# Enrichment of Tocopherols, Tocotrienols, and Oil in Barley Fractions by Milling and Pearling[1]

L. WANG,[2] Q. XUE,[2] R. K. NEWMAN,[2] and C. W. NEWMAN[3]

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## ABSTRACT

Tocopherols (T), tocotrienols (T3), and oil concentration were determined in the whole grain and milling and pearling fractions of two waxy hull-less barley cultivars. Pearling was more effective than milling as a means of concentrating total tocols and oil in barley flour. A pearling flour, 20% of kernel weight, had the highest ($P < 0.05$) concentrations of α-T3 (115.8 mg/kg), α-T (35.4 mg/kg), total tocols (205.3 mg/kg), and oil (81.5 g/kg): 2.7, 4.4, 2.9, and 2.9 times greater, respectively, than those of the whole grain. Among six milling fractions, the highest concentrations of α-T3 (50.2 mg/kg) and α-T (12.1 mg/kg) were observed in reduction shorts and fifth middling, respectively. Fifth middling, red dog, and reduction shorts were highest in total tocol concentration. Fifth middling was highest ($P < 0.05$) in oil concentration. The fifth middling, red dog, and reduction shorts were 1, 9, and 3%, respectively, of total weight of mill fractions. Flour and bran were lowest in total tocol and oil concentration, but they were the highest in total quantity of those components because they were the largest mill fractions. In pearling fractions, the pearling flour contained higher oil, total tocols, and α-T3 than did individual mill fractions. High α-T3, total tocol, and oil concentrations make the pearling flour a potential ingredient for food products to enhance human health.


Cereal grains are the major sources of vitamin E for humans. α-Tocopherol, the largest component of the vitamin E complex in most cereals, is a natural antioxidant that reportedly reduces the risk of ischemic heart disease and cataracts and enhances the immune function (Gaby and Machlin 1991). Total tocols (tocopherols and tocotrienols) in barley grain have 37% vitamin E activity (Hakkarainen et al 1984). Tocotrienols have 15–25% vitamin E activity. α-Tocotrienol reduces plasma cholesterol in experimental animals (Qureshi et al 1986). The tocopherols and tocotrienols, being fat soluble, are extracted with the oil of cereals.

Barley, one of the earliest cultivated cereal grains in the world, is now gaining renewed interest for food use due to its hypocholesterolemic property and other desirable nutritional and functional characteristics (Newman and Newman 1991). Barley grain contains about 21 g/kg of oil (Newman and McGuire 1985). The total tocol concentration reportedly ranges from 30.5 mg/kg (Barnes 1983) to 80.6 mg/kg (Työppönen and Hakkarainen 1985). The oil and total vitamin E levels in barley are lower than those in most other grains, but the concentration of tocotrienols in barley grain is higher (Barnes 1983).

Milling and pearling processing methods produce fractions of the barley kernel that are high in oil (Pomeranz and Chung 1983). The objective of this study was to determine the concentrations of tocopherols and tocotrienols in barley milling and pearling fractions.

## MATERIALS AND METHODS

### Milling and Pearling

Waxbar (two-rowed) and Azhul (six-rowed) waxy hull-less barley cultivars were selected for fractionation by milling and pearling because of their potential use for human food. Azhul was developed by R. T. Ramage, USDA-ARS, Department of Plant Sciences, University of Arizona, Tucson, AZ. Waxbar was bred by Western Plant Breeders, Bozeman, MT. These cultivars were grown during the winter of 1989 at the Arizona Agricultural Experiment Station, Marana, AZ, and harvested in May, 1990. The grains were tempered to 10% moisture for 12 hr before milling through a MIAG Multomat eight-roller experimental mill with a feed rate of 900 g/min at the USDA Regional Wheat Quality Laboratory, Washington State University, Pullman, WA. Fractions from the first break flour through the fourth middling were combined, mixed, and designated as flour. The six milling fractions obtained were flour, fifth middling, red dog, reduction shorts, break shorts, and bran. Pearling fractions were produced with a laboratory-type barley pearler several hours before oil extraction (model 6K572A, Dayton Electric Mfg., Chicago, IL). The whole barley grain and pearling and milling fractions were sampled and ground through a 0.5-mm screen with a cyclone sample mill (Udy, Fort Collins, CO) before analysis. Flow chart for the milling process is shown in Figure 1.

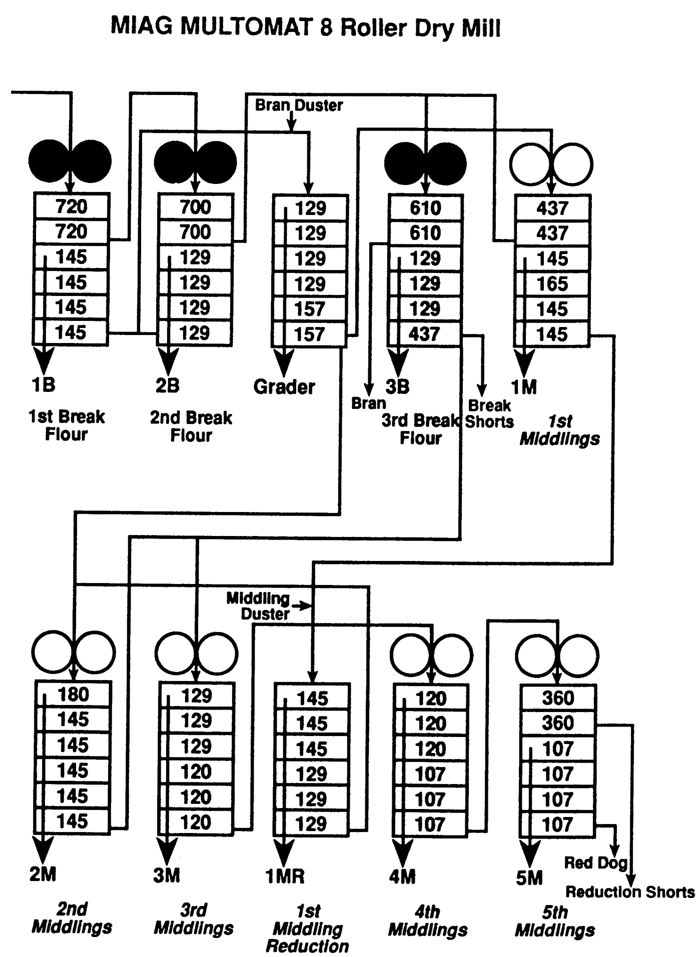


**Fig. 1.** Roller mill used for obtaining barley fractions.

[1]Contribution J-2722, Montana Agricultural Experiment Station, Bozeman.


[2]Graduate research assistants and professor, respectively, Department of Plant and Soil Science, Montana State University, Bozeman. Address correspondence to R. K. Newman.

[3]Professor, Department of Animal and Range Sciences, Montana State University, Bozeman.

### Oil Extraction

Oil content of all the barley materials was determined (AOAC 1980). Oil to be analyzed for tocopherols and tocotrienols was extracted with hexane (0.5 kg of sample per liter of hexane) for 1 hr with constant stirring at 27°C. The extract was filtered through glass-fiber filter paper. The extraction was repeated, and the two extracts were combined. Hexane was evaporated under vacuum at 45°C. The oil was stored at −20°C under nitrogen until analysis. Oil was extracted from ground whole grain and pearling fractions immediately after processing; it was extracted from the milling fractions after they were stored at 27°C in the dark for about two months.

### Tocotrienol and Tocopherol Determination

Free tocopherols and tocotrienols were extracted from barley oil after saponification. Barley oil (200 mg) was weighed into a brown glass Erlenmeyer flask, mixed with 50 mg of pyrogallic acid, 4 ml of $H_2O$, 4 ml of KOH (50%, w/v), and 40 ml of ethanol (high-performance liquid chromotography [HPLC] grade), and refluxed under nitrogen for 45 min. After being cooled in water for 20 min, the mixture was extracted with 30 ml of hexane three times, then washed with 100 ml of distilled water. Water was removed from the hexane extract by filtration through granular anhydrous sodium sulfate. Hexane was removed by vacuum rotary evaporation. Tocopherol standards were purchased from EM Science, Gibbstown, NJ. A barley oil with known concentration of tocotrienols was obtained from General Mills, Minneapolis, MN, and used as a standard. Tocotrienols and tocopherols were quantified by HPLC, using the method of Piironen et al (1984), with modifications in the composition of the mobile phase and flow rate. A silica column was used with a fluorescence detector at excitation 290 nm and emission 320 nm. Hexane with 0.5% 2-propanol was eluted through the column at a flow rate of 1 ml/min.

Data were statistically analyzed using one-way analysis of variance (SAS 1985). Significant differences in the concentrations of tocols and oil in barley fractions of cultivars used in the study were not detected; therefore, cultivar was not used as a second factor in statistical analysis.

**TABLE I**
**Pearling and Milling Fraction Yield of Two Waxy Hull-less Barleys**

| | Yield (% of total) | | |
|---|---|---|---|
| **Fraction** | **Waxbar** | **Azhul** | **Mean** |
| Milling | | | |
| Flour | 41.0 | 19.6 | 30.3 |
| Fifth middling | 1.5 | 0.9 | 1.2 |
| Red dog | 10.6 | 7.2 | 8.9 |
| Reduction shorts | 4.0 | 2.6 | 3.3 |
| Break shorts | 15.4 | 18.7 | 17.1 |
| Bran | 27.5 | 51.0 | 39.3 |
| Pearling | | | |
| Flour | 20.0 | 20.0 | . . . |
| Grain | 80.0 | 80.0 | . . . |

## RESULTS

Milling fraction yields were different ($P < 0.05$), with a range from 1% in fifth middling to 39% in bran (Table I). Greater cultivar variations were found in flour and bran, the predominant fractions, than in the other four fractions. The two pearling fractions were pearling flour (20%) and pearled grain (80%) in both barley cultivars. Azhul had much harder texture than Waxbar. The pearling time was 25 sec for Azhul and 19 sec for Waxbar.

Seven tocols, including α-, γ-, and σ-tocotrienol (T3) and α-, β-, γ-, and σ-tocopherol (T), were detected in the barley grain and its milling and pearling fractions (Table II). α-T3, γ-T3, α-T, and β-T concentration varied ($P < 0.05$) between whole grain and the milling and pearling fractions, but no significant differences were found in the concentrations of σ-T3, γ-T, and σ-T in barley grains and their fractions.

Pearling flour had the highest concentration of tocotrienols and tocopherols of all barley fractions and grain (Table II). The pearling flour had 115.8 mg/kg of α-T3, 28.1 mg/kg of γ-T3, 35.4 mg/kg of α-T, and 2.0 mg/kg of β-T, which were 2.7, 3.5, 4.4, and 8.0 times greater, respectively, than those of whole grain. Among the milling fractions, the reduction shorts and red dog were the highest in α- and γ-T3 concentration. Fifth middling was the highest in α- and β-T concentration. Total tocol concentration of the pearling flour was 205.3 mg/kg, which was 2.9 times greater than that of whole grain (Table III). High total tocol concentrations were found in fifth middling (76.9 mg/kg), red dog (77.4 mg/kg), and reduction shorts (78.9 mg/kg).

Concentration of α-T3 in total tocol was different for whole grain and milling and pearling fractions (Table III). α-T3 concentrations in total tocols were 640 g/kg and 620 g/kg, respectively, in reduction shorts and bran, which were the highest for the six milling fractions. In pearled grain and whole grain, α-T3 levels were 660 g/kg and 610 g/kg, respectively. It appears that α-T3 is either synthesized or accumulated in the starchy endosperm of barley grain during seed development, whereas other tocotrienols are not.

Oil concentration of the milling and pearling fractions varied from 9.7 g/kg in the pearled grain to 81.5 g/kg in the pearling flour (Table III). Oil concentration of pearling flour was 2.9 times greater than that of whole grain. The fifth middling had the highest oil concentration of all milling fractions: 1.6 times greater than that in the whole grain. Bran, flour, and break shorts contained the least amount of oil of the milling fractions. Pearled grain contained the least oil of all samples compared.

Distributions of oil, vitamin E, and α-T3, expressed as percent of the total of each component contained in the six milling fractions and two pearling fractions, are listed in Table IV. Because of higher milling yields, bran and flour fractions contained the largest proportions of total tocols, α-T3, and oil. Between two

**TABLE II**
**Tocotrienol (T3) and Tocopherol (T) Concentrations in Whole Grain and Milling and Pearling Fractions of Two Waxy Hull-less Barleys[a,b]**

| Sample | α-T3 | γ-T3 | δ-T3 | α-T | β-T | γ-T | δ-T |
|---|---|---|---|---|---|---|---|
| Milling Fraction | | | | | | | |
| Flour | 20.8 ± 8.0 ab | 3.3 ± 1.2 a | 0.5 ± 0.4 | 9.3 ± 3.1 abc | 0.8 ± 0.4 b | 7.8 ± 3.7 | 0.2 ± 0.1 |
| Fifth middling | 33.0 ± 10.3 bc | 5.5 ± 1.8 a | 0.7 ± 0.5 | 12.1 ± 7.0 c | 1.7 ± 0.6 c | 13.2 ± 7.1 | 0.8 ± 0.7 |
| Red dog | 41.7 ± 1.6 cd | 9.1 ± 1.8 a | 1.1 ± 0.6 | 11.4 ± 1.1 bc | 0.8 ± 0.0 b | 12.9 ± 3.0 | 0.4 ± 0.3 |
| Reduction shorts | 50.2 ± 4.2 d | 10.9 ± 0.5 a | 1.3 ± 0.7 | 2.9 ± 0.6 ab | 0.4 ± 0.2 ab | 13.0 ± 1.2 | 0.2 ± 0.1 |
| Break shorts | 27.7 ± 0.4 ab | 5.3 ± 0.0 a | 0.7 ± 0.4 | 6.2 ± 1.1 abc | 0.4 ± 0.1 ab | 13.7 ± 9.7 | 0.1 ± 0.1 |
| Bran | 28.8 ± 0.6 ab | 5.7 ± 0.2 a | 0.7 ± 0.6 | 3.3 ± 0.0 ab | 0.3 ± 1 ab | 8.0 ± 2.1 | 0.1 ± 0.1 |
| Pearling Fraction | | | | | | | |
| Flour | 115.8 ± 2.5 e | 28.1 ± 10.4 b | 2.1 ± 1.0 | 35.4 ± 9.1 d | 2.0 ± 0.3 c | 21.3 ± 5.1 | 0.8 ± 0.2 |
| Grain | 18.4 ± 3.3 a | 2.4 ± 0.4 a | 0.4 ± 0.1 | 1.0 ± 0.8 a | 0.1 ± 0.0 a | 5.1 ± 0.2 | 0.1 ± 0.0 |
| Whole grain | 43.3 ± 4.1 cd | 7.7 ± 2.1 a | 1.0 ± 0.7 | 8.1 ± 0.1 abc | 0.4 ± 0.1 ab | 10.0 ± 2.1 | 0.4 ± 0.3 |

[a] Means ± SEM (mg/kg) of cultivar Azhul and Waxbar with two determinations for each cultivar.
[b] Values in columns bearing different letters are significantly different ($P < 0.05$).

**TABLE III**
**Concentration of Oil, Total Tocols, and Tocotrienols (T3) in Whole Grain and Milling and Pearling Fractions of Two Waxy Hull-less Barleys[a,b]**

| Sample | Oil (g/kg) | Total Tocol[c] (mg/kg) | α-T3 (g/kg)[d] | γ-T3 (g/kg)[d] | δ-T3 (g/kg)[d] |
|---|---|---|---|---|---|
| Milling Fraction | | | | | |
| Flour | 24.4 ± 4.5 bc | 42.7 ± 16.9 ab | 490 ± 7 ab | 100 ± 28 | 10 ± 5 |
| Fifth middling | 45.4 ± 6.2 e | 76.9 ± 31.3 b | 430 ± 14 a | 80 ± 7 | 10 ± 3 |
| Red dog | 37.9 ± 1.6 de | 77.4 ± 8.3 b | 540 ± 42 abc | 120 ± 7 | 10 ± 7 |
| Reduction shorts | 29.7 ± 0.7 cd | 78.9 ± 2.5 b | 640 ± 35 cd | 140 ± 0 | 20 ± 10 |
| Break shorts | 21.8 ± 1.6 bc | 54.0 ± 11.5 ab | 530 ± 106 abc | 110 ± 21 | 10 ± 6 |
| Bran | 19.1 ± 0.8 ab | 46.9 ± 3.5 ab | 620 ± 3.5 cd | 130 ± 7 | 20 ± 11 |
| Pearling Fraction | | | | | |
| Flour | 81.5 ± 9.2 f | 205.3 ± 30.8 c | 570 ± 71 bcd | 140 ± 35 | 10 ± 6 |
| Grain | 9.7 ± 3.0 a | 27.5 ± 4.5 a | 660 ± 14 d | 100 ± 7 | 20 ± 6 |
| Whole grain | 27.8 ± 3.5 bc | 71.0 ± 9.5 b | 610 ± 28 cd | 110 ± 14 | 10 ± 8 |

[a] Means ± SEM (mg/kg) of cultivar Azhul and Waxbar with two determinations for each cultivar.
[b] Values in columns bearing different letters are significantly different ($P < 0.05$).
[c] Including tocotrienols and tocopherols.
[d] Of total tocol.

**TABLE IV**
**Percent Distribution of α-Tocotrienol, Total Tocols, and Oil in Milling and Pearling Fractions of Two Waxy Hull-less Barleys[a,b,c]**

| Sample | α-Tocotrienol | Total Tocol | Oil |
|---|---|---|---|
| Milling Fraction | | | |
| Flour | 21 ± 4 c | 23 ± 2 cd | 31 ± 7 bc |
| Fifth middling | 1 ± 0 a | 3 ± 0 a | 2 ± 0 a |
| Red dog | 14 ± 4 abc | 22 ± 5 cd | 15 ± 2 abc |
| Reduction shorts | 6 ± 2 ab | 7 ± 3 ab | 9 ± 5 a |
| Break shorts | 17 ± 1 bc | 17 ± 3 bc | 12 ± 7 ab |
| Bran | 41 ± 9 d | 28 ± 6 d | 32 ± 9 c |
| Pearling Fraction | | | |
| Flour | 61 ± 3 e | 93 ± 3 e | 80 ± 6 d |
| Grain | 39 ± 3 d | 7 ± 3 ab | 20 ± 6 abc |

[a] Means ± SEM of cultivar Azhul and Waxbar.
[b] Values in columns bearing different letters are significantly different ($P < 0.05$).
[c] Ratio of a component in each barley fraction to that in all fractions of the same type of processing.

pearling fractions, the pearling flour contained the larger percentages of total tocol (93%), α-T3 (61%), and oil (80%).

## DISCUSSION

Differences in milling yields among barley cultivars have been reported (Kent 1983). In the present study, the flour yield from Azhul was half that from Waxbar. Lower flour and higher bran yields in Azhul might be due to the harder texture of the outer grain surface, which contributes to the bran fraction.

In this study, pearling was more effective for processing barley grain to concentrate total tocol and oil than was the milling method. The pearling flour fraction (20% of kernel weight) contained the highest concentrations of vitamin E (including T and T3) and oil of all samples compared. Abrasive pearling has been used for many years to produce pearl barley for food and pet feed products; the pearling flour was a by-product. In this study, the pearling flour had 2.7–4.4 times more α-T3, α-T, total vitamin E, and oil concentration than did the barley grain suggesting possible use of this by-product as a nutrient-rich, health-promoting food ingredient.

Shelf life of the pearling flour is a concern because of possible deterioration of oil and oil-soluble components. Työppönen and Hakkarainen (1985) found that total tocol concentration in barley flour decreased at the rate of 5% per week when the flour was exposed to light at 25°C. Whether or not light was the only factor in that case is unknown. Concentration of tocols in the milling fractions of the present study may be underestimated because of reduction due to storage, in this case, in the dark at 27°C (Piironen et al 1988). Further investigations of bioavailability, shelf life, and storage treatments to stabilize barley tocols are suggested.


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