ALKYLRESORCINOLS IN WHEAT, RYE, AND TRITICALE[1]

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ABSTRACT

Samples of wheat, rye, and triticale, grown under the same agronomic and climatic conditions during four consecutive years, were analyzed for alkylresorcinol content. Rye contained the highest amounts and wheat the lowest of these compounds. Spring triticales showed intermediate values. Milling these grains into bran, shorts, and flour fractions and subsequent analysis showed the bran to contain the highest level of alkylresorcinols. Intermediate values were found in the shorts, while the flour fractions produced relatively low values. Baking of whole grain meals into breads resulted in losses of alkylresorcinols in the crust and crumb portions of the loaves. The losses were not attributed to heat destruction but were thought to have occurred as the result of fermentation.

A group of compounds thought to inhibit growth in several animal species has been isolated from cereal grains (1,2). Identification of these compounds led to a series of resorcinol derivatives with hydrocarbon chains (15 to 25 carbons) at the fifth position (1). Levels of these compounds were shown to vary among cereal grains, rye being consistently higher than wheat (1,3). Selection of cultivar progeny was effective in increasing or decreasing alkylresorcinol content (2).

Thus far, these compounds have only been mentioned as being detrimental factors in animal nutrition, although concern has been occasionally raised about possible ill-effects on humans (especially young children) in countries of Europe with high rye bread consumption (4). There are no established human toxicity levels for these compounds. The effect of the baking process on alkylresorcinols is unknown.

Triticale, a cross between wheat and rye, has found increased use in the U.S., not only as an animal feed (5,6), but also in the production of many food products (7–9). It is conceivable that the higher alkylresorcinol content in rye compared to that of wheat (2) has been inherited by triticale.

It was the purpose of this study: a) to compare the alkylresorcinol contents of wheat, rye, and triticale grown under the same agronomic and climatic conditions; b) to determine the variability due to crop year and stage of kernel development; c) to study the distribution of these compounds in various milling fractions; and d) to investigate the effect of baking on the retention of alkylresorcinols.

MATERIALS AND METHODS

Sample Identification

The cereal grains used in this study included two hard red spring wheats, one durum wheat, four triticales, and one rye. The wheat cultivar Chris was selected from the crop years 1972 and 1973, while Colano was used from the 1974 and 1975 harvests. Durum RF 710222, a spring semidwarf selection from the CIMMYT program, was grown in 1974 and 1975. Two of the four triticales, 6-

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TA-204 and 6-TA-206, are spring types obtained from the Jenkins Research Foundation, included in each of the 4 crop years of this study. Triticales TR 385 and TR 386 are winter types, grown during the 1972 and 1973 crop years. The rye was the spring cultivar Prolific grown each year from 1972 through 1975.

All grains were grown at the Colorado State University Agronomy Research Center, Fort Collins, Colo.

Harvest Date Comparisons

To study the relation between harvest date and alkylresorcinol content in grains, small samples of the 1974 crop of Prolific rye and the triticales 6-TA-204 and 6-TA-206 were harvested at different stages of kernel development for analysis. Analyses were done in triplicate.

Milling of Samples

For whole grain analyses, samples of each of the cultivars were ground in a micromill. To determine the distribution of alkylresorcinols within milling fractions of each of the different cereal grains, samples of Chris, Prolific rye, and triticale 6-TA-204 from the 1972 harvest were milled into bran and flour fractions on a Quadrumat Jr. mill. Prolific rye and triticale 6-TA-204 from the 1973 crop year were milled on a Quadrumat Sr. mill into bran, shorts, and flour fractions.

Wheat samples were tempered to 15% moisture, triticales to 14.5%, and the rye to 14% prior to milling. Preliminary milling experiments indicated these moisture levels to be optimum. Analyses of the milling fractions were conducted in triplicate.

Baking of Samples

Due to the uneven distribution of alkylresorcinols in grain kernels, which leaves only relatively small amounts of the compounds in the flour fraction after milling, whole grain meals obtained by grinding samples in a Wiley mill were used in baking experiments to determine the effects of temperature, reached during baking, on alkylresorcinol retention.

The following formulation was used to bake pup loaves: flour, 100%; sugar, 6%; shortening, 3%; yeast, 3%; salt, 2%; and yeast food, 0.5%. The doughs were fermented for 1.5 hr at 85° F and 85% r.h. They were divided (250 g/loaf), mechanically molded, and proofed for 55 min at 100° F and 95% r.h. Baking time was 18 min at 425° F. Samples of crust and crumb of each loaf as well as entire pup loaves were air-dried, ground, and blended prior to oven drying and analysis for alkylresorcinol contents.

Alkylresorcinol Determination

For the determination of alkylresorcinols, a modification of the method by Evans *et al.* (2) was used.

Samples were dried for 3 hr at 80° C after they were ground into a fine powder. There is no loss of alkylresorcinols at that temperature. Pyrex screw cap culture tubes were used for 0.13 g of sample in 5 ml of acetone. The tubes were covered with Teflon tape before they were capped. The use of Pyrex culture tubes and Teflon tape permitted thorough shaking while preventing evaporation of solvent and possible contamination from caps. Extraction time was 16 hr on a Burrell shaker, followed by centrifugation and transfer of the supernatant to a 20-ml

volumetric flask. The residue was washed with acetone and the transfer technique repeated. Flasks were made up to volume with acetone and shaken thoroughly.

Three aliquots of 0.5 ml were transferred to Pyrex culture tubes, placed in a 85° C water bath for acetone evaporation, and then cooled to room temperature. Chloroform (0.4 ml) was added, followed by the addition of 0.1 ml of 75% ethanol and 0.1 ml of 75% KOH. The reaction tubes were covered with Teflon tape, capped tightly, placed in a 45° C water bath, and agitated every 2 to 3 min. After 20 min, 1.0 ml of distilled water was added followed by 8.4 ml of 95% ethanol. The tubes were covered and shaken to assure a uniform solution, which is important because the presence of particles in the final solution was shown to cause a higher reading due to scattering of the incoming light. After 30 min, fluorescence spectra were run.

A standard curve was prepared using 5-pentadecylresorcinol. Spectra were interpreted by using peak height in relative intensity units, subtracting the blank and mathematically correcting all samples to the standard curve with regard to instrumental variation, using the standard fluorescence block readings.

Instrumentation

An Aminco-Bowman Spectrofluorometer (SPF 135) was employed for fluorescence measurements. The instrument uses both excitation and emission monochromators, allowing well-defined wavelength selection. A 150-W Xenon lamp for excitation and a R 446 photomultiplier for detecting emission provided high sensitivity. The optimal excitation wavelength was 420 nm, giving strong emission at 525 nm. In a typical analysis, the excitation was set at 420 nm using a 1-mm slit (band pass of 5.5 nm) and emission was scanned from 440 nm to 650 nm using a 2-mm slit (11.0-nm band pass). Immature grain samples were scanned to 800 nm to include the pigment peak.

Since both excitation and emission wavelengths are in the visible region of the spectrum, glass sample cells rather than quartz were employed. A standard fluorescence block of plastic impregnated with fluorescein was used to calibrate the instrument.

Thin-Layer Chromatography

Thin-layer chromatography (tlc) was used to confirm the presence of alkylresorcinols according to the method of Evans *et al.* (2).

Statistical Evaluation of Data

Significant differences between alkylresorcinol contents of the whole cereal grains were determined using analyses of varance.

RESULTS AND DISCUSSION

Fluorescence Spectra and tlc Analysis Interpretation

Fluorescence spectra showed that the alkylresorcinol emission peaked at 525 nm. Acetone was shown to fluoresce at 480 nm and chlorophyll at 665 nm, thereby causing no interference due to chlorophyll extracted from immature samples, as experienced in the study by Evans *et al.* (2) They employed a Turner fluorometer with a primary filter that had an excitation wavelength of 350 nm

and a secondary filter with a cutoff at about 470 nm. The chlorophyll emission peak at 665 nm was verified using a leaf extraction, run in comparison with immature samples of grain. A typical fluorescence spectrum is shown in Fig. 1.

Thin-layer chromatography of some of the samples indicated a single spot, well separated from chlorophyll, with a R_f value approximately the same as that of the 5-pentadecylresorcinol standard, as was reported by Evans *et al.* (2). The spot intensities of extracts from different cultivars were in the range of their respective fluorometric values, shown in Table I, permitting an estimation of the alkylresorcinol content by comparison with spot intensities of known amounts of the standard.

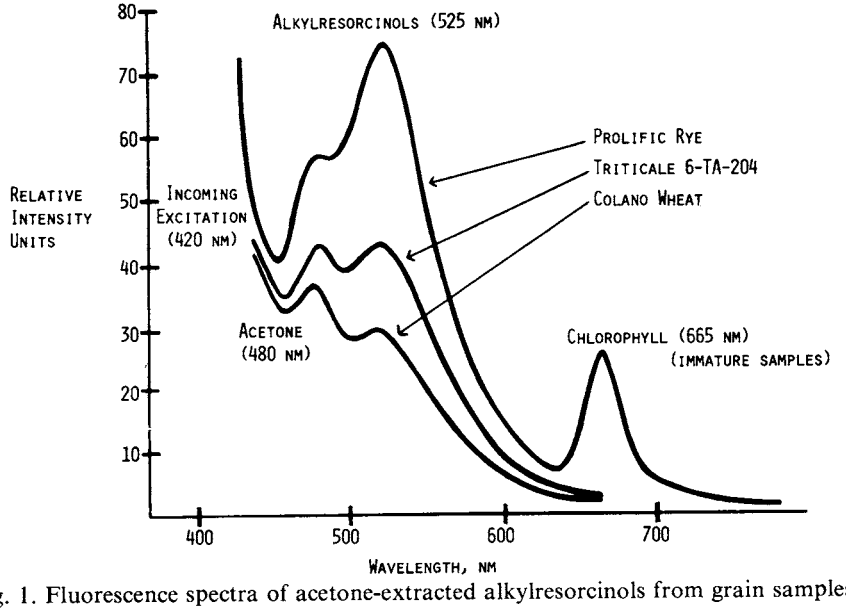

Fig. 1. Fluorescence spectra of acetone-extracted alkylresorcinols from grain samples.

TABLE I
Alkylresorcinols in Cereal Grains[a]
(% dry weight)

Grain Sample	Crop Year			
	1972	1973	1974	1975
HRS Chris Wheat	0.062 ± 0.004	0.065 ± 0.002	...	...
HRS Colano Wheat	...	...	0.067 ± 0.004	0.067 ± 0.003
Durum RF710222	...	...	0.067 ± 0.005	0.067 ± 0.004
Triticale 6-TA-204	0.085 ± 0.003	0.082 ± 0.001	0.095 ± 0.002	0.095 ± 0.006
Triticale 6-TA-206	0.070 ± 0.004	0.079 ± 0.005	0.077 ± 0.001	0.075 ± 0.002
Triticale TR 385	0.075 ± 0.003	0.066 ± 0.005	...	...
Triticale TR 386	0.067 ± 0.005	0.067 ± 0.004	...	...
Prolific Rye	0.124 ± 0.004	0.105 ± 0.001	0.101 ± 0.002	0.108 ± 0.003

[a]Averages of three separate determinations.

Alkylresorcinols in Cereal Grains

Alkylresorcinol values of whole grains from different crop years are presented in Table I. The values for wheat were relatively low and very similar to those reported by Evans *et al.* (2). Average alkylresorcinol contents of the winter triticales TR 385 and TR 386 were in the same range as those of the wheat cultivars. Rye values, however, were considerably higher, while those of the spring triticales were intermediate.

Alkylresorcinol contents of 0.097% have been reported for Prolific rye (2). Under our climatic and agronomic conditions, a value of 0.110% was obtained, averaging the results from the 4 different crop years of this study. A statistical evaluation of the data included significant differences ($\alpha = 0.05$) in alkylresorcinol content between wheats (Chris, Colano, and the durum) and rye, the wheats and the spring triticales (6-TA-204 and 6-TA-206), and all triticales and the rye, but not between the wheats and the winter triticales TR 385 and TR 386.

Statistically significant differences ($\alpha = 0.05$) due to crop year were found only between the 1972-1973 and the 1974-1975 spring triticale 6-TA-204 and between the 1972 and the 1973-1975 Prolific rye samples.

Effect of Harvest Date

Alkylresorcinol content in kernels of Prolific rye and the spring triticales 6-TA-204 and 6-TA-206 from the 1974 crop, harvested at various stages of development, are shown in Fig. 2. Moisture contents of the grain kernels at time of harvest were used as an indicator of maturity.

Alkylresorcinols are present quite early in kernel development. Their

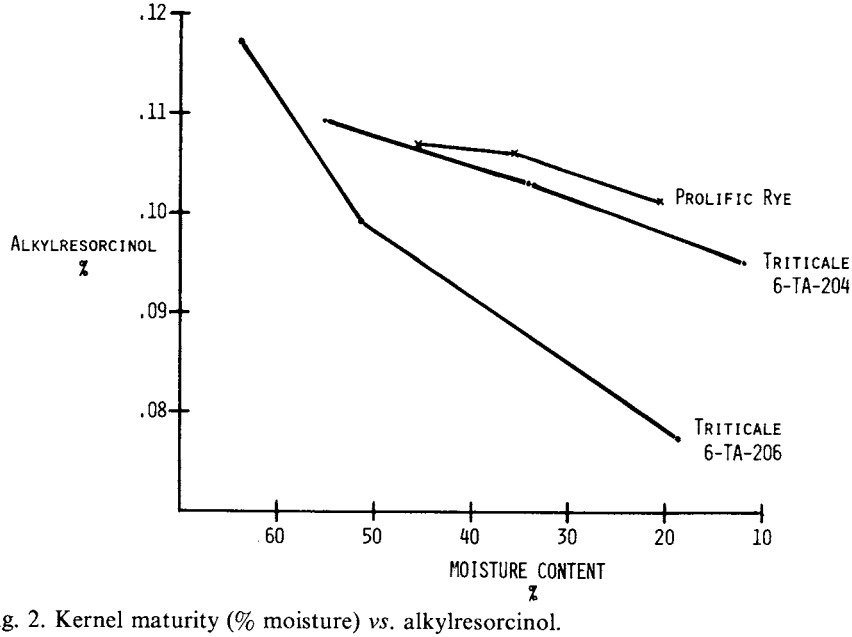

Fig. 2. Kernel maturity (% moisture) *vs.* alkylresorcinol.

amounts, expressed on a dry basis, decreased as kernel moisture decreased or as kernel maturity increased.

This apparent decrease in percentage of alkylresorcinols is a dilution effect, partly due to deposition of starch and protein in developing grain kernels, which causes an increase in solid matter.

Alkylresorcinols in Milling Fractions

Each of the cereal grains to be milled was conditioned to give optimum milling performance, defined as the highest flour extraction obtainable, as it is done commercially. Under those conditions, analyses of mill fractions—bran and flour—of Chris wheat, triticale 6-TA-204, and Prolific rye from the 1972 crop showed over 80% more alkylresorcinols in the bran than in the flour. The value of 0.187% for Prolific rye bran, as shown in Table II, appears to be low in relation to the triticale 6-TA-204 and Chris wheat values. This is due to the dilution effect of the bran by flour. The difficulties of milling triticale and rye on a Quadrumat mill to get a clean separation of bran from endosperm have been experienced by many researchers. Under optimum milling conditions for wheat and triticale, the percentages of bran always seem to be higher for triticale, which has been

TABLE II
Alkylresorcinols in Milling Fractions[a]
(1972 Crop)

	Milling Fractions, Quadrumat Jr. Mill			
	Bran		Flour	
Grain Sample	Milling fraction %	Alkylresorcinol %	Milling fraction %	Alkylresorcinol %
Wheat—Chris	21.7	0.211 ± 0.003	78.3	0.038 ± 0.002
Triticale 6-TA-204	30.0	0.271 ± 0.007	70.0	0.039 ± 0.009
Rye—Prolific	43.8	0.187 ± 0.008	56.2	0.029 ± 0.002

[a]Average of three separate determinations.

TABLE III
Alkylresorcinols in Milling Fractions[a]
(1973 Crop)

	Milling Fractions, Quadrumat Sr. Mill					
	Bran		Shorts		Flour	
Grain Sample	Milling fraction %	Alkyl-resorcinol %	Milling fraction %	Alkyl-resorcinol %	Milling fraction %	Alkyl-resorcinol %
Triticale 6-TA-204	27.7	0.213 ± 0.003	6.0	0.087 ± 0.001	64.8	0.026 ± 0.003
Rye—Prolific	33.6	0.186 ± 0.012	10.5	0.115 ± 0.001	52.0	0.031 ± 0.003

[a]Average of three separate determinations.

explained as the result of the shriveled condition of the triticale kernels.

Dissecting kernels of rye and analyzing the different fractions by tlc, Wieringa (1) detected alkylresorcinols in the combined pericarp and aleurone layers only and none in the endosperm or germ. Even with a flour extraction of only 56.2% for the rye, small amounts of the compounds were found, which is due to a greater sensitivity of the fluorometric method employed in this study compared to that of tlc. With the relatively low flour extraction for the rye, a complete separation of the pericarp and aleurone layers from the endosperm was assumed. It was concluded that the endosperm does contain a small amount of alkylresorcinols, not previously detected by less sensitive methods of analysis.

Wieringa (1) did not separate the rye pericarp from the aleurone layer for analysis. Therefore, to make a more precise statement as to the location of the highest percentage of alkylresorcinols in the grain kernel, samples of triticale 6-TA-204 and Prolific rye from the 1973 crop were milled on a Quadrumat Sr. mill to obtain bran, shorts, and flour fractions. Data of the analyses of these fractions are presented in Table III. Bran fractions contained the highest, shorts intermediate, and the flour fractions the lowest amounts of alkylresorcinols, which would indicate that a gradient exists with the highest amounts of the compounds in the pericarp, intermediate amounts in the aleurone layer, and relatively small but detectable amounts in the endosperm portion of cereal grain kernels.

Alkylresorcinols in Breads

The alkylresorcinol content of crust, crumb, and whole loaves baked from whole grain meals of Prolific rye, triticale 6-TA-204, and Chris wheat are shown in Fig. 3. Analyses of baking ingredients other than the whole grain meals did not indicate the presence of alkylresorcinols.

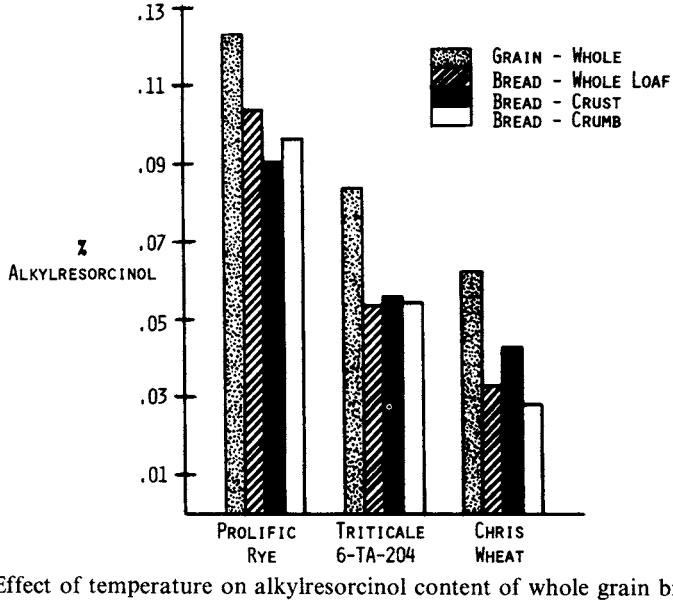

Fig. 3. Effect of temperature on alkylresorcinol content of whole grain breads.

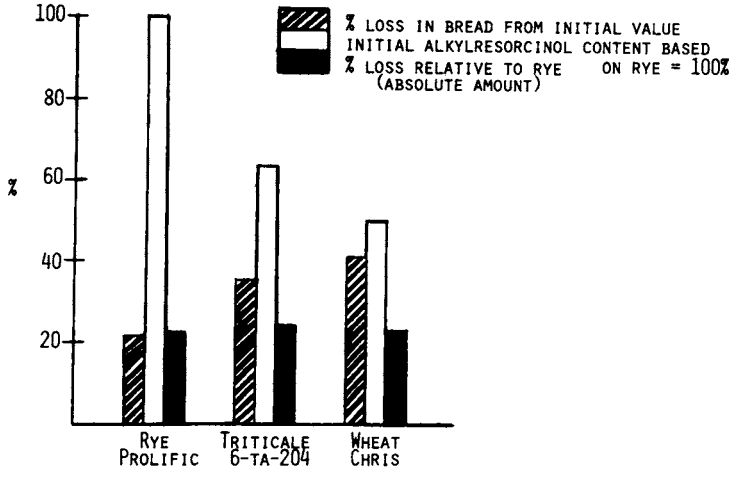

Fig. 4. Alkylresorcinol losses during the baking process.

There was a uniform loss of alkylresorcinols as shown for the triticale 6-TA-204 breads. The amounts of alkylresorcinols were lower in the breads and in the crusts and crumb portions of the breads than in the whole grain meal before baking. If temperature were a factor in the loss of these compounds, the crust of a loaf (which is exposed to a higher temperature and for a longer period of time at that temperature, than the crumb during the baking of bread) should consistently contain lower amounts of alkylresorcinols, which it did not. The small differences in alkylresorcinol content between crust, crumb, and the entire loaf of the Prolific rye and Chris wheat breads are believed to be due to sampling. Even though the ground and dried crusts and crumbs were blended thoroughly before an analysis sample was taken from each, this small sample could have contained either smaller or larger proportions of bran, which has the highest amounts of alkylresorcinols in grain kernels. It was concluded that the loss of alkylresorcinols was independent of the temperature reached during baking and probably occurred as the result of fermentation.

Percentage losses of alkylresorcinols during the baking process were lower the higher the initial amount in the whole grain meal, as illustrated in Fig. 4. However, when losses relative to rye were determined, it was clear that alkylresorcinol loss during the entire baking process was an absolute amount unrelated to the initial amount present in the whole grain meal, which led to the speculation that the loss occurs during fermentation. Absolute losses, under the conditions of this study, were 22% for rye, 24% for triticale 6-TA-204, and 23.5% for Chris wheat.

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