

## Alkylresorcinol Content and Homologue Composition in Durum Wheat (*Triticum durum*) Kernels and Pasta Products

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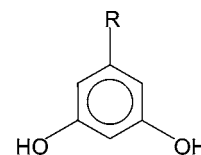
The total alkylresorcinol (AR) content and relative homologue composition of 21 durum wheat (*Triticum durum*) kernel samples, as well as 5 pasta products and the corresponding flour mixtures, were determined. Durum wheat contained on average 455  $\mu\text{g/g}$  ARs, and the average relative homologue composition was C17:0 (0.4%), C19:0 (14%), C21:0 (58%), C23:0 (21%), and C25:0 (6.5%). The homologue composition was found to be relatively consistent among samples, with durum wheat being different from common wheat by having a higher proportion of the longer homologues. No differences in content or homologue composition were observed in pasta products compared to flour ingredients, showing that alkylresorcinols are stable during pasta processing. The ratio of the homologues C17:0 to C21:0 was  $\leq 0.02$  for whole grain durum wheat products, which is different from those of common wheat (0.1) and rye (0.9).

**KEYWORDS:** Alkylresorcinols; durum wheat; *Triticum durum*; whole grain; pasta; cereals; biomarker

### INTRODUCTION

Alkylresorcinols (ARs) represent a group of phenolic lipids found in high contents in the outer parts of wheat and rye kernels (1). They are generally not found in refined flour or in refined products from cereals or in any other commonly consumed food (2). Chemically, they comprise 1,3-dihydroxy-5-alkylbenzene homologues with odd-numbered, mainly saturated hydrocarbon side chains in the range of 15–25 carbon atoms (C15:0 is present in only small amounts in rye) (Figure 1). ARs have been shown to be absorbed by humans, rats, and pigs, and methods for their analysis in cereal products, plasma, and erythrocytes have been developed in recent years (1–5). ARs are of interest both for their suggested physiological effects, often attributed to their amphiphilic nature [see reviews (5–7)], and for their possible use as specific biomarkers for whole grain wheat and rye intake (5).

Wheat and rye, commonly used in Swedish cereal foods, have previously been analyzed (2, 8, 9). Foods containing whole grain durum wheat have not been commonly available in the Swedish market. However, with the whole grain trend in Sweden and other countries, the consumption of pasta, with whole grain durum wheat, is rapidly increasing. The content and relative homologue composition of ARs of durum wheat have only been reported in a few samples using different extraction procedures and analysis techniques (10–13). In one study conducted recently by Zarnowski et al. (13), homologue composition and total AR content were estimated after acetone extraction in one sample of durum wheat grown in two different years. The average AR content was estimated to  $\sim 200 \mu\text{g/g}$  by using a



Alkylresorcinol	Abbreviation used	R	Molecular weight (g/mole)
5-n-Heptadecylresorcinol	(C17:0)	C <sub>17</sub> H <sub>35</sub>	348
5-n-Nonadecylresorcinol	(C19:0)	C <sub>19</sub> H <sub>39</sub>	376
5-n-Heneicosylresorcinol	(C21:0)	C <sub>21</sub> H <sub>43</sub>	404
5-n-Tricosylresorcinol	(C23:0)	C <sub>23</sub> H <sub>47</sub>	432
5-n-Pentacosylresorcinol	(C25:0)	C <sub>25</sub> H <sub>51</sub>	460

**Figure 1.** Structures of alkylresorcinols (ARs) commonly found in cereals.

colorimetric method with Fast blue, and the relative homologue composition was determined by gas chromatography–mass spectrometry (GC-MS) as C15:0 (0.3%), C17:0 (0.8%), C19:0 (15.8%), C21:0 (71.8%), C23:0 (11.1%), and C25:0 (0.1%).

The aim of this study was to investigate the content and relative homologue composition of ARs in durum wheat used in Sweden and pasta products containing whole grain durum wheat. Such information is necessary to make accurate estimation of intake levels, which in turn could be compared to levels in biological samples during the validation of ARs as biomarkers for whole grain intake of wheat and rye.

### MATERIALS AND METHODS

**Cereals and Cereal Products.** Twenty-one samples of durum wheat (*Triticum durum*) kernels, grown in Austria, France, Kazakhstan, Russia, Spain, and Sweden in the years 2003 and 2004, were provided by

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**Table 1.** Total Alkylresorcinol (AR) Content, Relative Homologue Composition, and Ratio of C17:0 to C21:0 in Samples of Durum Wheat Kernels Obtained from Different Countries

country	cultivar <sup>a</sup>	total AR <sup>b</sup> ( $\mu\text{g/g}$ of DM)	homologue composition (%)					ratio C17:0/ C21:0
			C17:0	C19:0	C21:0	C23:0	C25:0	
Austria	unknown	251	0.5	13.8	59.1	18.9	7.7	0.009
Austria	unknown	357	0.3	13.4	59.0	21.2	6.0	0.005
Austria	unknown	376	0.5	13.2	60.5	20.1	5.8	0.008
Austria	unknown	404	0.4	13.3	59.5	20.6	6.1	0.007
Austria	unknown	426	0.3	12.7	57.3	22.1	7.6	0.006
Austria	unknown	431	0.5	15.8	61.6	17.6	4.4	0.009
Austria	unknown	475	0.5	18.9	59.1	17.1	4.3	0.009
France	France Granit	467	0.4	15.1	61.9	18.6	4.0	0.007
France	Soccomac soufflet	447	0.3	13.7	59.2	21.4	5.4	0.006
Kazakstan	unknown	488	0.3	15.2	52.4	23.9	8.2	0.007
Kazakstan	unknown	555	0.4	13.9	51.6	24.9	9.2	0.007
Kazakstan <sup>c</sup>	unknown	618	0.3	15.9	48.1	24.7	11.0	0.005
Russia	Transcon	483	0.3	10.8	56.2	24.6	8.1	0.005
Spain	Leycegra	401	0.5	15.5	60.4	18.9	4.7	0.008
Spain <sup>c</sup>	unknown	492	0.4	15.7	61.0	18.5	4.3	0.007
Spain	unknown	526	0.4	17.0	59.0	19.3	5.3	0.008
Sweden	unknown	407	0.5	12.7	58.7	21.5	6.6	0.009
Sweden	Ambrodur <sup>d</sup>	470	0.5	13.8	59.1	18.9	7.7	0.009
Sweden	Ambrodur <sup>d</sup>	494	0.4	10.8	60.4	21.1	7.3	0.007
Sweden	Topdur <sup>e</sup>	494	0.4	10.8	60.4	21.1	7.3	0.007
Sweden	Topdur <sup>e</sup>	490	0.4	12.5	59.1	21.0	7.0	0.007
mean $\pm$ SD		455 $\pm$ 77	0.4 $\pm$ 0.1	14.0 $\pm$ 2.1	58.3 $\pm$ 3.5	20.8 $\pm$ 2.3	6.5 $\pm$ 1.8	0.007 $\pm$ 0.001

<sup>a</sup> Information of cultivar was not available for all samples. <sup>b</sup> The analytical precision expressed as coefficient of variation (CV) of total AR content was always below 6%. <sup>c</sup> Peak identity confirmed by GC-MS. <sup>d</sup> Fertilized with 180 kg of N/ha. <sup>e</sup> Fertilized with 100 kg of N/ha.

**Table 2.** Total Alkylresorcinol (AR) Content, Relative Homologue Composition, and Ratio of C17:0 to C21:0 in Whole Grain Durum Wheat Pasta and Corresponding Flour Ingredients

pasta product <sup>a</sup>	total AR <sup>b</sup> ( $\mu\text{g/g}$ of DM)	homologue composition (%)					ratio C17:0/ C21:0
		C17:0	C19:0	C21:0	C23:0	C25:0	
pasta 1	218	1.1	12.5	57.4	21.7	7.3	0.02
pasta 2	270	1.5	17.9	54.3	20.0	6.3	0.03
pasta 3	236	0.7	11.0	58.4	22.0	7.9	0.01
pasta 4	220	0.4	11.2	57.6	22.7	8.1	0.01
pasta 5	215	0.7	11.9	57.5	22.0	7.9	0.01
mean $\pm$ SD	232 $\pm$ 23	0.8 $\pm$ 0.4	12.9 $\pm$ 3	57.2 $\pm$ 2	21.7 $\pm$ 1	7.4 $\pm$ 0.7	0.02 $\pm$ 0.01
whole grain durum wheat flour <sup>c</sup>	457	0.9	12.7	57.8	21.6	7.0	0.02
refined durum wheat flour	traces <sup>d</sup>						

<sup>a</sup> Pasta products contained 55% whole grain durum wheat flour and 45% refined durum wheat flour. <sup>b</sup> The analytical precision expressed as coefficient of variation (CV) of the total AR content was always below 6%. <sup>c</sup> Used for all pasta products. <sup>d</sup> Limit of detection (LOD) = 5  $\mu\text{g/g}$ .

Nordmills (Uppsala, Sweden), Svalöf Weibull AB (Svalöv, Sweden), and Svenska Lantmännen AB (Järna, Sweden). Samples from different countries were selected to obtain a large variation. Five commercial pasta products (55% whole grain durum flour and 45% refined durum flour) and their corresponding flour mixtures were provided by Svenska lantmännen AB. Pasta products were milled to pass a 0.5 mm sieve using a Cyclotech 1093 sample mill (Tractor AB, Höganäs, Sweden).

**Chemicals and Reagents.** Methyl behenate (C22:0, fatty acid methyl ester, Larodan Fine Chemicals AB, Malmö, Sweden) was used as internal standard. Synthetic alkylresorcinols (C17:0, C19:0, and C21:0, ReseaChem GmbH, Burgdorf, Switzerland) were used for confirmation of peak identity by GC-MS.

All solvents and reagents were of analytical grade (Merck, Darmstadt, Germany) and were used without further purification.

**Extraction and Analysis of Alkylresorcinols.** ARs in intact kernels were extracted with ethyl acetate and quantified by gas chromatography (GC) as discussed before (2). The identity of peaks was checked for two samples by comparing retention time of molecular ions and base fragment ion ( $m/z$  124) with those of synthetic standards. Because no standards for C23:0 and C25:0 were available, those peaks were identified just by molecular ions and base fragment. Pasta products were extracted with a hot 1-propanol/water mixture to release ARs bound in starch-lipid complexes (8). Dry matter (DM) was determined

by drying the samples for 16 h at 105 °C and weighing them after cooling in a desiccator. All samples were analyzed in triplicates, and results are reported as mean values on a dry matter basis.

## RESULTS AND DISCUSSION

Alkylresorcinol content and relative homologue composition of 21 different samples of durum wheat, selected from different sources, were analyzed (Table 1). To confirm identity of peaks, two samples (noted in Table 1) were analyzed by GC-MS as described by Ross et al. (1, 8) (results not shown). The average total content of ARs in durum kernels was 455  $\mu\text{g/g}$  ( $n = 21$ ). This is numerically higher compared to the contents in spring and winter wheat varieties (*Triticum aestivum*) commonly grown in Sweden (412  $\mu\text{g/g}$ ) (9). In durum wheat, the lowest AR content (251  $\mu\text{g/g}$ ) was found in a sample from Austria and the highest content in a sample from Kazakhstan (618  $\mu\text{g/g}$ ), and their range resembled that of common wheat grown in Sweden (9). The AR homologue pattern of durum wheat is markedly different from the patterns commonly found in other wheat varieties (8). Durum wheat contains less of C17:0 (0.4%) and more of the longer homologues, especially C23:0 (20.8%),

compared to common wheat. Homologues with a long alkyl tail are probably more easily incorporated into cell membranes, because they are more lipophilic and have higher octanol–water partition coefficients (7). Such incorporation might lead to physiological effects, because many of the important metabolic processes are related to biological membrane structures (5). As for common wheat, the predominant homologue in durum wheat is C21:0, but in durum the average level is approximately 60% compared to around 50% in winter and spring wheats commonly used in Sweden (9).

In refined durum flour and refined pasta products, only traces of ARs were found. No differences in AR content or homologue composition between whole grain pasta products and ingredients were observed when using the hot 1-propanol/water extraction method (Table 2). This finding supports the suggestion made by Ross et al. (8) that the low yield after hydrothermal processing (10) is a matter of extraction inefficiency rather than degradation. ARs might form a complex with amylose upon heat treatment, which makes them more difficult to extract (8).

The C17:0 to C21:0 ratio in durum wheat was around 0.007 on average (Table 1). In pasta products, the ratio was a bit higher (Table 2), possibly due to contamination with flour from common wheat in the production line. The ratio for wheat varieties commonly grown in Sweden and for wheat products was around 0.1, and for rye and rye products, it was around 0.9 (9). These ratios might be of value when using ARs as biomarkers in determining the sources of the whole grain foods. According to this study, a ratio below 0.1 suggests the presence of durum wheat in products, whereas ratios of 0.1 and 0.9 indicate wheat and rye products, respectively (9). ARs in foods may be related to levels and homologue ratios in the plasma of subjects consuming these foods (14). Further studies comparing ratios in biological samples with ratios in the diet are needed to evaluate whether they could be used to identify the ingested whole grain(s).

In conclusion, the composition of ARs in durum wheat is different from that of common wheat, but the content is about the same. No changes were observed in AR content and composition upon pasta processing. Because the consumption of whole grain pasta is continuously increasing, information obtained in this study might be of importance in the estimation of the source of the ARs in the diet.

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