Detection of Bread Wheat Farina Adulterant in Durum Wheat Semolina and Pasta Dried at Low, High, and Ultra-High Temperatures[1]

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ABSTRACT

Methods were developed to detect adulteration in durum wheat pasta by bread wheat farina. Adulteration can be detected when sterol palmitates (campesterol and β-sitosterol) are found in farina but not in semolina, as has been reported for wheats grown in the United States. A new method using reverse-phase high-performance liquid chromatography (RP-HPLC) was developed to measure sterol palmitates. The sterol palmitates in farina adulterant become chemically bound during pasta making. They are found in low amounts in petroleum ether extracts previously used to determine adulteration of semolina. Three polar solvents (chloroform-methanol, chloroform-methanol-water [C-M-W], and water-saturated *n*-butanol) extracted the bound sterol palmitates from pasta. Only the extracts with C-M-W proved to be satisfactory for measuring sterol palmitates by gas chromatography (GC) or RP-HPLC. Adulterated pasta samples (0–100% farina), dried at low, high, and ultra-high temperatures, were prepared for testing the adulteration methods. Sterol palmitate content in the pasta samples was determined in C-M-W extracts using the new RP-HPLC method and the GC method of Hsieh et al (1981). Adulteration levels down to about 3% were detected in our pasta test samples made with U.S. wheat varieties. In farina from 97 bread wheat varieties, six soft cake wheat varieties currently grown in the U.S. and Canada, and two soft wheat varieties from Italy, sterol palmitate content varied from 17 to 44 mg/100-g sample; that of farina from the Canadian variety Glenlea was 8.9 mg/100-g sample. In semolina from 40 durum wheat varieties (29 from the U.S. and Canada, five from Mexico, and six from Italy), sterol palmitate content varied from 0 to 0.7 mg/100-g sample. Thus, our results confirm the observations of earlier workers that analysis for sterol palmitate content can be used to detect adulteration in pasta made from U.S. wheats.

Adulteration of bread wheat farina in pasta is illegal in some countries; in others it is illegal if the package is labeled as 100% durum product. The presence of campesterol and β-sitosterol palmitates in farina, and their absence in semolina, has been used to detect farina in semolina. Matveef (1952) and Montefredine and La Porta (1953) used a sterol ester precipitation method to detect adulteration. The intensity of the white precipitation and the color from a sterol reagent indicated the presence of soft wheat in durum wheat macaroni. Later, Gilles and Youngs (1964) used thin-layer chromatography on silica-gel G-coated plates, with carbon tetrachloride (100%) as a developing solvent, to determine β-sitosterol palmitate in petroleum ether extracts of farina-semolina mixtures. In semolina-farina mixtures, the spot densities of the sterol palmitates increased proportionately with the increase of farina. Hsieh et al (1981) used a gas chromatography (GC) method to determine sterol palmitates in petroleum ether extracts of bread wheat flour-semolina mixtures. Campesterol and β-sitosterol palmitates separated into two peaks. Hsieh et al (1980) observed that both of these sterol esters had R_f values the same as those found by Gilles and Youngs (1964).

Gilles and coworkers (Gilles and Youngs 1969, Berry et al 1968) surveyed the flour or semolina of bread and durum wheat varieties from the United States and Italy for sterol ester content. Six U.S. durum wheat varieties and one from Italy were free of β-sitosterol palmitates, whereas 33 bread wheat varieties grown in the United States contained 34–72 mg/100-g sample of β-sitosterol palmitate. However, seven varieties of Italian soft wheat contained only 5–8 mg/100-g sample of sterol ester; three others contained 53–58 mg/100-g sample. Hsieh et al (1981) also analyzed semolina or flour of 11 varieties of durum and 25 varieties of hard red spring (HRS) wheat grown in the United States. All the durum wheats were devoid of sterol esters except Produra variety with 2.4 mg/100-g sample of sterol ester. HRS wheat flours contained 24–37 mg/100-g sample of sterol ester. These results indicate that detecting adulteration by measuring sterol palmitates is an effective method in the United States but probably not in Europe because some bread wheat varieties are low in this sterol ester.

Adulteration has been detected, not only by measuring sterol palmitate content (Gilles and Youngs 1964, Hsieh et al 1981), but also by specific protein fractions (Pence et al 1954, Garcia-Faure et al 1969, Burgoon et al 1985, McCarthy et al 1990) and polyphenol oxidase activity (Feillet and Kobrehel 1975) on gel electrophoresis bands.

For a number of years, in our laboratory, we have detected adulteration using methods for measuring sterol palmitates (Gilles and Youngs 1964, Hsieh et al 1981). To date, these detection methods were used only on farina-semolina mixtures, not pasta. In the present study, methods for measuring sterol palmitates in pasta dried at low, high, and ultra-high temperatures were developed.

MATERIAL AND METHODS

Standards

Solid standards purchased for the study included: cholesteryl pentadecanoate (98% purity) and cholesterol (99% purity) from NU Check Products Inc. (Elysian, MN); β-sitosterol (98% purity) and campesterol (68% purity) from Sigma Chemical Co. (St. Louis, Mo); β-sitosterol containing 50% campesterol from Aldrich Chemical Co. (Milwaukee, WI).

A purified β-sitosterol and campesterol palmitate mixture was synthesized from the sterol mixture (Youngs 1965). The palmitate mixture had a melting point of 95°C after recrystallizing from 95% ethanol, acetone, ethyl ether/acetone (1:3, v/v), and again from ethanol. Hsieh et al (1980) reported a melting point of 94°C. The preparation gave only two peaks for the sterol palmitates using GC and RP-HPLC. From relative peak areas, the calculated mean ratios (w/w) of campesterol to β-sitosterol were 0.418 ± 0.022 to 0.582 ± 0.022 ($n = 4$) for GC and 0.425 ± 0.015 to 0.575 ± 0.015 ($n = 12$) for RP-HPLC.

Wheat Samples

In developing an extraction method, farina from the HRS wheat variety Len and semolina from the durum wheat variety Ward (11.25 and 13.2% protein, respectively) were used for preparing 50% adulterated samples of pasta. Farina from 26 varieties of HRS and semolina from 11 varieties of durum wheat (Sarwar 1992) were used for preparing semolina pasta samples, farina pasta samples, and 1–50% adulterated pasta samples for testing. The protein contents of adulterated farina-semolina mixtures used for making pasta ranged from 12.1 to 17.7% (14%, mb) randomly over the entire range of adulteration. Sterol ester content was surveyed in farina and semolina from 99 varieties of bread wheat, six varieties of soft cake wheat, and 40 varieties of durum wheat

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currently grown in United States, Canada, Italy, and Mexico (Sarwar 1992).

Milling Farina and Semolina

HRS and durum wheats used for preparing adulterated pasta were cleaned using a Carter Dockage tester (Carter Day Care Co., Minneapolis, MN). Durum wheat samples (4,000 g) were tempered to 17.5% moisture levels in three steps: 72 hr before milling to 12.5%, 24 hr before milling to 14.5%, and 45 min before milling to 17.5%. HRS wheat samples (4,000 g) were conditioned at 15.5% moisture for 24 hr before milling. Wheat samples were milled on a modified Buhler automatic laboratory mill (Buhler-Miag, Inc.) with all-corrugated rolls and two laboratory-size Miag purifiers. The laboratory mill is used for semolina production in the Durum Laboratory of the Cereal Science and Food Technology Department, North Dakota State University, Fargo.

Small wheat samples (125 g) for the survey of sterol ester in semolina and farina were tempered for 24 hr to 17.5% and 15.5% moisture (durum wheat and bread wheat, respectively) (Mousa et al 1983). Then they were milled on a Quadrumat Junior mill (C. W. Brabender Inc., South Hackensack, NJ); the no. 2 roll with 26 corrugations per inch was replaced with the no. 1 roll with 13 corrugations per inch, and the no. 3 and 4 rolls were replaced with no. 2 rolls as described by Mousa et al (1983). Bran particles were removed by bolting using a no. 30 sieve.

The ash content of all the farina and semolina mill products was near, or under, the recommended 0.80% (dmb) level for semolina (Winfield 1989).

Analytical Methods

The moisture, protein, and ash content in grain, semolina, farina, semolina-farina blends, and pasta were determined by AACC approved methods 44-15A, 46-11, and 08-01 (AACC 1983). A titanium dioxide catalyst (Williams 1973) was used instead of the mercury catalyst in method 46-11.

Pasta Processing

Farina-semolina mixtures of 1–50% farina were prepared with random protein levels of 12–17%, as described by Sarwar (1992). Farina from the 26 varieties of HRS wheat and semolina from the 11 varieties of durum wheat was used for these mixtures. They were well blended in a Cross Flow blender (Patterson-Kelley Co., East Stroudberg, PA) for 20 min. The semolina-farina mixtures and samples of semolina and farina were used to prepare spaghetti under vacuum with a Demaco semicommercial laboratory extruder. After case hardening for 15 min in air at room temperature, half of the spaghetti was dried at low temperature (conventional method), and the other half at high temperature in a high-temperature dryer (model 300, Standard Industries Inc., Fargo, ND). Low-temperature drying was done at 40°C by reducing the relative humidity from 95 to 65% over 18 hr. High-temperature drying was done at 56°C and 80% rh for 3 hr, followed by drying at 72°C and 76% rh for 6 hr. Temperature and relative humidity were then reduced to 28°C and 50% over 1.75 hr. Additional batches of spaghetti with adulteration levels of 0, 3, 6, 10, 20, 30, 50, and 100% were also prepared and dried at ultra-high temperature. The ultra-high drying cycle was 56°C and 85% rh for 0.5 hr, 76°C and 85% rh for 0.5 hr, 90°C and 85–80% rh for 2 hr, and reduced to 28°C and 50% rh over 3 hr.

The dried pasta was broken into small pieces in a mortar, preground in a Wiley mill (Arthur H. Thomas Co. Scientific Apparatus, Philadelphia, PA), to pass through a no. 10 mesh screen, and then ground in a Udy mill (Udy Corp., Boulder, CO) to pass through a 0.5-mm screen at a uniform feed rate of 2 g/sec using a Syntron Vibra feeder (Syntron, Homer City, PA).

Sterol Ester Extraction

Four solvent systems were used to develop the extraction method.

Petroleum ether extraction. For GC analysis, 5 g of finely ground sample, or 10 g of sample for RP-HPLC analysis, was shaken with 50 ml of petroleum ether in a 150-ml glass centrifuge tube for 15 min on a Burrel wrist-action shaker (model DD, Burrel Corporation, Pittsburgh, PA) at a speed setting of 7. After centrifugation of the mixture at 10,000 × g for 10 min at 0°C, the supernatant was filtered through Whatman No. 43 filter paper, and the filter paper was washed with a small amount of petroleum ether. The extract was evaporated to dryness under a stream of nitrogen at room temperature. After dissolving, the dried lipid was made up to 2 ml volume with petroleum ether in a calibrated 1–5-ml Reacti Vial (Pierce, Rockford, IL) for GC analysis and up to 1 ml volume in chloroform for RP-HPLC analysis.

Chloroform-methanol (C-M) extraction. Ten grams of finely ground sample in 100 ml of CM (2:1, v/v) was blended 3 min in a 200-ml beaker with a Virtis blender (model 6-10, AF Virtis Research Equipment, Gardiner, NY) set at medium speed. The blended sample was transferred to a 150-ml centrifuge glass tube and centrifuged at 10,000 × *g* for 10 min at 0°C. The supernatant was filtered through Whatman No. 43 filter paper and the filter paper was washed with a small amount of chloroform. The extract was evaporated to dryness at room temperature under a nitrogen stream. Dried lipid was dissolved in petroleum ether and made up to 2 ml for GC analysis or dissolved in chloroform and made up to 1 ml for RP-HPLC analysis.

Reversed phase C_8, C_{18}, and silica (normal phase) sample cleanup cartridges (Analtech, Inc., Newark, DE; Waters Assoc., Milford, MA; and Analtech, respectively) were used on the CM extracts (2:1, v/v) to remove nonlipid contaminants. The reversed phase C_8 or C_{18} cartridges were conditioned with 3 ml of methanol followed by 3 ml of water before the extracted sample was added. The polar lipids were eluted with 3 ml of methanol followed by 3 ml of chloroform. The nonpolar sterol palmitate was eluted with 3 ml of petroleum ether. When silica cartridges were used, the cartridge was washed with petroleum ether, and the sample was added in petroleum ether. The nonpolar lipids were eluted with 5 ml of chloroform.

Water-saturated n-*butanol extraction.* Ten grams of ground sample and 100 ml of freshly prepared water-saturated *n*-butanol were blended in a 200-ml beaker for 3 min at medium speed using the Virtis blender. After centrifugation at 10,000 × *g* for 10 min at 0°C, the supernatant was filtered through Whatman No. 43 filter paper. The extract was evaporated to dryness under vacuum (26 inches Hg) at <40°C using a Rotovapor model R (Brinkman Instruments, Westbury, NY). The lipid was dissolved in petroleum ether and made up to 2 ml for GC analysis or dissolved in chloroform and made up to 1 ml for RP-HPLC analysis.

C-M-W extraction. A ternary solvent method (Tsen et al 1962) was used for this extraction. Ten grams of ground sample, 25 ml of methanol, 12.5 ml of chloroform, and 10 ml of water (2:1:0.8, v/v) were blended in a 200-ml beaker with the Virtis blender at medium speed for 3 min. Chloroform (12.5 ml) was added, and the mixture was blended for another 30 sec. Water (12.5 ml) was added, and the mixture was blended 30 sec. The blend was transferred to a glass centrifuge tube and centrifuged at 10,000 × *g* for 10 min at 0°C. The top methanol-water layer was removed by vacuum and discarded. The bottom chloroform layer, containing the sterol palmitates, was removed with a Pasteur pipette and filtered through Whatman No. 43 filter paper. The paper was washed with a small amount of chloroform, and the extract was evaporated to dryness under a stream of nitrogen at <40°C. The lipid extract was dissolved in petroleum ether and made up to 2 ml for GC analysis and dissolved in chloroform and made up to 1 ml for RP-HPLC analysis.

Thin-Layer Chromatography

Silica-gel G-coated plates (Analtech) were conditioned by heating at 110°C for 30 min. A mixture of chloroform-carbon tetrachloride (5:95, v/v) was used for developing the plates twice (Hsieh et al 1980). The plates were dried under a stream of air at room temperature and sprayed with Rhodamine 6G (Supelco, Inc., Bellefonte, PA). The spot densities were observed under UV light at 3,000 Å. Cholesteryl pentadecanoate with the same

R_f as β-sitosterol palmitate (Hsieh et al 1980) was used to locate the sterol palmitate band.

Gas Chromatography

Sterol palmitates were determined on a Hewlett-Packard (HP) gas chromatograph instrument using a HP5840A GC integrator for measuring peak areas (Hsieh et al 1981). The column was a 48-cm × 2-mm i.d. glass column packed with 3% OV-17 on 80/100 Gas Chrom Q (Alltech Assoc., Deerfield, IL). A 1-μl aliquot of extract in petroleum ether, with cholesteryl pentadecanoate (3 μg) as an internal standard, was injected for the analysis. Detector response was linear with the internal standard of cholesteryl pentadecanoate and the external standard of the synthesized campesterol and β-sitosterol palmitate mixture as reported by Hsieh et al (1981). The weight and peak area ratios of external standard to internal standard by GC analysis were also linear (Sarwar 1992). Results (as mg per 100-g sample of sterol palmitate) were calculated by the internal standard method (Barbato et al 1966).

RP-HPLC

For RP-HPLC analysis, an HP1090L liquid chromatograph was used. This instrument had an autoinjector, a diode array detector interfaced with an HP jet printer for spectra scanning, and an HP3396A integrator for measuring peak areas. The peak absorption wavelength was 210 nm with a bandwidth of 4 nm. The reference wavelength was 550 nm with a bandwidth of 100 nm. The parameters for the RP-HPLC method developed for measuring sterol palmitates were as follows.

C_{18} Spherisorb 5 ODS-2, 4.6-mm × 25-cm (Phenominex, Torrance, CA), with a 15- × 3.2-mm C_{18} guard column (Chrom Tech Inc, Apple Valley, MN), and 7-μm C_{18} packing. Column temperature was 45°C. Mobile phase used acetonitrile-isopropanol (60:40, v/v) for isocratic elution. Flow rate was 1.2 ml/min of mobile phase. Sample injection was 5-μl in chloroform with cholesteryl pentadecanoate (10 μg) as an internal standard.

The solvents were filtered through a 0.45 filter (Schleicher and Schuell Inc., Keene, NH) and degassed by vacuum and sonication. Results (as mg per 100-g sample of sterol palmitate) were calculated by the internal standard method (Barbato et al 1966).

Statistical Analysis

Statistical analysis for the effect of methods of analysis and adulteration levels and the interactions on the samples of farina-semolina blends and low- and high-temperature-dried pasta were done using the general linear models procedure of SAS (release 6.06, 1991, SAS Institute, Cary, NC) run on the North Dakota State University mainframe computer. For extraction methods comparison, sterol ester contents were treated as a dependent variable; the effect of extracting solvent, measuring instrument, product being analyzed, and the interactions were also measured. Standard deviation of repeatability was calculated by the method of Youden (1978) on the means of duplicate GC or HPLC values measured on each of two replicate extractions of eight adulterated pasta samples. The method described by Bauer (1971) was used to calculate 95% confidence limits for the means of ratios of campesterol to β-sitosterol in the synthesized standard of the palmitate mixture.

RESULTS AND DISCUSSION

Development of RP-HPLC Method

RP-HPLC had not been not used to measure β-sitosterol and campesterol palmitates in detecting adulteration of farina in semolina and pasta. The RP-HPLC methods for measuring cholesterol fatty acid esters in blood plasma and other biological materials was modified for extracts of adulterated pasta. Cholesterol has a structure very similar to campesterol and β-sitosterol. Mixtures of standards (campesterol, β-sitosterol, campesterol-β-sitosterol palmitate mixture, cholesterol, and cholesteryl pentadecanoate internal standard) were tested with the isocratic method of Vercaemst et al (1989) and the method of Chu (1990), which used a mobile phase gradient. The former was selected because of its higher reproducibility and short turnaround time. This RP-HPLC method uses an isocratic mobile solvent of isopropanol-acetonitrile (50:50, v/v) at a flow rate of 1.2 ml/min and a column temperature of 45°C. Because eluted peaks of β-sitosterol and

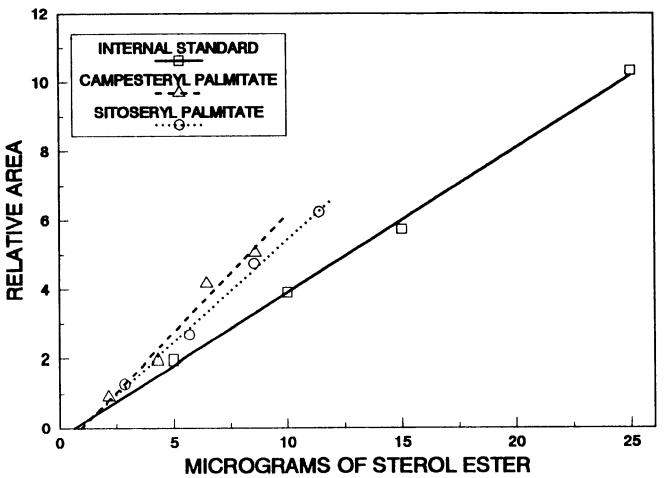

Fig. 2. Reverse-phase high-performance liquid chromatographic detector response for standards.

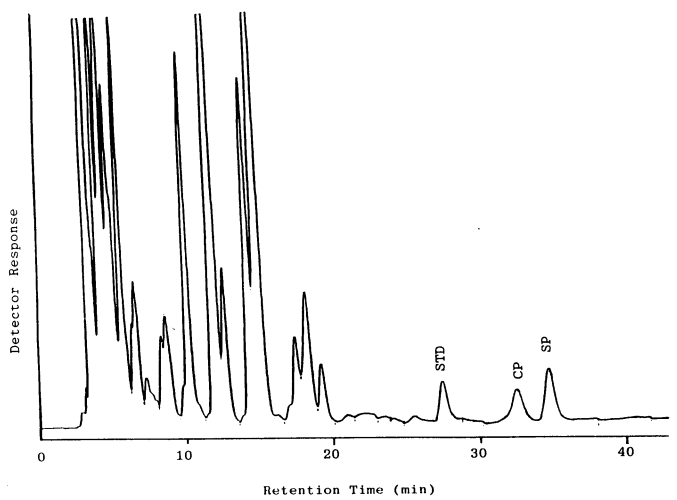

Fig. 1. Reverse-phase high-performance liquid chromatographic separation of campesterol palmitate (CP) and β-sitosterol palmitate (SP). STD = internal standard of cholesteryl pentadecanoate in farina extract.

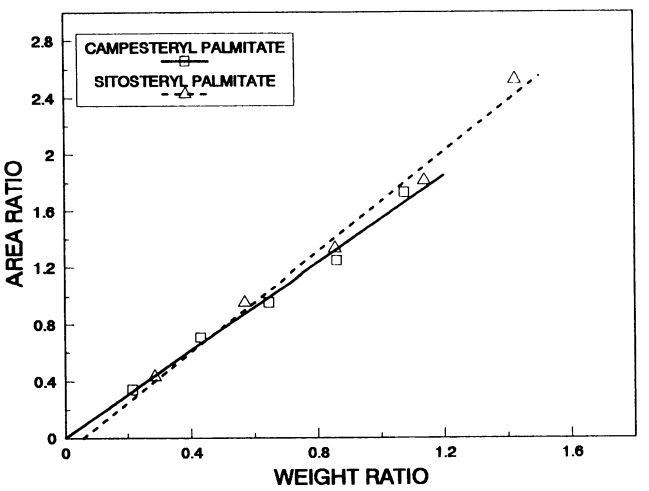

Fig. 3. Plot of weight ratio of campesterol palmitate and β-sitosterol palmitate to the cholesteryl pentadecanoate internal standard against the resulting area ratios for the respective standards.

campesterol palmitates were not completely separated, the concentration of acetonitrile in the mobile solvent was tested at 45, 50, 55, 60, and 65%. The 60% acetonitrile gave complete separation of the peaks of these esters at 31 and 34 min of elution (Fig. 1).

With the new HPLC method, detector response was linear with 5.0–25.0 μg of cholesteryl pentadecanoate internal standard and 5.0–20 μg of standard campesterol and β-sitosterol palmitate mixture (Fig. 2) added to a semolina extract with no sterol palmitates. The weight and peak area ratios of external standards (campesterol-β-sitosterol mixture) to internal standard were also linear (Fig. 3). The internal standard was eluted before the campesterol palmitate (Fig. 1).

Sterol Palmitate Extraction from Pasta

For the extraction of sterol esters from pasta, three polar solvent systems were tested on pasta made from a blend of farina-semolina (50:50). First, thin-layer chromatography was used in a preliminary study. The relative amount of sterol palmitates extracted from pasta by the nonpolar solvent petroleum ether was less than extracted by the three other polar solvent systems (Table I). The extracts with C-M and water-saturated *n*-butanol, even after drying and redissolving in chloroform, gave streaks all the way up to the palmitate ester spots. This streaking might have been caused by nonlipid contaminants that apparently cause problems on separation and quantification of sterol esters by GC and RP-HPLC. There was no streaking with C-M-W and petroleum ether extracts. In the C-M-W system, these nonlipid contaminants were probably removed in the discarded top M-W phase.

TABLE I
Thin-Layer Chromatographic Analysis for Campesterol and β-Sitosterol Palmitate Mixture[a]

	Spot Relative Density[b]		
		Pasta-Drying Temperature	
Extraction Solvents	Blend	Low	High
Petroleum ether	+++	++	+
Chloroform-methanol	++++	+++	+++
Chloroform-methanol-water	+++	++++	++++
Water-saturated *n*-butanol	++++	++++	++++

[a] Palmitates do not separate, they occur as one band.
[b] ++ = low density; +++ = medium density; ++++ = high density.

TABLE II
β-Sitosterol-Campesterol Palmitate Values[a] by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) and Gas Chromatography (GC) Analysis of Polar Solvent Extracts

	Sitosterol-Campesterol Palmitates (mg %)			
	RP-HPLC Extract		GC Extract	
Solvent	1	2	1	2
Semolina-farina blends				
Petroleum ether	22.12	22.62	18.96	18.42
Chloroform-methanol	24.10	23.71	22.13	21.68
Cleanup by C_8 cartridge	14.23	15.58	14.36	14.49
Cleanup by C_{18} cartridge	10.59	11.12	11.31	11.63
Cleanup by silica cartridge	15.83	13.97	14.11	14.72
Chloroform-methanol-water	20.78	19.73	21.73	20.98
Water-saturated *n*-butanol	24.63	24.72	23.42	23.86
Low-temperature dried pasta				
Petroleum ether	12.75	12.23	11.89	11.73
Chloroform-methanol	21.04	20.67	20.33	19.65
Cleanup by C_8 cartridge	15.93	16.15	14.22	13.49
Cleanup by C_{18} cartridge	10.70	11.35	11.09	11.27
Cleanup by silica cartridge	12.22	12.29	12.11	12.03
Chloroform-methanol-water	18.14	18.52	19.19	18.67
Water-saturated *n*-butanol	19.64	19.83	20.11	20.43
High-temperature dried pasta				
Petroleum ether	11.83	11.81	11.03	11.11
Chloroform-methanol	19.01	20.36	19.76	19.39
Cleanup by C_8 cartridge	10.88	11.39	10.20	10.79
Cleanup by C_{18} cartridge	10.98	10.18	11.20	10.88
Cleanup by silica cartridge	10.84	11.15	10.72	10.48
Chloroform-methanol-water	17.04	16.79	17.30	17.21
Water-saturated *n*-butanol	19.37	18.33	19.21	19.14

[a] 50:50 farina-semolina blend. Mean of duplicate determinations on each extract.

The sterol palmitates were determined quantitatively by HPLC and GC analysis from extracts of the 50% adulterated semolina and pasta (Table II). Petroleum ether extracted about the same amount of sterol palmitates from adulterated semolina as did the three polar solvents, but this solvent extracted only about 60–65% of the sterol palmitates from pasta compared to the the polar solvents. The amount of ester extracted from pasta by C-M and water-saturated *n*-butanol was about 2–3 mg/100-g sample higher than that extracted by C-M-W (Table III). These two solvents caused high-pressure problems in the HPLC analysis and ruined the peak resolution of the GC column, apparently because extracted nonlipid material that causes streaks in TLC analysis. Removal of nonlipid material from the C-M extract with C_8, C_{18}, and silica sample cleanup cartridges was not successful because of a large loss of sterol palmitates (Tables II and III). No attempt was made to clean up the water-saturated *n*-butanol because of its undesirably high boiling point and toxicity.

C-M-W extracted about as much sterol palmitate from pasta as did the other two solvents (Tables II and III) but did not ruin the HPLC and GC columns. Therefore, this solvent was selected for use in detecting pasta adulteration. The mean amount of sterol palmitates extracted from pasta by this solvent was 1–4 mg/100-g sample less than that from adulterated semolina (Table IV). This may be caused by some oxidation of sterols (Nourooz-Zadeh and Appelqvist 1992) during pasta drying or it may be due to the sterol palmitates being too tightly bound for extraction.

Analysis of Adulterated Pasta for Campesterol and β-Sitosterol Palmitates

The RP-HPLC and GC methods were tested on pasta adulterated with different levels of farina and dried at low, high, and

TABLE III
Mean Values[a,b] for Sterol Esters in Polar Solvent Extracts Analyzed by Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) and Gas Chromatography (GC) Methods

	RP-HPLC	GC
Extraction Solvent	Mean (mg %)	Mean (mg %)
Chloroform-methanol (C-M)	22.282 A	20.270 A
Cleaned by C_8 cartridge	12.175 C	13.587 C
Cleaned by C_{18} cartridge	11.300 D	10.803 C
Cleaned by silica cartridge	11.335 D	11.625 C
Water-saturated *n*-butanol	19.722 A	19.292 A
Chloroform-methanol-water	18.092 B	17.622 B
Petroleum ether	11.440 D	12.155 C

[a] 50% adulterated high- and low-temperature dried pasta.
[b] Means followed by a common letter are not significantly different (Duncan's new multiple range test, $P = 0.05$). Mean square error = 3.242 and 3.502 for RP-HPLC and GC, respectively.

TABLE IV
Mean (mg %)[a] Sterol Ester Values by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) and Gas Chromatography (GC) Analysis of Chloroform-Methanol-Water Extracts

Products	RP-HPLC	GC
Farina-semolina blends	23.905 A	21.905 A
Pasta, low-temperature dried	20.855 B	19.990 B
Pasta, high-temperature dried	19.685 B	19.575 B

[a] Means followed by a common letter are not significantly different. Duncan's new multiple range test, $P = 0.05$. Mean square error = 0.352 and 0.134 for RP-HPLC and GC, respectively.

ultra-high temperatures. The sterol ester (campesterol plus the β-sitosterol palmitate) values observed using GC and RP-HPLC for the C-M-W extracts from semolina-farina blends were higher than those for the petroleum ether extracts (Tables V, VI, and VII). The decrease in sterol ester content for the C-M-W extracts after processing pasta at low, high, and ultra-high drying temperatures was small; petroleum ether extracts gave markedly lower values for sterol ester content in pasta.

The mean values observed for C-M-W extracts by HPLC are a little higher than those for GC (Tables V, VI, and VII). This might be due to a small amount of contaminating components in the sterol ester peaks that could be absorbing at the same wavelength used for detection and are detected as sterol esters. Some sterol esters might also be destroyed at the high temperature in the GC column. Oxidation of some of the sterol ester may cause somewhat lower sterol ester contents in the low- and high-

TABLE V
Total Sterol Ester Values[a] (mg %) for Semolina-Farina Blends and Pasta Analyzed by Gas Chromatography

Adulteration (% Farina)	Pasta Drying Temp (mg %)					
	Petroleum Ether Extract			Chloroform-Methanol-Water Extract		
	Blend	Low	High	Blend	Low	High
0	0.42	0.00	0.00	0.52	0.46	0.35
1	0.00	0.00	0.00	0.48	0.37	0.39
2	1.28	0.88	0.56	0.82	0.75	0.67
3	0.81	0.45	0.43	2.01	1.71	1.55
4	1.31	0.67	0.68	3.41	3.18	3.16
5	0.35	0.26	0.33	2.97	2.78	3.22
6	1.78	0.61	0.45	3.86	3.52	3.35
7	1.56	0.84	0.81	4.55	4.41	4.18
8	1.63	0.81	0.89	3.89	3.66	3.84
9	2.75	1.62	1.50	5.03	4.80	4.67
10	2.19	1.35	1.39	4.68	4.93	4.79
12.5	3.05	1.87	1.92	8.03	7.66	7.57
15	3.54	1.80	1.78	7.95	7.74	7.47
17.5	4.98	2.67	2.90	7.28	6.39	6.48
20	5.29	2.54	2.66	8.47	8.06	7.92
22.5	6.12	3.40	2.81	10.24	9.79	9.40
25	6.51	3.52	3.42	11.62	11.21	11.87
30	7.66	4.51	4.40	14.17	13.71	13.95
35	9.00	5.61	5.77	15.48	13.96	13.81
40	9.75	4.94	6.29	13.64	13.84	14.19
45	13.10	7.30	8.12	19.25	19.11	19.29
50	15.52	11.94	12.21	22.11	21.38	21.05
100	22.41	13.23	13.73	32.38	30.86	30.20
Mean	5.26	2.83	3.18	8.82	7.85	8.40

[a] Mean of duplicate analysis on a single extract.

TABLE VI
Total Sterol Ester Values[a] (mg %) for Semolina-Farina Blends and Pasta Analyzed by Reverse-Phase High-Performance Liquid Chromatography

Adulteration (% Farina)	Pasta Drying Temp (mg %)					
	Petroleum Ether Extract			Chloroform-Methanol-Water Extract		
	Blend	Low	High	Blend	Low	High
0	0.00	0.00	0.00	0.43	0.58	0.41
1	0.00	0.00	0.00	1.05	1.53	0.39
2	0.82	0.56	0.23	0.72	0.17	0.48
3	1.91	0.73	0.47	3.06	4.61	3.22
4	3.41	1.52	1.29	3.43	3.87	2.97
5	2.96	2.25	1.50	3.51	2.44	2.62
6	3.81	2.18	2.06	3.79	3.64	3.68
7	4.19	2.44	1.76	4.09	3.05	4.06
8	3.74	1.98	1.88	2.90	3.33	3.63
9	4.90	3.95	2.48	5.79	5.33	5.84
10	4.97	3.80	3.59	5.03	3.71	4.11
12.5	7.72	6.26	5.76	7.86	5.51	5.09
15	9.29	5.93	5.58	8.50	5.78	8.79
17.5	7.45	5.66	5.64	10.62	7.78	7.51
20	8.69	7.56	6.85	10.24	7.23	10.28
22.5	10.61	7.75	7.14	11.28	10.67	10.02
25	11.79	9.15	8.55	12.84	10.34	10.18
30	13.64	9.80	9.60	16.40	13.38	17.02
35	14.38	10.10	10.19	15.90	17.44	16.78
40	13.95	11.10	11.25	16.80	14.65	17.72
45	18.04	14.75	14.71	23.94	21.56	18.93
50	19.50	16.70	16.43	26.91	25.09	29.27
100	27.38	23.27	21.55	40.96	36.32	34.35
Mean	8.40	6.41	6.02	10.26	9.04	9.45

[a] Mean of duplicate analysis on a single extract.

temperature-dried pasta than in the blends. The oxidation of β-sitosterol has been reported in wheat flour (Nourooz-Zadeh and Appelqvist 1992).

Analysis of covariance was used on data of Tables V and VI to evaluate the effect of extracting solvent, measuring instrument, and set of product being analyzed on the amount of sterol ester observed. With level of adulteration as a covariate, significant interaction between each of the factors and the adulteration level (Table VIII) indicates that the amount of sterol ester observed over the different levels of adulteration does not have the same pattern (slope) for the different levels of the main-effect factors (extracting solvent, measuring instrument, and set of product being analyzed). Factors tested individually indicated differences at the 5%, or lower, level of significance, except for the set of product (blends and pasta dried at low and high temperature). Adulteration interacts significantly with method of sterol ester analysis, set of product, and extraction solvent. Extraction solvent also interacted significantly with method of sterol ester analysis and set of product.

Percent of adulteration can be calculated from values for sterol palmitate using regression equations (Table IX). Much of the standard deviation in the equations is due to a variation in content of sterol palmitate in farina samples used for making the adulterated pasta. These farina samples were milled from 26 different cultivars of HRS wheat. This variation in sterol palmitate from different wheat cultivars was surveyed. However, varieties of wheat are mixed in the U.S. marketing system, so this variation caused by cultivar could be less than that observed here.

Standard deviation of repeatability for sterol palmitate analysis on petroleum ether extracts using GC and RP-HPLC is relatively low, but it is somewhat high for the C-M-W extracts analyzed by RP-HPLC (Table X). The highest value (0.53) for RP-HPLC analysis of C-M-W extracts has a coefficient of variation of 7.25, which is reasonably low for an analytical method on biological materials.

TABLE VII
Total Sterol Ester Values[a] for Pasta Dried at Ultra-High Temperature by Gas (GC) and Reverse-Phase High-Performance Liquid Chromatography (RRHPLC)

Adulteration (% Farina)	GC Extract, mg %		RP-HPLC Extract, mg %	
	PE[b]	CMW	PE	CMW
0	0.00	0.00	0.00	0.00
3	0.36	1.21	0.00	0.79
6	0.00	2.08	0.81	3.58
10	0.34	4.23	3.67	4.29
20	1.89	7.30	5.44	10.15
30	2.32	10.70	8.13	15.72
50	4.28	17.76	13.99	25.23
100	9.97	26.09	19.04	31.19
Mean	2.39	8.67	6.38	11.37

[a] Mean of duplicate analysis on a single extract.
[b] PE = Petoleum ether, CMW = Chloroform-methanol-water.

TABLE VIII
Analysis of Variance on Sterol Ester Values

Source[a]	*DF*	Mean Square	*F* Value[b]	Pr > *F*
Adulteration	1	23459.25	8331.15	0.0001
Solvent	1	58.36	20.72	0.0001
Method	1	22.32	7.93	0.0050
Product	2	7.22	2.57	0.0778
ADULT*METH	1	372.59	132.32	0.0001
ADULT*SET	2	70.93	25.19	0.0001
EXT*METH	1	144.27	51.23	0.0001
EXT*SET	2	30.98	11.00	0.0001
ADULT*EXT	1	1288.18	457.48	0.0001
ERROR	539	2.81		

[a] ADULT = adulteration level; EXT = extraction solvent; METH = method of analysis; SET = products of farina-semolina blend and low- and high-temperature-dried pasta.
[b] To test overall model, *F* is calculated at 833.64 with *P* value of less than 0.0001.

TABLE IX
Equations for Calculating Percent Adulteration

Analyses and Product[a]	Regression Equations[b]	*N*	SD	*R*
Gas Chromatography				
LT	% A = 2.48 SE − 1.75	22	2.56	0.986
HT	% A = 2.47 SE − 1.64	22	2.49	0.987
BLEND	% A = 2.42 SE − 2.03	22	2.70	0.984
UHT	% A = 2.83 SE − 0.51	7	0.76	0.999
Reverse-Phase High-Performance Liquid Chromatography				
LT	% A = 2.15 SE − 0.09	22	3.34	0.976
HT	% A = 1.96 SE − 0.42	22	3.45	0.974
BLEND	% A = 2.00 SE − 1.02	22	2.52	0.986
UHT	% A = 1.94 SE − 0.42	7	1.09	0.998

[a] LT, HT, and UHT = low, high, and ultra-high temperature dried pasta. Blend = farina adulterated semolina.
[b] A = adulteration, SE = total steryl palmitate. *N* = number of samples. SD and *R* = standard deviation and correlation coefficient for the equation.

Survey of β-Sitosterol and Campesterol Palmitate in Wheat Semolina and Farina

Total sterol palmitate was determined on semolina and farina, respectively, from durum and bread wheat samples of currently

TABLE X
Standard Deviation (SD) of Repeatability and Confidence Limits for Sterol Palmitate Determination[a]

Product and Solvent	Gas Chromatography (mg %)[b]		Reverse-Phase High-Performance Liquid Chromatography (mg %)	
	SD	Mean	SD	Mean
Farina-semolina blends				
Petroleum ether	0.158	5.52	0.192	8.70
Chloroform-methanol-water	0.219	8.69	0.528	9.23
Pasta, low-temperature dried				
Petroleum ether	0.318	3.75	0.301	5.80
Chloroform-methanol-water	0.274	7.93	0.436	8.64
Pasta high-temperature dried				
Petroleum ether	0.124	3.70	0.137	5.48
Chloroform-methanol-water	0.187	7.72	0.449	8.09
All products				
Petroleum ether	0.266	4.32	0.142	6.66
Chloroform-methanol-water	0.179	7.93	0.382	8.65

[a] Mean of duplicates on two replicate extracts of eight adulterated samples used for calculations. Individual values are given by Sarwar (1992).

TABLE XI
Summary of Survey of Total Sterol Ester Content (mg %) in Farina or Semolina

Wheats[a]	Origin	*n*	Mean	Range
Durum	United States	21	0.10	0.00 − 0.33
	Canada	8	0.24	0.00 − 0.68
	Mexico	5	0.10	0.00 − 0.25
	Italy	6	0.25	0.22 − 0.70
HRS	United States	45	28.87	16.88 − 38.29
	Canada[b]	17	23.95	20.67 − 44.26
HRW	United States	35	22.14	19.91 − 30.74
Soft	United States	6	28.05	27.01 − 30.95
	Italy	2	23.89	22.94 − 24.84

[a] HRS = hard red spring, HRW = hard red white.
[b] Not given is Canadian wheat variety Glenlea with 8.91 mg % of total sterol esters.

grown varieties collected from different locations in the United States, Italy, Mexico, and Canada. Semolina from durum wheat of current varieties, mostly from the United States with a few from other countries was devoid of, or very low in, campesterol and β-sitosterol palmitates (Table XI). Farina from HRS and HRW of the United States and Canada and two bread wheats from Italy contained substantial amounts of these sterol esters. One exception was the HRS bread wheat, Glenlea, a utility wheat from Canada, which had only 8.91 mg/100-g sample total content of sterol palmitate (Sarwar 1992). Farina from this wheat in small quantities in adulterated semolina or pasta is more difficult to detect than farina from other wheats based on amount of sterol esters. However, in the U.S. marketing system, varieties are mixed, and the farina would probably not be from a single variety. Values for each sample in the survey are given by Sarwar (1992).

CONCLUSIONS

A newly developed method for measuring sterol ester content of pasta detected adulteration of durum semolina or pasta down to a level of 3% in our test samples. The extracted sterol esters can be determined on commonly used GC or RP-HPLC instruments. A new method using RP-HPLC to measure sterol palmitates was developed in this study. In the varieties of wheat tested, the sterol palmitate content was very low in semolina and, with one exception, relatively high in farina.

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