Alkylresorcinols in Cereal Grains

Occurrence, absorption, and possible use as biomarkers of whole grain wheat and rye intake

Alastair Benjamin Ross
Department of Food Science
Uppsala

Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2003

Acta Universitatis Agriculturae Sueciae
Agraria 417

ISSN 1401-6249
ISBN 91-576-6445-5

Tryck: SLU Service/Repro, Uppsala 2003

Abstract

Ross, AB. 2003. Alkylresorcinols in Cereal Grains – occurrence, absorption, and possible use as biomarkers of whole grain wheat and rye intake. Doctoral thesis.
ISSN 1401-6249, ISBN 91-576-6445-5

Alkylresorcinols are phenolic lipids present at levels of up to 0.15% of whole grain wheat and rye, but little is known about their presence in food, absorption in animals and humans, and their *in vivo* biological effects. Because alkylresorcinols are present in the human diet in significant amounts only in products containing whole grain wheat or rye, they have potential to be biomarkers of whole grain wheat and rye intake. This thesis describes some of the research undertaken to investigate whether alkylresorcinols could be biomarkers of whole grain wheat and rye intake.

A rapid gas chromatographic method was developed to analyse alkylresorcinols in whole cereal grains. This method was then applied to detect the presence and amount of alkylresorcinols in several cereal grains. Wheat, rye and triticale all contain moderate to high amounts of alkylresorcinols (300-1500 μg/g), while barley contains low amounts (~50 μg/g). In these cereals, alkylresorcinols are present in the bran fraction. All other cereals analysed (rice, oats, maize, sorghum and millet) did not contain any detectable amounts of alkylresorcinols. Previous studies have suggested that alkylresorcinols are destroyed by the baking process. However, an extraction method using hot propanol:water was able to recover all alkylresorcinols from experimental breads, indicating that alkylresorcinols are not destroyed during baking.

The absorption of alkylresorcinols in rats, pigs and humans was determined, with values for absorption ranging from 34–79%, depending on the model and the amount of alkylresorcinols consumed. Alkylresorcinols in the plasma of pigs fed a single meal of rye, peaked at 3-4 hours, and remained elevated compared to the baseline levels after 16 hours. Preliminary studies to find alkylresorcinol metabolites in humans suggest that they have their alkyl chains shortened by β-oxidation.

The effect of purified rye alkylresorcinols on lipid parameters (tocopherols, cholesterol and fatty acids) was tested on a rat model. Alkylresorcinols did not appear to affect rat performance, but in high amounts they could decrease liver cholesterol, and moderately elevate γ-tocopherol levels. Overall, the results suggest that alkylresorcinols do not have a large effect on lipid absorption/metabolism in rats.

Keywords: Alkylresorcinols, whole grain cereals, biomarker.

Author's address: Alastair Ross, Department of Food Science, P.O. Box 7051,
Swedish University of Agricultural Sciences (SLU), S-750 07 Uppsala, Sweden.
E-mail: Alastair.Ross@lmv.slu.se

Sammanfattning

”Functional foods” har förändrat vår syn på livsmedel. Idag tänker man inte bara på att mat skall vara god och innehålla de näringsämnen som vi behöver utan också på att mat kan fungera som förebyggande medicin. Allt fler studier visar att det finns samband mellan intag av ”plant foods”, som till exempel fullkorn, frukt och grönt och minskad risk för utveckling av olika sjukdomar som hjärtsjukdom, diabetes och kanske vissa cancertyper.

I Sverige har livsmedelsindustrins egenåtgärdsprogram nyligen godkänt ett nytt hälsopåstående kring ökat intag av fullkorn och minskad risk för hjärtsjukdom. Liknande hälsopåståenden har också godkänts i bland annat USA och Storbritannien. En fullkornsprodukt är ett livsmedel som innehåller minst 51 % fullkorn med samtliga delar av spannmålskärnan (grodd, kli och mjöl) ingår. De flesta svenska fullkornsprodukter innehåller vete, råg och/eller havre.

Bakgrunden till det nya hälsopåståendet är att många epidemiologiska studier har påvisat ett mycket klart statistiskt samband mellan intag av fullkorn och minskad risk för hjärtsjukdom. Ett stort problem epidemiologiska studier av fullkorn är emellertid att avgöra vad som är fullkorn och vad som bara ser ut som fullkorn, till exempel ett mörkt bröd som färgats med sockerkulört. Ett annat problem är att kunna validera kostundersökningarna, det vill säga ta reda på att försökspersoner verkligen ätit det som de uppger. En biomarkör för kostintag är en substans som kan mätas i t ex blod eller urin och som kan härledas till intaget av en viss produkt. I den här avhandlingen har jag studerat en grupp fenoler, så kallade alkylresorcinoler, i spannmål och möjligheten att kunn använda dessa eller deras omvandlingsprodukter som biomarköerr för intag av fullkorn.

Metoder att analysera alkylresorcinoler i spannmålfraktioner och livsmedel har utvecklats. De kunde påvisas i relativt höga halter i råg och vete och i låga halter i korn men inte i andra spannmålsslag som majs, ris och havre. Alkylresorcinolerna kunde bara påvisas i de yttre delarna av kärnan och återfinns därför inte i det vita mjölet.

I studier med försöksdjur och i en humanstudie har vi visat att ca hälften av alkylresorcinolerna i kosten tas upp från tarmen och kan påvisas i blodet. Den högsta halten i blodet kunde påvisas 3-4 timmar efter intag men små mängder fanns kvar efter 5 dagar. Vi har även påvisat en trolig omvandlingsprodukt från alkylresorcinoler i urin. Dessa resultat tyder på att alkylresorcinoler eller någon omvandlingsprodukt från dessa har en stor potential att kunna utvecklats till biomarkörer för intag av fullkorn eller kli från vete och råg.

I ett försök med råtta visades att ett högt intag av alkylresorcinoler hade positiva effekter på kolesterolhalten i levern och halten gamma-tokeferol samt att inga negativa effekter på tillväxten kunde påvisas.

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Appendix

Papers I-VIII

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Ross, A.B., Kamal-Eldin, A., Åman, P. Dietary alkylresorcinols: absorption, bioactivities and possible use as biomarkers of whole grain wheat and rye rich foods. *Submitted for publication*.

II. Ross, A.B., Kamal-Eldin, A., Jung, C., Shepherd M.J., & Åman, P. 2001. Gas chromatographic analysis of alkylresorcinols in rye (*Secale cereale* L) grains. *Journal of the Science of Food and Agriculture* 81, 1405-1411.

III. Ross, A.B., Shepherd, M.J., Schüpphaus, M., Sinclair, V., Alfaro, B., Kamal-Eldin, A., Åman, P. 2003. Alkylresorcinols in cereals and cereal products. *Journal of Agricultural and Food Chemistry* 51, 4111-4118.

IV. Ross, A.B., Shepherd M.J., Bach Knudsen, K.E., Glitsø L.V., Bowey, E., Phillips, J., Rowland, I., Guo, Z.-X., Massy, D.J.R., Åman, P., Kamal-Eldin, A. 2003. Absorption of dietary alkylresorcinols in ileal-cannulated pigs and rats. *British Journal of Nutrition*. *In press*.

V. Ross, A.B., Kamal-Eldin, A., Lundin, E.A., Zhang, J-X., Hallmans, G., Åman, P. 2003. Cereal alkylresorcinols are absorbed by humans. *Journal of Nutrition* 133, 2222-2224.

VI. Linko, A-M., Ross, A.B., Kamal-Eldin, A., Serena, A., Bjørnbak Kjær, A.K., Jørgensen, H., Adlercreutz, H., Åman, P., Bach Knudsen, K.E. Kinetics of cereal alkylresorcinol uptake in pigs. *Manuscript*.

VII. Ross, A.B., Åman, P., Kamal-Eldin, A. A note on the preliminary identification of possible cereal alkylresorcinol metabolites in human urine. *Manuscript.*

VIII. Ross, A.B., Chen, Y., Kozubek, A., Frank, J., Lundh, T., Vessby, B., Åman, P., Kamal-Eldin, A. Cereal alkylresorcinols decrease liver cholesterol and weakly elevate tocopherol levels in rats. *Manuscript*.

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Introduction

The importance of whole grain cereals in the human diet

The potential benefits of whole grain cereal consumption for humans have been demonstrated in numerous epidemiological and clinical studies (see the Proceedings of the Nutrition Society, vol. 62, 2003, for recent reviews of the literature on whole grain cereals and associated health effects). Consumption of whole grain cereals has been linked to a decreased risk of diabetes, obesity, heart disease, and some cancers. Furthermore, the importance of whole grain cereals in human nutrition has now been recognised by the United States Food and Drug Administration, the British Joint Health Claims Initiative, and the Swedish Nutrition Foundation, all of whom allow certain health claims on the reduced risk of heart disease to be made for products containing more than 50% whole grain cereals (FDA, 1999; JHCI, 2002; SNF, 2003). However, methodology used in epidemiological studies for estimating dietary intake is weak, and is a problem for confirming the role of whole grain cereals in disease prevention. A biomarker of whole grain cereal intake would be a tool for linking together intake of whole grain cereals and the reported health benefits of their consumption.

A group of phenolic lipids, the alkylresorcinols, found in high levels in whole grain wheat and rye, but not in significant levels in other food plants, may prove to be suitable as biomarkers of whole grain wheat and rye intake. In this thesis, analysis of alkylresorcinols and their presence in cereals and cereal products was investigated, along with their absorption and metabolism, with a view to assessing their suitability as biomarkers of whole grain wheat and rye intake. A study was also carried out to test the effect of alkylresorcinols on certain lipid parameters of rats.

Possible use of alkylresorcinols as biomarkers of products rich in whole grain wheat and rye

What is a biomarker?

Generally speaking, a biomarker is a biological compound that can be objectively measured in blood or urine, and can be linked to a biological process – such as a disease or intake/digestion of a food. For example, a high level of cholesterol in plasma is a biomarker of an increased risk of coronary heart disease, and elevated blood glucose is a biomarker of diabetes (Biomarker Definitions Working Group, 2001).

A biomarker of whole grain cereal intake would allow assessment of the amount, and perhaps type, of whole grain cereal a person has consumed, from a plasma or urine sample, with or without using a dietary questionnaire. The biomarker acts as a surrogate measurement of dietary intake for a particular food group – in this case, whole grain cereals (Fig. 1). In epidemiological studies,

dietary questionnaires are used to assess dietary intake. However, there are a number of general problems associated with the dietary recall method for assessing intake (see Kantor *et al*., 2001 for a discussion). Moreover, there are also some specific problems related to assessing intake of whole grain cereals as many consumers have difficulty in identifying which products contain whole grain cereals and which do not (Slavin *et al*. 2001a). A biomarker of whole grain cereal intake is an objective way of checking dietary intake data. Biomarkers of intake are particularly useful in cases where there are interesting samples, but no dietary intake data available, for example with samples from blood banks.

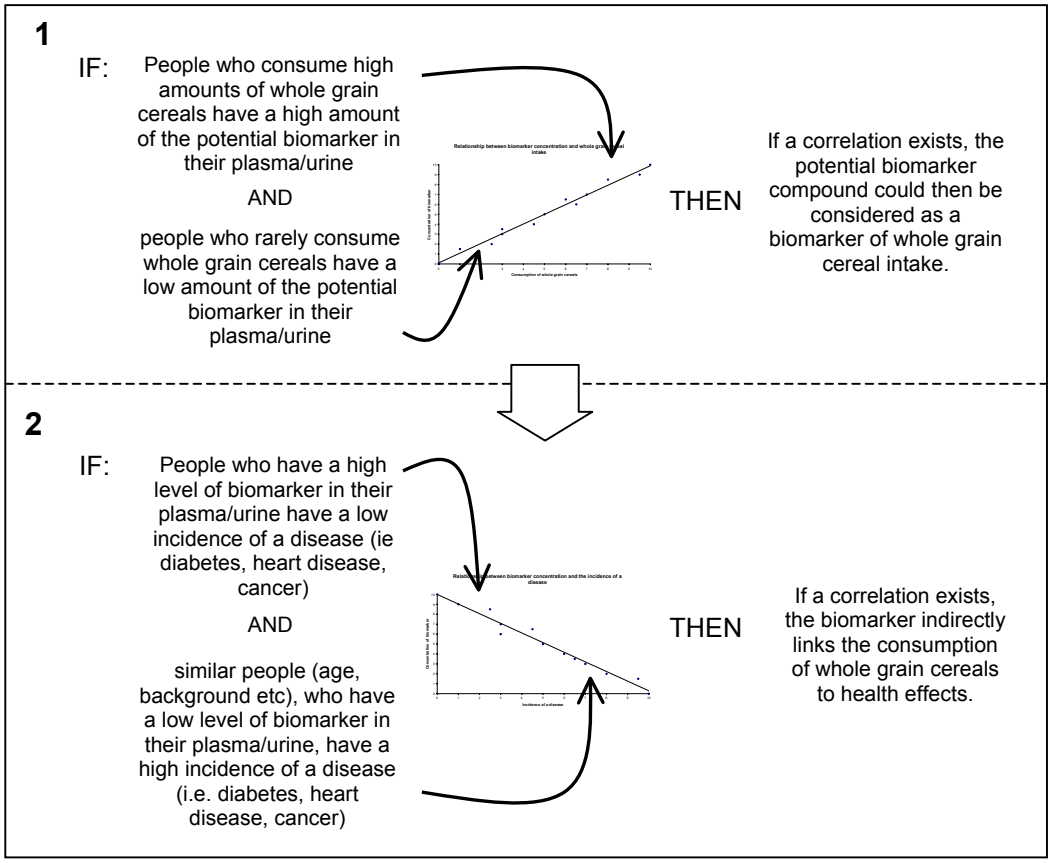

Figure 1. A simplified example of how a potential biomarker compound would be evaluated as a biomarker of whole grain cereal intake (**1**), and then used to link whole grain cereal intake to health benefits (**2**).

A biomarker of whole grain cereal intake does not need to be bioactive or related to a particular disease, but it should meet a number of other criteria (Weber, 2001).

- Present in whole grain, but not in refined cereals, and preferably not present in other foodstuffs.
- Not affected by food processing
- Absorbed by humans

- Possible to rapidly analyse levels of biomarker in cereals, food and biological samples (ie plasma or urine) in intact or metabolised form
- Persist in biological samples for a reasonable amount of time (ie not be completely excreted from the body within a short time)
- Limited effect of microflora on amounts of biomarker present in biological fluids

Previously, enterolactone, a mammalian lignan produced by microbial fermentation in the gut, has been proposed as a biomarker of whole grain cereal intake (Stumpf *et al*., 2000, Lampe, 2003). Enterolactone can be measured in plasma and urine, and increased plasma levels have been correlated with higher levels of whole grain cereal intake (Jacobs *et al*., 2002). However, many other plants and plant products also contain precursors of enterolactone *e.g*. consumption of alcoholic beverages and coffee also lead to increased plasma enterolactone (Horner *et al*., 2002). Thus, enterolactone is unspecific as a biomarker of whole grain cereal intake (though it may still be useful as a biomarker of diets rich in plant foods (Lampe, 2003)).

What are alkylresorcinols?

Alkylresorcinols (1,3-dihydroxy-5-alkylbenzene derivatives, Fig. 2) are phenolic lipids present in a number of plants and bacteria. Of plants commonly used for food, alkylresorcinols are present in high amounts in wheat (*Triticum aestivum*), durum wheat (*Triticum durum*) and rye (*Secale cereale*) kernels (~ 0.03-0.15% of dry kernel weight). Small amounts (<5 μg/g) have also been reported in maize (*Zea mays*) (Gembeh *et al*., 2001) and in garden peas (*Pisum sativum L.*) (Żarnowski and Kozubek, 1999). High levels are also present in triticale (X *Triticosecale*) and various *Triticum* species other than *T. aestivum*, and low levels (40-100 μg/g) are found in barley (*Hordeum vulgare*) but these cereals are not commonly used for human consumption.

Because alkylresorcinols have a polar 'head' (the dihydroxybenzene group) and a non-polar alkyl 'tail', they are amphiphilic. This is an important aspect with regard to their analysis, absorption, metabolism and potential bioactivity. The length of the alkyl tail of alkylresorcinols in cereal grains varies from C15 to C27. Normally this chain is saturated, but unsaturated and oxygenated chain analogues have also been reported (Seitz, 1992; Kozubek & Tyman, 1999) (Fig. 2). While only ~5% of alkylresorcinols in wheat have a modified alkyl chain, up to 20% of rye alkylresorcinols are present as either unsaturated or keto derivatives.

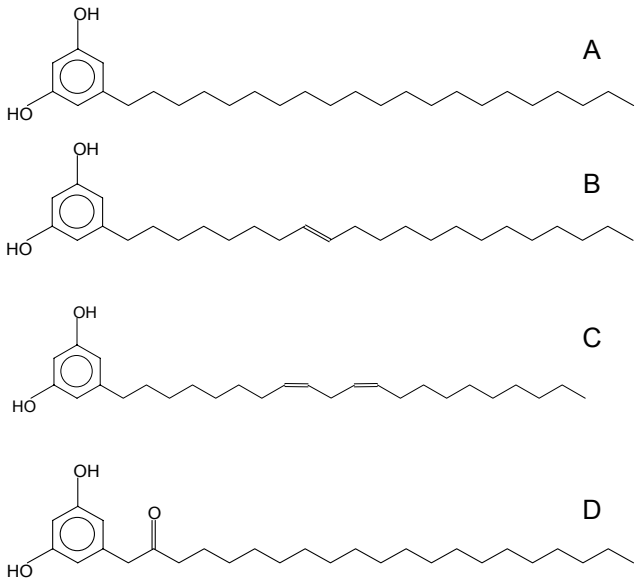

Figure 2. Basic structure of alkylresorcinols and some analogues. A is an alkylresorcinol with a saturated alkyl chain (C21:0); B, with a monounsaturated alkyl chain (C21:1), C, with a diunsaturated alkyl chain (C21:2); D, with a keto group substituted on the alkyl chain. In wheat and rye, the alkyl chain varies from 15 to 25 carbons long.

Throughout this thesis, alkylresorcinols are named according to their chain length, in a similar manner to the fatty acids. As keto- and unsaturated-alkylresorcinols are only present in low amounts in wheat and rye, their analysis and biological activities have not been studied in this thesis.

Literature Review

Paper I reviews the literature on alkylresorcinols in food and human nutrition, covering their occurrence in cereals, analysis, effects of food processing, absorption and possible metabolism, effect on animals, bioactivities, and their potential as biomarkers of whole grain wheat and rye. This review is intended to provide a comprehensive coverage of the literature related to the topics covered in this thesis. For more discussion of the chemical aspects of alkylresorcinols, the reader is referred to the review by Kozubek & Tyman (1999).

Levels of alkylresorcinols in cereal grains and cereal products

Analysis of alkylresorcinols in cereal grains

To investigate the content of alkylresorcinols in cereal grains, and the effects of processing on their content in food, a rapid analytical method was needed. Quantitative methods previously used for alkylresorcinol analysis include fluorescence, thin layer chromatography (TLC), gas chromatography (GC) and high performance liquid chromatography (HPLC). A method was developed to

analyse alkylresorcinols in cereal grains. It was possible to extract total alkylresorcinols from whole rye grains by extracting with ethyl acetate for 24 hours, eliminating the need to mill the grains. As previous GC methods offered the best combination of speed of analysis and peak resolution (Gohil *et al*., 1988; Mullin *et al*., 1992), a GC method was optimised to allow quantitative analysis with good repeatability (coefficient of variation <5%) without derivatisation (**Paper II**).

Presence of alkylresorcinols in cereals

Previous literature has reported that of the cereals commonly used for food and feed, alkylresorcinols are present in the kernels of wheat, rye, triticale, barley, maize, oats and millet, and that alkylresorcinol derivatives are present in rice and sorghum plants, but not the grains of these cereals (see **Paper III** for references).

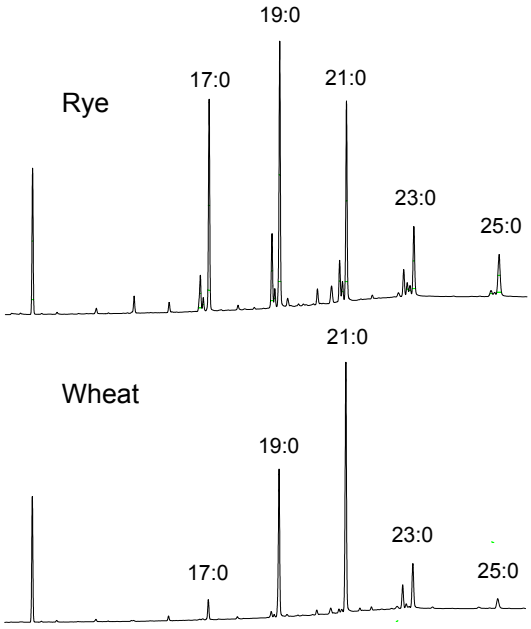

Figure 3. Gas chromatograms of alkylresorcinols in rye and wheat, with the different homologues indicated. Peaks immediately preceding the alkylresorcinol peaks in the rye sample are alkylresorcinol analogues. Note the difference in the proportion of different homologues for the cereals.

The presence of alkylresorcinols in selected cultivars of commonly cultivated cereals (barley, maize, millet, oats, rice, rye, sorghum, triticale and wheat) was checked using gas chromatography-mass spectrometry (GC-MS), a method not previously used for alkylresorcinol analysis for many of these cereal grains. Alkylresorcinols were found in all studied cultivars of wheat, rye, triticale, and barley, but not in maize, oats, millet, rice or sorghum. While this does not discount the possibility that alkylresorcinols or derivatives of alkylresorcinols could be present in these cereal grains, it does strongly suggest that in the human diet, the

main sources of alkylresorcinols are whole grain wheat and rye (triticale and barley are not commonly used for human consumption). The alkylresorcinol content of 125 samples of wheat (31 cultivars) grown in different locations was 595-1429 μg/g (mean ~900 μg/g) (**Paper III**). The alkylresorcinol content in Swedish rye samples varied from 568-1022 μg/g (**Paper II**). These results indicate that the content of alkylresorcinols can vary widely, probably depending on a combination of environmental and genetic factors.

The difference in homologue composition between wheat and rye is important as it can be used as a 'fingerprint' for each cereal: wheat has a low level of C17:0 compared to C21:0, while in rye the level of C17:0 is similar to C21:0 (Fig. 3), so by comparing the amount of each alkylresorcinol homologue, it is possible to identify the cereal of origin.

Effect of milling on alkylresorcinol content

Alkylresorcinols in semi-pure botanical fractions of rye (whole grain, pericarp/testa, aleurone layer and endosperm) were located in the outer layers of the rye grain (pericarp/testa and aleurone layer) (**Paper II**). Normally, cereal grains are separated into different components by milling (flour, shorts and bran). If alkylresorcinols are to be used as biomarkers of whole grains, it is important to know if they are present in the white flour (endosperm) fraction of milled grains, and if so, to what extent. Wheat and rye grains were milled into eight fractions (six white flour, one shorts, containing both a mix of bran, germ and endosperm, and one bran fraction) in an experimental mill, and the alkylresorcinol content in each fraction was determined. The white wheat flour fractions contained virtually no alkylresorcinols, while the white four of rye contained low levels (50-100 μg/g). The presence of trace amounts of alkylresorcinols in white rye flour is most probably due to the problem of separating the alkylresorcinol rich aleurone layer from the rest of the endosperm (Slavin *et al*., 2001b). Thus, products made from white wheat or rye flour should have very low levels of alkylresorcinols. For both cereals, alkylresorcinols were present in high concentrations in the shorts and bran milling fractions (0.1-0.3% of dry weight). Because alkylresorcinols are present in the outer layers of wheat and rye, they are closely correlated to the bran fraction of wheat and rye, they could be used as markers of the extent of bran contamination in white flour (**Paper III**).

Effect of baking on alkylresorcinol content

The baking process has been reported to reduce the alkylresorcinol content of flour by 42-100% (Wiepert & Al Bayâ; 1977, Winata & Lorenz, 1997). This was suggested to be due to a combination of fermentation and the high temperatures involved in bread baking. However, autoclaving conditions did not affect the alkylresorcinol content of rye grains (Michniewicz & Jankiewicz, 1988), so it is difficult to understand how baking conditions can alter alkylresorcinol content so drastically. A study was undertaken using a rye flour baked in three different ways (a loaf bread baked in a baking tin, a loaf bread baked without a baking tin, and small "pancakes" (rågkaka)) to test if differences in surface area make any

difference to the alkylresorcinol content of the breads. Bread samples were further divided into whole bread, crust, and crumb. Extraction with ethyl acetate or acetone yielded a ~ 20% lower recovery of alkylresorcinols after baking, but this difference was not evident with methanol extraction, indicating that the 'destruction' of alkylresorcinols may actually be an extraction problem. 100% recovery of alkylresorcinols from the breads was possible when using a method previously used for extracting total lipids from starch, hot propanol and water extraction (Morrison *et al*., 1984). This method was applied to extracting alkylresorcinols from the experimental breads. There were no differences in alkylresorcinol content between the three different breads, nor the whole bread, crust and crumb. As non-polar solvents (acetone and ethyl acetate) were not able to extract total alkylresorcinols, but more polar solvents such as methanol and hot propanol:water were able to extract 90-100% alkylresorcinols, it is probable that they are bound when the flour is wetted and heated. Alkylresorcinols probably form complexes with amylose starch, similar to amylose-fatty acid complexes, where the non-polar tail of the alkylresorcinols would be encased in the non-polar core of an amylose helix (Fig. 4), making extraction more difficult when extracting with non-polar solvents such as acetone or ethyl acetate (**Paper III**).

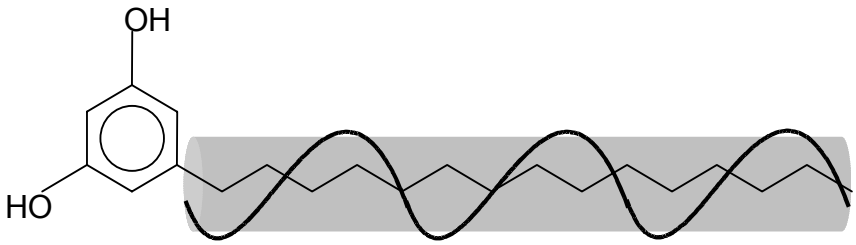

Figure 4. A schematic representation of the potential amylose-alkylresorcinol complex formed during the baking process. The grey area represents the non-polar (hydrophobic) core of the starch helix, which can incorporate the non-polar alkyl tail of the alkylresorcinol, making their extraction from bread more difficult.

The hot propanol:water extraction method was applied to various cereal products to help get an idea of the range of alkylresorcinol levels in products available to consumers. Products made from wheat bran contained the highest amount of alkylresorcinols (1784 µg alkylresorcinols/g), while products made from white wheat flour contained no alkylresorcinols. Products made from wheat or rye that were labelled 'whole grain' (ie containing >50% whole grains) contained 222-1007 µg alkylresorcinols/g, while those made from white wheat and/or rye flour contained <50 µg/g (**Paper III**).

Absorption and metabolism of alkylresorcinols

Prior to the work in this thesis, only one study had been carried out on the absorption of alkylresorcinols. Tłuścik *et al*. (1990) did a simple intake – excretion experiment with rats (intake of alkylresorcinols – amount of alkylresorcinols in faeces = amount of alkylresorcinols absorbed). It was estimated that rats absorbed 36-48% of alkylresorcinols, and that the amount absorbed depended on the

amount of alkylresorcinols consumed. In this thesis, three studies were carried out to determine the extent of absorption of alkylresorcinols in animals (rats and pigs) (**Paper IV**) and humans (**Paper V**). Further studies were carried on the kinetics of alkylresorcinol uptake in pigs (**Paper VI**) and on the possible nature of metabolites in human urine (**Paper VII**).

Absorption of a radiolabelled alkylresorcinol in rats

A pilot study feeding radiolabelled alkylresorcinol to rats was carried out. Radiolabelled C21:0 was fed as a single dose to rats, and the amount of radioactivity was determined in blood, urine and faeces. In selected rats, distribution of radioactivity in the body organs was also determined. Radioactivity peaked in blood between 7 and 12 hours, and decreased rapidly after this point, though a small amount of radioactivity (<1%) was still present after 144 hours. Radioactivity in the urine and faeces peaked after the peak in blood (~24 hours). Around 34% of total radioactivity was found in the urine – evidence that alkylresorcinols are absorbed and metabolised (as alkylresorcinols are essentially insoluble in water, they must be metabolised to be soluble in urine). Around 90% of the remaining radioactivity was detected as unchanged alkylresorcinol in the faeces. A small amount (<0.1%) remained in the carcass (**Paper IV**).

Absorption of rye alkylresorcinols in pigs

A study was carried out on ileostomy operated pigs fed diets enriched with different fractions of rye: whole grain rye, aleurone layer, pericarp and testa, and endosperm. The first three fractions contained varying amounts of alkylresorcinols, while the endosperm contained virtually none. Ileal absorption is determined in a similar way to faecal absorption, but the digesta does not pass through the large intestine. This method avoids the risk that colonic microflora could metabolise alkylresorcinols, giving a falsely elevated value for absorption. Ileal absorption of alkylresorcinols varied from 60 – 79%, depending on the diet. No alkylresorcinols were detected in the digesta of pigs fed rye endosperm. There were some differences in homologue intake observed, and though there was a general trend for shorter chained alkylresorcinols (C17:0 and C19:0) to be taken up to a greater extent, this was not consistent for all diets. Because alkylresorcinols disappeared from the small intestine, it is likely that they are taken up *via* the lymphatic system, similar to other fat soluble compounds (**Paper IV**).

Absorption of rye alkylresorcinols in humans

Ileal absorption was also determined in human ileostomy subjects fed either white wheat bread (no alkylresorcinols) or rye bread with added rye bran (rich in alkylresorcinols). Subjects ate their diets according to two dietary regimes – either an ‘ordinary’ diet, with three meals a day, or a ‘nibbling’ diet, with seven meals a day (with the same energy intake). The ileal absorption of alkylresorcinols in humans was calculated to be 60%. There was no difference in absorption according to whether the subjects ate three meals/day or seven meals/day, but

shorter chain alkylresorcinol homologues (C17:0-C21:0) were taken up to a greater extent than the longer chain homologues (C23:0 and C25:0) (**Paper V**).

Kinetics of alkylresorcinol absorption in pigs

The development of a method to analyse alkylresorcinols in plasma (Linko *et al*., 2002) has made it possible to study the kinetics of alkylresorcinol appearance in plasma. Alkylresorcinols as part of a rye diet, were fed to pigs as a single meal, and blood samples taken from the mesenteric artery and the portal vein over 960 minutes to determine the kinetics of alkylresorcinol appearance in plasma. As there was no difference between the levels of alkylresorcinols in the mesenteric artery and the portal vein, it was not possible to calculate their absorption. This provides further evidence that alkylresorcinols are absorbed *via* the lymphatic system, as if they were absorbed *via* the blood system, then there would be a difference between alkylresorcinol concentrations in the mesenteric artery and the portal vein. Levels of alkylresorcinols in plasma peaked at 3-4 hours, but remained 10 times higher than baseline levels at the end of 16 hours. The homologue pattern in plasma was similar to that of the rye diet. The fact that pigs still had low levels of alkylresorcinols in their plasma after a five day 'washout' period suggests that they may persist in the body long enough to be useful biomarkers of whole grain wheat or rye intake (**Paper VI**).

Preliminary remarks on the metabolism of alkylresorcinols

An important aspect that needs to be researched before alkylresorcinols are established as biomarkers of whole grain wheat and rye intake is the identification of their metabolites. The rat study showed that alkylresorcinols must be metabolised for excretion in urine (**Paper IV**). Other lipophilic compounds with a long aliphatic chain such as tocopherol (Birringer *et al*., 2001) and 4-nonylphenol (Zalko *et al*., 2003) undergo ω-oxidation, followed by β-oxidation mediated shortening of the alkyl chain. It is possible that alkylresorcinols are metabolised *via* a similar pathway.

A pilot study was carried out to find alkylresorcinol metabolites in human urine. An overnight urine sample from a human was taken after a meal rich in wheat bran, and compared to a 'blank' urine sample (an overnight urine sample after a five day alkylresorcinol free diet). Urine samples were deconjugated with β-glucuronidase/sulphatase, and run on TLC plates, which were sprayed with Fast Blue B (a dye that specifically stains phenolic compounds). Several spots were apparent in the urine sample taken after the wheat bran meal, that were not present in the 'blank' urine sample. These spots were scraped off, extracted, and run on GC-MS. Evidence for alkylresorcinols being metabolised to 3-(3,5-dihydroxyphenyl)-1-propanoic acid (Fig. 5) was found, as well as small amounts of native alkylresorcinols (**Paper VII**).

The finding of alkylresorcinol metabolites in human urine suggests that alkylresorcinols and/or their metabolites may be a candidate as a biomarker of whole grain wheat and rye, provided they meet the criteria outlined at the beginning of this thesis.

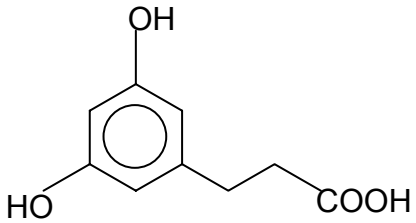

Figure 5. 3-(3,5-dihydroxyphenyl)-1-propanoic acid, a possible metabolite of cereal alkylresorcinols, identified in human urine.

Effect of alkylresorcinols on growth and lipid parameters in rats (Paper VIII)

Alkylresorcinols have been reported to have a wide range of bioactivities *in vitro* (see **Paper I**), but few have been tested *in vivo*. As alkylresorcinols are amphiphilic and are able to insert into and form membranes at physiological pH (Przeworska *et al*. 2001), they may have an effect on lipid absorption *in vivo*. A rat model was used to test the effect of alkylresorcinols on tocopherol levels in plasma, liver and lungs, and cholesterol levels in plasma and liver. Growing rats were fed either a control diet, or diets containing 0.1, 0.2 or 0.4% alkylresorcinols. The 0.4% alkylresorcinol in the diet decreased α-tocopherol levels, but increased γ-tocopherol levels in the liver and lungs, while the other two levels of alkylresorcinols only increased γ-tocopherol levels in the lungs, but these effects were small. The 0.4% alkylresorcinol diet also lead to a large decrease in liver cholesterol, whereas no effect was seen for the other two alkylresorcinol diets. No significant effects were seen in the plasma samples. The mechanism for the increase in γ-tocopherol is not known, but given that alkylresorcinols appear to be metabolised in a similar fashion to tocopherols (**Paper VII**), they may competitively inhibit γ-tocopherol metabolism. Alkylresorcinols do not appear to have a large biological effect on lipid parameters *in vivo* and appear to be benign with regard to the growth of rats. This limited study does not exclude the possibility that alkylresorcinols have other biological effects similar to those demonstrated *in vitro*. In this study, perirenal adipose tissue samples from rats fed alkylresorcinols contained intact alkylresorcinols, indicating that they are accumulated in the bodies of rats (**Paper VIII**).

Main findings

- Alkylresorcinols can be analysed in whole grains without milling, and can be analysed by GC without derivatisation.
- Wheat and rye are the major sources of alkylresorcinols in the human diet.
- Contrary to previous reports, alkylresorcinols were not found in oats, millet or maize.
- Levels of alkylresorcinols in analysed samples of wheat ranged from 489-1429 µg/g, and in rye from 568-1022 µg/g.
- As alkylresorcinols are located in the outer layers of wheat and rye grains, there are only minute quantities present in the milling fractions used for white flour.
- Bread baking does not destroy alkylresorcinols, but may lead to the formation of complexes with starch, making the alkylresorcinols difficult to extract using normal methods. Complete recovery of alkylresorcinols can be achieved by extraction with hot propanol:water (3:1 v/v).
- Alkylresorcinols are absorbed by rats, pigs and humans at levels ranging from 33–79%. Alkylresorcinols are probably taken up *via* the lymphatic system.
- Peak levels of alkylresorcinols in plasma occur at around 3-4 hours in pigs after ingestion of a single dose of rye, and around 24 hours in rats fed a radiolabelled alkylresorcinol. Alkylresorcinols persisted in pig plasma for five days after ingestion of a single dose.
- Intact alkylresorcinols and a metabolite (3-(3,5-dihydroxyphenyl)-1-propanoic acid) were detected in human urine after a meal rich in alkylresorcinols, and appear to be mostly present in conjugated form.
- Alkylresorcinols slightly elevate tocopherol levels, and in very high amounts, lower liver cholesterol in rats.
- Alkylresorcinols were found in the adipose tissue of rats.
- Alkylresorcinols appear to fit a number of the criteria for being biomarkers of whole grain wheat and rye intake.

Concluding Remarks

The potential of alkylresorcinols to be used as biomarkers of whole grain wheat and rye intake is exciting for researchers interested in linking a healthy diet rich in whole grains to health effects.

Alkylresorcinols appear to fit a number of criteria for a biomarker of whole grain wheat and rye intake:

- They are only present in whole grain products and the bran, and not in significant amounts in refined products.
- They do not appear to be affected by bread baking, one of the most common forms of processing cereal grains.
- They are highly absorbed by humans.
- They can be rapidly analysed in cereal grains, food products, and in intact form in plasma (Linko *et al*., 2002)
- In pigs, they can persist in low levels in plasma for at least five days after a habitual alkylresorcinol containing diet

However, there are a number of questions that remain to be answered before alkylresorcinols can be regarded as biomarkers of whole grain wheat and rye intake.

- Studies on the content of alkylresorcinols in wheat and rye grains suggest that their content varies widely, so estimation of the amount present in food products, and therefore intake, is complicated. The effect of crop growing conditions and genetic factors on alkylresorcinol content, as well as the effects of food processing on alkylresorcinol content. A greater knowledge about the amounts present in whole grain cereal products is important for estimating intake and linking levels in biological fluids to the amount of alkylresorcinols consumed.
- The kinetics of alkylresorcinol absorption and metabolism still need to be studied in more detail. Dose response studies are also needed in humans to check if there is a good correlation between intake of alkylresorcinols and the amount appearing in plasma/ metabolites in the urine.
- Urinary alkylresorcinol metabolites may present a good alternative to plasma alkylresorcinol levels as a biomarker, but much more needs to be done to isolate and characterise them. Then a method for their quantitative analysis can be developed and studies on the kinetics of their formation can be carried out.
- Little is known about the effect of intestinal microflora on alkylresorcinols, though little intestinal fermentation of alkylresorcinols was seen in rats. It is important to know if intestinal microflora affect alkylresorcinols and their absorption, or if alkylresorcinols themselves can affect the intestinal microflora.

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Acknowledgements

“He aha te mea nui i tenei ao? Maku e kī atu: ‘he tangata, he tangata, he tangata’.”
“What is the greatest treasure in this World? I say to you: ‘it is people, it is people, it is people’.” (Māori proverb)

Doing this thesis has been made easier and much more enjoyable by many great people, so I would like to say a big thank you to…

Afaf, my main supervisor – thank you for all the help and support (and firm words when needed!) you have given me. You have helped me grow as a person as well as a researcher.
Per, min professor – tack för alla dina diskussioner och ditt stöd och entusiasm för alkylresorcinoler!
Paresh – for showing me the ropes when I first arrived and for always having your door open.
Roger, tack för din glada hjälp med nästan allting när det gäller labb, statistik och datorer.
Janne, tack för alla hjälp i början av min tid här. Det fattas någonting här, nu när du inte är i labbet!
Till alla doktorander på institutionen, tack för att ni är så trevliga och hjälpsamma! Doktorandresan var en stor höjdare.
Lena R, det var jätte roligt att dela rum med dig i tre år. Jag saknar våra vetenskapliga diskussioner…hoppas att vi kan hålla kontakten! Stort tack också till familjen Rimsten för att jag fick fira jul och påsk hos er – det var alltid trevlig!
Jan Frank – thanks for being a good friend and always being willing to go out a beer or two…
Other friends that I have met at the department – Hanno, Mangala, Tuomo, Anders, Stalin, Anke und Sabine to name but a few – you’ve helped me laugh a lot during my time here.
Tack till alla på institutionen för livsmedelsvetenskap för att ni är så trevliga att jobba ihop med och att ni har varit så tålamod med min svenska. Speciellt tack till Maggan för alla hjälp, inte bara administrativt, och Einar för snabb hjälp med att laga grejer när det behövdes, och svenskalektioner under fikapausen .

I’ve not been alone in doing this work - a big thank you to all my co-authors! Special thanks Martin Shepherd, Knud Erik Bach Knudsen and Arkadiusz Kozubek for help and advice via e mail and telephone.
Anna Linko – it’s been great to have someone else also working on alkylresorcinols! Thanks to you and everyone at Folkhälsan for your friendship while I was in Helsinki.
Ulf Bondesson och Anna Franzén, vi lyckades inte, men tack för att ni var så trevliga och hjälpsamma.
Anne et Geraldine my two French summer students – I never realised there were so many nice French people!
Åsa Ramberg, tack för alla hjälpen med GC-MS:n!

Fortunately, life is not all about work, and many people have helped me relax and enjoy life outside of LMV…

Kristin och Carin, tack för att ni visade mig studentlivet i Uppsala och var min Svenska kontakt i början av min tid här.
Geoff Savage – it's your fault I'm here doing this, and I am eternally grateful.
Max – mate, cheers – you've been like an older brother to me.
Alors Stéphanie! Thank you that you are the World's best flatmate! I cannot say enough about your spirit, good humour, tolerance and excellent tiramisu!
Isabelle and Anuschka – you are excellent people to live with!
Thanks to my other friends in Uppsala/Stockholm that have nothing to do with work – Lesley Ann, Rob, Dave, Catherine, Calandra, the hardcore Ultuna innebandy players, and the Uppsala cricket team for letting me bowl my rank leg-spin and not complaining too much…
Karen B – thanks for being a great mate! I can't help but smile and laugh when I think about our travels together! You're a top Aussie if ever there was one.
All the exchange students that I met during my 'exchange student years' – Tracy, Tuure, Kristiina, Eva, the Canadians…I will never forget those times!
The people whom I've met on various courses on the Continent – Tim, Magali, Carsten, Erica…and many more. I am always amazed at how many excellent people there are in this World.
Friends back home – thanks for being the same mates as when I left. It's great to know that the important things about home don't change. And an extra big thanks to those who made the trip over to Sweden – always good times.

To my Whanau – thank you for all your support from near and far! I would never have made it this far without you all. And there is a lot to be said for surprise Red Cross parcels in the mail…

Och till min kära Carina – tack för att du är så underbar! Det var mer än värt en resa till andra sidan jorden och fyra års hårt arbete (?!) bara för att träffa dig. Jag ser fram imot att har en litet mer vanlig förhållande nu den här är klart!

And finally, thanks to Kalsec Inc (Kalamazoo, MI, USA) and the Swedish Nutrition Foundation for funding.

TACK!