

Effects of Fermentation and Baking of Whole Wheat and Whole Rye Sourdough Breads on Cereal Alkylresorcinols

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ABSTRACT

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Alkylresorcinol (AR) content was determined in multiple-stage whole wheat and whole rye flour sours, as well as in whole wheat and whole rye flour doughs and breads. AR content decreased considerably during fermentation and baking. AR content was reduced by 20 and 46%, respectively, at the end of sourdough starter fermentation of whole wheat

and whole rye flour sours. AR content, which was 512 and 210 µg/g in whole rye and whole wheat flour doughs, respectively, was 30 and 0 µg/g, respectively, after baking of breads. Synthetic AR added at different levels to doughs was also greatly reduced during fermentation and baking.

Alkylresorcinols (AR) are amphiphilic phenolic lipids found in plants from a number of different families notably the *Anacardiaceae* (such as poison ivy, cashew nut shell, poison oak), *Proteaceae* (such as *Grevilla pyramidalis*), *Gramineae* (such as cereal grains), *Araceae* (such as *Philodendron scandens*), and certain bacterial and algae sources (Madrigal et al 1977, Tyman 1979, Reffstrup et al 1982, Reffstrup and Boll 1985, Bandyopadhyay et al 1985, Hengtrakul et al 1990).

Wheat, rye, and triticale grains contain 5-*n*-alkylresorcinols with odd-number side chains of C15–C25 (Wenkert et al 1964, Wieringa 1967, Verdeal and Lorenz 1977, Gohil et al 1988, Lorenz and Hengtrakul 1990). A minor group of AR has also been reported (Wieringa 1967) with apparent side-chain lengths of C17–C25 (Gohil et al 1988). Kozubek (1984) reported separations of 5-*n*-alk(en)ylresorcinol homologs from rye and wheat grains by using silica gel thin-layer chromatography (TLC). Kozubek (1985) described a preparative-scale isolation of 5-*n*-alkyl-, 5-*n*-alkenyl, and 5-*n*-alkadienyl-resorcinol homologs from rye grain. Several homologs of 5-(2-oxoalkyl)-, and 5-(2-oxoalkenyl) resorcinols were identified in extracts of wheat and rye grains by Seitz (1992). Homologs of the 5-(2-oxoalkenyl) resorcinols included 5-(2-oxononadecyl)-, 5-(2-oxoheneicosanyl)-, 5-(2-oxotricosanyl)-, and 5-(2-oxopentacosanyl)-, resorcinol, with the heneicosanyl and tricosanyl homologs being predominant. The homologs of 5-(2-oxoalkenyl) resorcinols consisted of 5-(2-oxoheneicosanyl)- and 5-(2-oxotricosanyl)- resorcinol. The major alkyl and alkenyl homologs were isolated by TLC and high-performance liquid chromatography (HPLC), and then identified by TLC, HPLC, and gas chromatography coupled with infrared and mass spectroscopy, and proton magnetic resonance spectroscopy.

The amount of AR 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; Hengtrakul et al 1990, 1991). However, some varieties of wheat have an AR content similar to that of rye. Analysis subsequent to milling of wheat, rye, and triticale into bran, shorts, and flour fractions by Verdeal and Lorenz (1977) and Salek (1978) showed that the bran contained the highest levels of AR.

There is no established human toxicity level for these compounds. AR were shown to induce potassium release from erythro-

cytes and liposomes (Kozubek and Demel 1980) and to change the properties of the hydrophobic environment of the membrane (Kozubek et al 1988). AR also possess hemolytic activity, which is temperature-dependent and proportional to the side-chain unsaturation, and inversely proportional to the chain length (Kozubek 1984, 1987). Naturally occurring AR were found to mediate DNA strand scission (Scannel et al 1988).

AR content in cereals and cereal products is reduced during extrusion (Al-Ruqaie and Lorenz 1992) and baking processes (Verdeal and Lorenz 1977, Weipert and El Baya 1977). The purpose of this study was to determine the effect of fermentation on AR content in multiple-stage sours, doughs, and breads.

MATERIALS AND METHODS

Whole Grain Rye and Wheat Samples

Whole grain flour of rye and wheat were prepared by grinding rye and wheat grain samples in a laboratory mill (model 4, Thomas-Wiley, Philadelphia) with a mesh size of 0.5 mm. The hard red winter wheat was a 1994 Colorado composite from Fort Collins, and the rye sample was grown in Wray, CO, during the 1994 crop year.

Multiple-Stage Rye and Wheat Sours

A sourdough starter was prepared as described by Sultan (1965). A multiple-stage sour was then prepared using the overnight procedure as outlined by Lorenz (1983). Samples (2 g) were taken from the starter, to which 6 g of whole grain flour (wheat or rye) and 8 mL of water (Anfrischsour stage) were added. This was allowed to ripen for 6 hr at 26°C and 92% rh. At the end of the ripening time, the Anfrischsour was increased to a basic sour by adding 12 g of whole grain rye or wheat flour and 8 mL of water. Ripening time was again 6 hr at 26°C and 92% rh. The full sour was prepared by the addition of 162 g of whole grain rye or wheat flour and 128 mL of water with a third ripening time of 6 hr at 26°C and 92% rh. At the final step, 220 g of whole grain wheat or rye flour and 156 mL of water were added. At each stage, the initial and final pH were measured, and 16-g samples of sour were taken at the end of each sour stage. The sour dough samples were frozen and then placed into a freeze dryer (Labconco, Kansas City, MO). Dried samples were used for AR determination.

Whole Rye and Whole Wheat Flour Doughs and Breads

Doughs were mixed without and with added synthetic AR. Synthetic AR (5-*n*-pentadecylresorcinol) was incorporated into vegetable oil at concentrations of 0.05, 0.075, and 0.1% by weight of the flour and added to the doughs (rye and wheat). The formu-

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TABLE I
Average Alkylresorcinol (AR) Concentration and pH in Sours and Dough^{a,b}

Stages of Sour Development	Whole Wheat Flour		Whole Rye Flour	
	AR Concentration	pH	AR Concentration	pH
Whole wheat meal	250.0 ± 2.8a	6.01 ± 0.01aA	550.0 ± 2.8a	6.23 ± 0.03aA
Starter sour	200.0 ± 5.7b	4.91 ± 0.01bB	310.0 ± 5.7b	4.77 ± 0.05bB
Anfrischsour	210.0 ± 5.7b	5.38 ± 0.07bC	324.0 ± 2.8b	5.46 ± 0.04bC
Basic sour	224.0 ± 2.8b	5.27 ± 0.13bD	310.0 ± 2.8b	5.35 ± 0.05bD
Full sour	230.0 ± 5.7b	4.89 ± 0.01bE	300.0 ± 2.8b	4.86 ± 0.02bE
Dough	228.0 ± 2.8b	4.52 ± 0.03bF	274.0 ± 5.7b	4.21 ± 0.07bF

^a Values (µg/g, dry basis) ± standard deviation.

^b Means with common letters represent no statistical difference ($P < 0.05$). Lower case letters are used for statistical comparison between the control and each sour stage for AR concentration and pH using least significant difference. Upper case letters are used for statistical comparison between pH values and each sour stage. All measurements were made at the end of each stage.

TABLE II
Alkylresorcinol (AR) Concentration of Whole Wheat Flour Dough During Fermentation and in Bread After Baking^{a,b}

Treatment	Initial	Fermentation Treatment of Dough				Bread
		1 hr	2 hr	3 hr	4 hr	
Control	211	28 ± 4aA	30 ± 0aA	25 ± 1aA	14 ± 0bA	0 ± 0cA
AR (%)						
0.050	710	72 ± 0aB	60 ± 3bB	60 ± 3bB	38 ± 1cB	3 ± 0dA
0.075	960	110 ± 6aC	70 ± 0bC	64 ± 5bB	40 ± 2cB	4 ± 0dA
0.100	1,210	140 ± 14aD	90 ± 0bD	88 ± 6bC	44 ± 6cB	5 ± 1dA

^a Values (µg/g, dry basis) ± standard deviation. Initial concentration of the whole wheat flour and dough were calculated as 250 and 210.5 µg/g (dry basis), respectively.

^b Means with common letters represent no statistical differences ($P < 0.05$). Lowercase letters are used for statistical comparison between each hour of fermentation and bread within each treatment using least significant difference (LSD). Uppercase letters are used for statistical comparison of different treatments within each fermentation hour and bread.

TABLE III
pH ± Standard Deviation at Each Stage of Fermentation of Whole Wheat Flour Doughs^a

Treatment	Initial	Fermentation Treatment of Dough			
		1 hr	2 hr	3 hr	4 hr
Control	5.96 ± 0.02aA	5.76 ± 0.01bA	5.62 ± 0.03cA	5.63 ± 0.02cA	5.51 ± 0.07dA
AR (%) ^b					
0.050	5.76 ± 0.04aB	5.61 ± 0.03bB	5.56 ± 0.02bA	5.57 ± 0.02bA	5.38 ± 0.12cB
0.075	5.73 ± 0.02aB	5.58 ± 0.01bB	5.46 ± 0.09cB	5.46 ± 0.08cB	5.46 ± 0.02cC
0.100	5.74 ± 0.02aB	5.45 ± 0.06bC	5.49 ± 0.02bB	5.45 ± 0.02bB	5.37 ± 0.06cB

^a Means with common letters represent no statistical differences ($P < 0.05$). Lowercase letters are used for statistical comparison between each hour of fermentation within each treatment using least significant difference (LSD). Uppercase letters are used for statistical comparison of different treatments within each fermentation hour.

^b Alkylresorcinol concentration.

lation for whole wheat doughs was: flour (100%), yeast (3%), yeast food (0.5%), salt (2.25%), sugar (6%), shortening (3%), nonfat dry milk (4%), and water. The formulation for whole rye doughs was: flour (100%), yeast (2.5%), yeast food (0.25%), salt (2.5%), shortening (2%), and water. Doughs were mixed (N-50 mixer, Hobart, Troy, OH), then fermented at 26°C and 92% rh for 1, 2, 3, or 4 hr. Each hour, the pH of doughs was measured, and 16 g of dough was taken and immediately frozen. Breads were baked at 218°C for 18 min after 4 hr of fermentation. The samples (doughs and breads) were frozen prior to freeze drying. Freeze-dried samples were ground using a micro mill (Lab Apparatus, Cleveland, OH) to a particle size of 500–600 µm. The samples were stored in tightly closed container for AR determinations.

Fluorometric Determination of AR

The method used was a modification of that described by Verdeal and Lorenz (1977) and Al-Ruqaie and Lorenz (1992). Samples (2.5 g) of the whole grain flours as well as freeze-dried sours, doughs, and breads were combined at room temperature with an equal weight of acetone in a Pyrex screw-cap tube. After 24 hr, the extract was filtered through No. 4 Whatman paper and saved. The sample was again immersed in the same amount of acetone and filtered after another 24 hr. Filtrates were combined with previous extracts. Samples were dried and soaked again with acetone

(1:5, sample to acetone, by weight) and filtered after 24 hr. This filtrate was added to the previous extracts. 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 acetone was evaporated by heating in an 85°C water-bath. The residue was cooled to room temperature and dissolved in 0.4 mL of chloroform. Following this, 0.1 mL of 75% ethanol and 0.1 mL of 75% KOH were added; the tube was then tightly capped, placed in a shaker bath at 45°C, and agitated every 2–3 min. After 20 min, 1.0 mL of distilled water and 8.4 mL of 95% ethanol were added to each tube. The total volume of 10 mL was shaken and allowed to stand for 30 min. The tubes were shaken again before measuring the fluorescence on a spectrofluorometer (Hitachi Perkin-Elmer MPF-2A, Perkin-Elmer, Norwalk, CT). The excitation and emission wavelengths were 420 and 520 nm, respectively. A standard curve was prepared from 5-*n*-pentadecyl-resorcinol (Aldrich Chemical, Milwaukee, WI) with each set of samples. This method has an excellent (96%) AR recovery (Al-Ruqaie 1991, Al-Ruqaie and Lorenz 1992).

Statistical Design and Analysis

Completely randomized designs were used to determine the effects of fermentation (stage of sour development or dough processing) and baking on the AR content of whole rye or whole

TABLE IV
Alkylresorcinol Concentration (AR) ± Standard Deviation of Whole Rye Flour Dough During Fermentation and After Baking^{a,b}

Treatment	Initial	Fermentation Treatment of Dough				Bread
		1 hr	2 hr	3 hr	4 hr	
Control	513	150 ± 4aA	120 ± 0bA	80 ± 3cA	40 ± 6dA	29 ± 2eA
AR (%)						
0.050	1,013	182 ± 3aB	170 ± 10bB	160 ± 3bB	76 ± 3cB	40 ± 1dB
0.075	1,263	230 ± 6aC	176 ± 1bB	162 ± 6cB	80 ± 9dB	50 ± 3eB
0.100	1,513	264 ± 3aD	200 ± 14bC	185 ± 2cC	140 ± 17dC	53 ± 0eB

^a Values (µg/g, dry basis) ± standard deviation. Initial concentration of the whole rye flour and dough were calculated as 550 and 512.8 µg/g (dry basis), respectively.

^b Means with common letters represent no statistical differences ($P < 0.05$). Lowercase letters are used for statistical comparison between each hour of fermentation and bread within each treatment using least significant difference (LSD). Uppercase letters are used for statistical comparison of different treatments within each fermentation hour and bread.

TABLE V
pH ± Standard Deviation at Each Stage of Fermentation of Whole Rye Flour Doughs^a

Treatment	Initial	Fermentation Treatment of Dough			
		1 hr	2 hr	3 hr	4 hr
Control	5.73 ± 0.04 a A	5.65 ± 0.02 b A	5.54 ± 0.08 c A	5.66 ± 0.05 d A	5.53 ± 0.03 e A
AR (%) ^b					
0.050	5.53 ± 0.02 a B	5.46 ± 0.02 a B	5.36 ± 0.06 b B	5.36 ± 0.05 b B	5.34 ± 0.04 b B
0.075	5.55 ± 0.02 a B	5.46 ± 0.01 b B	5.37 ± 0.06 c B	5.33 ± 0.03 c B	5.28 ± 0.03 c B
0.100	5.61 ± 0.05 a B	5.38 ± 0.07 b C	5.39 ± 0.08 b B	5.36 ± 0.07 b B	5.34 ± 0.01 b B

^a Means with common letters represent no statistical differences ($P < 0.05$). Lowercase letters are used for statistical comparison between each hour of fermentation within each treatment using least significant difference (LSD). Uppercase letters are used for statistical comparison of different treatments within each fermentation hour.

^b Alkylresorcinol concentration.

wheat flours and doughs. All results are the average values of two replicates (duplicate extractions with duplicate aliquots). Tests of significance among treatment means were performed by least significant difference (LSD). All of the data were analyzed by using the SAS system (SAS 1987).

RESULTS AND DISCUSSION

Effect of Sourdough Fermentation on AR Content

Table I shows the effects of fermentation of multiple-stage whole wheat flour sour and whole rye flour sour on AR content. AR concentrations were 250 µg/g in whole wheat flour and 550 µg/g in whole rye flour, which are slightly lower than the range of values reported in the literature (Evans et al 1973; Verdeal and Lorenz 1977; Gohil et al 1988; Hengtrakul et al 1990, 1991). Differences in AR content are due to grain variety, maturity, climatic and soil conditions (Wieringa 1967, Hengtrakul et al 1990).

AR content and pH decrease during preparation of the starter. They increase at the Anfrischsour stage due to flour addition, but do not reach the levels determined in the flour. In whole wheat flour doughs, AR content also increased slightly again at the basic and full sour stages due to the rather large amounts of flour added.

Basic and full sour stages of whole rye flour sours show a progressive decrease in AR content and pH, even though substantial amounts of flour with an AR content of 550 µg/g are added at the beginning of these sour stages. The reduction in AR content due to fermentation was statistically significant ($P < 0.05$) comparing AR content in the flour with the AR content of sours at each multiple sour stage.

AR Concentration of Dough During Fermentation and After Baking

Tables II and III show AR concentration and pH values of whole wheat flour dough during fermentation and after baking. AR concentration was reduced during 1 hr of fermentation, as was the pH. The initial concentration of AR in the dough was 210.5 µg/g. Other baking ingredients did not contain AR. In the control, AR content was reduced by ≈86% during 2 hr of fermentation. There was a further reduction as the fermentation progressed. AR

content was reduced by 88–93% during 3–4 hr of fermentation. After the bread was baked, no AR was detected in the control bread. Similar reductions in AR were observed when additional synthetic AR was added to the doughs.

Verdeal and Lorenz (1977) reported a 23.5% reduction of AR in baking of a whole wheat bread, but they suspected the reduction was mainly due to fermentation rather than just baking. Resorcinol compounds sublime at temperatures of 196–250°C (CRC 1993); baking temperature was 218°C. Our results show that fermentation indeed reduces AR concentration.

Tables IV and V show the AR concentration and pH values during fermentation and after baking of the whole rye flour doughs. For the control, AR content was reduced significantly ($P < 0.05$) after 1 hr of fermentation. The whole rye dough contained 512.8 µg/g of AR. AR concentration was zero in all other baking ingredients. In the control, AR concentration was reduced by 71, 77, 85, and 92% during the first, second, third and fourth hour of fermentation, respectively. After baking, the AR concentration was reduced by 94%. Weipert and El Baya (1977) reported reduction in AR content of 86% during fermentation and baking of rye bread produced by a sourdough process. The results were similar for doughs with added AR with reduction of AR during fermentation and after baking being statistically significant ($P < 0.05$).

CONCLUSIONS

Both fermentation and the baking process cause a reduction of the AR content of whole wheat and whole rye doughs and bread. AR content was reduced at the end of the sourdough starter fermentation of whole wheat and whole rye flour sours by 20 and 46%, respectively. AR content was 512 µg/g in whole rye and 210 µg/g in whole wheat flour doughs; AR content was 30 and 0 µg/g, respectively, after baking.

LITERATURE CITED

- Al-Ruqaie, I. M. 1991. Alkylresorcinols in brans and breakfast cereals. MS thesis. Colorado State University: Fort Collins, CO.
Al-Ruqaie, I., and Lorenz, K. 1992. Alkylresorcinols in extruded cereal

- brans. *Cereal Chem.* 69:472-475.
- Bandyopadhyay, C., Gholap, A. S., and Mamdapur, V. R. 1985. Characterization of alkenyl resorcinol in mango (*Mangifera indica* L.). *J. Agric. Food Chem.* 33:377-379.
- CRC. 1993. *Handbook of Chemistry and Physics*, 74th ed. CRC Press: Boca Raton, FL.
- 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.
- Gohil, S., Pettersson, D., Salomonsson, A. C., and Aman, P. 1988. Analysis of alkyl- and alkenylresorcinols in triticale, wheat and rye. *J. Sci. Food Agric.* 45:43-52.
- Hengtrakul, P., Lorenz, K., and Mathias, M. 1990. Alkylresorcinol 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 Comp. Anal.* 4:52-57.
- Kozubek, A. 1984. Thin-layer chromatographic mapping of 5-*n*-alk(en)ylresorcinol homologues from cereal grains. *J. Chromatogr.* 295:304-307.
- Kozubek, A. 1985. Isolation of 5-*n*-alkyl-, 5-*n*-alkenyl-, and 5-*n*-alkdienylresorcinol homologs from rye grains. *Acta Aliment. Pol.* 9:185-197.
- 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. Poloniae* 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.
- Kozubek, A., Jezierski, A., and Sikorski, A. F. 1988. The effect of nonadec(en)ylresorcinol on the fluidity of liposome and erythrocyte membranes. *Biochim. Biophys. Acta* 944:465-475.
- Kubus, G., and Tluscik, F. 1983. Alkylresorcinols in grains from plants from the family *Graminea*. *Acta Soc. Bot. Poloniae* 52:223-230.
- Lorenz, K. 1983. Sourdough processes—Methodology and biochemistry. *Baker's Dig.* 47(4):41-45.
- Lorenz, K., and Hengtrakul, P. 1990. Alkylresorcinols in cereal grains—Nutritional importance and methods of analysis. *Lebensm. Wiss. Technol.* 23:208:215.
- Madrigal, R. V., Spencer, G. F., Plattner, R. D., and Smith, C. R., JR. 1977. Alkyl and alkenyl resorcinols in *Rapanea laetevirens* seed lipids. *Lipids* 12:402-406.
- Reffstrup, T., Hammershoy, O., Boll, P. M., and Schmidt, H. 1982 *Philodendron scandens* Koch et Sello subsp. *Oxycardium* (Schott) Bunting, a new source of allergenic alkylresorcinols. *Acta Chem. Scand.* B36:291-294.
- Reffstrup, T., and Boll, P. M. 1985. Allergenic 5-alkyl- and 5-alkenylresorcinols from *Philodendron* species. *Phytochemistry* 24(11):2563-2565.
- Salek, M. 1978. Determination of 5-alkylresorcinol contents in rye grain and milling products. *Roczniki Panstwowego Zakladu Higieny* 29:205-211.
- SAS. 1987. *Program for Microcomputers*, vers. 6. The Institute: Cary, NC.
- Scannel, R. T., Barr, J. R. and Murty, V. S. 1988. DNA strand scission by natural occurring 5-alkylresorcinols. *J. Am. Chem. Soc.* 110:3650-3651.
- Seitz, L. M. 1992. Identification of 5-(2-oxoalkyl) resorcinols and 5-(2-oxoalkenyl) resorcinols in wheat and rye grains. *J. Agric. Food chem.* 40:1541-1546.
- Sultan, W. J. 1965. *Practical Baking*. Avi: Westport, CT.
- Tyman, J. H. P. 1979. Non-isoprenoid long chain phenols. *Chem. Soc. Rev.* 8:499-512.
- Verdeal, K., and Lorenz, K. 1977. Alkylresorcinols in wheat, rye, and triticale. *Cereal Chem.* 54:475-483.
- Wenkert, E., Loeser, E. M., Mahapatra, S. N., Schenker, F., and Wilson, E. M. 1964. Wheat bran phenols. *J. Org. Chem.* 29:435-439.
- Weipert, D. and El Baya, A. 1977. 5-Alkylresorcin in Getreide und Getreide-produkten. *Getreide Mehl Brot* 9:225-229.
- Wieringa, G. A. 1967. On the occurrence of growth inhibiting substances in rye. Publication 156. Institute for Storage and Processing of Agricultural Produce: Wageningen, The Netherlands.

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