Determination of Alkylresorcinols in Cereal-Based Foods†

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"Ready-to-eat" breakfast cereals and raw, baked, and cooked cereal products were analyzed for alkylresorcinols. The finely ground, dried samples were extracted with acetone; the alkylresorcinols from each extract were separated by HPLC. A photodiode array detector was set at 280 nm to monitor the eluted compounds; subsequently, the spectrum of each compound was plotted from 240 to 340 nm to confirm the identity of the separated alkylresorcinols. The results indicate that, on a moisture-free basis wheat bran, wheat bran enriched, or whole wheat breakfast cereals contained much higher levels (343–1455 μg/g) than breads or bran muffins (61–217 μg/g). Total alkylresorcinols in a typical serving of cereal-based foods varied from 40 (wheat bran breakfast cereal) to 1 mg (1 slice of "7-grain bread" or rye bread).

INTRODUCTION

Alkyl- and alkenylresorcinols (ARs) have been found in many common cereal grains, e.g., rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), triticale, and millet (*Pennisetum typhoides* Stafp. et Hubb.) (Collins, 1986). They are also formed by some fungi (Reiss, 1989), as well as in shrubs (Barrero et al., 1991; Ridley et al., 1968) and in some fruits (Bandyopadhyay et al., 1985). There are many physiological and toxic effects attributed to resorcinol derivatives; they may cause appetite depression (Sedlet et al., 1984) or act as a hemolytic agent (Kozubec, 1984). Alk(en)ylresorcinols and catechols from cashew nut oil, poison ivy, and sap of the Japanese lac tree are strong vesicants (Symes and Dawson, 1953) and are frequently the cause of a strong allergic response. In mangoes the alkylresorcinols found in the outer skin may be part of a defense mechanism against microbial infection and are also a hazard to those harvesting the fruit (Bandyopadhyay et al., 1985).

Recently, more sensitive and accurate methods of analysis for the determination of alk(en)ylresorcinols in cereal products have been described (Gohil et al., 1988; Hengtrakul et al., 1991; Mullin and Collins, 1991; Mullin et al., 1992). These GC and HPLC methods are designed to measure the individual alkylresorcinols and are less prone to interference due to coextracted compounds than methods which measure "total" alkylresorcinols (Evans et al., 1973). In the study reported here GC and HPLC methods were used to measure the alkylresorcinols in a series of common "ready-to-eat" breakfast cereals and other grain products used as food. The five major alkylresorcinols were determined and used to calculate an estimate of the amounts ingested in an average serving. The total amounts in the diet may have increased in the past few years, due to the increased use of high-fiber products containing wheat, rye, or triticale.

EXPERIMENTAL PROCEDURES

Cereal Samples. Ready-to-eat breakfast cereals, cereal flours, barley, and other cereal products were purchased from local food stores. In preparation for extraction a 100-g sample of each dry product was ground in a small mill fitted with a 1-mm screen. The cooked samples (prepared according to manufacturer's directions), the muffins (home baked), and breads (store bought), were freeze-dried before grinding. The moisture content was calculated from the weights before and after freeze-drying.

Alk(en)ylresorcinols were extracted from a 1.0–5.0-g sample and weighed into a 250-mL Erlenmeyer flask with 50 mL of acetone which was stirred in a shaking water bath at 25 °C overnight. The mixture was filtered and the residue re-extracted twice by the same procedure. The filtrates were combined and the solvent removed under reduced pressure using a rotary evaporator. The residue was redissolved in methanol, filtered through a 0.45-μm filter, made up to 25 mL, and stored at 4 °C until analyzed. Two replicates of each sample were extracted.

HPLC Conditions. A Perkin-Elmer (Norwalk, CT) Series 4 pumping system, a Rheodyne (Cotati, CA) 7125 manual injector, and a Waters (Bedford, MA) 991 photodiode array detector with associated workstation were used for the analysis. The detector was set to record spectra between 220 and 400 nm every 2 s. The separation column was a Brownlee (San Jose, CA) RP 18 Spheri-5 4.6 mm i.d. × 25 cm fitted with a Brownlee 1.5-cm ODS 5-μm precolumn. The solvent gradient was as follows: solvent A, 95% water plus 5% methanol; solvent B, methanol; solvent C, hexane; isocratic 5 min, A = 25%, B = 75%; gradient 20 min to B = 100%, isocratic 10 min, B = 100%; gradient 5 min to B = 95%, C = 5%; gradient 5 min to B = 100%; gradient 5 min to A = 25%, B = 75%. For each extract data from two separate injections were used for calculations. A calibration curve, from 0.1 to 0.5 μg, was prepared using a suitably diluted stock solution of recrystallized pentadecylresorcinol (Aldrich Chemical Co., Milwaukee, WI) in methanol containing 1.0 mg/mL.

RESULTS AND DISCUSSION

The alkylresorcinols determined in this study were those with the saturated side chains ($C_{17}H_{35}$, $C_{19}H_{39}$, $C_{21}H_{43}$, $C_{23}H_{47}$, and $C_{25}H_{51}$) at the 5-position of the resorcinol ring corresponding to peaks 2, 4, 6, 8, and 9, respectively (Figure 1). These predominate in wheat and rye (Gohil et al., 1988) and occur together with some unsaturated analogues and minor amounts of $C_{15}H_{31}$ and $C_{27}H_{55}$ alkylresorcinols. A typical chromatogram of the extract from a sample of breakfast cereal containing wheat products is shown in Figure 1. The UV spectra of the eluted fractions were recorded between 240 and 400 nm; chromatograms are displayed at the monitoring wavelength of 280 nm. The spectrum of each eluted compound was compared with the spectrum of the standard 5-*n*-pentadecylresorcinol to confirm the presence of a resorcinol derivative. The observed spectrum for peaks 2, 4, 6, and 8 was attributable to the substituted phenolic ring structure; the saturated or unsaturated hydrocarbon side chain in the 5-position did not affect the UV absorption. Typical spectra from

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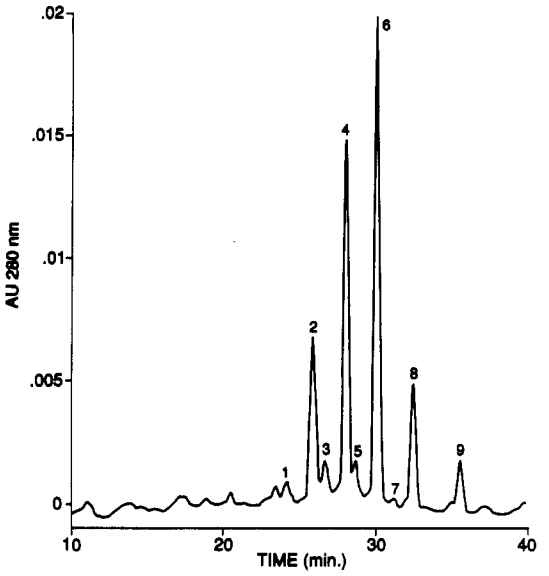

Figure 1. HPLC chromatogram from whole wheat breakfast cereal acetone extract. For conditions, see text.

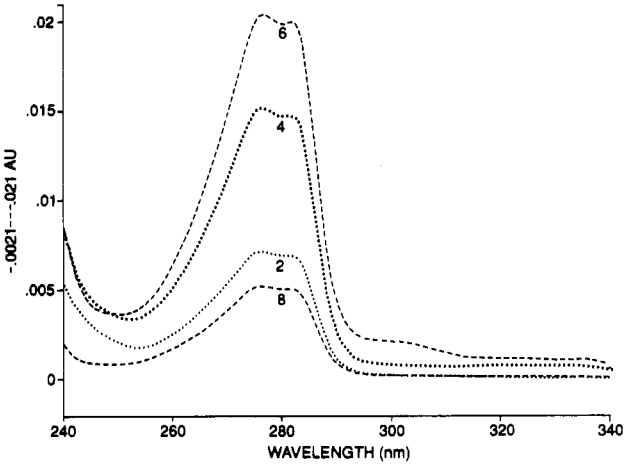

Figure 2. UV spectra of peaks 2, 4, 6, and 8.

240 to 340 nm of the eluting peaks, taken at the retention time of the chromatogram peak maxima, are shown in Figure 2 with absorption maxima at 276 nm and a shoulder at 281 nm, in agreement with data reported by other authors (Tyman, 1978; Su et al., 1981). Peak purity calculations, using systems in the photodiode array detector software, showed the peaks to be uncontaminated. Some of the minor peaks (3, 5, and 7; Figure 1), although much weaker, gave spectra similar to the major peaks and were attributed to alkenylresorcinols. The identities of the major peaks were confirmed by gas chromatography–mass spectroscopy using the same technique as reported earlier (Mullin and Collins, 1991).

The breakfast cereals in this study all contained wheat or wheat fractions, and one contained rye and flax. Alkylresorcinol content of the breakfast cereals is summarized in Table I. Breakfast cereal brand I contained the highest concentration of alkylresorcinols of all those tested; it was also the only product comprised entirely of wheat bran. The concentration was lower than was found in unprocessed bran (see Table II), which could be due to the wheat cultivar (Hengtrakul et al., 1991) or to the amount of purification, i.e., separation from the flour fraction, that the bran had undergone. Another factor in reducing the alkylresorcinol content could be the effect of processing, particularly heat treatment used in the preparation of cereals (Verdeal and Lorenz, 1977). The pattern of alkylresorcinols was typical of wheat extracts in this and other studies (Mullin and Collins, 1991; Gohil et al., 1988). Products II and III, which contained whole wheat as well as wheat bran, and products IV and V, comprised of whole wheat only, contained progressively less alkylresorcinols as the bran content was reduced. Only traces (<25 μg/g, dry basis) were detected in product VII, which was made up of wheat flour and wheat germ. Product VI, which included rye in its formulation, contained more alkylresorcinols than did whole wheat cereals; whole grain rye is known to be richer in alkylresorcinols than any other whole grains (Verdeal and Lorenz, 1977). With the exception of product III, the samples purchased 6 months apart showed <10% variation in alkylresorcinol concentration. Product VI, which required cooking before consumption, showed a reduction of 20% in total alkylresorcinol content after cooking.

Some common cereal grain products and baked goods were also analyzed, and the alkylresorcinol concentrations are summarized in Table II. The wheat bran contained approximately twice the amount of alkylresorcinol that was found in the bran breakfast cereal, by far the greatest concentration found in any of the samples tested. A range of concentrations due to the influence of cultivar and milling process can be expected (Verdeal and Lorenz, 1977). The flours in this study were within the range with wheat bran containing the highest concentrations of alkylresorcinols in the food products studied here. Whole wheat and rye flours also contain significant amounts of alkylresorcinols, which will be reduced in bakery products by fermentation or processing and by dilution with the other ingredients. No alkylresorcinols were detectable in the sample of "pot barley". There have been reports of them being present in low concentration in barley (Briggs, 1974; Evans et al., 1973), but since the sample was "polished", most of the pericarp would be removed, which may account for the lack of alkylresorcinols. The breads and bran muffins contained considerably less alkylresorcinols than the breakfast cereals on a dry weight basis.

The alkylresorcinol content of a typical serving of some common products on the "as is" basis, rounded off to the nearest milligram, are shown in Table III. These were calculated from the data in Tables I and II, taking into account the moisture content of each item. The breakfast cereals contain the largest amounts of alkylresorcinols per serving; the cooked products contain significantly less. Unprocessed wheat bran, sometimes sprinkled over breakfast cereals or desserts as an additional fiber source, will contribute approximately 13 mg of alkylresorcinols/15-mL serving. The two breads ("7-grain" and rye) contained only 1 mg of alkylresorcinols/slice, indicating that there were losses during processing.

The alkylresorcinol content of a normal diet will be influenced to some degree by the cultivars of the cereals used in the preparation of products for human consumption (Hengtrakul et al., 1990). However, in a diet with enhanced cereal fiber intake the alkylresorcinol content will be increased further. Nutritional and biochemical effects have been reviewed recently by Lorenz and Hengtrakul (1990), but levels toxic to humans have yet to be defined. The amphiphilic properties of alkylresorcinols are responsible for a number of observations that show activity at cell membranes; side-chain length and unsaturation are also important factors. While this study has defined the range of alkylresorcinols in the diet, there are still few data on their physiological effects, particularly

Table I. Alkylresorcinol Content of Ready-To-Eat and Cooked Breakfast Cereals

brand	ingredient declared on label	purchase[a]	mean alkylresorcinol ($n = 4$), μg/g dry basis; pentadecylresorcinol equivalent C_{17}	C_{19}	C_{21}	C_{23}	C_{25}	total ± SD[b]
I	wheat bran	A	99	368	651	146	55	1320 ± 36
		B	118	403	724	155	66	1455 ± 21
II	whole wheat, wheat bran	A	41	167	294	64	28	596 ± 21
		B	48	158	284	58	21	575 ± 80
III	whole wheat, wheat bran	A	34	139	232	49	19	473 ± 15
		B	51	172	265	57	23	568 ± 7
IV	whole wheat	A	36	130	229	48	17	460 ± 29
		B	41	131	241	47	17	476 ± 10
V	whole wheat	A	27	100	173	35	14	343 ± 14
		B	26	94	187	32	12	352 ± 5
VI	whole wheat, rye, flax	A	71	150	214	53	24	512 ± 12
		B	66	146	203	47	24	485 ± 6
VIa[c]	whole wheat, rye, flax (cooked)	B	58	113	152	36	19	380 ± 3
VII	wheat flour, wheat germ	A	tr	tr	tr	tr	tr	
		B	tr	tr	tr	tr	tr	
corresponding peak in Figure 1			2	4	6	8	9	

[a] B purchased 6 months after A. [b] SD, standard deviation. [c] Cooked VI.

Table II. Alkylresorcinol Content of Wheat Bran, Cereal Grains, and Baked Cereal Products

grain or grain product	mean alkylresorcinol ($n = 4$); μg/g dry basis; pentadecylresorcinol equivalent C_{17}	C_{19}	C_{21}	C_{23}	C_{25}	total ± SD[a]
whole soft wheat flour	41	145	210	41	16	454 ± 18
whole hard wheat flour	17	53	88	16	6	181 ± 5
wheat bran	198	756	1304	288	112	2658 ± 35
rye flour	287	290	187	73	57	897 ± 19
barley	ND[b]	ND	ND	ND	ND	
7-grain bread[c]	12	32	64	tr	tr	114 ± 3
bran muffins	15	61	113	21	8	217 ± 12
rye bread	13	16	22	4	5	61 ± 2
corresponding peak in Figure 1	2	4	6	8	9	

[a] SD, standard deviation. [b] ND, none detected. [c] Contains wheat flour, wheat bran, barley, millet, buckwheat, oats, and rye.

Table III. Alkylresorcinol Content of Some Cereal-Based Foods

product	typical serving, g	mg of AR[a] per serving
wheat bran breakfast cereal	30	40
whole wheat, wheat bran breakfast cereal	30	16
whole wheat, rye, flax, cooked breakfast cereal	125	14
wheat bran (unprocessed), 15 mL	5	13
bran muffin	35	3
7-grain bread, 1 slice	30	1
rye bread, 1 slice	30	1

[a] AR, total alkylresorcinols to nearest milligram.

with respect to unsaturation in the side chain. The influence of alk(en)ylresorcinols on the control of microbial food spoilage and food-borne molds has been reported (Reiss, 1989; Reusch and Sadoff, 1979). A positive consequence of more bran in a diet could be the increase in shelf life of high wheat bran products. This is a subject that requires much more attention, and with the recent advances in analytical methodology more detailed information should become available.

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Registry No. 5-Heptadecylresorcinol, 41442-57-3; 5-nonadecylresorcinol, 35176-46-6; 5-heneicosanylresorcinol, 70110-59-7; 5-tricosanylresorcinol, 70110-60-0; 5-pentacosanylresorcinol, 70110-61-1.