Bread volume and Alkylresorcinol content in rye bread baked with high and low levels of Alkylresorcinols

Sofie Hedkvist

Examensarbete

Institutionen för Livsmedelsvetenskap — Publikation nr 261

Swedish University of Agricultural Sciences — Uppsala 2009
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Supervisor: Annica Andersson
Examiner: Per Åman

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Abstract

Alkylresorcinols (AR) are phenolic lipids mostly found in the bran part of rye and wheat. The five main homologues differ in the length of the odd numbered alkyl chain, from 17 to 25 carbons long. Studies have shown that AR are bioactive and can be measured in blood or urine (Ross, 2004b) and the exclusive presence of AR in rye and wheat bran makes the AR to a possible biomarker for whole grain intake. The usage of a biomarker can objectively connect intake of wholegrain products and might relate intake to western diseases. The intake of whole grain bread in Europe is low and one reason for this could be the more compact structure of high fibre breads. Studies have shown that AR could possibly reduce the leavening volume, resulting in even more compact bread (Söderman, 2008).

The aim of this study was to investigate the influence of AR on the leavening properties of high fiber breads. The breads were baked with wheat flour and 30 or 20% non-extracted non-milled or finely milled rye bran and compared to breads baked with extracted rye bran (without AR), added in the same amounts to the flour. Breads with 20% added bran had a slightly larger volume and height than breads with 30% bran, but the difference between extracted and non-extracted bran was modest. After baking the breads was freeze-dried and total content as well as homologue composition of AR was determined by gas chromatography. The total amount of saturated AR in breads with 30% non-extracted bran was 1064-1068µg/g dm and 150-153 µg/g dm in breads baked with 30% extracted bran. The AR values for the breads with 20% non-extracted bran was 720-663µg/g dm and 100-99 µg/g dm for breds baked with 20% extracted bran. The proportional total saturated AR content in bread is generally comparable to the content in bran (4030.4-4144.5 µg/g non-extracted bran and 430.8-578.0 µg/g extracted bran), which shows that AR are not degraded during baking. Milling did not influence the total amount or homologue composition of AR in the bran but the composition in breads after baking differs slightly from the original rye bran. This can be a result of AR forming complexes during acetone extraction, which are not detected in the analysis. In this study naturally occurring AR in rye did not affect the leavening properties during baking and the reduction of AR gave no improvement. The volume, height and pore size was similar throughout the trials.

Key terms: Alkylresorcinols, rye, rye bran, wholegrain

Sammanfattning

Alkylrecorsinoler (AR) är fenoliska lipider som främst hittas i klidelarna hos råg och vete. De fem vanligaste homologerna har ett ojämnt antal kolatomer (17-25) i alkylkedjan. Studier har visat att AR är bioaktiva och går att mäta i blod och urin (Ross et al., 2004b), och vetskapen om att AR nästan bara finns i råg och vetekli gör att AR kan komma att användas som biomarkör för intag av fullkorn. Med denna markör kan man från ett blodprov skatta intaget av fullkorn från råg och vete. Intaget av fullkorn i Europa är lågt vilket kan bero på den kompakta strukturen hos fullkornsbröd. Tidigare studier har visat att AR kan påverka brödvolymen vid jäsningen vilket resulterar i ännu kompaktare bröd (Söderman, 2008).

Syftet med den här studien var att undersöka hur AR påverkar jäsningen hos fullkornsbröd med rågkli. Bröden bakades med vetemjöl där 30 och 20% omalet och finmalet rågkli tillsattes och jämfördes med bröd som bakats med extraherat rågkli (utan AR), tillsatt i samma mängder. Bröd med 20% tillsatt kli hade en lite större volym och höjd än bröd med 30% rågkli, medan skillnad mellan omalet och finmalet kli var ringa. Bröden frystorkades efter gräddning och AR-halten samt homologsammansättningen analyserades med gaskromatografi. Summan mättade AR i bröd med 30% icke-extraherat kli var 1064-1068 µg/g ts och 150-153 µg/g ts för bröd bakade med 30% extraherat kli. AR-halten för bröd med 20% ickeextraherat kli var 720-663 µg/g ts och 99-100 µg/g ts för bröd bakade med 20% extraherat kli. Det proportionella innehållet av mättade AR i bröden är generellt jämförbara med innehållet i kli (4030-4145 µg/g ts för icke-extraherat kli och 431-578 µg/g ts för extraherat kli), vilket visar att AR inte bryts ned under gräddning. Malning påverkade inte den totala mängden eller homolog -sammansättning av AR i kliet men innehållet efter gräddning skiljer sig något från kliet. Detta kan bero på att AR bildar komplex under extraktion med aceton vilket inte upptäcks i analysen.

I den här studien visades att naturligt förekommande AR i rågkli inte påverkar jäsningsegenskaperna och reducering av AR gav ingen förbättring. Volym, höjd och porstorlek skiljde sig inte åt under försöken.

Nyckelord: Alkylresorcinoler, råg, rågkli, fullkorn

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Abbreviations

AR, 5-alkylrecorsinol

C19, alkylchain with 19 carbons

IS, internal standard

EA, ethyl acetate

GC, gas chromatography

BU, Brabender Units

dm, dry matter

CV %, coefficient of variation

1 Introduction

1.1 Background

Cereals are one of the major staple foods in the world. They belong to the family grass (Gramineae) and produce dry one seeded grains or kernels that can be used as food and feed. The seed consist of starchy endosperm and different layers of protecting peel (Hoseney, 2004) (Figure 1). Many food products are made from cereals, e.g. bread, pasta, noodles and breakfast cereal products (Cauvain, 2003a). Cereals are also used in the food industry as a functional texturizer in many foods due to its gelling properties (Wrigley, 2003). One cereal that is an important part of the Scandinavian diet is rye. This cereal has low soil and fertilization requirements and good overwintering abilities which make it a good crop to cultivate in Scandinavia. Today high-yielding cultivars are available and previous problems with weak straw and low yield have decreased.

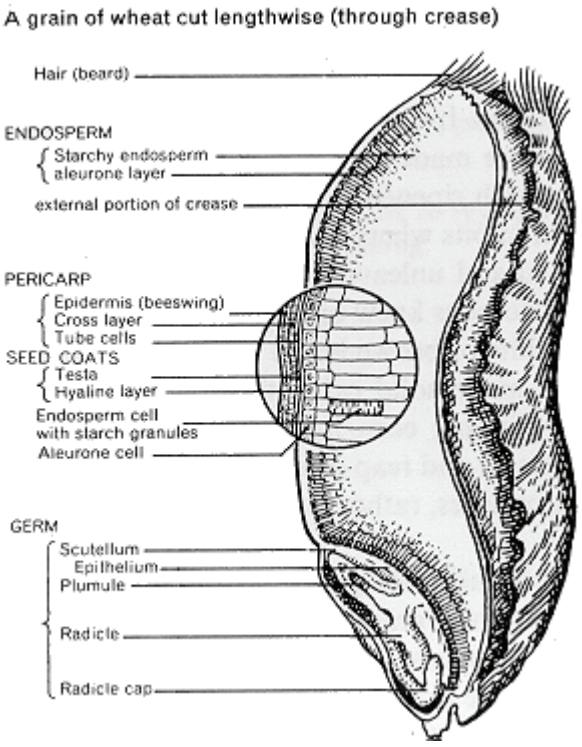

Figure 1. Morphology of a wheat kernel (www.botham.co.uk/bread/grain.htm)

Whole grain flour is often used, i.e. the bran and germ is present in the flour, to produce dark and sour bread and also crisp bread (Åman *et al*., 1997). Whole grain flour has a high content of dietary fibre, which is resistant to digestion and absorption in the human small intestine but completely or partially fermented in the large intestine (Katina, 2003). The bran fraction in rye has a unique composition and contains constituents that are considered nutritionally important such as vitamins, minerals and phenolic antioxidants.

The latter is believed to have health beneficial properties (Åman *et al*., 1997). One group of phenolic compounds that are found in particularly high levels in rye bran are alkylresorcinols (AR) (Ross *et* a*l*., 2003a).

1.2 The aims of this project

The addition of bran to a dough in adequate amounts to get health benefits often cause problems during baking, and some people consider the bread quality as poor. The dough gets wetter and non-elastic, leading to increase leavening volume, the crumb is tenser and CO_2 can escape from the dough (Katina, 2003). In many countries, it is usually considered desirable to have an open gas cell structure, leading to well-risen bread with a large volume, giving a light texture. Bread volume is increased by the incorporation of shortening (Smith & Johansson, 2005). Söderman (2008) added extracted AR in high levels (0.5-1%) to wheat flour dough with the purpose of improving the leavening properties and increase the volume of the rye bread. The AR were supposed to work as an emulsifier but the study showed on the contrary that addition of extracted AR decreased the bread quality and bread volume with 26-39%.

Since rye bran naturally contains a high amount of AR it could effect the baking of whole grain rye bread. The objective of this study was therefore to investigate if also natural levels of AR in rye could affect the bread volume, and if AR is contributing to the volume problem when baking with high levels of dietary fibre.

Breads were baked with wheat flour and added rye bran (20-30%) with high levels of AR, or with acetone-extracted rye bran with very low levels of AR, and bread volume, height and pore size were determined. The same types of breads were also baked with finely milled rye bran to study if particle size could be a factor of importance. All flours and breads were analysed for content and homologue composition of AR.

1.3 What are Alkylresorcinols?

Alkylresorcinols, or 1, 3-dihydroxy-5-alkylbenzene, are a group of phenolic lipids identified in several higher plants, algae and microorganisms in a wide range of derivates. Cereal AR are found in the outer parts of rye (*Secale cereale*), wheat (*Triticum aestivum*) (Ross *et al*., 2008; Zarnowski *et al*., 2004), triticale (× *Triticosecale*) (Hengtrakul *et al*., 1990) and barley (*Hordeum vulgare*) kernels (Zarnowski *et al*., 2004) in concentrations of about 700, 400, 500 and 30 μg/g respectively (Mattila *et al*., 2005; Ross *et al*., 2004a). The content of AR is therefore high in the bran fraction, more specifically in the outer cuticula of testa (seed coat)/ inner cuticula of pericarp (fruit coat) (Landberg *et al*., 2008). AR are not found in refined products from these grains (Ross *et al*., 2001) which was also confirmed by Chen *et al*. (2004) who showed that whole grain products and products with added rye or wheat bran contained a higher amount AR than products made from sifted rye, wheat flour or other cereal products. The content of AR is highest in early grain development and decreases as the kernel mature (Hengtrakul *et al*., 1990).

The backbone of the AR molecule is a resorcinol, and in cereals a straight hydrocarbon alkyl chain with an odd number (17-25) of carbon atoms is attached to position number 5 on the ring (Figure 2) (Kozubek & Tyman, 1999; Gohil *et al*., 2001; Ross *et al*., 2004a). In cereals, saturated alkyl chains are most common, but up to 20 % of the total AR in rye can consist of monoenoic-, dienoic- and oxygenated derivates (Kozubek & Tyman, 1999; Ross *et al*., 2004b). AR are amphiphilic due to the polarity of the dihydroxybenzene group and the hydrophobic alkyl carbon chain. These properties are central for many of the alkylresorcinol features like absorption, metabolism, analysis and bioactivity.

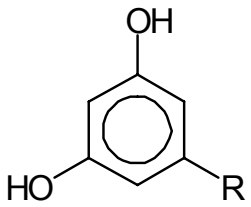

Figure 2. Structure of alkylresorcinols in cereals. R is a straight chain hydrocarbon with 17-25 carbon atoms.

Earlier studies have shown that fermentation and baking could reduce the amount of AR in the bread and therefore also affect the amount AR in the diet (Lorenz & Winata, 1997). However, later studies have indicated that this difference arises from poor extraction of AR rather than the baking (Parikka, 2007).

In food products AR form complexes with hydrophobic parts of the starch and stronger solvents are needed to extract the AR. The extractability using ethyl acetate (EA) or acetone is only 70% in bread and complete extraction (90-100%) can be achieved by extracting total lipids with 1-propanol:water (3:1, v/v). Acetone and EA are non-polar solvents and are not able to extract total AR in comparison to more polar solvents (Ross *et al*., 2003a). AR are absorbed by humans (Ross *et al*., 2003b) and can be quantified in human plasma (Linko *et al*., 2002) and urine (Ross et al., 2004b). Other reports indicate that AR homologues are found in human plasma in similar ratio as in the rye grain and that the content correlates with the intake of rye bread (Linko *et al*., 2002; Linko *et al*., 2005).

1.3.1 Alkylresorcinols as biomarker

A food product is considered as eligible for whole grain health claim if it contains > 51% whole grain ingredients (Chen *et al*., 2004). Epidemiological studies have shown health benefits with intake of whole grain cereals due to a decreased risk of welfare diseases such as obesity, diabetes and coronary heart disease (Jacobs *et al*., 2001). In one study Leinonen *et al*. (2000) showed that rye bread reduced total and LDL cholesterol concentrations in men with elevated serum cholesterol. The mechanisms behind these diseases are however multiple and the benefits of the whole grain and how they influence the body need to be investigated more (Ross *et al*., 2008). One proposed reason for the protective mechanism of whole grain is the effect of phenolic compounds. A direct link between consumption of whole grain and observed health benefits has not been established, partly due to the lack of biomarker of whole grain cereal intake (Branca *et al*., 2001; Jacobs *et al*., 2001; Ross *et al*., 2003b).

A biomarker is a substance that can be measured in body fluids like blood, plasma or urine and can be linked to a biological process. The exclusive presence of AR in rye and wheat whole grain products and the fact that AR are absorbed and measurable in blood, plasma and urine indicate that they can be used as a biomarker of intake. (Ross *at al*., 2003a).

1.4 Baking

There are few steps that form the basis of bread making. A dough is prepared by mixing flour, salt, yeast and water. During the mixing energy is applied, the flour is hydrated and a protein network is built up. The most important proteins responsible for the cohesive dough are gliadin and glutenin, proteins that are primarily present in wheat endosperm. Together they form a gluten network. When the dough is let to leaven, yeast produces CO_2 that is trapped in the protein network and flavours are developed. The pores expand with increasing CO_2 production. The dough is shaped to required form and let to proof before baking (Cauvain, 2003b).

1.4.1 Starch structure

Starch is present in almost all plant cells in form of granules but cereal seeds are particularly rich in starch (Hansen *et al*., 2008). The cereal starch is built up by α-D-glucopyranosyl residues mainly linked together with α-(1-4)-linkages (amylose), but also α-(1-6)-linkages (amylopectin). The two molecules vary widely in properties; amylose is a straight chain while amylopectin is highly branched (Cornell, 2003). Starch is involved in bread staling, starch-gluten interactions, water redistribution and gluten firming.

1.4.2 Starch gelatinization

When bread is baked, the product "set" at a certain temperature. This means that the dough can no longer expand under the gas pressure generated by the increasing temperature. The change starch undergoes is partly responsible for that. Starch gelatinization is known as the thickening of a liquid. When starch is heated with water the granules are hydrated and swell slightly. In the presence of water and heat, the intermolecular bounds are broken down, allowing the hydrogen bounding sites to hold more water. When water penetrates the starch molecule, the general structure gets more random and the crystalline regions increase in number and size. Starch is released into the solution when amylose chains start to pull out from each other. The viscosity is increasing and birefringence is decreasing. Amylopectin is also included in the gelatinization process and are influenced by lipids that form complex with starch, starch-water ratio and solute concentration. Bread has limited water content and the swelling and migration of starch into the aqueous phase is restricted (Hoseney, 1994).

1.5 AR extraction methods

Many different types of extraction methods to determine AR content and homologue composition in cereal grains and cereal food products have been used. Most of these methods rely on organic solvent extraction and subsequent analysis by chromatographic system (Kozubek & Tyman, 1999). Today gas chromatography (GC) is one of the most commonly used methods for quantification of individual and total AR in cereals and cereal products. The GC allows an easy way of determining total AR and homologue composition with separation of unsaturated-, saturated and oxygenated homologues (Mattila *et al*., 2005).

2 Material and Methods

2.1 Dry matter

To be able to compare the different trials with each other, all calculations and measurements were calculated on a dry matter basis. All samples were weighed in metal baking tins and put into an oven (Electrohelios, Sweden) that held 105°C over night (about 16 h), cooled in a desiccator and then weighed.

2.2 Extraction of AR from rye bran

The extraction process was made to remove lipids from the rye bran, including the AR. Acetone was used because it gives a high AR extraction yield and high purity, which means that the acetone does not remove too much of other components (Axelsson, 2007). For the extraction, 330 g bran was mixed with 1000 ml of acetone in an erlenmeyer flask and put on a shaking table (GFL 3015, Burgwedel, Germany) for 24 hours. The acetone was decanted and centrifuged for 12 minutes at 1500×g (Multifuge 3s, Heraeus, Sweden, 2500 G). The pellet was suspended in 50 ml acetone, mixed and returned to the bran in the Erlenmeyer flask. Another 500 ml of acetone was added to the bran and the extraction procedure was repeated, acetone was decanted again and centrifuged as above. The acetone extract was thereafter filtered two times with a 25 μm and 15 μm filter cloth to recover all material.
The bran was dried in room temperature and used for the non-AR baking trials. The content of AR in non-extracted and extracted rye bran was analysed (see below).

2.3 Material

The rye bran used was a mixture of 77% rye bran from Lantmännen and 23% rye bran from Wasabröd. The chemical composition of this mixed rye bran was analysed earlier and is shown in table 1 (Kamal-Eldin, 2008). The baking trials ware made with wheat flour (Kungsörnen) and 20 or 30% non-extracted or extracted rye bran. Baking was also performed with 20 or 30% finely milled rye bran (non-extracted or extracted) to investigate the effects of milling on bread volume.

Non-extracted and extracted rye bran was therefore milled twice, first through a 1 mm sieve and then a 0.5 mm sieve to produce finely milled rye bran. The particle size was determined by sifting 50 g rye bran and finely milled rye bran in a Buhler sieving machine (Bühler Miag, Milan, Italy) into six different fractions.

Table 1. Chemical composition of rye bran (% of dm) and wheat flour (%)

Sample	Protein	Fat	Starch	Total dietary fibre	Ash	Total AR (μg/g)
Rye bran (Lantmännen + Wasabröd)[a]	15.8	4.4	19.7	45.3	4.6	4144.5
Wheat flour (Kungsörnen) [b]	10	1.5	70	3	-	32.8

a) According to Kamal-Eldin, 2009

b) According to the wheat flour nutrition table on package

2.4 Baking tests

Breads were baked with wheat flour and 20 or 30% rye bran (non-extracted and extracted), as well as with 20 or 30% finely milled rye bran (non-extracted and extracted). The water content in the baking tests (table 2) were the optimal amount for each flour mixture, respectively, determined by adding water until a farinograph mixing curve peak reached 500 Brabender units (BU).

Table 2. Water amount added to different flour mixtures

Flour mixture		Water amount (ml)
Wheat +	30% non-extracted rye bran	141
	30% finely milled non-extracted rye bran	143
	30% extracted rye bran	142
	30% finely milled extracted rye bran	144
Wheat +	20% non-extracted rye bran	138
	20% finely milled non-extracted rye bran	141
	20% extracted rye bran	138
	20% finely milled extracted rye bran	141

For the baking tests doughs were prepared with 200 g flour mixture, 2.5 g dry yeast (Kronjäst, Orginal, Jästbolaget, Sollentuna, Sweden), 1.9 g sodium chloride (Falksalt, AB Hanson & Möhring) and tap water (48° C). The ingredients were mixed for 10 minutes in a farinograph at the highest speed and the doughs were left in a leavening cupboard (34° C, 60% RH, Elektro Helios, Stockholm, Sweden) for 60 minutes. The doughs were thereafter divided into pieces of 3×100 grams, moulded and placed in baking tins greased with cooking fat and placed in a second leavening cupboard (39° C, 85% RH, Elektro Helios, Stockholm, Sweden) for 60 minutes. The fermented doughs were baked for 12 minutes at 255° C in a rotation oven (Simon, Greenfield, England).

The inner temperature of the breads was measured when taken out from the oven and after 60 minutes of cooling. Weight, height, volume (by displacement with pearl "sagogryn") and porosity according to the Dallman scale were measured. The breads were thereafter put in sealed plastic bags, frozen and freeze-dried for analysis of AR.

2.5 Determination of AR in wheat flour and rye bran

To determine the amount of AR in wheat flour and non-extracted and extracted rye bran and finely milled rye bran, the samples were analysed with GC according to Ross *et al*. (2001). The samples were milled to a particle size of <0.5 mm and mixed (1 g for extracted rye bran and wheat flour, and 0.5 g for non-extracted rye bran) with 200 µl internal standard (0.500µg/ml methyl behenate) and 40 ml ethyl acetate in 50 ml tubes. The test tubes were thereafter put on a rolling mixer (Swelab 180) for 24 hours and centrifuged for 10 minutes at 1500×g. Portions of the extract (4ml) were transferred to 5 ml test tube and evaporated, using a centrifuge evaporator (Speedvac concentrator, Savant Instruments Inc, Farmingdale, NY, USA), for 40 min. Ethyl acetate was added (200µl) and the samples were mixed and filtered through 0.45 µm GHP Acrodisc® filters (VWR, Darmstadt, Germany) connected to 1 ml Omnifix®-F syringes (Braun, Germany) into GC vials for analysis.

2.6 Determination of AR in bread

The freeze-dried bread samples were crushed and milled (Retsch mill, Hannover, Germany) to a particle size of <0.5 mm. Since AR form complexes with amylose, starch in the breads makes the extraction of AR difficult with non-polar solvents such as acetone. Extraction was therefore performed with hot 1-propanol and water according to Ross *et al*. (2003). The milled samples (0.5 g for non-extracted and 1 g for extracted samples) were placed in 30 ml glass tubes with tight screw cap. Internal standard (200 µl methyl behenate) dissolved in ethyl acetate (0.5mg/ml) and 10 ml 1-propanol:water (3:1, v/v) was added. The tubes were put in water bath with boiling water for 2 h and mixed on a vortex every 30 min. After cooling to room temperature the tubes were centrifuged at 2300×g for 10 min. The supernatants were thereafter transferred to 50 ml tubes with tight screw cap. The procedure was repeated two times more with extraction for 2h and 1h, respectively, and all extracts were pooled in the 50 ml tubes. Portions of the sample extracts (5.0 ml) were transferred to 50 ml pear-shaped flasks. The samples were evaporated to dryness in a roto-evaporator at 60°C. To trap the water and get the samples dry, absolute ethanol was added. Ethyl acetate (600 µl) was added to the dried samples and mixed on a vortex. The ethyl acetate extracts were filtered twice trough 0.45 µm GHP Acrodisc® filters (VWR, Darmstadt, Germany) connected to 1 ml Omnifix®-F syringes (Braun, Germany) into GC vials for analysis.

2.7 GC analysis

GC was run with a split/splitless injector at following conditions:

1. Inlet temperature: 325 °C
2. Detector temperature: 350 °C
3. Temperature programme: 250 ºC (0 min), 320 ºC (20 min), 320 ºC (22 min), 330 ºC (30 min).
4. Carrier gas: helium, constant flow at 1.5 mL/min approx. (37 cm/s), split 1:10.
5. Detector gases: make-up gas (N_2) (46 mL/min), hydrogen gas (40 mL/min)
6. Column: HP-5 capillary column (30 m × 0.25 mm i.d. × 0.25µm) or equivalent.

3 Result and discussion

3.1 Baking

It is important that the baking conditions are the same throughout the experiments. To get use to the technique, test bakings were done before the real trials started to minimize risk for uneven results due to human source of error. The temperature in the leavening cupboard varied slightly and the relative humidity was difficult to keep at the required level, but was held as close as possible. After baking the inner temperature of the breads were measured and the breads were cooled to room temperature under a baking cloth for one hour. After cooling, the volume and height were measured and the breads were weighed and cut in two pieces to evaluate the pore size. The results are shown in table 3. The porosity of each bread was examined by visual observation and comparison with pictures from the Dallman scale. The Dallman scale ranges from 1 to 9, where 1 is a very open heterogeneous structure with large pores and 9 describes a homogenous compact structure with small pores. Generally, larger Dallman scale values are more acceptable than smaller values.

There was a difference in height and volume between the breads baked with 30 and 20% rye bran. The breads with 20% bran had a larger volume (172-175 ml) than the breads with 30% bran (158-165 ml) and they were slightly higher (4.5-4.9 mm) than the breads with 30% bran (4.1-4.2 mm). This was expected since the leavening process is reduced with increasing amount of bran. The bread with 30% bran had a compact structure and the porosity value was 9 on the Dallman scale. The pore structure of the 20% breads was a little less compact and was given 8 on the Dallman scale (Figure 3). There were no differences between non-extracted and extracted rye bran breads, or between finely milled and normal rye bran breads. These results showed that bread volume and height did not increase when the bran was milled or extracted with acetone.

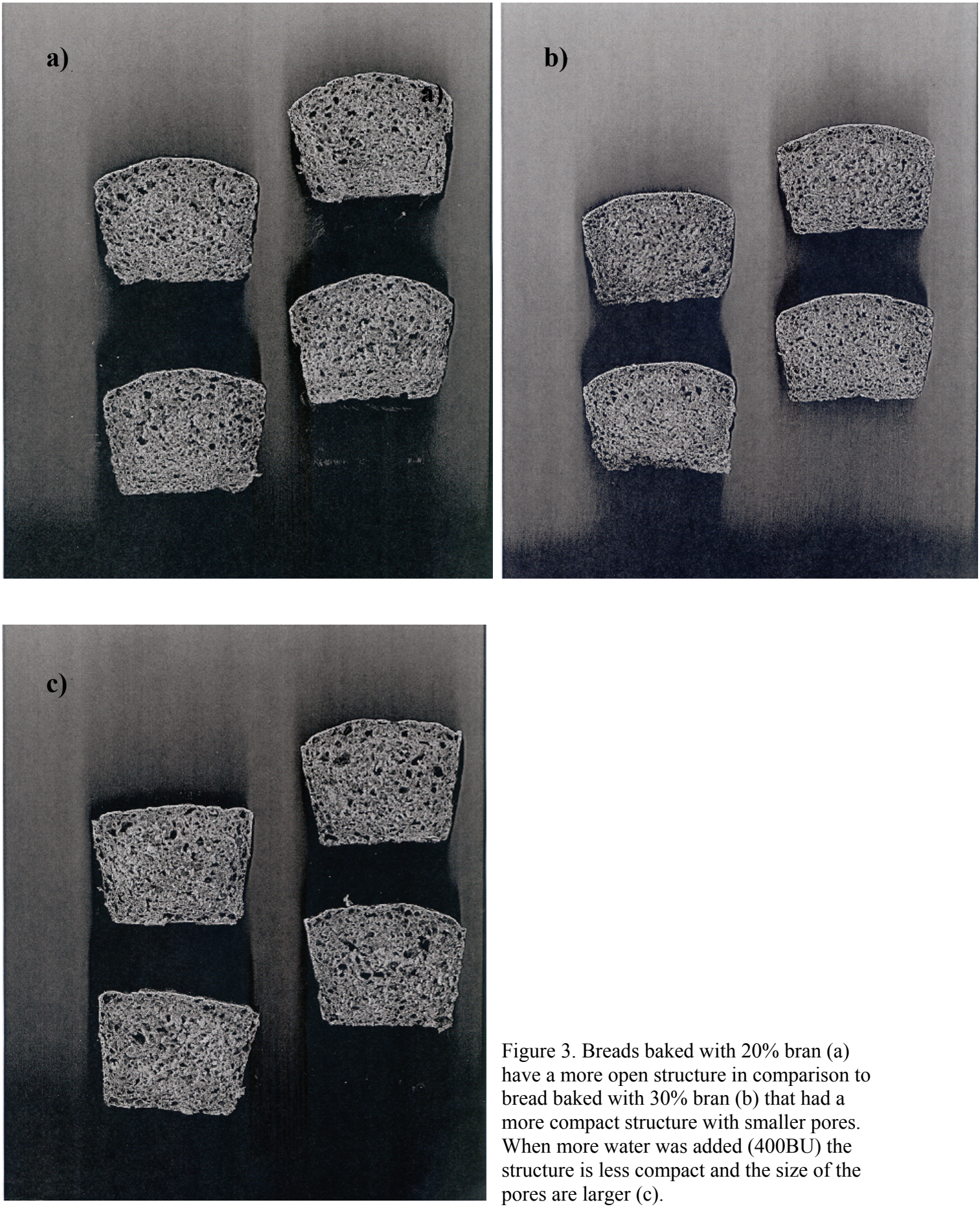

Figure 3. Breads baked with 20% bran (a) have a more open structure in comparison to bread baked with 30% bran (b) that had a more compact structure with smaller pores. When more water was added (400BU) the structure is less compact and the size of the pores are larger (c).

Table 3: Inner temperature (°C), weight (g), height (mm), volume (ml) and porosity according to the Dallman scale (1-9, where 1 is a very open structure and 9 is a compact structure with small pores) in breads baked with wheat flour and 20 or 30% rye bran

Trial	Temp (°C)[a]	Weight (g)[b]	Height (mm)[b]	Volume (ml)[b] (CV %)	Dallman scale (1-9)[b]
30 % non-extracted rye bran	96.3	90.4	41	165.0 (5.4)	9
30 % finely milled non-extracted rye bran	95.9	89.9	41	158.3 (3.8)	9
30 % extracted rye bran	96.5	89.3	41	158.3 (2.6)	9
30 % finely milled extracted rye bran	95.2	89.2	42	162.5 (2.6)	9
20 % non-extracted rye bran	96.7	90.1	45	175.0 (3.6)	8
20 % finely milled non-extracted rye bran	96.1	89.7	45	173.3 (2.4)	8
20 % extracted rye bran	96.7	90.6	49	174.2 (1.2)	8
20 % finely milled extracted rye bran	96.5	90.6	46	171.7 (3.0)	8

a) measured directly after baking

b) measured 1h after baking

During the 30% bran bakings, too little water was added due to miscalculations. The access of bran was limited and the 30 % trials could not be repeated. The miscalculation during the 30% trials explains the similar amounts of water added to the 30 and 20% trials.

The water addition was calculated to get a 500 BU curve on the farinogram. A couple of breads outside the experiment were baked with a larger water addition (400 BU). Those breads had a similar volume and height as the 500 BU breads but the porosity was different, see figure 3. The flour was fully hydrated and the cohesiveness of the dough was probably better.

3.2 Particle size

The regular and milled rye bran was sifted into 6 different milling fractions to measure particle size. The proportion of particles >219 μm was 49% for non-milled bran and only 22% for milled bran, while the proportion of particles <219 was 36 and 78%, respectively (Table 4). This shows that finely milled rye bran had smaller particle size than non milled rye bran, which was expected.

Table 4. The particle size distribution (relative %) for the different fractions in non-milled and milled rye bran. The results are given in gram and percentage

Sift	Proportion (%) non-milled rye bran	Proportion (%) finely milled rye bran
> 670 μm	3.4	0
<670 μm	12.4	0
<460 μm	33.6	22.2
<219 μm	32.4	31.4
<150 μm	1.6	23.6
<75 μm	0.6	21
<50 μm	1	1.8
Total	100	100

3.3 Alkylresorcinols in regular and extracted bran

The rye bran and wheat flour was extracted with acetone, and all AR was not completely extracted from the bran (table 5). The content of AR in non-extracted bran was 4030-4145 µg/g dm and in extracted bran 413-570 µg/g dm. The homologue composition was similar before and after extraction. The extraction procedure was repeated two times and could have been repeated one more time to decrease the amount of AR even more. The difference between regular and extracted bran was however quite large and another extraction would not necessary have given a decent effect.

The wheat flour contained only very small amounts of AR (33 µg/g dm) (Table 5), which has also been shown before (Hengtrakul *et al*., 1990). The wheat flour does not contain any bran parts due to the easy separation of wheat bran from starchy endosperm during milling. This explains the low amounts of AR in wheat flour (Slavin *et al*., 2001). In rye on the other hand, the bran is almost impossible to separate totally from the starchy endosperm, resulting in higher contents of AR in sifted rye flour (Gohil *et al*., 1988; Ross *et al*., 2001; Ross *et al*., 2003a).

Table 5. Content (µg/g dm) and relative homologue composition (%) of AR in flour and bran samples. All results are average of triplicates. The extraction of the bran was made in two sets; rye bran 1 is used for the 30% bran baking and extracted rye bran 2 for the 20% baking

Sample	Total saturated (µg/g) (CV %)	Relative homologue composition (%)				
		17:0	19:0	21:0	23:0	25:0
non-extracted rye bran	4144.5 (5.1)	20.6	30.1	26.5	11.9	10.9
finely milled non-extracted rye bran	4030.4 (1.5)	20.5	30.2	26.5	11.9	10.9
extracted rye bran	430.8 (3.3)	19.7	29.4	26.9	12.3	11.7
finely milled extracted rye bran 1	464.3 (2.3)	19.8	29.7	26.5	12.3	11.7
finely milled extracted rye bran 2	578.0 (4.5)	19.8	29.9	26.6	12.1	11.6
Wheat flour	32.8 (15.6)	0.0	40.6	52.3	7.1	0.0

3.4 Alkylresorcinols in bread

The content of AR in breads baked with 30 and 20% rye bran was 1064-1067 µg/g dm and 662-720 µg/g dm respectively. In bread baked with 30 and 20% extracted rye bran the content of AR was 153-150 µg/g dm and 99-100 µg/g dm respectively (Table 6). All breads tested in this study, contained AR and from the results it is seen that AR was not degraded during baking, which is in accordance with Ross *et al.* (2003a). The proportional content of total saturated AR in the breads baked with 30 or 20% rye bran is generally comparable to the content of AR in the rye bran. The homologue composition of the AR in the baked bread differed however slightly from the bran. There were no differences in AR content between breads baked with normal and finely milled rye bran.

The different homologue composition of AR between rye bran and breads is partly due to the different composition of AR in wheat flour, but may also be due to the complex that AR possible forms during the acetone extraction of the bran. The AR in the bran are not extracted during the EA preparation before GC analysis. This can explain why some samples diverge. The relative proportion of homologue C19:0 seems to increase in the baked bread after extraction whiles the homologue C25:0 decreases in the breads after extraction with 1-propanol. Both these homologues are present in equal amounts before and after extraction in rye bran (Table 6) but the possible complex formation in the bran during acetone extraction are not detected during the analysis. Earlier studies by Ross *et al*. (2003a) have shown that all AR are extracted from food products with 1-propanol and that no homologue is harder to extract than others. To extract all AR from the product and reduce the risk of complex formation, 1-propanol should have been used as solvent during all extractions, both bran and bread.

Even though the content of AR was ten times lower in breads baked with extracted rye bran, bread volume and porosity was not affected. These results suggest that natural AR levels in bread are too low to have any practical impact on the bread quality in comparison to the study made by Söderman (2008) where the negative effect of leavening properties was expressed in bread with high levels of added AR.

Table 6. Amount of saturated AR and the homologue composition of the different breads in percentage. All results are the average of duplicates. The extracted bran used for all 30% bakings is from extraction 1 and the bran used for all 20% breads is from extraction 2

Bread sample	Total saturated (µg/g) (CV %)	Homologue composition (%)				
		17:0	19:0	21:0	23:0	25:0
30 % non-extracted rye bran	1064.1 (4.2)	19.4	31.2	26.4	12.0	11.0
30 % finely milled non-extracted rye bran	1067.6 (3.4)	19.8	31.1	26.4	12.1	10.8
30 % extracted rye bran	153.3 (2.4)	15.2	44.1	25.0	11.1	4.6
30 % finely milled extracted rye bran	150.3 (4.9)	14.2	45.0	24.7	10.1	5.9
20 % non-extracted rye bran	719.6 (3.2)	19.0	31.9	26.9	11.9	10.4
20 % finely milled non-extracted rye bran	661.6 (1.5)	19.9	32.0	27.2	10.7	10.2
20 % extracted rye bran	98.7 (8.4)	15.6	40.4	24.8	11.5	7.6
20 % finely milled extracted rye bran	100.2 (4.9)	14.2	42.7	21.6	12.1	9.4

4 Conclusions

In this study eight baking trials were done using the same baking conditions with two different proportions of rye bran (20 or 30%) that was treated differently, e.g. no treatment, fine milling, extraction with acetone or extraction and milling.

The results showed that the naturally occurring AR in rye bran did not affect the baking properties in bread. The height, volume and pore size were more dependent on fibre content and water addition to the dough. The water addition to reach the 500 BU standard is not developed for high fibre doughs and the high fibre content make each dough absorb water differently making it difficult to get even results. Further investigations needs to be done to ensure these findings but due to this study extraction of AR to increase bread volume is not essential. The extraction procedure is time consuming and not only AR, but also other lipids are extracted which can have a negative effect on the baking properties.

Regarding future studies on the effect of AR on baking properties; AR should be fully extracted to eliminate the risk of remaining AR to influence the results. The bran should also be extracted with 1-propanol, instead of acetone, to eliminate the risk of AR forming complexes during the extraction.

5 Acknowledgement

This study has been a degree thesis at the Department of Food Science, Swedish University of Agricultural Science, Uppsala.

A warm thank to my supervisor Annica Andersson for her valuable guidance and help through this project. Thanks to Rikard Landberg for all the help with gas chromatography and laboratory procedures, Janicka Nilsson and Gunnel Fransson for all the help in the laboratory and general help with all conceivable things.

Many thanks to everybody at the Department of Food Science, making me feel welcome, Einar for the quick help with the equipment in the milling room and all people that contributed with information necessary to accomplish this project.

I also want to thank Ove Albertsson at Västmanlands forsknings- och utvecklingsråd for the economic support.

Lastly, special thanks to my family and friends that have been a great support during this project.

6 References

Printed references

Branca, F. Hanley, A.B. Pool-Zobel, B. Verhagen, H. (2001) Biomarkers in disease and health. *British Journal of Nutrition*. 86. 55-92

Cauvain, S. (2003a) Introduction. In: Cauvain, S (Ed) *Bread Making, improving quality*. Cambridge: Woodhead publishing limited. p.1-7

Cauvain, S. (2003b) Breadmaking:an overview. In: Cauvain, S (Ed) *Bread Making, improving quality*. Cambridge: Woodhead publishing limited. p. 8-27

Chen, Y. Ross, A.B. Åman, P. Kamal-Eldin, A. (2004) Alkylresorcinols as markers of whole grain wheat and rye in cereal products. *Journal of Agricultural and Food Chemistry* 52. 8242-8246.

Cornell, H. (2003) The chemistry and biochemistry of wheat. In: Cauvain, S (Ed) *Bread Making, improving quality*. Cambridge: Woodhead publishing limited.p.31-66

Gohil, S. Pettersson, D. Salomonsson, A-C. Åman, P. (1988) Analysis of alkyl-alkylresorcinols in triticale, wheat and rye. *Journal of the Science of Food and Agriculture* 45. 43-52.

Hansen, R.H. Blennow, A. Pedersen, S. Nørgaard, L. Engelsen, S.B. (2008) Gel texture and chain structure of amylomaltase-modified starches compared to gelatin. *Food Hydrocolloids* 22. 1551-1566.

Hengtrakul, P. Lorenz, K. Mathias, M. (1990) Alkylresorcinols in U.S. and Canadian wheats and flours. *Cereal Chemistry* 67 (5). 413-417.

Hoseney, R.C. (1994) Principles of cereal science and technology, 2nd edition. St. Paul, Minnesota: American association of cereal chemists, Inc. p. 229-273.

Jacobs, D. Marquart, L. Slavin, J.L. Wiemer, K. (2001) The role of whole grains in disease prevention. *Journal of the American Dietetic Association* 101. 780-785.

Katina, K. (2003) High-fibre baking. In: Cauvain, S (Ed) *Bread Making, improving quality*. Cambridge: Woodhead publishing limited. p.485-497

Kozubek, A. Tyman, J.H.P. (1999) Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity. *Chemical Reviews* 99. 1-25.

Leinonen, K.S. Poutanen, K.S. Mykkänen, H.M. (2000) Rye Bread decreasesserum total and LDL cholesterol in men with moderately elevated serum cholesterol. *The Journal of Nutrition* 130. 164-170.

Landberg, R. Kamal-Elden, A. Salmenkallio-Marttila, M. Rouau, X, Åman, P. (2007) Localization of alkylresorcinols in wheat, rye and barley kernels. *Journal of Cereal Science* 48. 401-406

Linko, A.M. Parikka, K. Wähälä, K. Adlercreutz, H. (2002) Gas chromatographic – mass spectrometric method for the determination of alkylresorcinols in human plasma. *Analytical Biochemistry* 308. 307-313.

Linko, A.M. Juntunen, K.S. Mykkänen, H.M. Adlercreutz, H. (2005) Whole-grain rye bread consumption by women correlates with plasma alkylresorcinols and increases their concentration compared with low-fiber wheat bread. *The Journal of Nutrition* 135. 580-583.

Lorenz, K. Winata, A. (1997) Effects of fermentation and baking of whole wheat and whole rye sourdough breads on cereal alkylrecorsinols. *Cereal Chemistry* 74. 284-287.

Mattila, P. Pihlava, J-M. Hellström, J. (2005) Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. Journal of Agricultural and Food Chemistry 53. 8290-8295.

Parikka, K. (2007) Antioxidative long chain Alkylresorcinols. Synthesis and deuterium labelling of bioactive compounds present in whole grains. Doctoral thesis, Faculty of Science, University of Helsinki, Finland.

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.

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

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

Ross, A.B. Åman, P. Andersson, R. Kamal-Eldin, A. (2004a) Chromatographic analysis of alkylresorcinols and their metabolites. *Journal of Chromatography A* 1054. 157–164.

Ross, A.B. Åman, P. Andersson, R. Kamal-Eldin, A. (2004b) Identification of cereal alkylresorcinol metabolites in human urine – potential biomarkers of wholegrain wheat and rye intake. *Journal of Chromatography B* 809. 125-130.

Ross, A.B. Kamal-Edin, A. Åman, P. (2008) Dietary alkylresorcinols: absorption, bioactivities and possible use as biomarkers of whole grain wheat and rye rich foods. *Nutrition Rewiews* 62 (3). 81-95

Slavin, J. L. Jacobs, D. Marquart, L. (2001) Grain processing and nutrition. *Critical reviews in biotechnology* 21. 49-66.

Smith, P.R, Johansson, J. (2005) Influences of the proportion of solid fat in a shortening on loaf volume and staling of bread. *Journal of Food Processing & Preservation*. 28(5). 359-367.

Söderman, T. (2008) *Effects of alkylrecorsinols on bread baking properties and starch gelatinisation.* Master of Science thesis, Department of Food Technology, Engineering and nutrirtion, Lund University, Sweden

Wrigley, C. (2003) Assessing bread quality. In: Cauvain, S (Ed) *Bread Making, improving quality*. 71-92. Cambridge: Woodhead publishing limited.

Zarnowski, R. Suzuki, Y. Yamaguchi, I. (2002) Alkylresorcinols in barley (Hordeum vulgare L. distichon) Grains. *Zeitcshrift für Naturforschung* 57c. 57-62.

Zarnowski, R. Suzuki, Y. Pietr, S.J. (2004) Alkyl- and Alkenylresorcinols of wheat grains and their chemotaxonomic significance. *Zeitcshrift für Naturforschung* 59c. 190-196.

Åman, P. Nilsson, M. Andersson, R. (1997) Positive Health Effects of Rye. *Cereal Foods World* 8, 684-688.

Personal Communication

Kamal-Edin, A, Department of Food Science, Swedish University of Agricultural Science, personal communication 2009-02-10 afaf.kamal-edin@lmv.slu.se

I denna serie publiceras examensarbeten (motsvarande 15-30 hp i agronomexamen) samt större enskilda arbeten (7,5-15 hp) vid Institutionen för Livsmedelsvetenskap, Sveriges lantbruksuniversitet.

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