Simultaneous Determination of Pyridoxine, Riboflavin, and Thiamin in Fortified Cereal Products by High-Performance Liquid Chromatography

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Following extraction, pyridoxine, riboflavin, and thiamin are separated from other sample components by an ion-pairing mechanism using a reverse-phase (ODS) column with an acidified methanol/water mobile phase containing sodium hexanesulfonate. Pyridoxine and riboflavin are quantitated by fluorescence detection with 288-nm excitation. The fluorescent thiochrome derivative of thiamin is formed by postcolumn addition of an alkaline ferricyanide solution, and the thiochrome is quantitated by a second fluorescence detector. A column-switching technique is used to minimize the required chromatographic time, allowing quantitation of all three vitamins in less than 25 min. Detection limits are 2 μg/g for pyridoxine and 1 μg/g for both riboflavin and thiamin. Relative standard deviations of the analytical procedure are 1.66%, 1.51%, and 2.06% for pyridoxine, riboflavin, and thiamin, respectively. Chromatographic specificity makes the high-performance liquid chromatographic method subject to fewer interferences than wet chemical techniques.

The need for rapid and reliable analytical methods for quantitation of vitamins in fortified cereal based food products is growing, due to increased fortification and emphasis on nutritional labeling. Although microbiological or bioassay procedures remain the ultimate reference standards, they have largely been replaced in quality control applications by manual or automated wet chemical procedures (Egberg and Potter, 1975; Pelletier and Madere, 1977). These procedures are more rapid than the microbiological techniques but remain subject to interferences. In recent years, high-performance liquid chromatography (HPLC) has shown promise as a more selective technique capable of determining vitamins at fortification levels.

The various forms of vitamin B_6 (pyridoxine, pyridoxal, pyridoxamine, and their phosphate esters) have been determined in foods by HPLC using ion-exchange (Vanderslice et al., 1981) or reverse-phase (Gregory, 1980) separation modes and in a model food system by ion pairing (Saidi and Warthesen, 1983). In all cases, fluorescence detection was used to maximize selectivity and sensitivity. Riboflavin has been determined in eggs and dairy products by reverse-phase HPLC with ultraviolet detection (Ashoor et al., 1983) and in various food products by using a normal-phase (Bognar, 1981) or reverse-phase separation mode (Woodcock et al., 1982) with fluorescence detection. In some cases, riboflavin has been converted to lumiflavin prior to analysis (Ang and Moseley, 1980). Thiamin in foods has been determined as its thiochrome derivative by chromatographic separation of oxidized sample extracts followed by fluorescence detection (Bognar, 1981; Ang and Moseley, 1980) and in chocolate by HPLC separation and postcolumn formation of thiochrome (Hurst et al., 1983).

Attempts to simultaneously determine riboflavin and thiamin in fortified cereal products have been made using ion-pairing chromatography with ultraviolet (UV) detection (Toma and Tabekhia, 1979; Kamman et al., 1980). However, UV detection techniques suffer from lack of sensitivity and encounter serious UV-absorbing interferences. Methods for simultaneously determining riboflavin and thiamin (as thiochrome) in oxidized extracts using fluorescence detection have been reported (Fellman et al., 1982; Skurray, 1981). Mauro and Wetzel (1984) have determined riboflavin and thiamin simultaneously by use of a two-stage postcolumn reaction system for thiochrome formation, thereby allowing detection conditions to be optimized for each vitamin.

The research described below represents the development of a rapid, reliable, and practical method for simultaneously determining pyridoxine, riboflavin, and thiamin in fortified cereal based food products.

EXPERIMENTAL SECTION

Preparation of Standards. Individual stock solutions containing 100 μg/mL pyridoxine monohydrochloride, riboflavin, or thiamin hydrochloride (Sigma Chemical Co., St. Louis, MO) were prepared. A total of 100.0 mg of desiccated vitamin was weighed into a 1-L volumetric flask, dissolved, and diluted to volume with an appropriate solvent. Pyridoxine monohydrochloride was dissolved in 0.1 N HCl, riboflavin in 0.02 N acetic acid, and thiamin hydrochloride in 25/75 (v/v) ethanol/water. Working standards were prepared by diluting the stock solutions with distilled water to obtain the desired concentrations. Standards were stored in low actinic glassware under subdued light.

Standard samples of fortified flour or other cereal products were prepared by adding carefully weighed amounts of the vitamins of interest to 300 g of flour or ground cereal product and mixing in a rotary tumbler for 48 h.

Extraction of Pyridoxine, Riboflavin, and Thiamin from Fortified Cereal Products. Samples of wheat or corn flour, semolina, or corn meal were extracted without further particle size reduction. Prior to extraction, ready-to-eat (RTE) cereal breakfast foods were ground through a Wiley mill equipped with a 1 mm diameter screen. A 2.000-g sample of fortified cereal product was weighed into a 40-mL centrifuge tube, to which 35 mL of 0.1 N H_2SO_4 was added. After the sample was slurried with the dilute acid, the tube was placed into a boiling water bath for 30 min.

Upon removal from the boiling water bath, the sample extract was cooled by placing the tube under running tap water. A total of 5.0 mL of a 2% (w/v) suspension of Clarase fungal amylase preparation (Miles Laboratories, Elkhart, IN) in 2.5 M aqueous sodium acetate solution was added to the tube, and the extract was incubated in a 55

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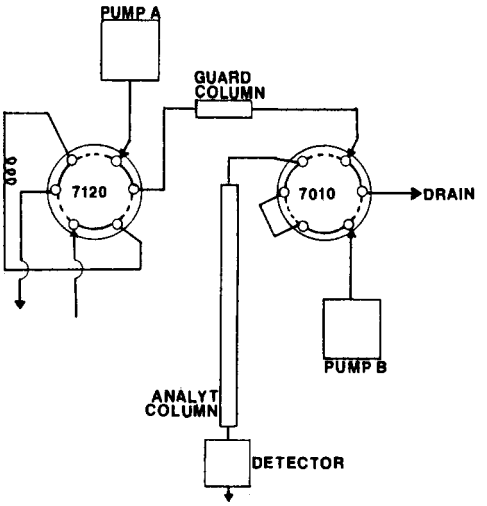

Figure 1. HPLC system in column-switching mode, as used for simultaneous determination of pyridoxine and riboflavin in fortified cereal product extracts.

°C water bath for 60 min with periodic agitation. After removal from the water bath, the sample was centrifuged for 15 min at a minimum of 2000*g*. The supernatant was decanted into a 50-mL volumetric flask and diluted to volume with distilled water. Prior to injection into the chromatograph, an aliquot of the extract was pressure-filtered through a 0.45-μm microporous filter (HAWP 013 00, Millipore Corp., Bedford, MA) by use of a Swinny adapter fitted to a 10-mL hypodermic syringe.

To minimize photodegradation of pyridoxine and riboflavin, extraction was carried out under subdued light using gold fluorescent lamps (Sylvania F40G0) as the room lighting source. Exposure of extracts to light was minimized by storing extracts in low actinic glassware or in standard laboratory glassware covered with aluminum foil.

Chromatography of Sample Extracts. The chromatographic system used consisted of a Waters M6000 dual-piston reciprocating pump (Waters Associates, Milford, MA), a Waters M45 dual-piston reciprocating pump, a Rheodyne Model 7120 six-port injection valve (Rheodyne Corp., Cotati, CA) equipped with a 20-μL injection loop, a Rheodyne Model 7010 six-port switching valve, a 4.6 mm i.d. × 250 mm analytical column packed with μBondapak C_{18} (10-μm diameter) packing material (Waters Associates), a 4.6 mm i.d. × 50 mm column packed with the same material, a Kratos FS 970 fluorescence detector with variable excitation wavelength (Kratos Analytical Instruments, Ramsey, NJ) for detection of pyridoxine and riboflavin, and a Hewlett-Packard 3385A recording integrator. A 30/69/1 methanol/water/acetic acid mobile phase containing 0.005 M hexanesulfonate ion was used.

The chromatograph was configured in a column-switching mode (Figure 1). Each solvent pump was set to deliver mobile phase at 1.0 mL/min. Samples were injected onto the short column. At time of injection the switching valve was set so that eluent from the short column was diverted directly to waste, with solvent flow through the analytical column and detector maintained independently by the second pump. At 0.60 min after injection, the switching valve was rotated so that components eluting from the short column were directed onto the analytical column. At 3.50 min into the chromatographic run, after pyridoxine, riboflavin, and thiamin had eluted onto the analytical column, the valve was switched back to its initial position. During the remaining time, as the three vitamins of interest were eluting from the analytical column, other more highly retained components were eluted from the short column directly to waste. The FS 970 fluorescence detector was equipped with a deuterium source lamp to allow excitation of pyridoxine and riboflavin in the ultraviolet region. A 288-nm excitation wavelength was used in conjunction with a 418-nm emission cutoff filter. Quantitation was obtained by measurement of chromatographic peak areas.

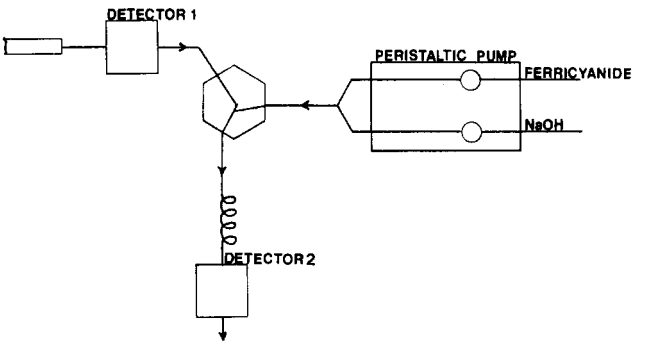

Figure 2. Postcolumn reaction system for formation and detection of the fluorescent thiochrome derivative of thiamin.

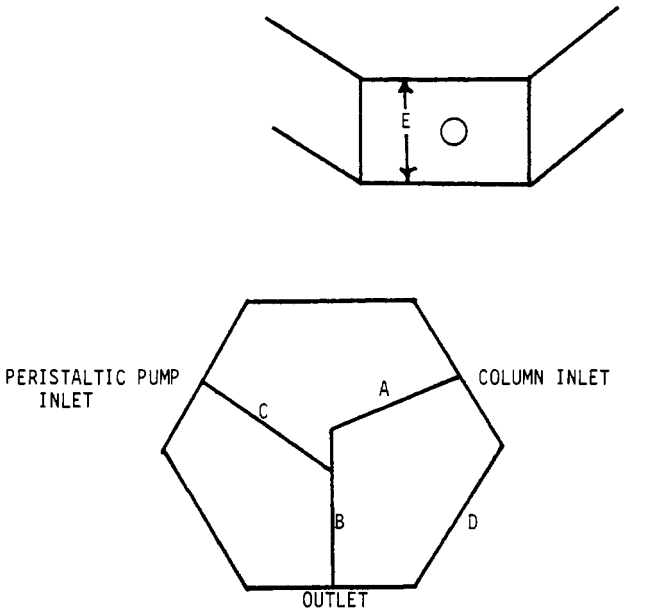

Figure 3. Reaction tee for postcolumn production of thiochrome. (A) 0.51 mm × 13 mm; (B) 0.51 mm × 15 mm; (C) 0.51 mm × 15.5 mm; (D) 16 mm; (E) 10 mm.

Postcolumn formation of the thiochrome derivative of thiamin was achieved by individually pumping NaOH and $K_3Fe(CN)_6$ solutions with a Technicon AutoAnalyzer multichannel peristaltic pump (Technicon Instruments Corp., Tarrytown, NY) and combining the reagents through a glass tee (Technicon No. 116-0200-00) just prior to their mixing with the chromatographic eluent (Figure 2). The concentrated NaOH solution was prepared by dissolving 50.0 g of NaOH pellets/100 mL of distilled water. The 0.1 mg/mL $K_3Fe(CN)_6$ solution was prepared by diluting 1.0 mL of a 1% (w/v) aqueous $K_3Fe(CN)_6$ solution with 99.0 mL of distilled water. This solution was prepared fresh daily and stored in a brown bottle to prevent photodecomposition. Prior to use, the solutions were sonicated to expel dissolved gases and minimize bubble formation. The NaOH and $K_3Fe(CN)_6$ solutions were pumped at flow rates of 0.23 and 0.16 mL/min, respectively.

The combined alkaline ferricyanide reagent stream was added to the chromatographic eluent through a mixing block as described by Mauro (Figure 3). The eluent-

reagent stream was then passed through a 3.0 m × 0.76 mm i.d. 316 stainless steel delay/mixing coil, prior to entering a second fluorescence detector. A Varian Fluorochrom filter fluorometric detector (Varian Instruments Group, Walnut Creek, CA) was used to measure thiochrome fluorescence. The detector was equipped with a tungsten/quartz/halogen source lamp, a Corning 7-54/7-60 combination filter providing a 360-nm excitation maximum, and a Corning 3-71 emission filter with a 460-nm cutoff. Peak areas were obtained with a Hewlett-Packard 3390 recording integrator.

RESULTS AND DISCUSSION

Chromatography. Simultaneous HPLC determination of water-soluble vitamins (including niacin, niacinamide, pyridoxine, riboflavin, thiamin, and folic acid) in pharmaceutical preparations and multivitamin tablets has become a routinely used analytical procedure (Kwok et al., 1981; Jenkins, 1982). An ion-pairing mechanism using a reverse-phase column, followed by ultraviolet detection, can successfully separate and quantitate the high levels of vitamins found in these relatively simple mixtures. However, determination of water-soluble vitamins in fortified cereal based foods requires quantitation of ppm levels in a complex matrix. Ultraviolet detection frequently lacks sufficient sensitivity to detect these low levels. Also, UV-absorbing interferences often cannot be resolved chromatographically from the vitamins of interest, making reliable quantitation difficult.

Fluorescence has proven to be a useful alternative detection technique for certain water-soluble vitamins, offering improved sensitivity and selectivity over UV detection. Native pyridoxine in an acidic medium can be made to fluoresce by excitation with UV radiation over a relatively narrow wavelength range, with a maximum occurring in the 290–300-nm region. Riboflavin is an extremely strong fluorophore that can be excited over a wide spectral range in both the UV and visible regions, with a maximum occurring near 280 nm. A 288-nm excitation wavelength was selected for the simultaneous determination of pyridoxine and riboflavin, allowing excitation of pyridoxine close to its maximum, while also providing good sensitivity for riboflavin. Under the instrumental conditions used, the response factor for riboflavin was ca. 5 times that of pyridoxine. Coupling of fluorescence detection with an ion-pairing separation mode allowed successful simultaneous determination of pyridoxine and riboflavin in fortified cereal product extracts.

We found that the surface chemistry of the column packing material significantly affected the separation of the water-soluble vitamins when using the ion-pairing mode. The μBondapak C_{18} packing material, or materials with similar surface characteristics, provided the best peak symmetries for the vitamins of interest. Use of a μBondapak C_{18} column with an optimized mobile phase containing 30% methanol provided sufficient selectivity to satisfactorily separate pyridoxine and riboflavin from background interferences. Since pyridoxine hydrochloride is the form of vitamin B_6 normally used to fortify cereal products and was the only B_6 vitamin found to be present in significant quantities, the separation was optimized for this form. Figure 4A depicts the chromatogram obtained from an extract of a fortified bran flakes sample. This sample matrix provided the most serious background interferences of any sample encountered during the course of our study. Although neither pyridoxine nor riboflavin was base-line resolved, quantitation of the respective peak areas was highly reproducible.

Although the vitamins of interest eluted from the column in less than 20 min, some sample extracts were found to contain components requiring up to 50 min to elute under the conditions used. Since a 50-min chromatographic analysis time was considered too long for use in routine quality control applications, we investigated methods for decreasing the time. Use of a step gradient reduced the time required to elute all components from the analytical column. However, actual time savings over the isocratic method were small, as additional time was required to reequilibrate the column to the initial mobile phase conditions prior to each injection.

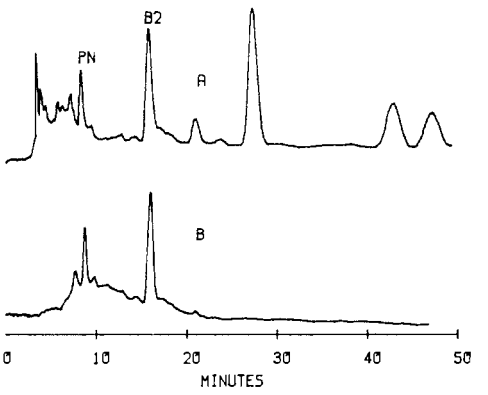

Figure 4. (A) Chromatographic separation of a fortified bran flakes extract using a μBondapak C_{18} column and 30/69/1 methanol/water/acetic acid mobile phase containing 0.005 M hexanesulfonate ion. Fluorescence with 288 nm excitation was used as the detection mode. (B) Chromatogram of the fortified bran flakes extract obtained by use of the optimized ion-pairing separation mechanism with a column-switching technique.

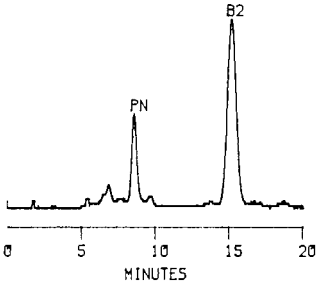

Figure 5. Chromatographic separation of pyridoxine and riboflavin in a typical fortified cereal product extract using an optimized ion-pairing separation mechanism and column switching, with fluorometric detection.

A column-switching technique was found to be most successful in minimizing the required chromatographic time. By use of column switching, the required analysis time was reduced to ca. 20 min (Figure 4B), representing a time saving of 50%, while obtaining excellent reproducibility of results. The column-switching step can be automated easily by use of a pneumatically actuated valve controlled by the chromatography data system. Switching times were determined by connecting the short column directly to the detector and observing the elution times of the vitamins of interest, after which over 200 sample injections could be made onto the system without requiring replacement of the short column or alteration of switching times. Use of column switching also aided in prolonging the lifetime of the analytical column, as early eluting sample components, such as inorganic ions, are not eluted onto the analytical column. Over 500 injections have been made onto a single analytical column without incurring significant deterioration of resolution.

Figure 5 is representative of the separation obtained for a majority of cereal product extracts using the optimized

chromatographic conditions and column switching. For most samples, base-line resolution of riboflavin was achieved, while near-base-line resolution was achieved for pyridoxine.

Postcolumn Formation of Thiochrome. Since thiamin does not fluoresce in its native form, a postcolumn reaction system was utilized to produce the fluorescent thiochrome derivative of thiamin, which was then quantitated by use of a second fluorescence detector. The system used was a modification of the two-stage postcolumn reaction system developed by Mauro.

A peristaltic pump was used in place of a syringe infusion pump, thereby eliminating frequent syringe refillings and making unattended, automated operation practical. $K_3Fe(CN)_6$ was stable in concentrated NaOH solution for only short periods of time; therefore, to provide for several hours of unattended operation, the $K_3Fe(CN)_6$ and NaOH solutions were prepared and pumped individually and mixed together on-line just prior to their combining with the chromatographic eluent.

Initial attempts to quantitate thiamin using postcolumn thiochrome formation resulted in very poor reproducibility (>10% RSD) of results. The lack of reproducibility of the fluorescence response was attributed largely to the quenching effect of excess $K_3Fe(CN)_6$ in the reaction mixture as it passed through the detector flow cell. This quenching effect has been previously documented (Ellinger and Holden, 1944) and most likely occurs from the overlap of the thiochrome spectral emission band (λ_{max} = 444 nm) with the ferricyanide absorption band (λ_{max} = 436 nm), resulting in reabsorption of emitted radiation. In the manual and semiautomated fluorescence procedures, this quenching effect is overcome by extracting the thiochrome away from the excess $K_3Fe(CN)_6$ into 2-butanol. However, application of such an extraction technique is not practical for HPLC postcolumn thiochrome formation.

To minimize the quenching effect, the concentration of ferricyanide added for postcolumn thiochrome formation was decreased significantly from levels used in previous procedures. The final ferricyanide concentration in the reaction mixture was 3.5×10^{-5} M. Decreasing the excess of ferricyanide in the reaction mixture significantly improved the reproducibility of response without significantly affecting the signal to noise ratio or linearity of response. Increasing the length of the delay/mixing coil improved the reproducibility of the thiochrome determination. A coil providing a 1.0-min reaction time was found to be optimum. Incorporation of this delay coil into the chromatographic flow adds significantly to the system dead volume and thereby results in slight peak broadening. However, the ability to quantitate the thiochrome peak was not affected by this broadening. Thermostating of the delay coil in a water bath did not improve reproducibility of the determination, indicating that temperature changes due to heat of reaction are not significant.

Figure 6 is a representative chromatogram obtained from a fortified cereal product extract by using the postcolumn thiochrome reaction. Neither pyridoxine nor riboflavin fluoresce under the strongly alkaline conditions used to form the thiochrome derivative, and base-line resolution of thiamin is achieved for most samples. The postcolumn reaction system is fully compatible with the chromatographic conditions and column-switching technique used for determination of pyridoxine and riboflavin, thereby allowing determination of all three vitamins from a single injection in less than 25 min.

Extraction of Water-Soluble Vitamins from Cereal Products. Initial attempts to simultaneously extract

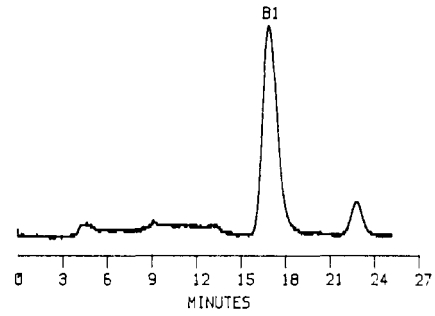

Figure 6. Chromatographic separation of a fortified cereal product extract using an optimized ion-pairing separation mode and column switching, with postcolumn formation of thiochrome and fluorometric detection.

pyridoxine, riboflavin, and thiamin from fortified cereal products involved application of a technique developed for extraction of pyridoxine (Gregory, 1980), in which the sample is sonicated in 0.5 M aqueous sodium acetate solution. However, we found that use of this technique resulted in low (<70%) recoveries of riboflavin. The technique we found to be most successful for simultaneously extracting the three vitamins of interest was a modification of the AACC-approved extraction procedure for thiamin (American Association of Cereal Chemists, 1981), in which a sample is extracted with dilute acid, followed by clarification of the solution by enzymatic digestion with an amylase preparation. The procedure was modified by increasing the acid digestion time to 30 min, thereby providing increased recovery of pyridoxine. Elevation of the temperature to 55 °C during the enzymatic digestion step, as recommended by Mauro, allowed the required digestion time to be decreased to 1.0 h. Also, the filtration step used in the official method was replaced by centrifugation, which was faster and exposed the extracts to light for a shorter period of time.

Average recoveries of pyridoxine, riboflavin, and thiamin were 90%, 91%, and 92%, respectively. To compensate for the less than complete recovery, samples prepared by adding known quantities of the vitamins to an unfortified cereal product matrix were carried through the extraction procedure and used as standards.

As some degradation of riboflavin was noted in samples stored for 24 h, even when refrigerated and in the dark, it is recommended that analysis be completed within 12 h following extraction.

Reproducibility and Accuracy of Analytical Procedure. Replicate injections of a given sample extract were made to evaluate the reproducibility of the chromatographic technique. Relative standard deviations of 1.50%, 1.07%, and 2.02% were obtained for pyridoxine, riboflavin, and thiamin, respectively. Reproducibility of the overall analytical procedure, including extraction and chromatography, was evaluated by analyzing seven replicate samples of a fortified RTE cereal breakfast food. Relative standard deviations of 1.66%, 1.51%, and 2.06% were obtained for pyridoxine, riboflavin, and thiamin, respectively. Practical detection limits for the analytical procedure were found to be 2 μg/g for pyridoxine and <1 μg/g for both riboflavin and thiamin.

To evaluate the accuracy of the simultaneous HPLC procedure, results obtained from a set of fortified commercial RTE cereal breakfast food samples were compared to results obtained from the same samples by a second laboratory using other standard analytical techniques. A comparison of the analytical results obtained for pyridoxine by the two laboratories is given in Table I. Excellent agreement existed, as evidenced by a correlation

Table I. Comparison of Analytical Results Obtained for Pyridoxine in Fortified Cereal Products

	μg/g		
sample no.	KSU-HPLC		collaborator
1	21.8		21.1
2	43.2		44.6
3	17.9		16.9
4	21.9		23.1
5	19.7		20.6
6	26.3		27.4
mean	25.1		25.6
difference		0.50	
SD of diffs		1.05	
correlation		0.996	

Table II. Comparison of Analytical Results Obtained for Riboflavin in Fortified Cereal Products

	μg/g		
sample no.	HPLC		wet chemical fluorescence
1	17.3		19.1
2	38.1		41.7
3	72.0		81.3
4	12.4		15.9
5	18.0		19.1
6	15.5		20.1
7	20.5		25.4
mean	27.7		31.8
difference		4.1	
SD of diffs		2.7	
correlation		0.998	

coefficient of 0.996, with no significant bias.

A comparison of riboflavin results obtained on a set of fortified RTE cereal breakfast food samples is given in Table II. An automated wet chemical fluorescence procedure (Association of Official Analytical Chemists, 1981) was used by the collaborating laboratory for riboflavin determination. Excellent correlation (r^2 = 0.998) was obtained between the HPLC and wet chemical procedures; however, an obvious bias existed. The results obtained by HPLC were consistently lower than those obtained by the wet chemical technique. The most likely cause for this bias is lack of total specificity for riboflavin in the wet chemical method. Fluorescent components other than riboflavin have been found to undergo reduction to nonfluorescent species during the hydrosulfite treatment step. These substances are measured as riboflavin in the difference technique, resulting in artificially high riboflavin values. The HPLC technique derives its specificity from a chromatographic separation rather than chemical reaction and, therefore, provides a more accurate value.

A comparison of thiamin results is given in Table III. An automated wet chemical procedure for determining thiamin as its fluorescent thiochrome derivative following cleanup on a Decalso column was used by the collaborating laboratory. Excellent correlation (r^2 = 0.996) existed between the HPLC and wet chemical procedures. However, it was observed that for four samples containing added sucrose as a sweetener, the HPLC analytical values were significantly higher than the wet chemical analytical values. For three unsweetened samples, there were no significant differences.

To evaluate the effect of added sucrose on the HPLC procedure, replicate 2.000-g samples of an unsweetened RTE cereal breakfast food were analyzed. To half of the samples, 0.5 g of sucrose was added prior to extraction. No significant difference in response was obtained between the unsweetened samples and those to which sucrose was added.

Table III. Comparison of Analytical Results Obtained for Thiamin Hydrochloride in Fortified Cereal Products

	μg/g		
sample no.	HPLC		wet chemical fluorescence
1	8.5		7.1
2	49.1		45.1
3	69.1		61.0
4	11.2		10.7
5	21.1		21.2
6	17.8		18.1
7	36.9		37.2
mean	30.5		28.6
difference		1.9	
SD of diffs		3.0	
correlation		0.996	

The differences between the two analytical methods can be attributed to the effect of reducing sugars, formed by hydrolysis of sucrose during acid extraction, on the response of the wet chemical technique. The reducing sugars compete with thiamin for the oxidizing agent, thereby shifting the equilibrium of the thiochrome reaction to produce less thiochrome (Wehling and Wetzel, 1984). Thiamin values obtained by wet chemical fluorescence techniques from sucrose-containing samples thus will generally be lower than those obtained from unsweetened samples containing equivalent amounts of thiamin. However, in the HPLC determination, the reducing sugars are chromatographically separated from thiamin. Therefore, any interference from reducing sugars is eliminated, and the HPLC technique provides a more accurate analytical value for thiamin in sweetened samples than does the wet chemical procedure.

High-performance liquid chromatography provides a relatively rapid means for simultaneously determining pyridoxine, riboflavin, and thiamin in fortified cereal products, giving significant time savings over individual manual assays. Use of column switching and selective detection eliminates the necessity of sample cleanup or preconcentration. In addition, the HPLC analytical procedure provides excellent reproducibility of results and is less subject to interferences from the sample matrix than are the wet chemical techniques. These factors combine to make simultaneous HPLC determination of pyridoxine, riboflavin, and thiamin a practical technique for routine, day-to-day usage in quality control applications.

Registry No. Pyridoxine, 65-23-6; riboflavin, 83-88-5; thiamin, 59-43-8.

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Iron Binding of Wheat Bran at Human Gastric pH

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In vitro measurements on several wheat brans demonstrate that iron binding in ~0.1 N HCl is dependent upon time and concentration of iron in solution and is similar for all brans examined. Ferric iron is bound more than ferrous iron during exposure times of ≤4 h; at longer times, >16 h, there is little difference in amounts bound. Binding of ferric iron by wheat brans can be greatly altered by various treatments and may involve mechanisms other than interaction with phytate. The iron content of wheat bran remnants recovered after passage through the human gastrointestinal tract is equivalent to or less than that associated with initial pericarp tissue.

Research efforts currently are directed to examination of effects of dietary fiber consumption upon human health and nutrition. Reports claim both beneficial and adverse effects of dietary fiber in human diets. One area of major interest is the relationship between fiber consumption and mineral requirements. Earlier work had established that iron balance was lowered when whole-meal wheat bread was eaten rather than white bread (Widdowson and McCance, 1942). Recent works have amply verified that wheat bran in the diet lowers iron absorption (Björn-Rasmussen, 1974; Simpson et al., 1981). Thus, Simpson et al. report a sharp decrease in iron absorbed from a light meal that contains 12 g of wheat bran. However, reports from longer term studies show that a modest increase of about 25 g/day of wheat bran added to a low-fiber diet may increase the apparent requirements of humans for several minerals but not for iron (Sandstead et al., 1979). A discussion of the complex multitude of factors involved in physiological mechanisms and chemical properties concerned with iron absorption is presented in a review by Forth and Rummel (1973). A recent review addresses aspects of iron fortification of foods, bioavailability, and behavior in food systems (Lee and Clydesdale, 1979). Clinical studies provide ample evidence that a variety of diet components, including fruit juices and egg protein, also affect iron absorption in humans (Elwood et al., 1968; Monsen and Cook 1979; Rossander et al., 1979). Thus, wheat bran is one of many items in the diet that may influence mineral absorption.

Because wheat bran is a natural, whole diet component and is being used as a supplementary dietary fiber source, we consided it worthwhile to examine some iron binding properties of this food ingredient. Recent work has convincingly demonstrated that various dietary fiber components and sources, including wheat brans, do bind minerals in vitro under a variety of conditions (Reinhold et al., 1976;, 1981; Ismail-Beiji et al., 1977; Reilly, 1979; Camire and Clydesdale, 1981). Although dietary fiber components have the ability to act as a mineral sink, it is not yet directly demonstrated whether or not this binding is sufficiently strong under physiological conditions to be a major mechanism by which mineral bioavailability is reduced. In this work are examined some aspects of iron binding behavior of wheat brans under simulated human gastric conditions of low pH and then are considered a few treatments that affect iron binding. This report concludes with some data that allow comparison of the iron binding content of as-is bran pericarp with that of bran remnants retrieved after having been baked in bread, consumed, and passed through the human digestive system.

MATERIALS AND METHODS

Brans of Waldron variety, a hard red spring wheat grown in North Dakota, and durum wheat were obtained from the Spring and Durum Wheat Quality Laboratory, North Dakota State University, Fargo. AACC wheat bran, a blend made from soft white winter wheats, was purchased from the American Association of Cereal Chemists. Eagle variety, a hard red winter wheat, was purchased from the Department of Grain Science and Industry, Kansas State University, Manhattan. All brans used in these binding experiments were of similar particle size distributions obtained by sieving to pass a No. 18 U.S. standard sieve (0.98-mm opening) onto a No. 30 U.S. standard sieve (0.52-mm opening). A few measurements were made on similar distributions of a commercial dry-milled corn bran.

Measurements of tracer counts were made in a Tracor analytic automatic gamma system equipped with a TN-1710 multichannel analyzer. Activities were kept at a low level, with counting periods of 10 min used for sample counting. Control blanks that did not contain bran substrates were run with all measurements.

Experiments were designed to obtain balance measurements of radioactivities under conditions of high gastric acidity. Solutions were made 0.1 N in HCl to contain 10 mequiv/L of K^+ added as KCl and 30 mequiv/L of Na^+ added as NaCl. Radioactive iron, ^{59}Fe, for tracer use was purchased either as $FeCl_3$ in 0.1 N HCl (Amersham, Arlington Heights, IL) or as $FeSO_4$ in 0.05 M H_2SO_4 (New England Nuclear, Boston, MA). Iron in the appropriate form of $FeCl_3$ or $FeSO_4$ was added as a carrier

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