Phenolic Compounds in Oats

Effects of Steeping, Germination and Related Enzymes

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Abstract

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This research project examined how to treat raw oat material for oat-based food products in order to sustain or increase the levels of phenolic compounds. The focus was mainly on the avenanthramides, which are potentially health beneficial bioactive components found exclusively in oats. A proposed enzymatic decrease in avenanthramide levels when non heat-treated milled oats are steeped in water was investigated. The decrease was strongly suggested to be caused by a polyphenol oxidase. Although the avenanthramides are only found in oats, the polyphenol oxidase enzyme that acted on avenanthramides was also found to be present in wheat, barley and rye. The effects of a highly controlled steeping and germination process on levels of phenolic compounds and related enzyme activities in oats were studied. The process resulted in increased levels of avenanthramides and some unknown compounds to various extents depending on cultivar. This increase was suggested to be partly due to enzymatically catalysed *de novo* biosynthesis. Whether germination was the reason behind elevated levels of avenanthramides in one harvest year compared with another, in the same oat genotypes, were investigated. The differences in avenanthramide levels between the two years could not be explained by preharvest sprouting of the oat grains in the field. The content and location of tricin was studied in various oat samples. Tricin was found to be localised to oat hulls and was detected and quantified in a minority of all oat samples analysed.

The overall conclusion was that germination of oats can be a good method to sustain or increase avenanthramides and other potentially health beneficial phenolic compounds. It is important to inactivate the polyphenol oxidase present in oats and other cereal grain ingredients included in oat-based food products, since it may otherwise decrease the levels of avenanthramides in these products. Oat hulls may be a good source of tricin if high-tricin cultivars are chosen.

Keywords: avenanthramides, *Avena sativa*, germination, hydroxycinnamic acids, oats, phenolic compounds, polyphenol oxidase, tricin

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Populärvetenskaplig sammanfattning

Havre har under lång tid ansetts vara hälsosamt för både människor och djur. Havrens unika protein- och fettsammansättning har bidragit till denna syn. Havre innehåller även en stor mängd vattenlösliga kostfibrer som har visat sig kunna sänka blodkolesterolhalten hos människor. En följd av att havre är så rikt på fett, dock ett hälsosamt sådant, är att mängden antioxidanter också är högt. Antioxidanter bidrar till att förhindra fettet från att härskna men är också bra för hälsan. Studier har påvisat samband mellan intag av livsmedel med hög fenolhalt och lägre risk för vissa sjukdomar t.ex. cancer, hjärtkärlsjukdomar och stroke. Många antioxidanter i havre tillhör gruppen fenoler. Fenolerna i havre finns främst i klidelen av kärnorna. Eftersom havre nästan alltid konsumeras som en fullkornsprodukt anses den vara en utmärkt källa av fenoler som kan komma människor till godo genom kosten. Målet med den här studien var att undersöka hur havre ska behandlas för att kunna bevara, eller till och med öka, mängden fenoler i havreråvara till livsmedel. Huvudfokus har legat på två olika fenoliska föreningar; avenantramider samt tricin.

Avenantramider är en grupp ämnen som är unika för havre. De finns i störst mängd i klidelen av havrekärnan men även i skalet. Avenantramiderna har visat sig ha goda antioxidativa egenskaper och studier har också indikerat att de kan ha anti-inflammatoriska och anticancerogena egenskaper. Avenantramiderna har visat sig vara relativt stabila under beredning av livsmedel vilket är en förutsättning för att de ska kunna komma människor till nytta. Eftersom avenantramider är potentiellt hälsobefrämjande kan det vara önskvärt att öka halten av dessa i havrebaserade livsmedel. Den här studien undersökte bl.a. hur en kontrollerad groningsprocess påverkade halten avenantramider i havre. Resultatet visade att halten ökade under groning i alla sorter som analyserades men att graden av ökning var beroende av havresort. Under groningen ökade även aktiviteten av ett enzym som deltar i bildandet av avenantramider vilket tyder på att ökningen av avenantramiderna kunde bero på nybildning. Det undersöktes också om den skillnad i halt avenantramider som fanns mellan två årsskördar havre kunde förklaras av groning i fält. Dock kunde ingen sådan koppling göras utan skillnaden var snarare ett resultat av andra omgivande odlingsfaktorer. Tidigare studier har visat att halten avenantramider minskar när man blötlägger havremjöl i vatten vilket är en icke önskvärd reaktion. Den här studien visade att minskningen beror på att enzymet polyfenoloxidas bryter ned avenantramiderna under vissa betingelser. Även om avenantramider bara finns i havre så kunde aktivitet av detta enzym, mot just avenantramider, även detekteras i vete, korn och råg.

Tricin är en fenol som tidigare har studerats som en komponent i riskli. Det finns många studier som indikerar att tricin besitter anticancerogena egenskaper mot vissa typer av cancer. Den här studien undersökte förekomst samt lokalisering av tricin i havre. Resultaten visade att tricin fanns i varierande halter främst i utländska havresorter och att det endast kunde detekteras i skalet.

Sammanfattningsvis visar resultaten att groning av havre kan vara en bra metod för att bevara eller öka halten fenoler, inklusive avenantramider, i havre. Den grodda havren kan sedan användas som ingrediens i livsmedelsprodukter. Valet av havresort måste dock övervägas eftersom effekten av groning skiljde sig åt mellan sorterna. Enzymet polyfenoloxidas, som finns i havre såväl som i andra spannmål, bör inaktiveras för att bibehålla den potentiellt goda nutritionella kvaliteten som avenantramiderna bidrar till i livsmedlet. Havreskal kan vara en god källa till tricin om havresorter med hög halt tricin väljs och om havreskal i större utsträckning än idag används som ingrediens i t.ex. spannmålsbaserade livsmedel.

Contents

Appendix

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

Papers I-IV

I. Skoglund, M., Andersson, R., Sunnerheim, K., Ebbers, M. & Dimberg, L.H. Avenanthramides as substrates for oat polyphenol oxidase. *Submitted for publication*.

II. Skoglund, M., Peterson, D.M., Andersson, R., Nilsson, J. & Dimberg, L.H. Avenanthramide content and related enzyme activities in oats as affected by steeping and germination. *Accepted for publication in Journal of Cereal Science*. DOI: 10.1016/j.jcs.2007.09.010.

III. Skoglund, M., Andersson, R., Scholz, I., Ajithkumar, A., Jonsson, R. & Dimberg, L.H. Annual differences in oat avenanthramide content not explained by preharvest sprouting. *Submitted for publication*.

VI. Skoglund, M., Andersson, R., Jonsson, R. & Dimberg, L.H. Tricin in oats. *Manuscript to be submitted*.

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Author's main contributions to the papers:

I. Planned the experiments together with the supervisors and co-authors, independently performed the laboratory work, evaluated the results and performed statistical analyses, prepared the manuscript, acted as corresponding author.

II. Planned the experiments together with the supervisors and co-authors, independently performed the laboratory work, evaluated the results and performed statistical analyses, prepared the manuscript, acted as corresponding author.

III. Planned the experiments together with the supervisors and co-authors, performed parts of the laboratory work, evaluated the results and performed statistical analyses, prepared the manuscript, acted as corresponding author.

IV. Planned the experiments together with the supervisors and co-authors, performed parts of the laboratory work, evaluated the results and performed statistical analyses, prepared the manuscript.

List of abbreviations

ANOVA   analysis of variance
HHT   hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase
HPLC   high performance liquid chromatograpgy
LC-MS   liquid chromatography-mass spectrometry
NMR   nuclear magnetic resonance
PCA   principal component analysis
PPO   polyphenol oxidase
a   cinnamic acid
p   *p*-coumaric acid
c   caffeic acid
f   ferulic acid
s   sinapic acid
1a   *N*-(cinnamoyl)-anthranilic acid
1p   *N*-(4'hydroxy-(E)-cinnamoyl)-anthranilic acid
1c   *N*-(3', 4'-dihydroxy-(E)-cinnamoyl)-anthranilic acid
1f   *N*-(4'-hydroxy-3'-methoxy-(E)-cinnamoyl)-anthranilic acid
1s   *N*-(4'-hydroxy-3',5'-dimethoxy-(E)-cinnamoyl)-anthranilic acid
2a   *N*-(cinnamoyl)-5-hydroxyanthranilic acid
2p   *N*-(4'hydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid
2c   *N*-(3', 4'-dihydroxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid
2f   *N*-(4'-hydroxy-3'-methoxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid
2s   *N*-(4'-hydroxy-3',5'-dimethoxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid
3a   *N*-(cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid
3p   *N*-(4'hydroxy-(E)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid
3c   *N*-(3', 4'-dihydroxy-(E)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid
3f   *N*-(4'-hydroxy-3'-methoxy-(E)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid
3s   *N*-(4'-hydroxy-3',5'-dimethoxy-(E)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid

Introduction

Background

Intake of whole grain cereals has long been considered to be beneficial to human health. In the early 1970s, it was reported that a high intake of whole grain foods may be protective against hyperlipidaemia and ischaemic heart disease (Trowell, 1972). Since then, several epidemiological studies have shown an inverse correlation between intake of whole grains and risk of cardiovascular disease, diabetes, some forms of cancer and obesity (Chatenoud *et al.*, 1998; Anderson, 2003; Montonen *et al.*, 2003; Slavin, 2005), although the mechanisms behind these effects are poorly understood (Seal, 2006). It has also been shown that consumers of whole grains have a significantly better nutrient intake profile than non-consumers, with a higher intake of vitamins and minerals and a lower intake of fat and added sugars (Cleveland *et al.*, 2000).

Oats are a cereal that is commonly consumed as whole grains and known to provide healthy nutrients to humans. Oats have been grown for thousands of years, mainly as an animal feed crop, but during the 19th century oats won acceptance as part of the human diet (Webster, 1986). At that time oats were generally consumed as oatmeal, but today oats can be found in various food products, for example breakfast cereals, beverages, bread and infant foods (Webster, 1986; Yao *et al.*, 2006; Johansson *et al.*, 2007; Zhang *et al.*, 2007a). Many studies have shown important physiological effects of oats. The attenuation of postprandial plasma glucose and insulin levels and the control of cholesterol can be ascribed to the partly soluble and viscous dietary fibres found in oats (Anttila, Sontag-Strohm & Salovaara, 2004). Oats are also a good source of various bioactive compounds, for example antioxidants such as vitamin E, phytic acid and phenolic compounds (Dimberg, Theander & Lingnert, 1993; Peterson, 2001). It has been shown that some phenolic compounds found in oats may have potential health beneficial properties (Dimberg, Theander & Lingnert, 1993; Peterson, Hahn & Emmons, 2002; Ji *et al.*, 2003, Chen *et al.*, 2007). These circumstances together emphasise the importance of oat-based food products as part of the human diet. Furthermore, in many countries oats are one of few grains that can be recommended in the diet of patients suffering from coeliac disease, since oats lack the provocative gluten proteins (Garsed & Scott, 2007; Rashid *et al.*, 2007).

The beneficial nutritional properties of oats have attracted attention from researchers and have resulted in the food industry wishing to use oats as a food ingredient more extensively than today and therefore more research is needed in this area. The overall aim of the present project was to obtain new knowledge on how to treat the raw oat material for oat-based food products in order to sustain or increase the levels of phenolic compounds, in this case mainly the avenanthramides, which are a group of potentially health beneficial bioactive compounds unique to oats. A tentative avenanthramide degrading enzyme found in oats was investigated. The effects of steeping and germination of oat samples on content of avenanthramides were studied in combination with activity of related

enzymes affecting the avenanthramide levels. We also investigated whether elevated levels of avenanthramides found in combination with low average molecular weight of β-glucan in one harvest of oats could have been related to preharvest sprouting. In addition, the flavone tricin, which has been described as having potential anticarcinogenic properties, was studied in oats.

Oats

Among the cereals cultivated in Sweden, wheat is the largest crop, followed by barley, with oats in third place. In 2007 the total production of oats in Sweden was 883 000 tons, cultivated on an area of 208 483 ha (Jordbruksverket, 2007). The average yield was 4290 kg/ha, which is more than the average yield for the EU (3500 kg/ha). The average consumption of oats in Sweden is somewhere around 2.5 kg/person and year with a large individual variation, whereas the average for the EU is approximately 1.2 kg/person and year (European Commission, 2005). In Europe, the largest producers of oats are Finland, Germany and Sweden (Foreign Agricultural Service, USDA). Other large oat producers are North America, Canada, Russia and Australia. The most cultivated oat species in the world is *Avena sativa* L. (common covered white oat) (White, 1995). A variety of *A. sativa* is *A. sativa var. nuda* (a naked variety), which is commonly cultivated and has a hull which is loosely attached to the groat and which is removed in threshing. The naked oat variety has good grain quality, although it is more prone to mechanical damage than covered oats and generally has lower yields compared with covered oat varieties (Lászity, 1998). Oats can be grown on many different soil types and are considered to be one of the most versatile cereals regarding soil type. The nutrient requirements (N, P, K) for oats are less than those for wheat or maize. The optimal conditions for cultivation of oats are cool and moist climates, since oats need more moisture to produce a given unit of dry matter than all other cereals except rice (Forsberg & Reeves, 1995).

Morphology and location of nutrients in oat grain

A mature oat grain has one primary function, which is to generate a new plant by developing shoots and roots during germination, providing sufficient stores of nutrients during the first days of growth, releasing these nutrients and protecting itself from hostile environmental conditions (Fulcher, 1986). The outermost part of the oat grain is the hull or husk, which tightly encloses the oat groat (Figure 1). The hull structure consists of two layers, the lemma and the palea. The structure is leaflike and provides the grain with protection and contains for example photosynthetic and vascular structures during development of the grain. In mature oat grains the hull lacks significant metabolic activity and exists primarily as layers of cell wall material (Welch, Hayward & Jones, 1983; Fulcher, 1986). The hull is thereby of great importance for the total dietary fibre content of the oat grain. The hull constitutes on average approximately 25% of the total grain weight, which is a large proportion compared with barley where the hull only constitutes about 10% of the grain weight (White, 1995; Browne, White & Burke, 2002). The chemical composition of the hull can differ with variety and cultivation

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environment (Welch, Hayward & Jones, 1983). The oat hull is composed mainly of cell wall material with almost equal amounts of cellulose and hemicellulose (30-35%). Lignin and ash contents are 2-10% and 3.5-9% respectively. Protein, oil, starch and water-soluble carbohydrate levels are overall relatively low. A large number of bioactive phenolic compounds can be found in the hull, among them *p*-coumaric acid, ferulic acid, vanillic acid, tricin and avenanthramides (Collins, 1986; Dimberg, Theander & Lingnert, 1993).

The dehulled oat groat, which is covered with hollow trichomes, is composed of three morphologically and chemically distinct commercial milling fractions, the bran, starchy endosperm and germ. These three components are all composed of several different tissues. The bran acts as an envelope for the groat and is composed of the outermost pericarp, testa, nucellus, aleurone layer and sub-aleurone layer. The aleurone layer is considered to be the most important component of the bran, surrounding the starchy endosperm and comprising approximately half the germ. The aleurone layer contains a high concentration of phenolic compounds, *e.g.* ferulic acid, which is suggested to be involved in the plant's defence system against microorganisms. The aleurone layer is also rich in proteins and overall the bran contains approximately 50% of the total groat protein (Fulcher, 1986). The vitamins and minerals are mainly present in the bran fraction and to a lesser extent in the starchy endosperm and germ (Lockhart & Hurt, 1986).

The amount of starchy endosperm constitutes 55-70% of the total weight of the groat depending on cultivar (Youngs, 1972). The starchy endosperm is considered to be metabolically inactive but serves as a major reserve of various nutrients to be provided to the growing embryo during germination. Starch is mainly found in the starchy endosperm and in parts of the sub-aleurone layer, while β-glucan is the main component of the starchy endosperm cell walls. The thickness of the cell wall tends to increase in the outer part of the endosperm and β-glucan is mainly enriched in this part of the oat grain (Fulcher, 1986). The largest proportion of the oil in oats has been reported to be found in the endosperm (86-90%), followed by the bran (13%) and the germ (2.4%) (Price & Parsons, 1979; Banaś *et al*., 2007).

The germ, where a mature plant arises, consists of several structurally and functionally distinct tissues of which the scutellum represents more than 80% of the germ weight. The germ is highly metabolically active during germination and transports nutrients released from the starchy endosperm to the embryonic axis. During development of the oat grain the germ and the starchy endosperm expand as two independent structures and near maturity considerable pressure is exerted at the border between them. This results in damage to the cells in that region, which is usually referred to as the depleted layer (Fulcher, 1986).
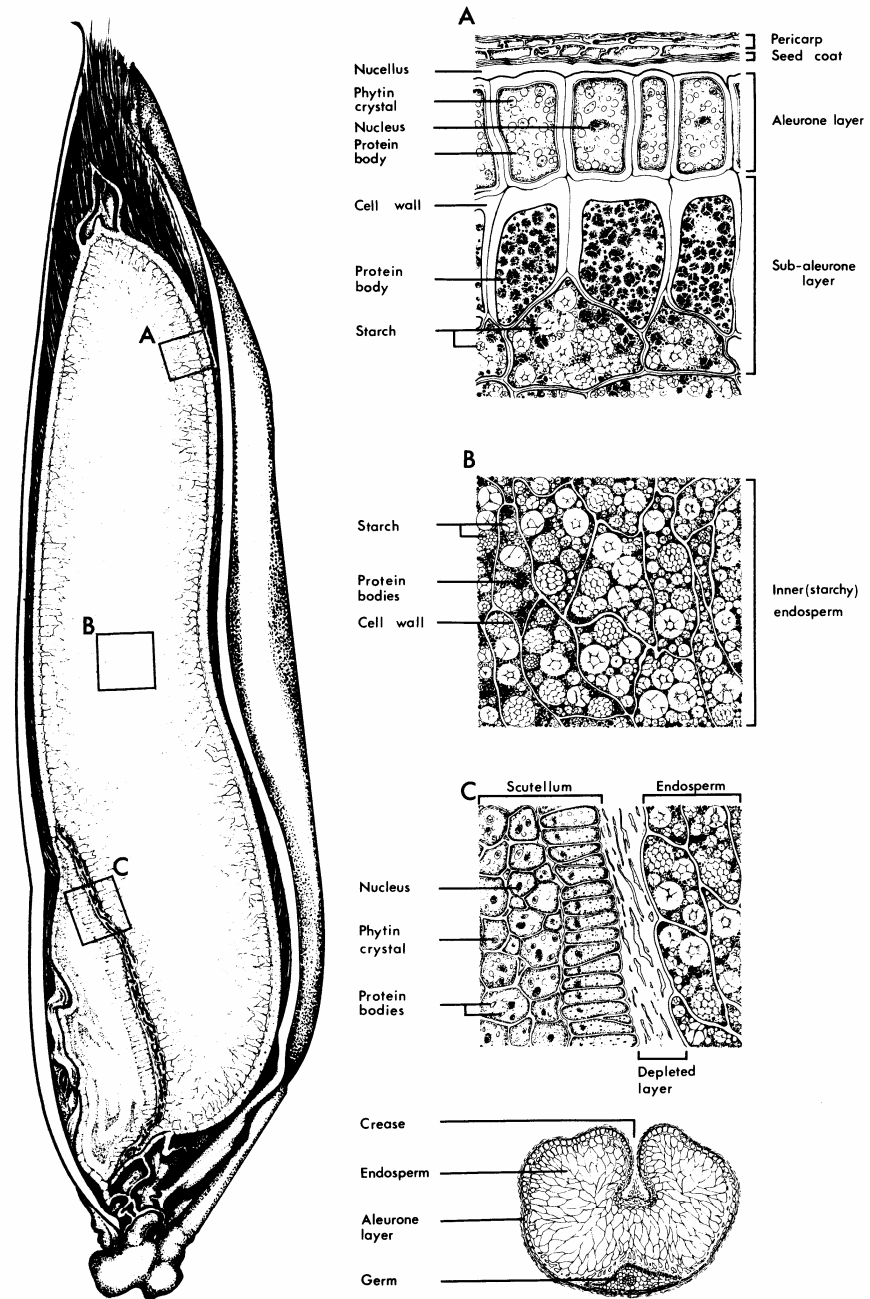
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Figure 1. Major structural features of the oat grain presented as a cross-section of the grain. (A), (B) and (C) are higher magnifications of portions of the bran, starchy endosperm and germ respectively. Reprinted from an original by Fulcher (1986) with kind permission from the author.

Content and quality of nutrients in oat grain

The content and quality of nutrients in oat grain depend on several factors. It has been shown that the growth environment affects the nutritional composition to a large extent. There are also significant interactions between environment and genotype for content of some nutrients (Peterson *et al.*, 2005). The main constituents of oat grains are starch (39-55%), protein (9-16%), lipids (4.5-7%) and dietary fibre (20-39%, including non-starch polysaccharides and Klason lignin) (Åman, 1987). Fertilisation with nitrogen can increase the protein content (Welch & Yong, 1980; Lásztity, 1998) and high-oil oat lines have been developed where the oil content can reach as high as 18% (Peterson & Wood, 1997; Frey & Holland, 1999; Banaś *et al.*, 2007). The protein and β-glucan concentrations may increase with increased oil concentration, while the concentration of starch decreases (Peterson & Wood, 1997). Micronutrients found in oats include minerals such as calcium, phosphorus, potassium, magnesium, iron, zinc and copper, and vitamins such as thiamine, pantothenic acid, niacin, folic acid and vitamin E (Lockhart & Hurt, 1986). Other micronutrients found in oats include antioxidants, of which many are phenolic compounds (Collins, 1986; Dimberg, Theander & Lingnert, 1993; Dimberg *et al.*, 1996).

Triglycerides account for the major content of lipids in oats (Youngs, 1986; Zhou *et al.*, 1999). The most common fatty acids are oleic (18:1), linoleic (18:2) and palmitic (16:0). These three fatty acids account for about 90-95% of the total fatty acids in oats (Welch, 1995). The fatty acid composition differs between different tissues, with a higher proportion of linoleic (18:2) and linolenic (18:3) acid in the germ compared with the whole grain (Banaś *et al.*, 2007). The content of oleic and linoleic acids in particular is much higher in oats compared with wheat and barley (Cuddeford, 1995). Linoleic and linolenic acids are considered to be essential fatty acids in mammalian nutrition and are therefore important constituents of oat lipids (Stryer, 1995). The lipid content in oats is more affected by variety than by cultivation conditions and location (Zhou *et al.*, 1999). The majority of the proteins in oats can be found in the starchy endosperm and the bran, but the amino acid composition varies between the different tissues (Peterson & Brinegar, 1986). The protein quality of oats is unusually high compared with other cereals. Oats contain the amino acids lysine, threonine and methionine, which are considered to be nutritionally limiting to humans and thus are referred to as essential amino acids (Peterson & Brinegar, 1986). Increased levels of protein are accompanied by a decline in protein quality, although this decline is less pronounced in oats compared with other cereals (Welch, 1995). The protein content varies primarily with environmental conditions such as available soil nitrogen. Oat starch granules are generally polygonal in shape and occur in the grain as compound granules that are large and spherical (Hoseney, 1994). The gelatinisation temperature for oat starch is somewhere around 55 °C, which is considered to be a low temperature compared with *e.g.* rice, which gelatinises at approximately 70 °C. The amylose content has been suggested to be in the range 16-34% but can vary with cultivar, genotype, climate and cultivar conditions (Paton, 1986; Welch, 1995; Rhymer *et al.*, 2005). The proportion of amylose to amylopectin in oat starch can have effects on starch functionality. A positive correlation between content of amylose and gelatinisation temperature has been

found, which could possibly be explained by an inhibition of swelling (Wang & White, 1994). The dietary fibre includes a range of chemical components, of which β-glucan (mixed-linkage $(1→3),(1→4)$-β-D-glucan), a soluble non-starch polysaccharide, is one of the most important. Content of β-glucan in oats is reported to be related to the effects on improvement of glucose and insulin regulation in humans, as well as on lowering serum cholesterol level (Wood *et al.*, 1989; Wood, 1994; Önning *et al.*, 1999). The content of β-glucan varies with genotype and environmental conditions (Welch, 1995). Vitamin E, which is a term used to describe activity exerted by tocopherols and tocotrienols, is an important antioxidant found in oats. The tocols consist of four homologues each of tocopherol and tocotrienol, with α-tocotrienol and α-tocopherol being the most abundant forms found in oats (Peterson, 2001). The remaining six forms are present in very small amounts in oats, if present at all. The tocotrienols can be found mainly in the starchy endosperm, while the tocopherols are concentrated to the germ and the concentrations vary with genotype and location (Peterson & Qureshi, 1993; Peterson, 1995).

Bioactive compounds in oats

Antioxidants

In humans, radicals are formed from normal metabolism as well as from environmental radiation. These radicals can cause changes in DNA, which may lead to certain diseases such as cancer or atherosclerosis (Peterson, 2001). The human body has a natural defence system against these reactions but dietary antioxidants also play an important role in the defence. Vegetables, fruits and grains are the most important sources of dietary antioxidants. Commercial breakfast cereal products have significant levels of antioxidants which may be important dietary antioxidants (Yu *et al.*, 2002). Health promoting effects reported for whole grain cereal products have been suggested to be partly related to the content of dietary antioxidants, which are often enriched in the outer part of the cereal grain (Miquel, 2001; Visioli & Galli, 2001). Oxidation of lipids is a major cause of food spoilage since it generally leads to off-flavours and colour changes. Therefore, antioxidants also play an important role in contributing to the stability and taste in processed food products by preventing rancidity. Oats are rich in total lipids and also have a large proportion of unsaturated fatty acids. A wide spectrum of active phytochemicals can be found in oats, which may act as antioxidants in different ways and combinations and even synergistically (Peterson, 2001). It has been proposed that a suspension of oat flour can exert higher antioxidative activity compared with an oat extract, which may be explained by the presence of additional antioxidants that are not extractable in the oat flour matrix (Miller *et al.*, 2000). Since oats are normally consumed as a whole grain cereal, these proposed additional synergistically antioxidative effects are important characteristics of oat products. The main sources of antioxidants found in oats are phenolic compounds of various classes such as tocopherols and tocotrienols (Vitamin E), hydroxycinnamic acids and avenanthramides and to lesser extent flavonoids (Peterson, 2001).

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Phenolic compounds

Phenolic compounds are composed of one or more aromatic rings with one or more hydroxyl groups (Collins, 1986). They can vary greatly in their complexity from simple to highly polymerised phenols. They are products of secondary metabolism in plants and can act for example as defence mechanisms against pathogens, parasites and predators and contribute to the colour of plants (Liu, 2007). The vast majority of dietary phenolic compounds originate from plant foods (Scalbert & Williamson, 2000). Various epidemiological studies have suggested that consumption of plant food products containing high concentrations of phenolic compounds can prevent certain cancers, stroke and coronary heart diseases (Steinmetz & Potter, 1996; Ness & Powles, 1997; Tijburg *et al*., 1997). A wide variety of bioactive phenolic compounds have been identified from oats. The main focus in the present work was on the hydroxycinnamic acids, avenanthramides and tricin.

Hydroxycinnamic acids

The main hydroxycinnamic acids found in oats are caffeic, *p*-coumaric and ferulic acid and, to a lesser extent, sinapic acid (Figure 2). Hydroxycinnamic acids are derived from phenylalanine or tyrosine via the phenylpropanoid pathway, which is ubiquitous in plants (Kroon & Williamson, 1999). They can be covalently bound to the plant cell wall, as well as found in free form in the cytoplasm (Faulds & Williamson, 1999). In their free form they can be found in both oat groats and hull, with caffeic acid found to a larger extent in the oat groat while *p*-coumaric, ferulic and sinapic acid are more concentrated to the hull fraction (Xing & White, 1997; Emmons & Peterson, 1999). The hydroxycinnamic acids play important roles in the life of the plant cell wall in that they are principal components that govern cell wall integrity, shape and defence against attack of pathogens (Faulds & Williamson, 1999; Kroon & Williamson, 1999). A proportion of the ferulic acid found in cell walls is present as dehydrodimers, which function to crosslink and strengthen the cell wall, a physiologically significant strategy in the cell wall defence system (Iiyama, Lam & Stone, 1994). In oats they can be found in free form in low concentrations of between 1-10 mg kg^{-1} (Dimberg *et al*., 1996; Emmons & Peterson, 1999; Peterson, 2001), while acidic or alkaline hydrolysis can release larger quantities of bound hydroxycinnamic acids, in the range 4-363 mg kg^{-1} depending on hydroxycinnamic acid (Mattila, Pihlava & Hellström, 2005).

Reports on the antioxidative activities of hydroxycinnamic acids show that sinapic or caffeic acid generally has the highest activity, followed by ferulic and *p*-coumaric acid (Chen & Ho, 1997; Bratt *et al*., 2003). Studies performed *in vivo* have shown that hydroxycinnamic acids are bioavailable and can be absorbed by humans to a high degree (Olthof, Hollman & Katan, 2001; Kern *et al*., 2003; Nardini *et al*., 2006) and that they can undergo extensive metabolism in the human digestive tract (Chesson *et al*., 1999). This uptake of hydroxycinnamic acids in humans may induce biological effects in the blood circulation and caffeic acid has been found to inhibit LDL oxidative modification *in vitro* (Nardini *et al*., 1995) and to inhibit formation of mutagenic and carcinogenic N-nitroso compounds which can form *N*-nitrosamines (Kono *et al*., 1995). In addition, some

hydroxycinnamic acids have been shown to inhibit formation of platelets, which play an important role in thrombosis and may lead to cardiovascular disease (Hubbard *et al*., 2003). Another study showed that caffeic and ferulic acid can be important compounds for use as topical protective agents against UV radiation-induced skin damage, which may otherwise lead to pre-cancerous and cancerous lesions (Saija *et al*., 1999). These studies taken together indicate that there is good evidence that hydroxycinnamic acids have health beneficial effects in humans and are therefore important constituents of oats and oat-based food products.

Avenanthramides

Avenanthramides are a group of substituted *N*-cinnamoylanthranilic acids that have only been reported from oats among the cereals and are constitutive components in oat groats and hulls (Collins, 1986, 1989; Dimberg, Theander & Lingnert, 1993; Dimberg *et al*., 1996; Emmons & Peterson, 1999; Bryngelsson *et al*., 2002) (Figure 2). The avenanthramides are all composed of an anthranilic acid part and a cinnamic acid part, where the substitution pattern on the two parts is what distinguishes the different avenanthramides from each other. In the present work, the nomenclature for avenanthramides follows that of Bratt *et al*. (2003), although several different alternative systems for naming the avenanthramides can be found in the literature (Collins & Mullin, 1988; Dimberg, Theander & Lingnert, 1993; Dimberg *et al*., 1996; Dimberg *et al*., 2001). The anthranilic acid part can consist of one anthranilic acid (1), 5-hydroxyanthranilic acid (2), 5-hydroxy-4-methoxyanthranilic acid (3) or 4-hydroxyanthranilic acid (4) and the cinnamic acid part can consist of caffeic (c), *p*-coumaric (p), sinapic (s), ferulic (f) or cinnamic acid (a) (Bratt *et al*., 2003; Jastrebova *et al*., 2006). The dominant avenanthramides found in oats are 2c, 2p and 2f but at least 24 different avenanthramides have been described in the literature as being present in oats, including cis- and trans- structures, although some of these have not been structurally characterised and are found in very low concentrations (Collins, 1989; Emmons & Peterson, 1999; Bratt *et al*., 2003). It is not yet known whether avenanthramides derived from sinapic and cinnamic acid are present in oats.

The total concentrations of avenanthramides in oat grains reported in the literature vary to a large extent and are in the range 2-289 mg kg^{-1} (values found in the following references: Dimberg, Theander & Lingnert, 1993; Dimberg *et al*., 1996; Emmons & Peterson, 1999; Emmons, Peterson & Paul, 1999; Matsukawa *et al*., 2000; Emmons & Peterson, 2001; Peterson, Emmons & Hibbs, 2001; Bryngelsson *et al*., 2002; Bryngelsson, Dimberg & Kamal-Eldin, 2002; Bratt *et al*., 2003; Bryngelsson, Ishihara & Dimberg, 2003; Dokuyucu, Peterson & Akkaya, 2003; Dimberg, Gissén & Nilsson, 2005; Mattila, Pihlava, & Hellström, 2005; Peterson *et al*., 2005; Peterson & Dimberg, 2008). The content of avenanthramides in oats has been shown to vary with cultivar, year, location, cultivation conditions and interactions between these parameters (Emmons & Peterson, 2001; Dimberg, Gissén & Nilsson, 2005; Peterson *et al*., 2005). Levels of avenanthramides are negatively affected by a higher nitrogen fertilisation rate and there is no difference in levels of avenanthramides between organic and conventional cropping systems (Dimberg, Gissén & Nilsson, 2005). The

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avenanthramides are present in spikelets of oat plants shortly after heading and the concentration increases throughout maturation of the grain (Peterson & Dimberg, 2008). Although the avenanthramides are constitutive components of oat grains and are present in oat hulls, the majority can be found in the groats, with a higher concentration in the bran (Dimberg, Theander & Lingnert, 1993; Emmons & Peterson, 1999; Emmons, Peterson & Paul, 1999; Peterson, Emmons & Hibbs, 2001). Avenanthramides are also present in oat leaves at approximate concentrations in the range 5-120 mg kg^{-1} with large variations between cultivars and cultivation conditions and the concentrations are even higher in oat leaves treated with elicitors (Mayama *et al*., 1981; Ishihara *et al*., 1997, 1998; Peterson & Dimberg, 2008).

Long-term storage of oats does not affect the concentrations of avenanthramides significantly (Dimberg *et al*., 1996). Stability studies of avenanthramides (2c, 2p and 2f) have shown that 2p and 2f are stable during changes of pH (from acidic to alkaline), while 2c is sensitive to alkaline pH and especially in combination with heat treatment (Dimberg *et al*., 2001). Some studies have indicated that avenanthramides isomerise, similarly to the cinnamic acids, when treated with UV-light (Collins & Mullin, 1988; Collins 1989), while another study did not see any effects of UV-radiation on isomerisation of 2c, 2p and 2f (Dimberg *et al*., 2001). The concentrations of avenanthramides have also been studied before and after processing of food products in which oats are included as an ingredient (breads, muffin, fresh pasta, macaroni) (Dimberg *et al*., 2001). This study showed that the concentration of total avenanthramides (2c, 2p and 2f) increased in all products tested. This increase was explained as a possible *de novo* synthesis, release of bound forms, increased extractability after processing or combinations of those factors (Dimberg *et al*., 2001). A heating and drying process did not significantly affect the concentrations of 2c or 2f in samples with or without hull, while the concentrations of 2p decreased to a large extent (Dimberg *et al*., 1996). The effects of commercial processing on levels of avenanthramides was studied in Bryngelsson, Dimberg & Kamal-Eldin (2002), who found that 2p decreased during processing of rolled oats while 2c and 2f remained stable, that 2c and 2p decreased during autoclaving of oat grains and that all three avenanthramides decreased during drum-drying of milled rolled oats. That study highlights the importance of analysing the content of avenanthramides not only in the raw material but also in the finished oat-based food product. When levels of 2c, 2p and 2f were analysed in oat products available on the Finnish market, the levels found were slightly higher than those reported in Bryngelsson, Dimberg & Kamal-Eldin (2002) and there was no difference in levels between regular or pre-cooked oat flakes (Mattila, Pihlava & Hellström, 2005). The losses of avenanthramides during some processing methods may be decreased by refinement of the processing methods, for example by reducing temperatures during heat treatment. Another option is to compensate for the losses by inclusion of processing steps that increase the levels of avenanthramides in the raw material. Studies of oats have shown that the levels of avenanthramides can increase during germination, sometimes to a high degree (Matsukawa *et al*., 2000; Pihlava & Oksman-Caldentey, 2001; Bryngelsson, Ishihara & Dimberg, 2003), and this may be one process to consider during processing of oat-based food products.
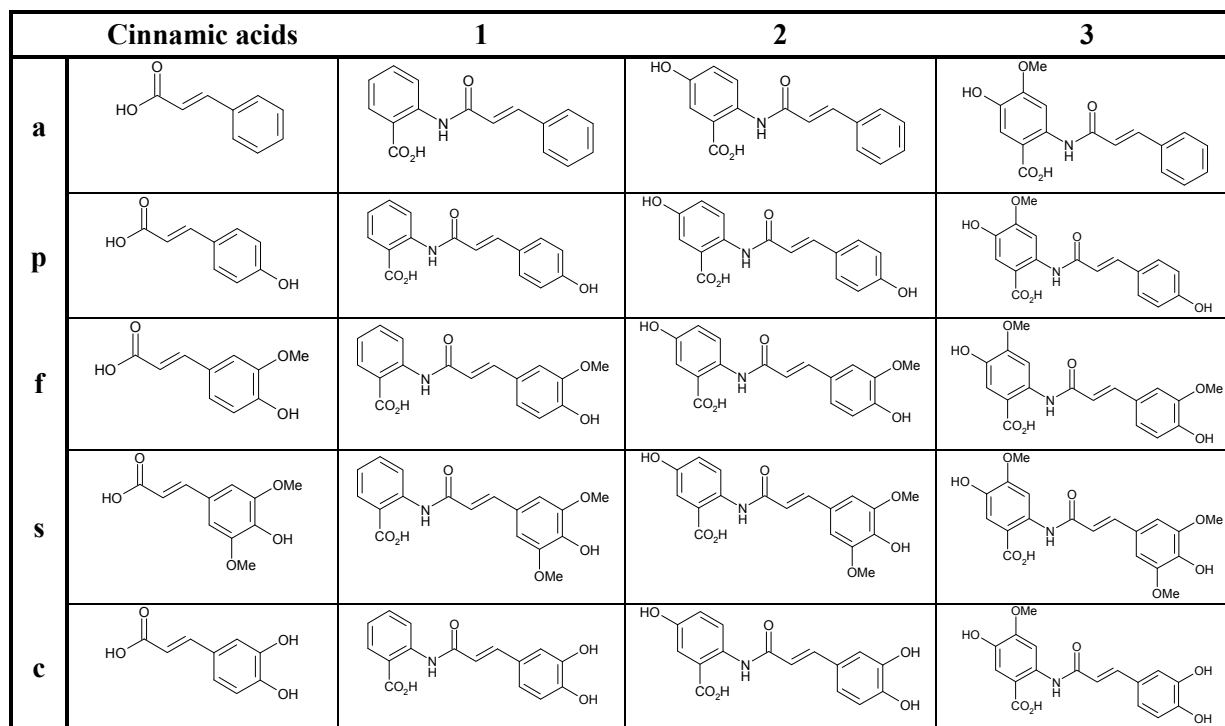
Figure 2. Chemical structure of cinnamic acids and avenanthramides. The numbers refer to the different anthranilic acids; anthranilic acid (1), 5-hydroxyanthranilic acid (2) and 5-hydroxy-4-methoxyanthranilic acid (3) and the letters refer to the different cinnamic acids; cinnamic acid (a), *p*-coumaric acid (p), ferulic acid (f), sinapic acid (s) and caffeic acid (c).

The avenanthramides have been characterised as antioxidants *in vitro* in several studies (Dimberg, Theander & Lingnert, 1993; Peterson, Hahn & Emmons, 2002; Bratt *et al*., 2003). Analyses of the antioxidative activity of 2f and 3f in a linoleic acid system showed that 2f had approximately 20% of the activity exerted by α-tocopherol, while 3f had 60% (Dimberg, Theander & Lingnert, 1993). The three major avenanthramides found in oats, 2c, 2p and 2f, were studied for antioxidative activity in two separate systems, the DPPH free radical system and the inhibition of β-carotene bleaching system (Peterson, Hahn & Emmons, 2002). It was found that all three avenanthramides exerted antioxidant activity in both systems and that 2c was the most effective. In another study eight different avenanthramides (1c, 1p, 1s, 1f, 2c, 2p, 2s, 2f) were studied for antioxidant activity in a DPPH free radical system, as well as in a linoleic acid system (Bratt *et al*., 2003). All avenanthramides except 1p exerted activity in the DPPH system, while all avenanthramides exerted activity in the linoleic acid system. Overall it was concluded that avenanthramides containing caffeic and sinapic acid were more effective as antioxidants than the other avenanthramides. Studies on the antioxidative activity of avenanthramides *in vivo* have indicated that the avenanthramide 2c can attenuate reactive oxygen species production in some tissues of exercised rats and enhance the activity of antioxidative enzymes in various tissues of rats (Ji *et al*., 2003). Furthermore, the avenanthramides in an avenanthramide-enriched mixture have been suggested to increase the antioxidant capacity in humans and to interact synergistically with vitamin C to protect LDL-oxidation in hamsters (Chen *et al*., 2004, 2007).

The avenanthramides have also been suggested to exert bioactivity in other ways in addition to antioxidative activity. Recent studies have indicated that avenanthramides may have potential anti-atherogenic and anti-inflammatory effects since they can inhibit the adhesion of monocytes to human aortic endothelial cell monolayers and the secretion of proinflammatory compounds from macrophages (Liu *et al*., 2004). The avenanthramide 2c has been shown to inhibit the proliferation of smooth muscle cells from humans and to increase the production of nitric oxide, in a dose-dependent manner, which are two key factors in preventing atherosclerosis (Nie *et al*., 2006a). The mechanism behind this protective effect is based on upregulation of a specific protein pathway in combination with inhibition of phosphorylation of a protein essential to the cell cycle transition (Nie *et al*., 2006b). The avenanthramide 1f has been patented as an *in vitro* inhibitor of lipoxygenase (Wakabayashi *et al*., 1986), a key enzyme in the biosynthesis of leukotrienes, which are substances that participate in allergic and inflammatory responses (Celotti & Durand, 2003) and for the first time, a recent report describes the avenanthramides as having anti-proliferative effects on colonic cancer cell lines (Nie *et al*., 2007). Research performed to date on the physiological effects of avenanthramides strongly suggests that the health benefits of oat consumption in preventing certain diseases extend beyond the known effects on lowering blood cholesterol. Another reason why avenanthramides are especially interesting to study is because of their similarity in structure to the commercial drug Tranilast. Tranilast has proven physiological and pharmacological properties and is intended for use in treating certain types of allergies (Isaji, Miyata & Ajisawa, 1998). The safety of Tranilast has been

extensively investigated in many studies and the compound is considered to be safe in many different areas of application (Namazi & Soma, 2005; Cooper *et al*., 2007; Wang *et al*., 2007). The safety of avenanthramides is still unknown but considering its structural similarities to Tranilast, there is reason to believe that the avenanthramides may also be safe, although further studies are needed to prove this.

The avenanthramides have been well-characterised as phytoalexins and they are accumulated in oat leaves during fungal attack and treatment with various elicitors (Mayama *et al*., 1981; Miyagawa *et al*., 1995, 1996). Studies on the biosynthesis of avenanthramides in oats show that avenanthramides in oat leaves are biosynthesised from anthranilic acid and phenylalanine via the phenylpropanoid pathway (Ishihara, Ohtsu & Iwamura, 1999a). In the final step of the biosynthesis the condensation of an anthranilic acid and a hydroxycinnamoyl-CoA ester is catalysed by an enzyme, hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase (HHT) (Ishihara *et al*., 1997, 1998; Matsukawa *et al*., 2000). This enzyme, along with many other enzymes, is induced by treatment of oat leaves with elicitors (Ishihara, Ohtsu & Iwamura, 1999a, 1999b; Matsukawa, Ishihara & Iwamura, 2002). Activity of HHT has also been detected in both endosperm and germ of oat seeds. In oat grain samples the HHT activity has been found to increase during germination (Matsukawa *et al*., 2000; Bryngelsson, Ishihara & Dimberg, 2003). HHT activity has been detected in oat grains at approximately 20 days after heading and increased during maturation of the grain (Peterson & Dimberg, 2008). HHT partially purified from dry seeds and elicitor-treated oat leaves has been found to consist of at least two isoforms, where the substrate specificity in oat seeds differed for the two isoforms of HHT (Matsukawa *et al*., 2000).

In a previous study where milled oat samples were steeped in water, the levels of avenanthramides decreased (Bryngelsson, Ishihara & Dimberg, 2003). This phenomenon was further investigated in Bryngelsson *et al*. (2003), where it was found that the decrease in levels of synthetic avenanthramides in a buffered slurry of milled oats was dependent on pH and temperature, with the largest decrease being obtained at pH 9 and 30 °C. Different synthetic avenanthramides were studied as substrates and the decrease was the most pronounced on avenanthramides containing caffeic or *p*-coumaric acid. The decreasing reaction was inhibited by 2-mercaptoethanol, acetic acid and high temperatures, which indicates the activity to be enzymatic, but no further attempts have been made to characterise the tentative enzyme.

Tricin

Tricin (4', 5, 7-trihydroxy-3', 5'-dimethoxyflavone) (Figure 3) is a compound classified as a flavone belonging to the larger group of flavonoids (Martens & Mithöfer, 2005). The flavones are derived from chalcones via the anthocyanidin/proanthocyanidin pathway and the final step in biosynthesis of flavones is catalysed by either of two enzymes, flavone synthase I or flavone synthase II. The flavones can be found in various parts of plants and have been

suggested to contribute to UV-protection and to be essential compounds in various plant defence systems (Harborne & Williams, 2000; Simmonds, 2003; Martens & Mithöfer, 2005). In humans, flavones such as apigenin and luteolin may be important health beneficial food compounds since they have been proposed to have antioxidative, antibacterial, antiviral, antiatherosclerotic, anti-inflammatory and anticarcinogenic properties (Martens & Mithöfer, 2005). Flavones can be found either in free form or as glycosides in plants. Tricin is mainly described as a constituent of rice bran but has also been reported to be present free or as a glycoside in various parts of other plants such as *Medicago sativa* (alfalfa), *Phyllostachus nigra* (bamboo) and *Trichosanthes kirilowii* (Chinese cucumber) (Jiao *et al*., 2007; Rahman & Moon, 2007; Stochmal, Kawalska & Oleszek, 2007), as well as in wheat, barley and maize (Cai, Steward & Gescher, 2005). In an early report on anticariogenic activity, tricin was identified in oat hulls (Vogel, Thompson & Phillips, 1962).

Several studies have reported the potential of tricin in cancer prevention. Significant chemopreventive effects of tricin have been found on breast and colon cancer cells (Hudson *et al*., 2000; Cai *et al*., 2004, 2005; Al-Fayez *et al*., 2006), as well as on inhibition of cyclooxygenase 1 and 2, which are enzymes involved in carcinogenesis (Al-Fayez *et al*., 2006). Tricin has also been suggested to possess antineoplastic properties in mice with leukaemia (Lee *et al*., 1981) and potent antihistamine release activity in leukaemia cells of rats (Kuwabara *et al*., 2003). A safety evaluation performed on tricin tentatively demonstrated that tricin may be considered sufficiently safe for clinical development as a cancer chemopreventive agent (Verschoyle *et al*., 2006).
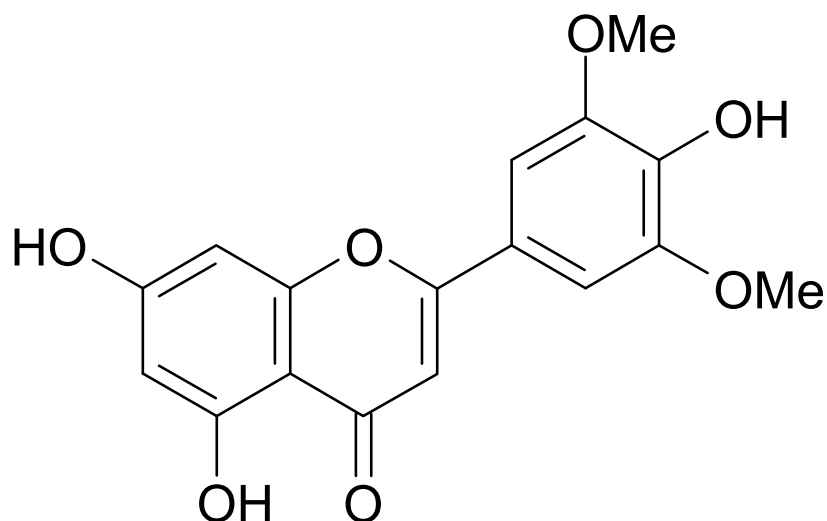

Figure 3. Chemical structure of tricin (4', 5, 7-trihydroxy-3', 5'-dimethoxyflavone).

Processing of oats

Processing of oats is essential in order to produce oat-based food products with attributes that will attract consumers to the products. These attributes include for example overall appearance, colour, smell, taste, shelf-life and price. Many food processes impair the nutritional properties of food products but during recent years it has become increasingly important to have processes that contribute to sustained or possibly enhanced nutritional value of the products.

The processing of oats is mainly based on a flaking procedure of the oat groats. The majority of oats included in oat-based food products are oat flakes that may have been further processed in some way before inclusion in the final food product. Processing involves the main steps cleaning, heat treatment, dehulling, cutting and flaking (or milling). These steps can vary depending on final oat product and also on variety of oats (covered or naked). The cleaning of oats is performed to remove coarse field trash, dust, *etc*. that may disrupt further processing. Oats contain high levels of lipids, with the majority of the fatty acids being polyunsaturated. The lipids may be hydrolysed to fatty acids by lipases found in the oat grain, and lipoxygenases may oxidise the free fatty acids, causing rancidity of the final oat product. Therefore oats intended for food purposes are heat treated in order to inactivate the enzymes responsible for changes in oat lipids (Deane & Commers, 1986). During heat treatment the moisture is increased to approximately 18% and the grains are kept at a temperature just above 100 °C for 90-120 minutes (Ganssmann & Vorwerck, 1995). Additional effects of heat treatment include destruction of bacteria and fungi and development of oat aroma (Molteberg *et al*., 1996). However, heat treatment is not completely positive but can reduce protein solubility and give rise to oxidation of lipids, which results in a higher content of volatile oxidation products (Lehtinen *et al*., 2003).

Prior to dehulling, oats are often graded to have similarly sized grains in order to improve the efficiency of the dehulling process. At dehulling the oat grains are forced centrifugally against an impact ring (rubber liner) which makes the hulls loosen from the groat, while air aspiration subsequently separates the dehulled groats from the hulls (Deane & Commers, 1986). The normal procedure is then to polish the groats to remove remaining pieces of hull material and the trichomes. During flaking of oat groats, steam is added to increase the moisture content, in order to soften the groats so that they form flakes with a minimum of breakage. This steaming process (performed at 99-104 °C) also completes the inactivation of undesired enzyme systems and contributes to the development of the characteristic oat flavour. Flaking of intact oat groats produces rolled oats, which are about 0.5-0.8 mm thick. By cutting the groats into pieces thinner flakes can be produced (0.25-0.40 mm), which are often used for instant cooking oatmeal. After flaking the rolled oats are cooled with air to about 45 °C and the moisture content is approximately 9-11.5% (Deane & Commers, 1986). Oat flour can be produced by milling oat flakes or groats using an impact-type hammer mill where exhaust air is drawn through the system to prevent the relatively high fat oat flour sticking to the sides of the mill. Milling is also performed to separate the anatomical parts of the grain (Hoseney, 1994; Wang, Koutinas & Campbell, 2007). Drum drying of oats

is a process where rolled oats are milled and mixed with water. The oat slurry is applied to steam-heated rotating rollers and the thin film produced is milled and used for example in instant baby-foods (Bryngelsson *et al*., 2002).

Oat-based food products

Oats are unique among the cereal grains in that they are almost always consumed as whole grains, while for most other grains the germ and significant amounts of the bran are often removed during processing. Typical oat products are rolled oats, oat bran and oat flour (Webster, 1986). These products are used in a large number of applications, where hot and cold cereals are two of the most important. For hot cereals, where rolled oats is the main ingredient, the addition of oat bran in order to increase the content of the health beneficial β-glucan has become a big success. The hot cereals can be flavoured in various ways to attract interest from consumers. The major ingredient in oat-based cold cereals is oat flour. The food industry has great interest in developing new cold cereals with nutritionally functional ingredients such as β-glucan. Therefore new oat lines with high levels of β-glucan are being evaluated (Yao *et al*., 2006). Oats are added to many baked goods such as bread and biscuits. The addition of oat flour to bread can keep the bread fresh for a long time, since oats have excellent moisture retention properties (Webster, 1986). Intact rolled oats can be added to bread to achieve a chewy texture. Known effects of baking that are of importance for the nutritional value of the end product include an increase in the content of some free phenolic compounds (Dimberg *et al*., 2001) and an undesirable decrease in the average molecular weight of β-glucan (Andersson *et al*., 2004). Increased fibre levels in biscuits are associated with an increase in tenderness and moistness and a decrease in spread (Jeltema, Zabik & Thiel, 1983). Beverages based on oats are quite new on the market. Thin rolled oats are processed by wet-milling and enzyme treatment and the product is ultra high temperature (UHT)-treated and packed aseptically (Zhang *et al*., 2007b). Studies have shown that these beverages can be of high nutritional quality with *e.g.* a large proportion of retained vitamins and a high bioavailability of iron (Zhang *et al*., 2007a, 2007b). Oats are also included as an ingredient in cereal bars, as a thickener in soups and as meat extenders (Webster, 1986; Aleson-Carbonell *et al*., 2005). Oat hulls have long been considered to be an agricultural by-product from production of rolled oats and oat flour. However their high fibre content has led to processed oat hulls now being used as a fibre source in some cereal products (Stephen *et al*., 1997; Galdeano and Grossman, 2006). Intake of oat hull fibre can significantly increase faecal mass and as a result reduce constipation (Stephen *et al*., 1997).

Germination of oats

The interest in processing methods that can alter the nutritional value of food products in a positive way has increased. Malting of barley for the brewing industry is one example of a process that not only enhances functional quality but also enhances the nutritional quality of barley, for example by increasing the bioavailability of nutrients such as proteins, vitamins and minerals (Bamforth &

Barclay, 1993). Germination of rye has been shown to result in increased levels of some plant sterols, folates, lignans and phenolic compounds (Katina *et al.*, 2007). Oat malt is also produced for the brewing industry, although to a lesser extent than barley, and it has been discussed whether malted or germinated oats could be used in food products such as speciality breads, biscuits, confectionary and prepared breakfast cereals (Valentine, 1995), as well as in novel foods with functional properties. The malting process consists of steeping, germination and kilning. All of these steps can be varied to obtain the desired result of the malting process. During steeping, dry grains absorb water to a moisture content of 43-45%, and the metabolic activity resumes (Bewley & Black, 1994). The steeping process is usually performed at 14-18 °C for up to 48 h with intermittent air rests (Bamforth, 2000). These air rests are important in order to control the levels of carbon dioxide and ethanol, which are produced by respiratory metabolism in the embryo and the aleurone tissues and by microorganisms (Bamforth & Barclay, 1993). During the subsequent germination process, which can continue for approximately 4-5 days at 16-20 °C, enzyme synthesis and grain modification take place (Kunze, 1999; Bamforth, 2000). To prevent moisture levels from decreasing during the germination step, the grains are often sprayed with water to maintain the rate of the reactions involved during germination (Bamforth & Barclay, 1993). The kilning step (20-24 h) performed at temperatures around 50-220 °C aims to dry the grains to a moisture content of approximately 3-5%. This is to stop or retard any biochemical reactions, to minimise the risk for microbial attack and to develop flavour compounds and colour (Bamforth, 2000). The germination conditions favour microbial growth and it is important to consider this when optimising the germination process. A lower temperature (approximately 15 °C) can help to avoid excessive growth of microorganisms (Wilhelmson *et al.*, 2001).

During germination of oats starch degradation is limited, although α-amylase activity can reach levels almost as high as in malted barley (Peterson, 1998). However, the diastatic power, which is the combined effects of α-amylase and β-amylase, is lower in oats than in barley. The activity of β-glucanase increases during germination of oats, resulting in almost total degradation of β-glucan (Peterson, 1998). Since β-glucan is known to have health beneficial effects in humans, the degradation during germination is not desirable if the oats are intended for use in food products rather than for brewing. A shorter germination process (72 h) at a relatively low temperature (15 °C) showed that 55-60% of the β-glucan content can be retained (Wilhelmson *et al.*, 2001). The total protein content in oats increases slightly during germination (Peterson, 1998). Even though the increase is small it is important, since the essential amino acids lysine and tryptophan increase and therefore improve the nutritional value of the oat proteins (Dalby & Tsai, 1976; Wilhelmson *et al.*, 2001). The lipid content in oats decreases slightly during germination while the content of free fatty acids increases, although there are differences between cultivars as well as between hulled and hulless cultivars (Peterson, 1998). The activity of lipase in oats increases in early germination to decrease gradually after approximately 24 h of germination (Urquhart, 1984). However, the wide variation between the different cultivars can allow selection of appropriate cultivars for specific applications. Many micronutrients in oats are also affected by germination. Phytates are

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considered to reduce the bioavailability of minerals, and germination is one way of reducing the phytate content in oats (Hall & Hodges, 1966; Larsson & Sandberg, 1992). Phenolic compounds such as the avenanthramides, which are well-described antioxidants (Dimberg, Theander & Lingnert, 1993; Peterson, Hahn & Emmons, 2002; Bratt *et al*., 2003), increase during germination, as does the avenanthramide synthesising enzyme (Matsukawa *et al*., 2000; Bryngelsson, Ishihara & Dimberg, 2003).

Objectives

The objectives of the present study were:
To evaluate whether the decrease in avenanthramide levels found when non heat-treated oat flour is steeped in water is a result of polyphenol oxidase (PPO) activity and to determine whether a similar decrease to that found in oats also could be detected in wheat, barley and rye (Paper I).

To study the effects of a highly controlled steeping and germination process on the content of avenanthramides, activity of the avenanthramide synthesising enzyme (HHT) and activity of the avenanthramide degrading enzyme (PPO) (Paper II).

To evaluate whether elevated contents of avenanthramides and low average molecular weight of β-glucan in oats may be a result of pre-harvest sprouting in the field (Paper III).

To quantify and localise the flavonoid tricin in oat grains from cultivars originating from Europe and North America (Paper IV).

Results and discussion

Table 1 gives a brief description of the samples and the analyses performed in the studies. A more detailed description is given in Papers I-IV.

Table 1. Summary of samples used and analyses performed in the studies

	Samples	Analyses
Paper I	Cultivars of oats, wheat, barley and rye[1]	• Activity of PPO on different substrates[2, 3] • Inhibition of PPO activity[2, 4] • Activity of PPO in cereals[2, 5]
Paper II	Oat grains from three North American cultivars[6]	• Total avenanthramides[7] (2c, 2p and 2f) • Hydroxycinnamic acids[7] (caffeic, *p*-coumaric and ferulic acid) • Detection of unknown compounds[7] • Isolation and identification of the avenanthramide 3f[8] • Activity of HHT[9] • Activity of PPO[2,10]
Paper III	Oat grains from twenty Swedish genotypes[11]	• Content and molecular weight of β-glucan[12] • Total avenanthramides[7] (2c, 2p and 2f) • Activity of alpha-amylase[13] • Activity of β-glucanase[14] • Activity of β-glucanase in oat hulls[14, 15]
Paper IV	Oat samples (total of 132) originating from Europe and North America[16]	• Tricin in 132 oat samples[7] • Tricin in developing oats[17] • Tricin as affected by germination[6, 7] • Location of tricin in the oat grain[18]

[1] Creal samples grown in southern Sweden and provided by Svalöf Weibull AB.
[2] Oat slurry of milled oat sample and phosphate buffer (pH 7) incubated with synthetic substrate at 30 °C for 2 h. The decrease in concentration of substrate was analysed by reversed phase HPLC.
[3] The substrates were caffeic acid, *p*-coumaric acid, sinapic acid, ferulic acid, cinnamic acid, and the avenanthramides 1c, 1p, 1s, 1f, 1a, 2c, 2p, 2s, 2f, 2a, 3c, 3p, 3s, 3f and 3a.
[4] The inhibitors were tropolone, potassium disulphite, sodium diethyldithiocarbamate and ascorbic acid. The substrates were caffeic acid, *p*-coumaric acid, 1c, 1p, 2c and 2p.
[5] Activity on 2p (pH 9, incubation for 1 h) was measured in oats, wheat, barley and rye. Activity on caffeic acid, *p*-coumaric acid, sinapic acid and ferulic acid was measured in oats, spring wheat, winter wheat, spring barley and rye.
[6] North American oat cultivars grown in 2001 in Madison, Wisconsin, USA. Samples were steeped (10-14 h) and germinated (72-120 h) at 16 or 20 °C.
[7] Extracted using 80% ethanol and analysed by reversed-phase HPLC.
[8] Purification of 3f followed by analysis using ^{1}H-NMR, 2D-NMR and LC-MS.
[9] Crude enzyme extract mixed with *p*-coumaroyl-CoA and 5-hydroxyanthranilic acid. The enzymatically produced 2p was analysed by reversed-phase HPLC.
[10] The avenanthramide 2p was used as a substrate.
[11] Fifteen covered genotypes, whereof four classified as early maturing, and five naked from the harvest of 2000 and 2001, provided by Svalöf Weibull AB.
[12] Data taken from Ajithkumar, Andersson & Åman, 2005.
[13] Amylazyme alpha-amylase assay procedure kit used with some modifications.
[14] Malt & bacterial beta-glucanase & cellulose assay procedure kit used with some modifications.
[15] Oat grains dehulled by hand and hulls extracted for β-glucanase.
[16] European oat samples (123) grown in southern Sweden in 2000 and 2001 and North American oat samples (9) grown in Madison, Wisconsin, USA.
[17] Two North American oat cultivars harvested at different stages of maturity extracted for content of tricin.
[18] Three European oat genotypes dehulled by hand and hulls and groats extracted for tricin.

Paper I

A previous study showed that when non heat-treated oat flour was steeped in water the levels of avenanthramides, especially 2c and 2p, decreased (Bryngelsson *et al.*, 2003). This decrease was suggested to be caused by enzymatic activity. The current study evaluated whether the proposed enzyme activity on avenanthramides in oats was caused by a polyphenol oxidase (PPO). The enzyme activity on fifteen different avenanthramides and five cinnamic acids as substrates was studied, as was the effect of four different known PPO inhibitors on the enzyme activity. In addition, it was investigated whether the activity found on the avenanthramides in oats also could be observed in wheat, barley and rye.

In the assay the enzyme activity was measured as the decrease in substrate concentration. This was performed since no products of the enzyme activity could be detected for any but *p*-coumaric acid derived substrates. It was shown that the highest activity was found on caffeic acid-containing compounds (c, 1c, 2c and 3c), followed by *p*-coumaric acid-containing compounds (p, 1p, 2p and 3p). The enzyme activity on sinapic, ferulic and cinnamic acid and on the avenanthramides containing those cinnamic acids was low overall. The products formed from enzyme activity on p, 1p, 2p and 3p were c, 1c, 2c and 3c respectively. The concentrations of the c-compounds formed were lower than the decrease in concentrations of the p-compounds, indicating that the reaction continued, *i.e.* that the c-compounds were further oxidised. The inhibition effect of the four known PPO inhibitors (tropolone, potassium disulphite, sodium diethyldithiocarbamate and ascorbic acid) on the enzyme activity was large overall. Tropolone, potassium disulphite and sodium diethyldithiocarbamate were found to inhibit the average enzyme activity on p, 1p and 2p more than on c, 1c and 2c, while the opposite result was found for ascorbic acid. When the original concentration of ascorbic acid used was doubled, caffeic acid produced from *p*-coumaric acid was stoichiometrically related to the decreased amount of *p*-coumaric acid, indicating that ascorbic acid stopped further reactions. It was also found that there was enzyme activity on the avenanthramide 2p not only in oats, but also to various extents in wheat, barley and rye (Figure 4). It should be noted that the cereal samples studied were only of one cultivar from each cereal type and that variations in enzyme activity between cultivars and harvest years were not evaluated in the current study.

It is well known that PPO can catalyse both the hydroxylation of monophenols to form *o*-diphenols and the removal of hydrogen from *o*-diphenol to form *o*-quinones (Whitaker, 1994). The former reaction can be exemplified by the hydroxylation of *p*-coumaric acid to form caffeic acid, which is further oxidised to quinones. In the current study it was found that the avenanthramides containing *p*-coumaric acid also formed avenanthramides containing caffeic acid, which supports the theory that a PPO was responsible for the decrease in avenanthramide concentration (Figure 5). It was not possible to detect what compound was formed from the oxidation of avenanthramides containing caffeic acid, but it is reasonable to believe that some kind of quinone would be formed. The overall low enzyme activity on sinapic, ferulic and cinnamic acid-containing compounds is in

agreement with the literature on substrate specificity for PPO (Whitaker, 1994). Actually, PPO activity described for some other plant species has been suggested to be inhibited by ferulic acid, as well as by cinnamic acid (Kihara *et al*., 2005; Neves & Da Silva, 2007).
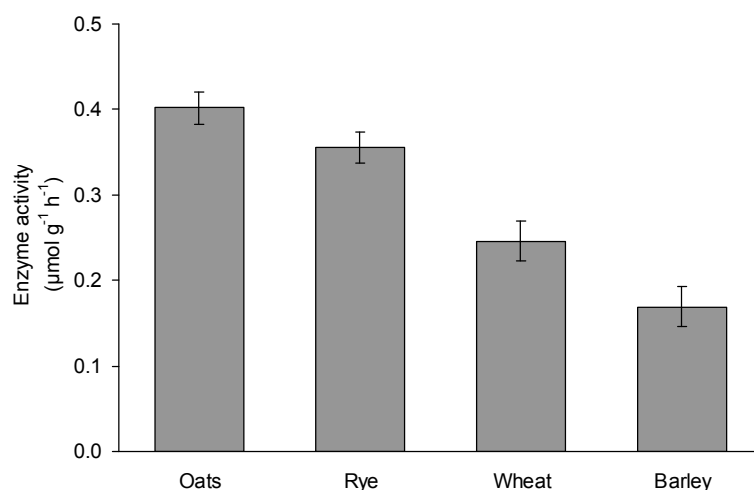

Figure 4. Enzyme activity on the avenanthramide 2p in samples from oats, rye, wheat and barley. The bars are means of duplicate analyses and the error bars indicate standard deviations.

Troplone, which is a known competitive inhibitor of PPO activity, can inhibit PPO activity by competing with the substrate for binding to the copper at the active site of both mono- and diphenol oxidase (Kahn & Andrawis, 1985). Tropolone highly inhibited the enzyme activity on all substrates used in the current study. The effect of potassium disulphite on the enzyme activity may be explained by the reducing ability of sulphite, which has been suggested as being an effective inhibitor of enzymatic browning (Sapers, 1993). Sulphites may irreversibly inhibit PPO directly through modification of the enzyme structure but may also react with intermediate products of PPO activity to prevent pigment formation (Sayavedra-Soto & Montgomery, 1986; Yoruk & Marshall, 2003). The inhibiting effect of sodium diethyldithiocarbamate can be explained by the ability of the compound to chelate with the copper at the active site of the enzyme, thereby inactivating the enzyme (Whitaker, 1994). The reason for the differences in inhibition of the enzyme activity on p, 1p or 2p and c, 1c or 2c may depend on the rate of the reaction. The monohydroxylation reaction has been described as being slower than the oxidation of *o*-diphenols, thereby enabling the inhibitors to act for longer (Wong, 1995). Ascorbic acid is known as a reducing compound in that it can reduce the product of PPO activity (quinones) back to the diphenol directly as it is formed (Whitaker, 1994; Yoruk & Marshall, 2003). Ascorbic acid can also affect PPO activity through a decrease in pH or have a direct effect on PPO by acting through its site-directed specificity toward histidine residues on PPO, thereby inactivating the enzyme (Golan-Goldhirsh *et al*., 1992; Osuga, Van der Schaaf &

Whitaker, 1994; Yoruk & Marshall, 2003). Ascorbic acid inhibited the enzyme activity on caffeic acid, 1c and 2c, to a large extent and with high concentration of ascorbic acid a total recovery of caffeic acid produced from *p*-coumaric acid was obtained, indicating that ascorbic acid reduced the oxidation product of caffeic acid. The enzyme activity found on p, 1p, 2p, 3p, c, 1c, 2c and 3c, the production of caffeic acid-containing compounds from *p*-coumaric acid compounds and the action of the known PPO inhibitors strongly support the hypothesis that PPO was responsible for the decrease in avenanthramide concentration when non heat-treated oat flour was steeped in water. Although the enzyme was not readily extractable, as also indicated in some previous studies (Marsh & Galliard, 1986; Fuerst, Anderson & Morris, 2006) and although an oat slurry containing many other enzymes, which theoretically could have acted on the avenanthramides, was used as enzyme source, the hypothesis is still valid. The proposed reactions are summarised in Figure 5.
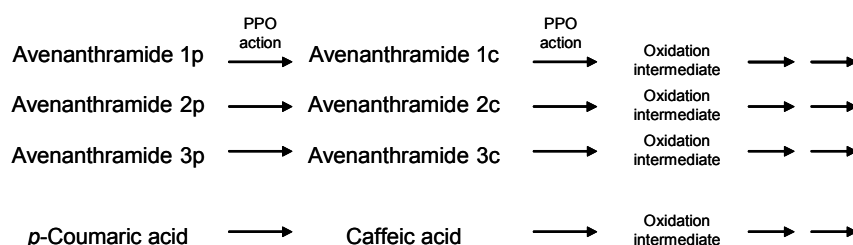
Figure 5. Proposed reaction caused by PPO where the *p*-coumaric acid-containing avenanthramides are hydroxylised to caffeic acid-containing avenanthramides, similarly to the known reaction where free *p*-coumaric acid is hydroxylised to caffeic acid (Whitaker, 1994), which is shown in the lower diagram.

The avenanthramides can only be found in oats among the cereals. Therefore, it was interesting to find that a similar enzyme activity to that found in oats on avenanthramide 2p could also be found in wheat, barley and rye. Oats intended for food production are almost always heat treated and the enzymes, including PPO, are possibly inactivated and thereby unable to act on the avenanthramides. However, when cereal-based food products are produced with a mixed content of cereal grains which are not heat-treated in combination with oats, the PPO present in the non heat-treated grains could act on the avenanthramides and thereby decrease their concentration in the final food product. As this is an undesirable reaction, since the avenanthramides have been shown to possess potential health beneficial properties, precautions must be taken to limit the action of PPO in such food products.

Paper II

Previous studies on germination of oats have indicated increased avenanthramide levels with germination (Matsukawa *et al*., 2000; Bryngelsson, Ishihara & Dimberg, 2003), but in those studies the germination process was performed under

relatively uncontrolled conditions. In the current study three North American oat cultivars were steeped and germinated under highly controlled conditions using a pilot plant malting system (Joe White, Melbourne, Australia). The whole process (steeping and germination) was performed at two different temperatures (16 and 20 °C). The samples were steeped in water until about 45% of moisture level was reached and thereafter drained and germinated for a maximum of 120 h. Samplings were performed at regular occasions during both steeping and germination. The samples were analysed for content of avenanthramides (2c, 2p and 2f), activity of the avenanthramide biosynthesising enzyme HHT and activity of the enzyme PPO. Measurements of PPO activity were included in the study since it was shown in Paper I that PPO can oxidise avenanthramides.

The steeping and germination process had effects in various ways depending on cultivar and measured variable, although many phenolic compounds were positively affected (Figure 6). The levels of avenanthramides increased in all cultivars included in the study but to various extents. The largest increase in total avenanthramides (2c, 2p and 2f) during the steeping and germination process was found in cv. Dane, followed by cv. Gem and cv. Vista (Figure 7). The individual avenanthramides contributing to the increase in total avenanthramides were 2p and 2f, while the content of 2c was not significantly affected in any of the cultivars (Figure 7). Furthermore, the levels of avenanthramide 3f increased to a large extent, especially in cvs. Dane and Gem at late germination times.
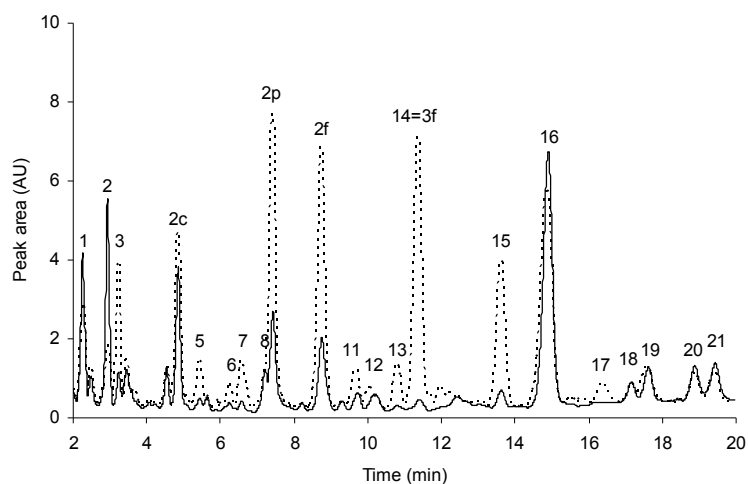

Figure 6. Chromatogram of avenanthramides and other phenols from the oat cultivar Dane (16 °C treatment). Raw grains (continuous line) and sample after 120 h of germination (dotted line) are shown. The peaks for the avenanthramides 2c, 2p, 2f and 3f are identified. The most significant unknown peaks are numbered in order of appearance (from 1-21).

The content of the hydroxycinnamic acids caffeic, *p*-coumaric and ferulic acid were measured in raw grains and in samples germinated for 120 h. The levels of caffeic and *p*-coumaric acid decreased in all three cultivars to almost undetectable

levels, while ferulic acid was only affected in cv. Vista (Figure 7). Many unknown compounds detected in the chromatograms were highly affected by the steeping and germination process (Figure 6). For several of these there was a significant interaction between cultivar and germination, indicating that the effect of germination differed between the different cultivars. The compounds corresponding to peaks 11, 13 and 15 increased during germination. Preliminary studies indicated that those compounds are avenanthramides. Further studies showed that peak 16 consists of two compounds. One of these, which is also suggested to be an avenanthramide, increased to a large extent during germination in all three cultivars. The other compound was identified as tricin and was not affected by germination (see Paper IV). This preliminary finding emphasises the fact that the unknown compounds may also be of nutritional importance.

The activity of the avenanthramide synthesising enzyme HHT increased significantly during steeping and germination only for cv. Dane, while in cvs. Gem and Vista the levels remained relatively unchanged. The activity of the avenanthramide oxidising enzyme PPO decreased during the germination process in cvs. Gem and Vista, while for cv. Dane there was no significant change.

The results suggest that the increase in activity of HHT and avenanthramide content during germination is highly dependent on choice of cultivar. Previous studies have shown even larger increases in avenanthramide levels than those found in the current study, although dependent on steeping and germination times, procedures and temperatures (Matsukawa *et al.*, 2000; Bryngelsson, Ishihara & Dimberg, 2003). However, these studies cannot be directly compared with the current study or with each other since the experimental design differed between studies, especially regarding oat material and germination conditions such as temperature and duration of the steeping and germination procedure. There was no indication that the increase in avenanthramide content had reached a plateau for any of the cultivars at 120 h of germination, indicating that further increase could take place. In the current study only covered oat cultivars were included, while in the study by Bryngelsson, Ishihara & Dimberg (2003) naked oats were steeped and germinated. It would be interesting to compare the effects of steeping and germination on both covered and naked oats in the same study in order to determine whether there are any differences between these two types of oats.

The decrease in the hydroxycinnamic acids caffeic and *p*-coumaric acid, which has also been reported from malting of barley (Goupy *et al.*, 1999), could be due to further metabolism of these compounds. For example, they could be involved in biosynthesis of hydroxycinnamic acid-containing compounds such as the avenanthramides. For this to take place the hydroxycinnamic acids must first be transformed to Co-enzymes, such as *p*-coumaroyl-CoA (used in the HHT assay). These pathways were not investigated in the current study. Ferulic acid was less affected by the treatment, which could be an effect of simultaneous release of ferulic acid bound to the cell wall structure (Besle, Cornu & Jouany, 1994; Faulds & Williamson, 1999). Insoluble *p*-coumaric and ferulic acid in barley malt have been suggested to be important potential antioxidants of beer if released during the kilning step (Maillard & Berset, 1995; Bartolomé, Faulds & Williamson, 1997).
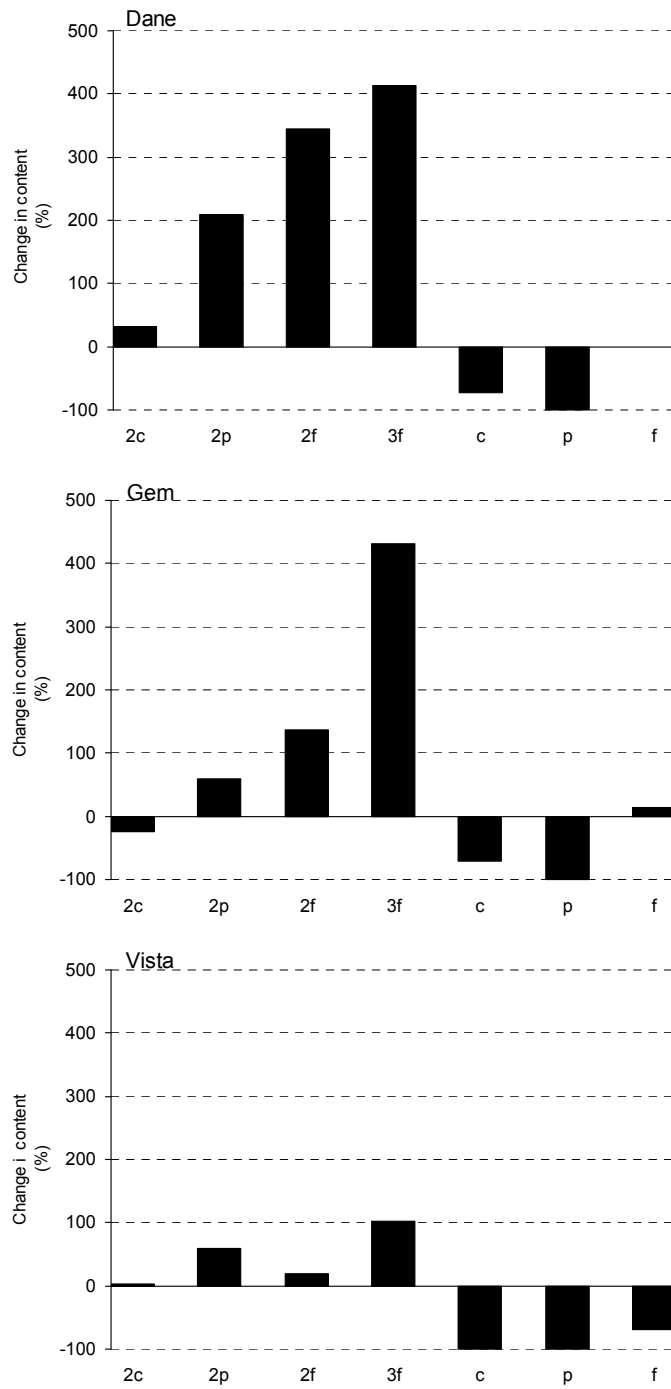
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Figure 7. Change in content of the avenanthramides 2c, 2p, 2f, 3f and the hydroxycinnamic acids c, p, f, during germination for 120 h at 16 °C of the oat cultivars Dane, Gem and Vista, expressed as % of content in raw grains.

The levels of avenanthramides increased in all three cultivars studied while the increase in HHT activity was only significant for cv. Dane. This result suggested that the activity of HHT may not have to increase to obtain increases in avenanthramide levels if the levels of HHT activity are already sufficiently high, as in cv. Gem. The difference in activity between HHT and PPO was large, with PPO having the higher activity (expressed as nmol g^{-1} h^{-1}). Although the activities of the two enzyme were measured in different ways (HHT activity was measured in an enzyme extract and PPO activity was measured in an oat slurry), making a comparison in many ways inequitable, it should be noted that despite the higher PPO activity the content of avenanthramides increased for all cultivars during the germination process. This indicates that HHT and the majority of the avenanthramides are compartmentalised from PPO in the oat grain even during the germination process, when the grain tissue theoretically could soften and compartmentalised constituents could be released.

The effect of temperature on the contents of the major measured parameters (2c, 2p, 2f and activities of HHT and PPO) was less important than the effect of cultivar and process time (both steeping and germination). However, most unknown compounds were highly affected by the temperature, indicating the importance also of this factor. The temperature is also an important factor to consider when it comes to the microbial quality of the germinated grain, since the germination process is favourable to growth of microorganisms. It has been reported that germination of oats at 25 °C increases the incidence of many different fungi and bacteria compared with germination at 5 °C (Wilhelmson *et al.*, 2001). In the present study, it was not possible to visually detect any microbial growth in any of the samples during the steeping and germination process, but the possibility cannot be excluded that microorganisms were present. It is unknown whether microorganisms can act on avenanthramides, causing a decrease in their levels during the germination process. Nevertheless, the total germination time is dependent on the temperature, resulting in an increased germination time with decreased temperature, suggesting that a balance must be found between the germination parameters in order to optimise the germination process in the best way possible.

Paper III

A preliminary study showed that there were elevated avenanthramide levels in oats harvested in 2000 compared with the 2001 harvest. This finding, in combination with a previous study showing that the average molecular weight of β-glucan was lower in 2000 compared with 2001 (Ajithkumar, Andersson & Åman, 2005), led to the hypothesis that preharvest sprouting of the oats had occurred in the field in the harvest of 2000. This hypothesis was based on studies showing that the levels of avenanthramides can increase during germination of oat grains (Matsukawa *et al.*, 2000; Bryngelsson, Ishihara & Dimberg, 2003; Paper II) and that the activity of the β-glucan degrading enzyme β-glucanase is upgraded during germination (Doehlert & McMullen, 2003). The avenanthramide content and the activity of alpha-amylase and β-glucanase, the latter two as indicators of preharvest

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sprouting, were measured in 20 Swedish oat genotypes grown in the south of Sweden in 2000 and 2001. The measured parameters were evaluated in combination with results on the average molecular weight of β-glucan from the same oat material (Ajithkumar, Andersson & Åman, 2005).

A significant difference in avenanthramide content was found between the two years for the 20 genotype samples, while there was no difference in avenanthramide content between these genotype samples (Figure 8A). The methods used to measure alpha-amylase and β-glucanase activity in the oat samples, which were based on commercial assay kits, had to be refined in order to detect any enzyme activity. The alpha-amylase (Figure 8B) and β-glucanase activities detected were found not to be significantly affected by harvest year or by genotype. However, it was found that a large proportion of the β-glucanase activity detected was exerted by microorganisms located on the surface of the oat hulls. When the results from the current study were compared with the average molecular weight of β-glucan in the same oat material (Ajithkumar, Andersson & Åman, 2005) it was found that there was no correlation between alpha-amylase activity and β-glucanase activity or between alpha-amylase activity or β-glucanase activity and content of avenanthramides or average molecular weight of β-glucan. The hypothesis that the oat samples from 2000 had been preharvest sprouted in the field could therefore not be confirmed.

The differences found in avenanthramide content between the two harvest years for the 20 genotype samples were large and consistent for all genotypes but two. Similar results have been reported previously when the avenanthramide levels were compared for three consecutive years (1998-2000) in three oat cultivars, also grown in the south of Sweden (Dimberg, Gissén & Nilsson, 2005). It was found that the levels of total avenanthramides were highest in 2000 for all three cultivars and that the effect of harvest year did not differ between the three cultivars, indicating that environmental factors may be responsible for the fluctuation in avenanthramide levels between years. Additional studies have also suggested that the levels of avenanthramides in oats are significantly affected both by genotype and growing environment (Emmons & Peterson, 2001; Peterson *et al*., 2005). Many oat constituents other than avenanthramides have been reported to be mainly affected both by genotype and environment, for example protein, oil and β-glucan (Humphreys, Smith & Mather, 1994; Doehlert, McMullen & Hammond, 2001). The differences in average molecular weight of β-glucan between the two harvest years could not be explained by preharvest sprouting since the levels of β-glucanase activity were low. However, using β-glucanase activity as an indicator of preharvest sprouting was found to be somewhat misleading. A correlation between preharvest sprouting and β-glucanase activity must be based on endogenous β-glucanase activity. In the current study it was found that exogenous β-glucanase activity exerted by microorganisms located on the oat hulls was also present. Therefore, alpha-amylase activity was determined to be a more reliable indicator of preharvest sprouting, since alpha-amylase activity only was found to be endogenous. The differences in avenanthramide content and average molecular weight of β-glucan between 2000 and 2001 were possibly an effect of environmental conditions.
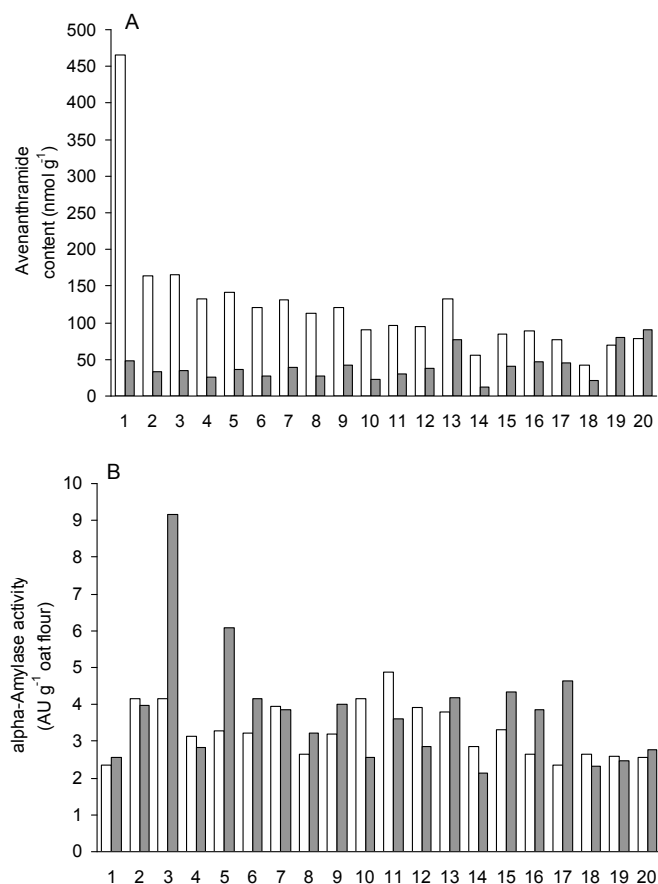
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Figure 8. A) Content of avenanthramides (nmol g^{-1}) and B) activity of alpha-amylase (AU g^{-1} oat flour) in samples from the harvest of 2000 (□) and 2001 (■) for the 20 oat genotypes (numbered 1-20) included in the study. For the avenanthramides the bars are means of duplicate analyses, while for the activity of alpha-amylase the bars are means of triplicate analyses.

Paper IV

Preliminary studies indicated that peak 16 in the chromatograms from oat extracts which had been steeped and/or germinated (Figure 6 and Paper II) consisted of two compounds. This was confirmed when the same samples were analysed using a slower gradient and peak 16 was separated into two peaks. The compound corresponding to the larger of these two new peaks was isolated and structurally identified from oat grains by Dimberg & Peterson (unpublished results) to be the flavone tricin. Tricin in oats is an interesting compound to study since it has been suggested to exert bioactivity *in vitro* and *in vivo* (Hudson *et al.*, 2000; Cai *et al.*, 2005). The content of tricin in selected European and North American oat genotypes was evaluated, as well as the location of tricin in the oat grain. Changes in the levels of tricin in oat samples (spikelets) at different stages of maturity of

the oat grain were studied, as was the effect of steeping and germination of oat grains on content of tricin.

A total of 132 oat samples were screened for content of tricin. In 28 of these samples tricin was detected and quantified, while in the remaining 104 samples the content of tricin, if present at all, was below the determination limit of 10 μg g^{-1} which was set by the possibility of evaluating the UV-spectrum of tricin (Figure 9). Even when the samples were concentrated to have a concentration of tricin above the determination limit, tricin could still not be detected. In the samples where tricin was detected, the content varied between 10.1 and 41.2 μg g^{-1}. Tricin could only be detected in oat hulls. In the groats there was a peak in the chromatograms at a retention time corresponding to that of tricin, but it was not possible to determine by the UV-spectra that this peak corresponded to tricin. The levels of tricin in developing oats decreased significantly according to a logarithmic scale for the two genotypes studied. The content of tricin in raw oat samples compared to germinated oat samples (16 °C for 120 h) did not change significantly for any of the three cultivars studied.
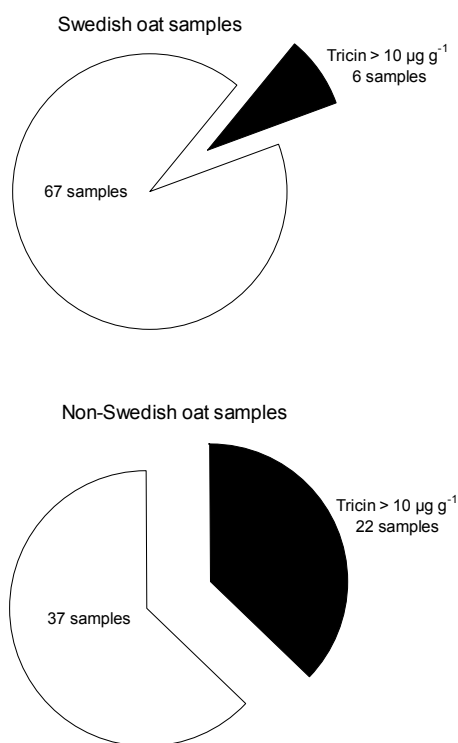
Figure 9. Proportion of samples where the content of tricin could be determined (>10 μg g^{-1}) (■) and not determined (<10 μg g^{-1} if present at all) (□) in Swedish and non-Swedish oat samples. The total number of samples was 132.

Of the 28 samples with levels of tricin that could be quantified, only six (of 73) were of Swedish origin while 22 samples (of 59) were non-Swedish (Figure 9). This may indicate that the presence of tricin in oats could be related to genotype-specific qualities, where tricin is either present or not in the different oat genotypes. However, it must be noted that the genotypes studied were not selected in a random way, resulting in a larger proportion of samples of Swedish origin than of non-Swedish origin. Compared with the tricin content in rice, which has been reported to be approximately 0.07 µg g^{-1} (Hudson *et al.*, 2000), oats appear to be a good source of tricin, with levels well above that reported in rice. However, account must be taken of the fact that the method used for extracting tricin and the quantification method used in Hudson *et al.* (2000) deviated from the method used in the current study. Furthermore, in the current study, no attempts were made to purify and isolate tricin from oat hulls and groats separately. Such an experiment would determine if tricin is present also in oat groats although not detected in the method used in the current study. The finding that tricin could only be detected in oat hulls was supported by the finding that all naked genotypes included in the study had tricin levels below the determination limit. Another result supporting this was that the levels of tricin did not change significantly during germination of oat grains. It is known that hull constituents remain unaffected during germination (Welch *et al.*, 1983; Fulcher, 1986). The decrease in levels of tricin during maturation of the oat grain is probably due to dilution caused by other grain constituents as the grain grows and reaches maturity. The present study did not analyse whether tricin was present in hulls of immature grains where the tricin levels were below the determination limit in the mature grains.

Since tricin could only be detected in oat hulls and not in oat groats, humans consuming oat-based food products would not benefit from the potential anticarcinogenic properties of tricin, since oat hulls are rarely used in food products. However, there are reports of processed oat hulls being used in cereal-based food products as a source of fibre (Stephen *et al.*, 1997; Galdeano & Grossman, 2006) and this is probably only the start of a new area of usage for high fibre food products. The current study indicates that oat hulls may be a good source of tricin if high-tricin genotypes are chosen and if the use of oat hulls in the food industry were to be further explored, for example in the area of cereal-based food products.

Important outcomes of the thesis

The degrading enzyme activity on avenanthramides in oats when oat flour is steeped in water is strongly suggested to be a result of PPO activity. Although the avenanthramides are only found in oats, PPO activity on avenanthramides was also found in grains of wheat, barley and rye (Paper I).

The levels of avenanthramides may be increased by steeping and germination but the increase is dependent on choice of oat cultivar and germination conditions (Paper II).

A germination process may increase the avenanthramide content and decrease the molecular weight of β-glucan in oat grain. However, the differences in avenanthramide concentrations and average molecular weight of β-glucan between the harvest years of 2000 and 2001 could not be explained by preharvest sprouting (Paper III).

The flavone tricin seems to be a hull constituent of oats and its presence is highly dependent on cultivar (Paper IV).

Germination of oats can result in a raw material with sustained or elevated levels of avenanthramides and other potentially health beneficial phenolic compounds. Germinated oats can be used as an ingredient in novel oat-based food products, but if oat grains are milled there is risk of PPO action on the avenanthramides resulting in decreased levels of avenanthramides. PPO activity acting on avenanthramides is also present in wheat, barley and rye. Therefore, in order to maintain the nutritional value of the avenanthramides, it is important to inactivate PPO in oats but also in other cereal grain ingredients if included in oat-based food products. It is also highly essential to use oat cultivars that have high and stable levels of avenanthramides regardless of cultivation conditions, to ensure the nutritional quality of the final oat-based food product. Characterisation of other compounds, such as tricin and additional avenanthramides, is a crucial task since other unknown compounds in oats may also be potentially health beneficial and therefore of great interest for humans. Although the concentrations of the phenolic compounds discussed here are relatively low in oats, continuous intake of oats in the daily diet might prevent the development of disease in humans.

Concluding remarks and future research

The main objective of this research project was to obtain new knowledge on how to treat the raw oat material of oat-based products in order to sustain or even increase the levels of endogenous phenolic compounds, with emphasis on the avenanthramides, in the final food product. Germination of oats proved to be a potential processing method for use on oats since it is relatively easy to perform, although time-consuming. However, the germination process needs to be further optimised in order to provide conditions that are favourable for all the health beneficial compounds found in oats, such as the fatty acids, amino acids, vitamins, β-glucan and phenolic compounds. Parameters such as temperature, time, pH and the effects of kilning, which are poorly described, are important factors to consider in order to obtain an oat product with as high nutritional quality as possible. It is also essential to consider PPO activity, since it can highly affect the nutritional quality of phenolic compounds of grain-based food products where oats is used as an ingredient. Fortunately, knowledge on how to inhibit PPO is good and many different methods are used today with great success on different food products to reduce enzymatic browning.

Knowledge about the raw material, in this case oats, is of priority since a raw material of low nutritional quality is difficult to improve. More research is needed on multiple nutrients in a wide perspective, considering how different oat cultivars react to different stimuli, such as cultivation conditions and processing, in order to be able to make a good choice of cultivar. Suitable cultivars should be stable and have high and constant levels of nutrients between years and cultivation sites. Identifying the factors that affect annual differences in levels of avenanthramides has proven to be a difficult task, since multiple cultivation factors are involved *e.g.* temperature, rainfall, sunshine, and microorganisms. It is not only the nutritional quality of oats that is important, but also the functional quality. It must be considered from a technological point of view whether a cultivar is functional or not, for example regarding hulling and flaking procedures, in order to be able to use the oats developed for their high nutritional quality when producing oat-based food products.

Furthermore, as regards the phenolic compounds in oats, more studies need to be performed to evaluate the physiological effects in humans of phenolic compounds, including avenanthramides, tricin and hydroxycinnamic acids, as well as the so far unidentified compounds, in order to know more of the actual value and importance of consuming these.

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