Influences of oil and soluble fiber of barley grain on plasma cholesterol concentrations in chicks and hamsters
by Linji Wang

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
β-Glucans and α-tocotrienol (&apha;-T3) from barley grain are hypocholesterolemic in animal models. To further identify the hypocholesterolemic components in barley, whole grain, oil, and soluble fiber were tested for their hypocholesterolemic effects. Barley cultivars were analyzed for comparative oil and &aplha;-T3 concentration and for the influence of environment and processing methods. From seven barley cultivars, the average oil and vitamin E (tocotrienols plus tocopherols) concentration were 22 g/kg and 63 mg/kg, respectively. α-T3 accounted for 52% (33 mg/kg) of vitamin E. Variation due to cultivar differences was significant in these traits. The cultivar differences were mainly due to the difference of hulled versus hull-less cultivars with the hull-less cultivars being higher in the concentrations of oil, vitamin E and α-T3 with lighter kernel weights. Pearling was the more effective processing method compared to milling to concentrate oil, vitamin E and α-T3. A pearling flour contained approximately 3-fold greater oil, vitamin E and &aplha;-T3 than whole barley grain. Barley oil was not hypocholesterolemic in Leghorn chicks fed no cholesterol and in hamsters fed with cholesterol (3 g/kg diet), however, barley oil was hypocholesterolemic in a feeding trial with broiler chicks fed cholesterol (5 g/kg diet). Barley soluble fiber was hypocholesterolemic in hamsters. Chicks fed barley diets with a high β-glucan concentration had a higher small intestinal viscosity. The viscosities were negatively correlated with plasma total cholesterol and low density lipoprotein cholesterol levels, lipid and protein digestibility and body weight gain, but positively correlated with excreta lipid concentration. Small intestinal viscosity may mediate the effects of barley soluble fiber by reducing the absorption of dietary nutrients including cholesterol, triacylglycerol and protein.

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Doctor of Philosophy

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Crop and Soil Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

April 1992

APPROVAL

of a thesis submitted by

Linji Wang

This thesis has been read by each member of the graduate committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ACKNOWLEDGEMENTS

I thank Drs. Rosemary K. Newman and C. Walter Newman for their guidance, support, patience and friendship throughout this project. I also thank Drs. Larry L. Jackson, Charles F. McGuire and R. Thomas Ramage for their advice, encouragement and help. I love my friends in our laboratory who are Petrea Hofer, Jill Abbott, Kibbie Horsley, Qi Xue and Alan Danielson. Their help was invaluable. I am indebted to Dr. Nancy Roth for her assistance in my laboratory work, and the faculty and the secretaries of our department for providing an excellent study environment. Finally, I thank my wife, my son, and my parents for their love and support.

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ABSTRACT

β-Glucans and α-tocotrienol (α-T3) from barley grain are hypocholesterolemic in animal models. To further identify the hypocholesterolemic components in barley, whole grain, oil, and soluble fiber were tested for their hypocholesterolemic effects. Barley cultivars were analyzed for comparative oil and α-T3 concentration and for the influence of environment and processing methods. From seven barley cultivars, the average oil and vitamin E (tocotrienols plus tocopherols) concentration were 22 g/kg and 63 mg/kg, respectively. α-T3 accounted for 52% (33 mg/kg) of vitamin E. Variation due to cultivar differences was significant in these traits. The cultivar differences were mainly due to the difference of hulled versus hull-less cultivars with the hull-less cultivars being higher in the concentrations of oil, vitamin E and α-T3 with lighter kernel weights. Pearling was the more effective processing method compared to milling to concentrate oil, vitamin E and α-T3. A pearling flour contained approximately 3-fold greater oil, vitamin E and α-T3 than whole barley grain. Barley oil was not hypocholesterolemic in Leghorn chicks fed no cholesterol and in hamsters fed with cholesterol (3 g/kg diet), however, barley oil was hypocholesterolemic in a feeding trial with broiler chicks fed cholesterol (5 g/kg diet). Barley soluble fiber was hypocholesterolemic in hamsters. Chicks fed barley diets with a high β-glucan concentration had a higher small intestinal viscosity. The viscosities were negatively correlated with plasma total cholesterol and low density lipoprotein cholesterol levels, lipid and protein digestibility and body weight gain, but positively correlated with excreta lipid concentration. Small intestinal viscosity may mediate the effects of barley soluble fiber by reducing the absorption of dietary nutrients including cholesterol, triacylglycerol and protein.

CHAPTER 1

INTRODUCTION

Cholesterol Metabolism

Cholesterol is an essential compound for growth and physiological function of the human body. Approximately one half of the molecules in erythrocyte plasma membranes is cholesterol and most of the cells in the human body are capable of synthesizing cholesterol. Synthesis of cholesterol occurs in the endoplasmic reticulum prior to transport to cell membranes. Cholesterol reduces ion permeability and activity of some enzymes in cell membranes by interaction with phospholipids and membrane proteins, and inhibits the membrane phase change from liquid crystalline to the gel crystalline state. Cholesterol is also a precursor of many metabolically active molecules including bile acids, steroid hormones and vitamin D.

Total body cholesterol comes from two sources, endogenous biosynthesis and exogenous dietary foods. The main site of cholesterol biosynthesis is the liver which produces half to two thirds of total body cholesterol. Other sites are the skin and gut (Mayes, 1988). Animal fat, butter and eggs are the most cholesterol-rich food ingredients, while plant foods contain very little cholesterol. After a meal, dietary cholesterol is mixed with other lipids into oil droplets in the stomach and the oil droplets

become micelles as they are mixed with bile salts and pancreatic lipase in the small intestine. Cholesterol is absorbed in the jejunum by passive diffusion and from 80% to 90% of the cholesterol will be esterified with free fatty acids within the enterocytes (Mayes, 1988). The cholesterol esters and the free cholesterol either absorbed or newly synthesized by enterocytes are then incorporated into chylomicrons within the enterocytes and released. The chylomicrons are transported via the lymphatic system and enter the blood circulation through the thoracic duct. In the circulation, cholesterol in the chylomicrons is removed by the liver and peripheral tissues with LDL receptors anchored in cell membranes (Goldstein and Brown, 1984). Cholesterol, either absorbed from the diet or synthesized, is packed with lipoproteins in liver cells and excreted by the cells as very low density lipoprotein (VLDL) into blood circulation. As VLDL particles move along in the blood stream they lose some components, particularly triacylglycerol, and the particles become smaller in size and more dense. The remnants of VLDL are low density lipoproteins (LDL). These lipoproteins are the major cholesterol carriers in the circulation, accounting for 70% of total plasma cholesterol in the human body. It is thought that LDL is the most atherogenic carrier of cholesterol. Low density lipoprotein cholesterol is removed from blood by both liver and peripheral tissues by LDL receptors. High density lipoprotein (HDL) is synthesized and secreted from both intestine and liver (Mayes, 1988). Circulating HDL in the blood has the higher protein and lower lipid composition which confers on HDL a higher density than other lipoprotein fractions (Fielding and Fielding, 1985). Apolipoprotein A-I found in HDL is an activator of lecithin:cholesterol acyltransferase (LCAT) which catalyzes the reaction from free

cholesterol to cholesterol ester. This is believed to create a flow of free cholesterol from VLDL, LDL and epithelium of blood vessels to HDL and cholesterol ester from HDL to VLDL and LDL. More free cholesterol is removed away from blood circulation by HDL when VLDL and LDL are taken up by liver and peripheral tissues. Uptake of HDL is mainly by the liver. The cholesterol absorbed is used as a precursor for biosynthesis of bile acids in the liver cells. By carrying out a reversed cholesterol transport, HDL levels in the plasma are negatively correlated with the incidence of coronary heart disease. Plasma cholesterol concentrations are indicators of body cholesterol supply since all available cholesterol for the body is transported in the blood.

The main excretion route of body cholesterol is through fecal loss of bile acids and free cholesterol which are later oxidized by gut bacteria to fecal steroids. Human bile contains 6.1 mmol/L free cholesterol (Cheillan et al., 1989). Small amounts of cholesterol are lost by excretion through the skin (Bhattucharyya et al., 1972) and urine (Vela and Acevedo, 1969).

The human body has an elaborate mechanism for maintaining a sufficient cholesterol level in the blood for normal growth of children and maintenance of adults. Cholesterol input may be greater than its output in children because cholesterol is used to build an increasing number of cells. However in adults, cell number appears to be constant, with cholesterol input balanced with output. An average adult was reported to excrete 1,100 mg cholesterol through fecal loss to counterbalance 850 mg cholesterol from biosynthesis and 250 mg from absorption of dietary cholesterol every day (Zubay, 1988).

Cholesterol biosynthesis is recognized as the center of regulation in cholesterol

homeostasis. Cholesterol is synthesized from acetyl-CoA in the mevalonate pathway with 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase being a rate-limiting enzyme. The activity of HMG-CoA reductase is inhibited by cholesterol intake level and reduction of dietary cholesterol lessens its inhibitory effect (Goldstein and Brown, 1984; Sabine, 1977). Increase in bile acid synthesis as the result of increased bile acid excretion also activates HMG-CoA reductase (Goldfarb and Potit, 1972).

Hypercholesterolemia and Diseases

Oversupply of cholesterol in the body in susceptible individuals may cause atherosclerosis. Atherosclerosis is related to coronary heart diseases (CHD) and brain stroke. The coronary arteries of patients suffering from CHD are blocked by plaque which is composed of cholesterol, cholesterol esters, other lipids, LDL lipoprotein and some dead cells. The deposit of plaque is assumed to start with the damage of artery wall by high blood pressure, bacterial infection or other unknown causes, followed by aggregation of smooth muscle cells and monocytes in the inflamed areas and uptake of LDL lipoprotein by the cells in these areas.

Hypercholesterolemia is one major risk factor of CHD. Based on clinical studies, plasma total cholesterol higher than 6.72 mmol/L (260 mg/dL), LDL cholesterol higher than 4.14 mmol/L (160 mg/dL) and HDL cholesterol lower than 0.91 mmol/L (35 mg/dL) are considered high risk factors of CHD (Anderson et al., 1987). Among lipoprotein fractions, HDL cholesterol levels are negatively correlated with the incidence of CHD (Anderson et al., 1987). Familial hypercholesterolemia (HF) is a genetic disease

of deficient or missing LDL receptors. In these patients LDL cholesterol cannot be removed by peripheral or liver cells and blood cholesterol concentration can be as high as 25.8 mmol/L (1000 mg/dl). Severe CHD development at the age of 4 to 6 years has been observed in these patients (Marinetti, 1990).

Dietary Prevention of Hypercholesterolemia

Cholesterol and saturated fats are hypercholesterolemic dietary components. In a seven country survey, Keys (1970) found that the proportions of saturated fat and cholesterol in the diets are positively correlated with plasma cholesterol levels and CHD incidence in humans. The main dietary sources of saturated fat are animal meat and dairy products which are also generally rich in cholesterol. Polyunsaturated oils (Keys, 1988; Keys et al., 1965) and monounsaturated oils (Grundy, 1986; Grundy et al., 1988) were reported to lower plasma cholesterol levels compared to saturated fats. Monounsaturated oils are more favorable to health due to their stability in lipid oxidation which is believed to be related to carcinogenesis.

Some soluble dietary fibers (SDF) have hypocholesterolemic effects (Topping, 1991). Soluble dietary fiber is a mixture of water soluble organic polymers including plant gums, algal polysaccharides, microbial polysaccharides and cellulose derivatives (Sandford and Baird, 1983) not hydrolyzed by human enzymes. Common SDF in human diets are plant gums from fruits, cereal grains and vegetables. Pectin, guar gum and oat gum are three plant gums found in plant cell walls. Pectin (Durrington et al., 1976) and guar gum (Miettenen and Tarpila, 1989; Superko et al., 1988) have hypocholesterolemic

effects. Pectin lowered plasma LDL cholesterol concentration by 18% (Durrington et al., 1976) and increased fecal excretion of bile acids (Stasse-Worthius et al., 1980). Oat gum containing 80% β-glucans, was responsible for the hypocholesterolemic effects of oat bran (Wood et al., 1989).

Other plant hypocholesterolemic components are saponins, plant proteins and nonsaponifiable lipids. Saponins are sterols or triterpene glycosides found in a variety of plant species including soybean, chick peas, peanuts, garden peas and spinach (Oakenfull, 1981). Saponins were found to inhibit cholesterol absorption in the small intestine (Oakenfull et al, 1984; Sidhu and Oakenfull, 1986). Plant proteins have also been reported to have hypocholesterolemic effects (Beynen et al., 1989; Sautier et al. 1979; Sirtori et al. 1979), however with many controversial results (Mol et al., 1982; Munoz et al., 1979). Additionally, nonsaponifiable lipid components such as oryzanol in rice oil (Seetharamaiah and Chandrasekhara, 1988) and tocotrienols in palm oil (Qureshi et al., 1991) were reported to be hypocholesterolemic.

Based on these findings, adults in North America have been advised to modify their diet composition by reducing their intake of animal fats, eggs and high-fat dairy products and increasing the intake of plant fiber from the current 11-23 g/d to 20-35 g/d (Life Sciences Research Office, 1987). Hypercholesterolemia can be reversed by treatments with either diets or diets plus drugs (Virkkunen, 1985), thereby reducing the risk of CHD. The general goal of dietary therapy is to reduce elevated levels of plasma cholesterol while maintaining a nutritionally adequate diet. Two diets have been developed by National Cholesterol Education Program (The Expert Panel, 1988) for this

purpose. The Step One Diet (total fat < 30% of total calories, saturated fatty acids <10% of total calories, and cholesterol <300 mg/d) is tried first. If it is not effective, the Step Two Diet (total fat <30% of total calories, saturated fatty acids <7% of total calories and cholesterol <200mg/d) is initiated (The Expert Panel, 1988). Dietary therapy regimes are suggested before drug therapy is initiated.

Barley consumption has been shown to have a hypocholesterolemic effect in humans (Newman et al., 1989; McIntosh et al., 1991). Soluble β-glucans were found to be one of the responsible components for this effect (Oakenfull et al., 1992; Klopfenstein and Hoseney, 1987). Soluble β-glucans and arabinoxylans are polysaccharides found in the cell walls of barley grains and are the major components of the SDF. Barley grain contains about 40 to 110 g/kg β-glucans with one third of it being water soluble and 44 to 78 g/kg arabinoxylans with 20% being water soluble (Ahluwalia and Fry, 1986). Likewise, soluble fiber from barley grain may also be hypocholesterolemic, but this needs to be verified experimentally.

Oil from brewers' grain has been shown to have hypocholesterolemic effects in chickens (Burger et al., 1984), swine (Qureshi et al., 1987) and humans (Robinson and Lupton, 1990). α-Tocotrienol of barley oil was found to lower serum cholesterol levels by inhibiting HMG-CoA reductase activity in the liver of chicks (Qureshi et al., 1986). Barley grains have relatively high concentrations of α-tocotrienol, although its total oil concentration is lower than that of most cereal grains.

Anitschkow (1913), a Russian scientist, fed rabbits diets high in cholesterol and induced atheromas very similar to arterial plaque found in humans. Since then numerous

cholesterol feeding studies have been done with other laboratory animals including chickens (Chandler et al., 1979), hamsters (Singhal et al. 1983), rats, mice, guinea pigs (Behr et al., 1963), dogs (Mahley et al., 1974), calves (Wigger et al., 1973), pigs (Link et al., 1972) and monkeys (Malinow et al., 1972). Hypercholesterolemia and arterial plaques were induced in all species except that rats are recalcitrant (Singhal et al., 1984). Chicks and hamsters have been popular animal models in the studies of lipid metabolism. In both species, arterial plaques can be induced and HMG-CoA reductase activity is inhibited by cholesterol feeding. The liver is the main site of cholesterol biosynthesis, and plasma cholesterol concentrations of 100 to 200 mg/dl are reached, close to that of humans. In addition, hamsters consume relatively less diet and chicks are easier for handling than other laboratory animals such as pigs and rats.

The objectives of this study were (1) to characterize the influence of cultivar, environment and processing on oil, α-tocotrienol and tocopherol concentrations in barley grain, (2) to determine fatty acid, tocopherol and tocotrienol concentrations of barley oil, (3) to examine the possible hypocholesterolemic effects of oil and soluble fiber of barley grain and their interaction, and (4) to characterize the modes of action by which barley oil and soluble fiber affect plasma cholesterol levels.

CHAPTER 2

VITAMIN E AND OIL CONCENTRATION OF SEVEN BARLEY CULTIVARS GROWN IN THREE DIFFERENT ENVIRONMENTS

Introduction

Barley oil concentration varies among barley cultivars and environments (Fedak and de la Roche, 1977) with an average of 2.1% (Newman and McGuire, 1985). Prowashonupana, a high-protein, high-lysine mutant of Compana (CI 5438) and the high-lysine mutant of Bomi, Risϕ 1508 were reported to contain 7.0% and 5.3% oil, respectively (Åman and Newman, 1986; Bhatty and Rossnagel, 1980). Both of these cultivars have shrunken endosperms and therefore low starch levels, which accounts in part for the higher oil concentration.

Vitamin E, a group of oil soluble compounds composed of tocopherols and tocotrienols, is essential for human health. Vitamin E acts as an antioxidant, preventing damage to cell membranes by free radicals. Rats fed a vitamin E deficient diet were found to have surface blebbing of the femoral artery endothelium (Hubel et al., 1989). According to Qureshi et al. (1986) α-tocotrienol inhibits the activity of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase in the liver of chicks and therefore has a hypocholesterolemic function. Qureshi et al. (1989) reported that in contrast to α-

T3, α-tocopherol stimulates HMG-CoA reductase activity.

Barley grain has a higher α-tocotrienol concentration (Barnes, 1983), but lower vitamin E and oil concentration (Newman and McGuire, 1985) than most cereal grains. The objective of this study was to examine vitamin E and oil concentration of seven barley cultivars grown in three environments.

Materials and Methods

Barley Cultivars and Growing Environments

The seven barley cultivars investigated in this study were WPB 501, Clark (CI 15857), Betzes (CI 6398), Nubet (CI 16559), Franubet, Wafranubet and Waxbar. WPB 501 is a six-rowed, hulled spring feed barley released by Western Plant Breeders (WPB) (811 Timberline Drive, Bozeman, MT 59715). Clark and Betzes are two-rowed hulled malting cultivars. Nubet is a hull-less cultivar selected from a backcross of Betzes x Sermo (Hockett, 1981). Franubet, a chemically induced mutant selected from Nubet has "fractured" starch granules (Chung 1982). Wafranubet is a waxy hull-less selection from a backcross of waxy Betzes on Franubet (R. F. Eslick, Personal Communication). Waxbar is a two-rowed, hull-less, short-awned cultivar selected from Washonupana crosses to Hector (CI 15514), released by WPB. These cultivars were planted in Scottsdale and Marana, AZ in November 1989 and harvested in May, 1990. The same set of cultivars was planted in Bozeman, MT in early June and harvested in September, 1990. Table 1 contains a description of these three environments.

Table 1. Description of the three growing environments

	Location		
Parameter	Scottsdale	Marana	Bozeman
Longitude	111°55' W	111°13' W	111°03' W
Latitude	33°30' N	32°27' N	45°40' N
Elevation (m)	368	597	1482
Planting date	11/14/89	11/14/89	6/1/90
Harvesting date	5/11/90	5/14/90	9/13/90
Length of daylight (h)[a]	12	12	15
Temperature (°C)[a]	13	13	18
Precipitation (cm)[b]	3.1	4.2	3.5
N fertilizer (kg/ha)	308	193	193
Irrigation[c]	Yes	Yes	No

[a] Average values over the time from planting to harvesting.
[b] Total precipitation over the time from planting to harvesting.
[c] Equal to 0.9-1.2 cm precipitation.

Chemical Analysis

Barley grain was first ground in a laboratory mill (Laboratory Construction Co. Kansas City) producing particle sizes smaller than 2 mm, and then reground in a cyclone sample mill (Udy Corporation, Fort Collins, Colorado) through a 0.5 mm screen. Oil content was determined by ether extraction (AOAC, 1980). Oil was extracted from ground barley with hexane, and total tocotrienols and tocopherols were extracted from the oil using a modification of the method of Piironen, et al. (1984). Barley oil (200 mg) was weighed into a brown glass Erlenmeyer flask, mixed with 50 mg pyrogallic acid, 4 ml of H_2O, 4 ml KOH (50% w/v) and 40 ml ethanol (absolute, HPLC grade) and refluxed under nitrogen for 45 min. After being cooled in water for 20 min, the mixture

was extracted with hexane and washed with distilled water four times. Water was removed by filtration of the extract through granular anhydrous sodium sulfate. Hexane was removed by vacuum rotary evaporation. Standard α-, β-, γ- and δ-tocopherol were purchased from Em Science (480 Democrat Road, Gibbstown NJ). A barley oil with known concentration of tocotrienols was obtained from General Mills (Minneapolis, MN 55427). Tocotrienols and tocopherols were quantified by HPLC with a silica column (250 mm x 4.6 mm) using a fluorescence detector at ex 290 nm and em 320 nm. Hexane with 0.5% 2-propanol was eluted through the column at 1 ml/min flow rate.

Statistical Analysis

Data were analyzed by analysis of variance and Pearson's correlation using the General Linear Models Procedure (SAS, 1985).

Results

Concentration of vitamin E including tocopherols and tocotrienols of barley grain was 63 mg/kg on the average with a range from 48 mg/kg in WBP 501 to 92 mg/kg in Wafranubet (Table 2). Variation in vitamin E concentration was significant ($P<0.05$) among cultivars but not environments (Table 3). Oil concentration of these barley cultivars averaged 22 g/kg with a range from 16 g/kg in Betzes to 31 g/kg in Wafranubet (Table 2). Cultivar variation in oil concentration was significant ($P<0.01$) but not by environment (Table 3).

Barley kernel weight was 35 mg (dry matter basis) on the average with a range from 31 mg in Franubet to 39 mg in WBP 501. Both cultivar and environment affected kernel

Table 2. Mean values of vitamin E, oil concentration and kernel weight of seven barley cultivars averaged over environments[a,b]

Cultivar	Ear type[c]	Vitamin E[d]	Oil	Kernel wt
		mg/kg	g/kg	mg[e]
WBP-501	6RH	48±5 a	19±5 a	39±5 d
Clark	2RH	50±16 ab	19±11 a	38±4 cd
Betzes	2RH	53±16 ab	16±8 a	37±2 bc
Nubet	2RHL	72±14 bc	23±3 ab	34±4 ab
Franubet	2RHL	59±23 ab	20±8 ab	31±4 a
Wafranubet	2RHL	92±19 c	31±3 c	32±3 a
Waxbar	2RHL	68±7 ab	26±3 bc	35±5 abc
Mean	--	63	22	35
Hulled vs hull-less[f]	--	265.4**	83.7**	61.3**

[a] Mean±SD, n=3.
[b] Values in each column sharing a common letter are not different at $P<0.05$.
[c] R: row; H: hulled; HL: hull-less.
[d] Including tocotrienols and tocopherols.
[e] Dry matter basis.
[f] Contrast; ** $P<0.01$.

Table 3. Mean values of vitamin E, oil concentration and kernel weight of barleys grown in three environments averaged over seven cultivars[a]

Environment	Vitamin E	Oil	Kernel Weight
	mg/kg	g/kg	mg[b]
Scottsdale	52±13	21±6	35±3 a
Marana	69±14	25±4	39±3 b
Bozeman	69±26	20±7	33±4 a

[a] Mean±SD, n=7.
[b] Values in each column sharing a common letter are not different at $P<0.05$.

weight ($P<0.05$) (Table 2).

Contrast of hulled versus hull-less cultivars was significant ($P<0.01$) in vitamin E, oil and kernel weight (Table 2), with the hull-less cultivars containing more oil and vitamin E than the hulled cultivars, but with lighter kernel weight.

α-Tocotrienol (α-T3) concentration in barley grain averaged 33 mg/kg, with a range from 23 mg/kg in Clark to 53 mg/kg in Wafranubet (Table 4). α-Tocotrienol accounted for 52% of vitamin E. γ-Tocotrienol (γ-T3) was the second largest tocotrienol fraction (7 mg/kg), slightly smaller than α-tocopherol (α-T) (11 mg/kg) and γ-tocopherol (γ-T) (11 mg/kg). Total tocotrienols accounted for 64% of total vitamin E on the average in these barley cultivars. Cultivar variation was significant ($P<0.05$) in tocotrienols and tocopherols except α-T. Contrast of hulled versus hull-less cultivars was significant ($P<0.05$) in α-T3, γ-T3 and γ-T concentration. The hull-less cultivars were higher in these compounds than the hulled types. Environmental variation was not significant in these fractions except for γ-T (Table 5). Kernel weight was negatively correlated with α-tocotrienol concentration of barley oil ($r=-0.46$, $P=0.03$) but not with α-T3, total vitamin E and oil concentration of barley grain ($P>0.05$) (Table 6). Values of the correlations between kernel weight and kernel α-T3, vitamin E and oil concentration of barley grain were affected by environments with a tendency for higher correlation in barleys grown in Bozeman and Marana, compared to those grown in Scottsdale.

Table 4. Mean values of tocotrienol (T3) and tocopherol (T) concentration of seven barley cultivars averaged over three environments[a,b]

Cultivar	α-T3	γ-T3	δ-T3	α-T	β-T	γ-T	δ-T
				mg/kg			
WBP 501	25±4 a	4±1 a	0.3±0.1 a	12±1	0.3±0.1 a	8±1 a	0.3±0.1 a
Clark	23±8 a	5±2 ab	0.8±0.4 cd	10±3	0.6±0.2 abc	10±1 ab	0.3±0.1 a
Betzes	25±8 a	6±2 abc	0.7±0.3 abc	10±2	0.5±0.1 ab	11±4 abc	0.2±0.1 a
Nubet	34±6 ab	8±2 bcd	1.1±0.2 d	12±2	0.6±0.1 abc	16±4 c	0.4±0.3 ab
Franubet	29±10 ab	8±4 cd	0.7±0.3 bcd	8±4	0.4±0.1 a	12±5 abc	0.3±0.0 a
Wafranubet	53±10 c	11±3 d	0.7±0.2 bcd	12±3	0.7±0.1 bc	13±4 bc	0.6±0.3 b
Waxbar	39±3 b	6±1 abc	0.4±0.1 ab	12±3	0.8±0.1 c	9±3 ab	0.2±0.1 a
Mean	33	7	1	11	0.6	11	0.3
Mean % of Vitamin E[c]	52	11	1	17	0.9	18	0.5
Hulled vs Hull-less	175.7**	42.6**	1.6	---	1.6	2.4*	0.6

[a] Average values, n=3.
[b] Values in each column sharing a common letter are not different at $p<0.05$.
[c] Vitamin E including tocotrienols and tocopherols.
* $P<0.05$ and ** $P<0.01$.

Table 5. Mean values of tocotrienol (T3) and tocopherol (T) concentration of barleys grown in three environments averaged over barley cultivars[a]

Environment	α-T3	γ-T3	δ-T3	α-T	β-T	γ-T[b]	δ-T
				mg/kg			
Scottsdale	28±9	5±2	0.5±0.3	10±3	0.5±0.2	8±3 b	0.2±0.1
Marana	37±11	8±3	0.8±0.3	11±2	0.6±0.1	12±2 b	0.3±0.1
Bozeman	34±15	8±4	0.7±0.3	12±3	0.6±0.2	13±5 a	0.5±0.3

[a] Mean±SD, n=7.
[b] Values in each column sharing a common letter are not different at $P<0.05$.