WMWF was shown to consume 10 times higher amounts of O_2 compared with freshly milled WMWF. Very similar results have been reported by Galliard (1986). The O_2-uptake capacity of WMWF can be ascribed to the synergistic effect of enzymes in the bran and germ fractions.

Interestingly, the scalding inhibited the lipase activity by more than 99% and probably also lipoxygenase activity, although this enzyme was not measured directly. Though these enzymes were inhibited, remarkable losses of α-tocopherol occurred during scalding—of both freshly milled and stored WMWF. In contrast, scalding during breadmaking has been reported to retain all vitamin E activity (Wennermark and Jägerstad, 1992). In that study, however, the scalding was performed on cut rye grain, flaked rye, and wheat germ, offering a much better protection of vitamin E by retaining most of the original cell structure. According to Håkansson and Jägerstad (1990), slurries of stored WMWF lost as much as 60% of vitamin E activity during 15 min in cold water and even more at higher temperatures. Thus, it is quite probable that enzymatic lipid oxidation took place during scalding initiated by the added water. The lipoxygenase-catalyzed oxidation takes place within seconds or minutes after the water is added to the flour mixture (Galliard, 1986), while the inhibition of lipase and lipoxygenase activity occurs more slowly. Vitamin E is partly destroyed by the co-oxidation of lipoxygenase (Nicolas and Drapron, 1983; Galliard 1989; Gordon and Barimalaa, 1989).

The mechanism behind the α-tocopherol losses during drum-drying is most probably due to both enzymatic and nonenzymatic lipid oxidation catalyzed by pro-oxidantia, e.g., iron and copper. Copper is supplied with the process water, which was shown to increase the copper content by about 50%. The iron content as measured in the stored WMWF almost doubled during mild drum-drying and increased even more under severe drum-drying. This iron probably originates by leakage from the steel material of the process equipment. Addition of chelating agents during processing might have reduced the loss.

A third lipid oxidation route that might have occurred in parallel during scalding and the subsequent drum-drying is the so-called "pseudo-enzymatic" lipid oxidation. According to Galliard (1986), many enzymes and other proteins in WMWF contain transition metal ions, especially iron and copper. When such proteins are denatured by heating, their prosthetic groups become exposed and can act catalytically, although the protein itself has been inactivated.

An extraction procedure, described by Bourgeois et al. (1985) and modified by the National Food Administration in Sweden, was adopted in the present study. This very neat extraction method included hydrolysis, which facilitated the quantitative extraction.

The present study gives further support to the view that lipid oxidation taking place during processing of WMWF destroys vitamin E. The most encouraging result of the present study was that, when using freshly milled WMWF, as much as 42% of the original α-tocopherol was retained when the drum-drying was performed under mild conditions. However, it is also important to study the fate of vitamin E when processed cereal products after storage are prepared as meals, often by supplying cold or hot water. Such studies are required to ensure that the vitamin E actually persists until the product is consumed.

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