Alkylresorcinols in Extruded Cereal Brans

I. AL-RUQAIE and K. LORENZ[1]

Cereal Chem. 69(5):472–475

ABSTRACT

Rye, wheat, and triticale grains and their milling fractions were analyzed for alkylresorcinol content. Rye grain was the highest, triticale intermediate, and wheat grain the lowest in alkylresorcinol content. The bran of these cereals contained the highest amounts of alkylresorcinols, shorts intermediate amounts, and flour the lowest amounts. Extrusion reduced alkylresorcinol content in bran by 53.7–76.9%, depending on the type of cereal and extrusion conditions (moisture, barrel temperature, and screw speed) used. The highest reduction was observed in rye bran and the lowest in wheat bran, possibly due to differences in alkylresorcinol homolog composition. The alkylresorcinol content of extruded bran samples was still several times higher, however, than that found in flour samples of wheat, rye, and triticale. Human toxicity levels of these compounds have not yet been established.

A group of compounds known to inhibit growth in several animal species has been isolated from cereal grains (Wieringa 1967, Evans et al 1973, Pawlik 1979, Sedlet et al 1984) and identified as resorcinol derivatives with hydrocarbon chains at the fifth position. There is no established human toxicity level for these compounds. Cereal alkylresorcinols were found to be mixtures of saturated, monoenoic, and dienoic homologs with 13–29 carbon side chains (Wieringa 1967; Kozubek 1984, 1987; Seitz and Love 1987). The C21, C23, C25, C27, and C29 homologs were observed in rye oil (Briggs 1974). In wheat bran, the C25 and C27 components were found to be predominant (Wenkert et al 1964). Wieringa (1967) states that factors such as soil type, fertilization, and weather may influence the content of alkylresorcinols. Cereal grains grown under the same agronomic and climatic conditions during consecutive crop years showed only slight variations from year to year. However, Verdeal and Lorenz (1977) found that alkylresorcinol content decreases in rye and triticale as the grains mature. Alkylresorcinols are present quite early in kernel development, and the apparent decrease in proportion of alkylresorcinols may be a dilution effect, partly due to deposition of starch and protein in developing grain kernels. Generally, the amount of alkylresorcinols in cereals is highest in rye, lower in wheat and triticale, and very low in other cereals such as oats, barley, and corn (Verdeal and Lorenz 1977; Musehold 1978; Kozubek and Demel 1980; Hengtrakul et al 1990, 1991). However, some varieties of wheat have an alkylresorcinol content similar to that of rye.

By milling wheat, rye, and triticale into bran, shorts, and flour fractions and by subsequent analysis, Verdeal and Lorenz (1977) and Salek (1978) showed bran to contain the highest alkylresorcinol level. Intermediate values were found in the shorts, whereas the flour fractions produced relatively low values. This indicates 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.

Today, with the increased emphasis on higher fiber intake, cereal brans are being used in various food products, including high-fiber breakfast cereals. It is known that alkylresorcinol content is substantially reduced during baking because of fermentation and/or heat (Verdeal and Lorenz 1977, Weipert and El Baya 1977). It is not known whether the extrusion process, which is used to make many of our breakfast cereals, affects the alkylresorcinol content of cereals.

The purpose of this study was to investigate the effect of extrusion at different moistures, temperatures, and screw speeds on alkylresorcinol content of brans from wheat, rye, and triticale.

[1]Department of Food Science and Human Nutrition, Colorado State University, Fort Collins 80523.

MATERIALS AND METHODS

Grain Samples

The study included a composite of the rye varieties Maton and Bonel, grown on field plots at the Irrigated Desert Research Station, Brawley, CA, the wheat variety Vona, and a composite of the winter triticales TR385 and TR386, grown at the Colorado State University Agronomy Research Farm, Fort Collins. The grain samples (15 kg each) were milled into bran, shorts, and flour on a Quadrumat Sr. mill (C. W. Brabender, South Hackensack, NJ).

Proximate Analysis

Before analysis, all samples were ground in a micromill (The Lab Apparatus, Cleveland, OH) to 2.0-mm mesh. Moisture, protein, crude fat, and ash were determined according to AACC methods 44-15A, 46-13, 30-20, and 08-01, respectively (AACC 1983). Each analysis was done in triplicate.

Extruder

A single-screw Brabender Plasticorder extrusion model PL-V500 with a 19.05-mm barrel diameter, a 20:1 length-to-diameter ratio, and a 0.79- × 3.18-mm longitudinal groove was used. All samples were extruded using a screw with a compression ratio of 3:1. The barrel was equipped with two electrically heated, compressed-air-cooled collars controlled by thermostats. The extruder was equipped with a variable speed drive, allowing all samples to be extruded at two screw speeds, 100 and 150 rpm. A die with a 4.7-mm exit diameter opening was used in all trials. Torque was read from the torque indicator as each sample was extruded under various extrusion conditions.

Sample Conditioning Before Extrusion

After determination of moisture, samples of each bran were adjusted to 20 and 30% moisture by adding appropriate amounts of distilled water to the samples of bran in glass jars. The samples were allowed to equilibrate at room temperature (22° C) by shaking them for 5 min twice each day for three days before extrusion.

Extrusion Conditions

Replicate samples of wheat and rye bran were extruded at one set of barrel temperatures, 80°C at the feed section and 100°C at the compression section. Another group of replicate samples of wheat, rye, and triticale bran was extruded at a different set of barrel temperatures, 110°C at the feed section and 150°C at the compression section. Samples were extruded at two screw speeds, 100 and 150 rpm. Average residence times were 28 and 15 sec, respectively, at 100 and 150 rpm.

To check loss and recovery of synthetic 5-pentadecylresorcinol (Aldrich Chemical Co., Milwaukee, WI), three samples in replicates of rye bran alone, rye bran plus 0.1% 5-pentadecylresorcinol, and rye bran plus 0.2% 5-pentadecylresorcinol were extruded at one screw speed (150 rpm) with one set of barrel temperatures—110°C at the feed section and 150°C at the compression section—and a moisture content of 20%.

Fluorometric Determination of Alkylresorcinol Contents

The method used was a modification of that described by Verdeal and Lorenz (1977). A sample of 1.25 g of whole grain, shorts, flour, and bran before and after extrusion was soaked at room temperature with an equal amount of acetone by weight in a Pyrex screw-cap tube. After 24 hr, the extract was filtered through Whatman no. 1 paper and then saved. The sample was again immersed in the same amount of acetone and filtered after 24 hr. Filtrates were combined with previous extracts. Samples were dried and ground, soaked again with acetone in the proportion of 1:5, filtered after 24 hr, and added to the previous extract. The volume of collected extracts was made up to 25 ml with acetone. Two aliquots of 2.0 ml each were transferred to Teflon-lined Pyrex screw-cap tubes, and the acetone was removed in a water bath at 85°C. The residue was cooled to room temperature and then dissolved in 0.4 ml of chloroform. After the addition of 0.1 ml of 75% ethanol and 0.1 ml of 75% KOH, the tube was capped tightly, placed in a shaker bath at 45°C, and agitated every 2–3 min. After 20 min, 1.0 ml of distilled water was added, followed by 8.4 ml of 95% ethanol. The total volume of 10 ml was shaken and allowed to stand for 30 min. Before measuring the fluorescence on a Hitachi Perkin-Elmer MPF-2A spectrofluorometer (Perkin-Elmer, Norwalk, CT), the tube was shaken again. The excitation and emission wavelengths were 420 and 520 nm, respectively. A standard curve was prepared from 5-pentadecylresorcinol with each set of samples. All values are reported as means of eight determinations of two replicates. The method has been shown to have an excellent (96%) alkylresorcinol recovery (Al-Ruqaie 1991).

Statistical Design and Analysis

A factorial design was used to determine the effect of feed moistures, barrel temperatures, screw speeds, and their interaction on alkylresorcinol contents of the extruded brans. Analysis of variance was performed using the SAS General Linear Models procedure (SAS 1987). Tests of significance among treatment means were performed by Tukey's studentized range test.

RESULTS AND DISCUSSION

Alkylresorcinols in Milling Fractions and Bran Proximate Composition

Extraction percentages for wheat, rye, and triticale and alkylresorcinol contents for the fractions are given in Table I. As in previous studies (Verdeal and Lorenz 1977, Salek 1978, Seitz and Love 1987), bran fractions contained the highest levels of alkylresorcinols and flour contained very low amounts. These results support all previous studies that reported that alkylresorcinols are present mainly in the bran fraction and that rye contains higher levels of alkylresorcinols than wheat or triticale (Verdeal and Lorenz 1977, Tluscik 1978, Kaczmarek and Tluscik 1985).

TABLE I
Extraction Rates and Alkylresorcinol Contents of Whole-Grain Cereals and Their Milling Fractions

Cereal	Milling Fractions	Extraction (%)	Alkylresorcinols[a] (% db)
Rye	Whole grain	...	0.120 ± 0.002
	Bran	47.0	0.277 ± 0.003
	Shorts	1.9	0.164 ± 0.003
	Flour	51.1	0.017 ± 0.000
Wheat	Whole grain	...	0.045 ± 0.002
	Bran	28.0	0.188 ± 0.003
	Shorts	11.6	0.018 ± 0.002
	Flour	60.4	0.012 ± 0.003
Triticale	Whole grain	...	0.058 ± 0.002
	Bran	28.0	0.101 ± 0.001
	Shorts	9.5	0.049 ± 0.003
	Flour	60.3	0.006 ± 0.000

[a]Results are averages ± standard deviation of four determinations.

The proximate compositions of the bran samples, which were highest in alkylresorcinol content and therefore used in the extrusion experiments, are given in Table II.

Alkylresorcinols in Extruded Brans

Alkylresorcinol contents of rye bran, tempered to 20 and 30% moisture and extruded at two different sets of barrel temperatures with two screw speeds for each sample, are presented in Table III. Alkylresorcinol content was significantly reduced because of extrusion. Unextruded rye bran contained 0.277% alkylresorcinols. Alkylresorcinol content in extruded rye bran samples ranged from 0.064 to 0.074%, which is a loss of 73.3–76.9%. Substantial reductions in alkylresorcinol content due to fermentation and/or heat during baking (22–43%) have been reported by Verdeal and Lorenz (1977) and Weipert and El Baya (1977).

Extruded samples tempered to 20% moisture showed a higher reduction of alkylresorcinol content than the extruded samples with 30% moisture. Samples tempered to 30% moisture content passed through the extruder with less stress, as indicated by their torque values. Torque decreased with increase in moisture content and temperature. Overall, Tukey's test showed no significant differences between the means of the samples. The analysis of variance indicated that moisture did not affect alkylresorcinol content significantly ($P < 0.05$). The interaction of moisture with screw speed had a significant affect on alkylresorcinol content. Barrel temperature, moisture content, and other interactions had no effect on alkylresorcinol content.

Extrusion of triticale bran produced reductions in alkylresorcinols similar to those observed in rye bran. Alkylresorcinol contents of triticale bran, tempered to 20 and 30% moisture and extruded at two different screw speeds, are presented in Table IV. Alkylresorcinol content in extruded triticale bran samples ranged from 0.029 to 0.031%, which is a loss of 69.3–71.3%, compared with that of the unextruded triticale bran, which contained 0.101% alkylresorcinols. Alkylresorcinol content for samples extruded with 30% moisture was a little lower (0.029%) than that found in extruded samples tempered to 20% moisture. The differences, however, were not statistically significant. The analysis of variance indicated that screw speed, moisture content, and their interaction did not significantly affect alkylresorcinol content.

TABLE II
Proximate Composition of Brans[a]

Brans	Ash	Ether Extract	Nitrogen	Protein[b]
Wheat	4.3	3.76	2.02	11.52
Rye	3.0	3.34	2.02	11.52
Triticale	4.8	2.44	1.98	11.29

[a]Values are percent dry basis.
[b]N × 5.7.

TABLE III
Alkylresorcinol Content of Rye Bran Tempered to 20 and 30% Moisture and Extruded at Different rpms and Barrel Temperatures

Moisture Content (%)	rpm	Barrel Temperature[a] (°C)	Torque (lb-in.)	Alkylresorcinol Content[b] (% db)
20	100	80,100	255	0.064 ± 0.007 a
20	100	110,150	180	0.066 ± 0.008 a
20	150	80,100	270	0.069 ± 0.006 a
20	150	110,150	105	0.074 ± 0.008 a
30	100	80,100	115	0.074 ± 0.006 a
30	100	110,150	95	0.070 ± 0.009 a
30	150	80,100	150	0.071 ± 0.006 a
30	150	110,150	90	0.071 ± 0.007 a
Unextruded rye bran				0.277 ± 0.003 b

[a]Feed and compression section temperatures, respectively.
[b]Results are averages ± standard deviation of eight determinations. Means followed by the same letter are not significantly different.

Alkylresorcinol contents of wheat bran, tempered to 20 and 30% moisture and extruded at two different sets of barrel temperatures with two screw speeds for each sample, are presented in Table V. Again alkylresorcinol content was significantly reduced because of extrusion. Unextruded wheat bran contained 0.188% alkylresorcinols. Alkylresorcinol content in extruded wheat bran samples ranged from 0.061 to 0.087%, which is a loss of 53.7–67.6%. The percent loss of alkylresorcinols was less, however, than that found in extrusion experiments with rye and triticale bran, possibly in part because of a different alkylresorcinol homolog composition (Hengtrakul et al 1991). Alkylresorcinol homolog composition is very similar in rye and triticale but different in wheat. The presence of some gluten-forming proteins in the bran fraction of wheat also might have had an effect causing different viscoelastic properties of the extrudates compared with those of rye and triticale. Tukey's test of the means of wheat bran samples extruded at 20% moisture indicated no significant differences. However, samples extruded at 30% moisture content differed.

The reduction of alkylresorcinol content in samples tempered to 30% moisture content and extruded at both 100 and 150 rpm screw speeds was higher in samples extruded at the lower temperature (80 and 100°C). Lawton et al (1972) reported previously that during extrusion the greatest changes occurred when the two variables (moisture and temperature) were at extremes—either high moisture and low temperature (39% and 90°C, respectively) or low moisture and high temperature (27% and 150°C, respectively). In our study, reduction of alkylresorcinol content was most effective with 30% moisture content, barrel temperatures of 80 and 100°C, and screw speed of 100 rpm.

The analysis of variance of the variables affecting alkylresorcinol content in all extruded wheat bran samples indicated that moisture, screw speed, barrel temperature, and the interaction of moisture with barrel temperature were significantly affecting the alkylresorcinol content ($P < 0.05$).

TABLE IV
Alkylresorcinol Content of Triticale Bran Tempered to 20 and 30% Moisture and Extruded at Different rpms

Moisture Content (%)	rpm	Barrel Temperature[a] (°C)	Torque (lb-in.)	Alkylresorcinol Content[b] (% db)
20	100	110,150	180	0.030 ± 0.002 a
20	150	110,150	130	0.031 ± 0.001 a
30	100	110,150	80	0.029 ± 0.002 a
30	150	110,150	88	0.029 ± 0.002 a
Unextruded triticale bran				0.101 ± 0.001 b

[a]Feed and compression section temperatures, respectively.
[b]Results are averages ± standard deviation of eight determinations. Means followed by the same letter are not significantly different.

TABLE V
Alkylresorcinol Content of Wheat Bran Tempered to 20 and 30% Moisture and Extruded at Different rpms and Barrel Temperatures

Moisture Content (%)	rpm	Barrel Temperature[a] (°C)	Torque (lb-in.)	Alkylresorcinol Content[b] (% db)
20	100	80,100	290	0.081 ± 0.013 ab
20	100	110,150	105	0.075 ± 0.003 ab
20	150	80,100	280	0.079 ± 0.014 ab
20	150	110,150	65	0.086 ± 0.009 a
30	100	80,100	185	0.061 ± 0.008 c
30	100	110,150	125	0.079 ± 0.004 ab
30	150	80,100	133	0.070 ± 0.006 bc
30	150	110,150	128	0.087 ± 0.008 a
Unextruded wheat bran				0.188 ± 0.003 d

[a]Feed and compression section temperatures, respectively.
[b]Results are averages ± standard deviation of eight determinations. Means followed by the same letter are not significantly different.

TABLE VI
AR[a] Content of Rye Bran Spiked with 0.0, 0.1, and 0.2% AR[b]

AR Added (%)	AR Content in Unextruded Samples[c] (% db)	AR Content in Extruded Samples[c] (% db)	AR Loss (%)
0.2	0.477	0.133	72.1
0.1	0.377	0.110	70.8
0.0	0.277	0.074	73.3

[a]Alkylresorcinol.
[b]Samples were tempered to 20% moisture and extruded at 150 rpm and barrel temperatures of 110 and 150°C for feed and compression sections, respectively.
[c]Results are averages of eight determinations.

Alkylresorcinol Content of Spiked Extruded Rye Bran

Alkylresorcinol content data of rye bran spiked with 0.1 and 0.2% 5-pentadecylresorcinol, tempered to 20% moisture, and extruded at one set of barrel temperatures and one screw speed for each sample are presented in Table VI. The results showed that the percentages of alkylresorcinol content reduction in extruded samples are approximately the same with either synthetic or natural alkylresorcinols.

CONCLUSIONS

Extrusion reduced the alkylresorcinol content in bran by 53.7–76.9%, depending on the type of cereal bran and extrusion conditions (moisture, barrel temperature, and screw speed) used. However, the alkylresorcinol content of extruded bran samples was still several times higher than that found in flour samples of wheat, rye, and triticale. Human toxicity levels of these compounds, which have been shown to have detrimental effects in animal nutrition, have not yet been established.

LITERATURE CITED

AL-RUQAIE, I. M. 1991. Alkylresorcinols in brans and breakfast cereals. M.S. thesis. Colorado State University: Fort Collins.

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 08-01, approved April 1961, revised October 1976 and October 1981; Method 30-20, approved April 1961, revised October 1975, reviewed October 1982; Method 44-15A; Method 46-13, approved October 1976, reviewed October 1982, revised October 1986. The Association: St. Paul, MN.

BRIGGS, D. E. 1974. Hydrocarbons, phenols and sterols of the testa and pigment strand in the grain of *Hordeum distichon*. Phytochemistry 13:987-996.

EVANS, L. E., DEDIO, W., and HILL, R. D. 1973. Variability in the alkylresorcinol content of rye grain. Can J. Plant Sci. 53:485-488.

HENGTRAKUL, P., LORENZ, K., and MATHIAS, M. 1990. Alkylresorcinols in U.S. and Canadian wheats and flours. Cereal Chem. 67:413-417.

HENGTRAKUL, P., LORENZ, K., and MATHIAS, M. 1991. Alkylresorcinol homologs in cereal grains. J. Food Compos. Anal. 4:52-57.

KACZMAREK, J., and TLUSCIK, F. 1985. Variability of alkylresorcinol content in rye (*Secale cereale*) grains: A comparative analysis with several species of the genus *Triticum*. Genet. Pol. 25:349-358.

KOZUBEK, A. 1984. Thin-layer chromatographic mapping of 5-*n*-alk(en)ylresorcinol homologues from cereal grains. J. Chromatogr. 295:304-307.

KOZUBEK, A. 1987. The effect of 5-(*n*-alk(en)yl)resorcinols on membranes. I. Characterization of the permeability increase induced by 5-(-*n*-heptadecenyl)resorcinol. Acta Biochim. Pol. 34:357-367.

KOZUBEK, A., and DEMEL, R. A. 1980. Permeability changes of erythrocytes and liposomes by 5-(*n*-alk(en)yl) resorcinols from rye. Biochim. Biophys. Acta 603:220-227.

LAWTON, B. R., HENDERSON, G. A., and DERLATKA, E. I. 1972. The effect of extruder variables on the gelatinization of corn starch. Can. J. Chem. Eng. 50:168-172.

MUSEHOLD, J. 1978. Thin-layer chromatographic separation of 5-alkylresorcinol homologs from cereals. Z. Pflanzenzuecht. 80:326-332.

PAWLIK, J. 1979. Growth of chickens as influenced by alkylresorcinols of rye. Rocz. Nauk. Zootech. Monogr. Rozprawy. 13:121-136.

SALEK, M. 1978. Determination of 5-alkylresorcinol contents in rye grain and milling products. Rocz. Panstw. Zakl. Hig. 29:205-211.

SAS INSTITUTE, INC. 1987. SAS Program for Microcomputers, version 6 ed. The Institute: Cary, NC.
SEDLET, K., MATHIAS, M., and LORENZ, K. 1984. Growth-depressing effects of 5-*n*-pentadecylresorcinol: A model for cereal alkylresorcinols. Cereal Chem. 61:239-241.
SEITZ, L. M., and LOVE, G. R. 1987. Identification and determination of alkylresorcinols and related compounds in cereal grains and milling fractions. (Abstr.) Cereal Foods World 32:651.
TLUSCIK, F. 1978. Localization of alkylresorcinols in rye and wheat caryopses. Acta Soc. Bot. Pol. 47:211-218.
VERDEAL, K., and LORENZ, K. 1977. Alkylresorcinols in wheat, rye, and triticale. Cereal Chem. 54:475-483.
WEIPERT, D., and EL BAYA, A. 1977. 5-Alkylresorcin in Getreide und Getreideprodukten. Getreide Mehl Brot 9:225-229.
WENKERT, E., LOESER, E. M., MAHAPATRA, S. N., SCHENKER, F., and WILSON, E. M. 1964. Wheat bran phenols. J. Org. Chem. 29:435-439.
WIERINGA, G. A. 1967. On the occurrence of growth inhibiting substances in rye. Publication 156. Inst. Storage Process. Agric. Produce: Wageningen, Netherlands.

[Received December 23, 1991. Revision received February 25, 1992. Accepted March 3, 1992.]