Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences

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Abstract

Interest in oats has increased during recent years because of its health beneficial effects, mainly as foodstuff but also as a pharmaceutical. Oats contain unique phytochemicals, like the oat specific antioxidants called avenanthramides. Oats also contain high levels of unsaturated fatty acids which can lead to oxidation problems during processing. Antioxidants are currently added to oat products for this purpose. Malting of oat grains prior to processing might replace the added antioxidants since some studies have found that the avenanthramide amount has increased by the process. However, some other health beneficial compounds like tocols (Vitamin E) and β-glucan (fiber) might decrease by malting. In the present study, qualitative and quantitative properties of avenanthramides and tocols (tocopherols and tocotrienols) of 10 different oat cultivars were investigated, both before and after malting. Some of the oat groats (dehulled kernels) were malted before extraction but none of them germinated. Although, malting resulted in a significant avenanthramide increase by 238%, whereas tocol amount did not change significantly. This suggests that some food additives in oats could be avoided by malting. Avenanthramide concentration varied a lot between cultivars. Amount increased most in the cultivars Belinda (+2943%) and SW151304 (+454%) but decreased in the cultivars Matilda (-71%) and Fatima (-63%). Belinda and SW151106 had the highest levels of avenanthramides after malting. However, large variations were observed for the malting duplicates of some cultivars regarding avenanthramide content. Generally, avenanthramide concentration was a bit lower than recorded by previous studies whereas tocol concentration was a bit higher. Antioxidant activity was higher after malting and largely it seemed to be attributed to the avenanthramides. In further studies, more cultivar analyses during controlled conditions are required and environmental and genotypic factors must be taken into consideration in order to assure cultivar variations. It is also of interest to analyse malting effects on β-glucan.

Keywords: Oats, oat cultivars, avenanthramides, tocols, malting

Sammanfattning

Intresset för havre har under de senaste åren ökat. Detta på grund av dess hälsofrämjande egenskaper, främst som livsmedel men även som läkemedel. Havre innehåller unika fytokemikalier, till exempel de havrespecifika antioxidanter som kallas avenantramider. Havre innehåller höga halter av omättade fettsyror vilket kan orsaka problem med oxidation under industriella processer. Vanligtvis tillsätts antioxidanter för att undvika detta. Mältning av havrekärnor före den industriella processen skulle kunna ersätta de tillsatta antioxidanterna eftersom vissa studier har visat att avenantramidhalten ökar under mältningen. Halten av andra hälsofrämjande ämnen som tokoler (Vitamin E) och β-glukan (fiber) kan dock minska under mältningsprocessen. I denna studie undersöktes kvalitativa och kvantitativa egenskaper hos avenantramider och tokoler (tokoferoler och tokotrienoler) i 10 olika havresorter, både före och efter mältning. Vissa av havrekärnorna (skalade) mältades före extraktionen men ingen av dessa grodde. Ändå ökade avenantramidhalten signifikant efter mältningen med 238%, medan tokolhalten inte ändrades signifikant. Resultaten tyder på att tillsatta antioxidanter skulle kunna ersättas av mältning. Avenantramidhalten varierade mycket i havresorterna. Halten ökade mest i sorterna Belinda (+2943%) och SW151304 (+454%) men minskade i sorterna Matilda (-71%) och Fatima (-63%). I sorterna Belinda och SW151106 uppmättes högst halt av avenantramider efter mältningen. Stora variationer inom mältningsduplikaten observerades dock i vissa av sorterna. Generellt var den uppmätta avenantramidhalten lägre än vad man funnit i tidigare studier medan tokolhalten var lite högre. Antioxidantaktiviteten var högre efter mältningen och verkade till största del bero på avenantramiderna. I framtida studier behöver det analyseras fler havreprover under kontrollerade förhållanden och omgivande och genotypiska faktorer behöver även tas i beaktning för att några slutsatser gällande sortvariationer ska kunna dras. Det är också av intresse att analysera mältningens effekter på β-glukan.

Nyckelord: Havre, havresorter, avenantramider, tokoler, mältning

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Abbreviations

AO	Antioxidant activity
AU	Area units
AVA	Avenanthramide
DM	Dry matter
HCA	Hydroxycinnamic acid
HHT	Hydroxycinnamoyl-CoA:hydroxyanthranilate N-hydroxycinnamoyl transferase
HPLC	High Performance Liquid Chromatography
MeOH	Methanol
Tocols	Tocopherols and tocotrienols
TP	Tocopherol
T3	Tocotrienol

1 Introduction

1.1 Background

Oats and oats products have gained interest during recent years because of its health beneficial effects, mainly as foodstuff but also as a pharmaceutical (Chu *et al.*, 2013). Oats, compared to other cereals, has superior nutrient profile and contains high amount of dietary fibre as well as unique phytochemicals (Ames *et al.*, 2013; Liu, 2007). Oat phytochemicals include tocopherols and tocotrienols (tocols), phenolic acids and avenanthramides. Avenanthramides are oat specific antioxidants (Gangopadhyay *et al.*, 2015) and their synthesis may be enhanced by germination of the oat grains. Probably, the increased amount may function as protection of the seedling from radicals during germination (Bryngelsson, 2002; Skoglund *et al.*, 2008). Knowledge about the unique qualities of oats might be utilized for different industry purposes in the future. Whole oat as foodstuff remains as an interesting subject for different food applications and for research (Ames *et al.*, 2013).

1.2 Project description

The present study is a part of a project with the purpose of investigating antioxidants in malted oats and its potential application in the food industry. In this project, it is of interest to investigate weather flour from malted oat groats with elevated levels of antioxidants can replace added antioxidants in processed oat products. Oats contain high levels of unsaturated fatty acids, compared to other cereals. This leads to problems, e.g. during extrusion, since the fat become prone to oxidize when it is exposed to air. Antioxidants are currently added to extruded oat products for this purpose (Delcour & Hoseney, 2010c; Head *et al.*, 2011).

Malting of oat grains prior to processing might replace the added antioxidants since some studies have found that malting, especially during germination, results in an increase of total free phenolics (Xu *et al.*, 2009) as well as avenanthramides (Bryngelsson, 2002; Skoglund *et al.*, 2008). However, some other health beneficial compounds, e.g. β-glucan, might decrease during the process (Tiwari & Cummins, 2009).

If addition of antioxidants to oat products could be avoided by malting, it would prevent consumer concerns regarding food additives (E-numbers) and the products would seem more natural from a consumer's perspective. Furthermore, it might provide with beneficial effects of the produced avenanthramides.

1.3 Objectives

In the present study, a screening of different oat cultivars for high content of antioxidants (avenanthramides, tocopherols and tocotrienols) before and after malting was performed. The aim was to investigate qualitative and quantitative antioxidant properties of the oat cultivars as well as detect cultivars responding to malting with elevated levels of avenanthramides without reducing the amount of tocols.

2 Literature review

Relevant literature was collected using scientific databases like Pubmed, Web of Knowledge and Google Scholar. Mainly publications from scientific journals were used as material for the literature review as well as general information from books.

2.1 Oats

Oats (*Avena sativa*) are a cereal crop used mainly as forage for horses, poultry and other animals. Oats are also used for malting and brewing as well as for different human food applications, like flakes (e.g. for muesli and oatmeal), breakfast cereals, dairy substitutions and baby foods (Ames *et al.*, 2013; Delcour & Hoseney, 2010b; Gangopadhyay *et al.*, 2015). They are cultivated all over the world (Gangopadhyay *et al.*, 2015) and world production is estimated to 22.8 million tons for the marketing year 2016/17 (FAS, 2017). Oats can also be used for industrial oil extraction (Ames *et al.*, 2013).

2.1.1 Morphology

Oats are harvested with the hulls attached to the kernel. The hulls enclose the caryopsis closely which prevents them from being removed from the grain during threshing. Most cultivars of rice and barley are also harvested with the hulls attached whereas other cereals become naked, i.e. hulls are removed, by thrushing (Delcour & Hoseney, 2013c). However, some oat cultivars are also classified as naked (Tiwari & Cummins, 2009).

The hull consists of floral envelopes and typically they constitute about 25-30% of the kernel weight (Ames *et al.*, 2013). After removing the hull, the oat kernel is

called groat. Oat groats are composed of a germ, starchy endosperm, epidermis, aleurone- and sub aleurone layers, seed coat and a pericarp (Ames *et al.* 2013; Delcour & Hoseney, 2013c). The bran is a milling fraction and comprises the outer layer of the groat. The fraction is not as distinct as in e.g. wheat but largely it constitutes of the seed coat, pericarp and aleurone- and sub aleurone layer (Ames *et al.*, 2013).

2.1.2 Nutritive composition

Compared to other cereals, oats contain higher amount of protein and fat with good quality. Protein concentration of oats has in previous studies been found to about 13-20% and lipid concentration about 2-12% of the dry matter (DM) (Tong *et al.*, 2014). The largest component of the oat macronutrients is the carbohydrates (Gulvady *et al.*, 2013).

Oats are usually consumed as a whole grain cereal (Delcour & Hoseney, 2013c). Since they only contain traces of gluten proteins, oat products can be ingested by people with celiac disease (Yan *et al.*, 2013).

Oats constitute a good source of dietary fibre, particularly the soluble fibre β-glucan, as well as many vitamins, antioxidants and enzymes. They also contain important minerals and favourable proportions of some of them, e.g. high amount of potassium and low amount of sodium (Delcour & Hoseney, 2013c; Kris-Etherton *et al.*, 2013). Most vitamins, minerals, phytates and phenolic compounds are located in the bran (Gulvady *et al.*, 2013). Antioxidants are to a large extent located in the groats, with highest concentration in the outer parts of the groats (Bryngelsson *et al.*, 2002). The largest part of the groats constitutes of the starchy endosperm which stores nutrients like starch, lipids and proteins (Gulvady *et al.*, 2013).

Amount of nutrients in oats depend on growth environment, cultivar and production year (Ames *et al.*, 2013).

2.1.3 Health beneficial compounds

Health benefits of oats have largely been ascribed to their nutrients and various phytochemicals such as phenolic compounds, avenanthramides, tocols (Vitamin E) and lignans as well as β-glucan, carotenoids and phytosterols (Cui & Liu, 2013; Liu, 2007; Yan *et al.*, 2013). Some health benefits have also been attributed to proteins and lipids (Gangopadhyay *et al.*, 2015).

β-Glucan can prevent coronary heart disease by reducing total cholesterol levels as suggested by European Commission (2016) as well as Berg *et al.* (2003) and Liu (2004). Oat antioxidants might also reduce the risk of CVD by reducing blood serum

cholesterol levels and prevent LDL oxidation and peroxidation (Chu *et al.* 2013). Other chronic diseases like type II diabetes and cancer may also be reduced by oats, as indicated by Gangopadhyay *et al.* (2015), Liu (2004) and Slavin (2003). Caloric regulation, blood pressure and weight maintenance are other positive effects which dietary oat could provide with (Kris-Etherton *et al.*, 2013). Many phenolic amides present in oats possess antioxidant capacity as well as anti-inflammatory, antigenotoxic and antiproliferative abilities (Chu *et al.* 2013).

Several bioactive compounds of oats have been suggested to act synergistically, both with each other but also with other compounds, regarding prevention of oxidative damage of cells (Chu *et al.*, 2013; Lee-Manion *et al.*, 2009; Liu, 2004; Sur *et al.*, 2008; Trombino *et al.*, 2004). Synergistic effects between whole grain phytochemicals and phytochemicals in vegetables and fruits have been studied by Liu (2004). Results from Liu (2004) as well as several other studies indicate that health benefits of oat phytochemicals are a result of complex combinations of compounds. This implies that ingestion of mixtures of phytochemicals of oats are more beneficial compared to separate compounds individually (Chu *et al.*, 2013; Gangopadhyay *et al.*, 2015; Lee-Manion *et al.*, 2009; Liu, 2004; Sur *et al.*, 2008; Trombino *et al.*, 2004).

Health claims

Oats as foodstuff have several health claims approved by the European Commission through European Food and Safety Agency (EFSA); Ingestion of oats contributes to normal blood cholesterol levels, reduces blood glycaemic responses and contributes to an increase in the faecal bulk. Preceding health claims are a result of the β-glucan content. Additionally, Vitamin E (tocols) contributes to health benefits of oats since it functions as antioxidant and thereby protects human cells-, DNA-, as well as proteins and lipids from oxidative damage (European Commission, 2016).

2.2 Antioxidants in oats

The main oat antioxidants comprise phenolic compounds, tocols (Vitamin E) and phytic acid (Delcour & Hoseney, 2013c; Gangopadhyay *et al.*, 2015). The antioxidants have abilities to prevent oxidation and donate hydrogen to free radicals which leads to scavenging of their radical activity (Bratt *et al.*, 2003). Radicals in human cells increase the risk of developing many of the major chronic diseases. Ingested antioxidants may thereby contribute to immune systems in the body (Gangopadhyay *et al.*, 2015).

2.2.1 Phenolic acids

Phenolic molecules are composed of aromatic rings and hydroxyl groups. They are produced by plants, often in response to pathogens and parasites. Flavonoids, phenolic acids and their derivates are the most common phenolic compounds in oat plants (Gangopadhyay *et al.*, 2015). Phenolic composition of oats is similar to that of some fruits e.g. apple and cranberry (Liu, 2007). Most of the phenols are concentrated in the bran part (Gangopadhyay *et al.*, 2015).

Phenolic acids are divided into hydroxybenzoic acids and hydroxycinnamic acids (Gangopadhyay *et al.*, 2015). They are present both as bound forms and free forms, though, in oats about 75% is bound. Bound phenolic acids are attached to cell wall polysaccharides and their bioavailability is lower than that of the free phenolics (Liu, 2007; Xu *et al.*, 2009).

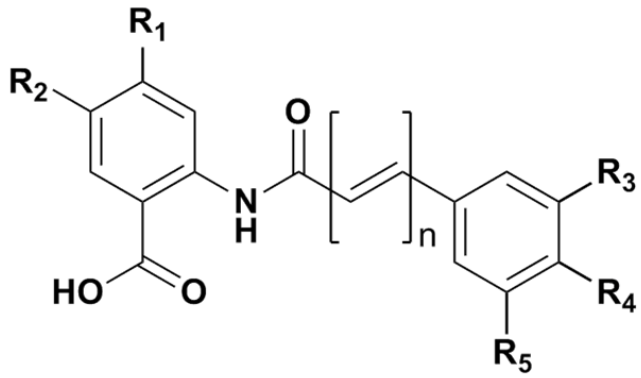
Concentrations of phenolic compounds in oats have varied between studies. Tong *et al.* (2014) reported a phenolic content ranging from 0,1 to 0,15 mg g^{-1} from analysis of 21 oat cultivars, and Aldieri and Redaelli (2015) reported similar results. Chu *et al.* (2013) reported a higher concentration ranging from 0,57 to 0,94 mg g^{-1}.

Li *et al.* (2017) found that total phenolic content is significantly affected by environmental and genotypic factors as well as their interactions.

2.2.2 Avenanthramides

Avenanthramides are oat specific, ethanol soluble polyphenolic amides with low molecular weight. They function as phytoalexins (antimicrobial substances) produced by oats against e.g. fungi, and are a part of the plants defence system (Boz, 2015; Gangopadhyay *et al.*, 2015; Wise *et al.*, 2016). Avenanthramides are a fundamental oat compartment of leaves and grains (Peterson & Dimberg, 2008) and are present in both groats and hulls (Gangopadhyay *et al.*, 2015).

Avenanthramide synthesis in oats is initiated by hydroxycinnamoyl-CoA:hydroxyanthranilate N-hydroxycinnamoyl transferase (HHT) which starts the reaction between anthranilic acids and hydroxycinnamoyl-CoA esters (Bryngelsson, 2002). They can also be synthesised by treatment of the oat plant with plant defence activators like BTH (benzothiadiazole). Treatment of oat plants with BTH have also resulted in enhanced crown rust resistance of oats (Wise *et al.*, 2016).



	R_1	R_2	R_3	R_4	R_5
2c	H	OH	OH	OH	H
2p	H	OH	H	OH	H
2f	H	OH	OCH_3	OH	H

Figure 1. Chemical structure of oat avenanthramides. The left part represents different anthranilic acids; e.g. anthranilic acid (1) or 5-hydroxy-anthranilic acid (2). The avenanthramides listed contain (1). The right part represents different hydroxycinnamic acids; e.g. p-coumaric acid (p), caffeic acid (c) and ferulic acid (f) (Modified from Wikimedia commons, Structure_s_of_AVA).

Avenanthramides consist of an anthranilic acid linked by amide bonds to hydroxycinnamic acids. Different avenanthramides distinguish from each other by number of- and position of hydroxyl-, methoxy- or hydrogen substitutions on specific carbons on the aromatic anthranilic moiety and the cinnamic acid moiety of the avenanthramide molecule. Different substitutions might be the reason of different biological effects. Avenanthramides can be classified according to the abbreviations described in Figure 1. According to this classification, hydroxycinnamic acids can be named with letters as following examples; p-coumaric acid (p), caffeic acid (c), and ferulic acid (f). Before each letter, a number representing different anthranilic acids is present. Number one represent anthranilic acid (1), number two represent 5-hydroxy-anthranilic acid (2), number three represent 5-hydroxy-4-methoxy-anthranilic acid (3), number four represent 4,5-di-hydroxy-anthranilic acid (4) and number five represent 4-hydroxy-anthranilic acid (5) (Skoglund *et al.*, 2008; Yang *et al.*, 2014). Structurally, avenanthramides are similar to the antiallergenic drug named Tranilast (N-3,4-dimethoxy-cinnamoylanthranilic acid) (Boz, 2015).

About 40 different avenanthramides have been discovered (Boz, 2015) but not all are present in oats. Fifteen avenanthramides have been identified in oat kernels whereof the most abundant ones are 2c, 2f and 2p (Boz, 2015; Cui & Liu, 2013; Dimberg, personal communication; Skoglund *et al.*, 2008; Tong *et al.*, 2014). Also, corresponding dienes of all avenanthramides have been detected. They distinguish by double bonds in the hydroxycinnamic acid moiety and are named with the letter *d* after the usual abbreviation, e.g. 2fd and 2pd (Dimberg, personal communication).

Avenanthramides have been reported to exert anti-inflammatory and radical scavenging effects in several studies (Chu *et al.*, 2013; Guo *et al.*, 2008; Kris-Etherton *et al.*, 2013; Sur *et al.*, 2008). Some studies have indicated that avenanthramides

decrease the risk of developing certain cancer forms (Guo *et al.*, 2010; Sur *et al.*, 2008; Wang *et al.*, 2012). They may also be beneficial for different skin afflictions and general skin health (Kris-Etherton *et al.*, 2013; Sur *et al.*, 2008).

In a study by Li *et al.*, (2017), total concentration of avenanthramides ranged from 22.1 to 471.2 mg kg^{-1} which is higher than reported by Chu et. al. (2013) and lower than reported by Tong *et al.* (2014) who measured concentrations as high as 718.5 mg kg^{-1}. In the study by Li *et al.* (2017), concentration of 2f (7.3-222.8 mg kg^{-1}) was higher than that for 2p (6.1-112.3 mg kg^{-1}) and for 2c (6.2-136.2 mg kg^{-1}) whereas 2c was the main avenanthramide in a study made by Xu *et al.*, (2009). Li *et al.* (2017) found that concentration of avenanthramides is highly influenced by environmental and genotype factors.

2.2.3 Tocols

Vitamin E is the generic name for eight lipophilic antioxidants which are divided into two groups; tocopherols (TP) and tocotrienols (T3). Together they are called tocols or E-vitamers. Each group consist of four homologues; α β ɣ and δ. The homologues distinguish from each other by amount of- and positions of methyl groups on the chromanol structure. The activity of the tocols is depending on the structure (Gangopadhyay *et al.*, 2015). The basic structure of tocols is displayed in Figure 2.

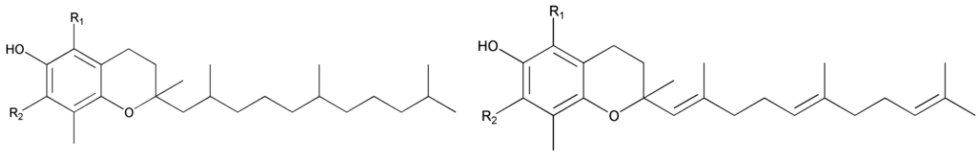


Figure 2. Basic structure of Tocopherols (a) and Tocotrienols (b) (Gangopadhyay *et al.*, 2015).

Tocols are synthesized from homogentisic acid and phytyl pyrophosphate. They are natural compounds of cereal grains that can prevent oxidation of lipids (Bryngelsson, 2002).

All tocols have been detected in oat grains (Gangopadhyay *et al.*, 2015). α-Tocopherol and δ-Tocopherol are mainly present in the germ whereas tocotrienols are concentrated in the endosperm and bran of the groat (Peterson, 1995). α-Tocotrienol is the most common tocol in oats followed by α-Tocopherol. The two tocols represent approximately 90% of the total oat tocols (Bryngelsson, 2002).

Vegetable oils represent the main tocol source of human diets, although most cereal grains have been found to contain substantial amounts (Gangopadhyay *et al.*, 2015).

Tocols possess antioxidant capacities. The α-compounds possess the highest activity followed by β ɣ and δ in decreasing order (Bryngelsson, 2002). α-Tocopherol has been found to possess the highest antioxidant activity but activity of α-Tocotrienol has been recorded higher in some cases (Gangopadhyay *et al.*, 2015).

Concentration of tocols in oats depends on cultivar and location of growth. In one study, tocol amount within 12 oat cultivars was found to range between 19 to 30 mg kg^{-1} (Gangopadhyay *et al.*, 2015). Chu et al. (2013) and Bryngelsson (2002) have reported similar results; 26.2 to 37.1 mg kg^{-1} and 13 to 43 mg kg^{-1}, respectively.

2.3 Processing

After harvesting, the oat kernels are cleaned and heat treated and/or dried. Drying is important as the hulls become easier to remove afterwards. Heat treatment in different forms during the oat processing is necessary for the inactivation of lipolytic enzymes. Steam is also commonly used during processing which provide a desirable flavour of the oats (Delcour & Hoseney, 2010b; Head *et al.*, 2011).

In different parts of the oat production chain, various attributes of the oat cultivar are preferred. Growers prefer to cultivate oats with stable grains, high yield, high resistance to diseases and microorganisms etc. whereas millers want uniform kernels which are easy to dehull, white groat color, low breakage percentage of the groats etc. (Yan *et al.*, 2013). Processing parameters need to be fulfilled too and the oat raw material should result in an attractive product with a favourable taste, shelf-life as well as appearance (Bryngelsson, 2002).

2.3.1 Products

Groats are processed by steaming, flaking and milling and useful products include rolled flakes, hulls and flour. Flakes are one of the major food products and from flakes products like muesli and oatmeal can be manufactured. From oat flour, breakfast cereals and baby foods can be produced (Ames *et al.*, 2013; Delcour & Hoseney, 2010b).

Extrusion is a commonly used method for the production of cereal flake products. The principle of extrusion is to mix oat flour with water and determine the final shape by controlling temperatures and pressure (Ames *et al.*, 2013).

Since oats do not contain gluten, possibilities to produce products like pasta and bread with good quality are limited (Ames *et al.*, 2013).

2.3.2 Malting

Malting is based on steeping, germination and kilning (drying) of cereal seeds. Steeping of the oat grains is the first step where the grains are soaked in water to a moisture content of about 42-44%. Water is thereafter drained and the grains are germinated on beds with high humidity. During this step, many enzymes are produced or activated. Kilning is a drying step which stops the germination and protects the groats from microbial growth by reducing the water content. If elevated temperatures are applied, kilning inhibit enzyme activity. Preferably it is done without a complete inactivation of the enzymes. During this step, the grains also develop characteristic flavour and colour (Delcour & Hoseney, 2010a; Skoglund *et al.* 2008; Xu *et al.* 2009).

Effects on nutritive composition

Germination and steeping alter the chemical composition of the grains. For example, avenanthramide concentration has been found to increase during controlled steeping and germination (Bryngelsson, 2002; Skoglund *et al.* 2008). Li *et al.* (2016) also found that avenanthramide content was increased by processing like flaking, kilning and steel cutting. Probably, *de novo* synthesis of avenanthramides are one of the reasons for the increase (Bryngelsson, 2002) and HHT activity have been suggested by Bryngelsson (2002) and Skoglund *et al.* (2008) to play a significant role in the increase. The increase may also be a result of the release of avenanthramides and its precursors from different structures in the grain (Skoglund *et al.*, 2008). Xu et al. (2009) found that germination resulted in an increase of free and total phenolic compounds. Simultaneously, the amount of bound phenolic compounds decreased. This is probably a result of an increase of enzymes during germination which have the ability to break down cell walls (Xu *et al.*, 2009).

Although malting seems like a beneficial step for oat nutrition, some nutritional beneficial substances are degraded due to the increased enzyme activity. For example, β-glucanase production is increased, which can lead to the degradation of β-glucan (Skoglund, 2008). Tiwari & Cummins (2009) have reported a decrease of β-glucan levels as a result of germination.

2.3.3 Shelf life

Oats have higher oil content compared to other cereals, which is a good quality for oil extraction and animal feed purposes. However, as the lipid fraction contain a high proportion of unsaturated fatty acids, it makes them prone to oxidize during processing methods which includes a lot of air, e.g. extrusion and milling. The oxidation can be initiated by reactions between oxygen and unsaturated fatty acids and/or degrading enzymes like lipases and lipoxygenases. These enzymes are normally denaturated during heating processes, e.g. during kilning. Oxidation can cause problems with rancidity, off flavours and unwanted aromas in food products. Added preservative agents like antioxidants may be added to increase the shelf life (Ames *et al.*, 2013; Delcour & Hoseney, 2010c).

Some health beneficial compounds, like tocols, are sensitive to heating processes. As a result, they may degrade during processing of oats which is not wanted as it leads to removal of antioxidants which could protect the lipids and other oat compounds as well as contribute with health beneficial effects (Gangopadhyay *et al.*, 2015; Peterson, 1995). Avenanthramides have been found to be relatively stable during processing methods like heat treatment (Dimberg, personal communication; Li *et al.*, 2016).

In this thesis, it was investigated weather avenanthramides and tocols are affected by malting, although heat treatment with high temperatures was not applied in the method.

3 Materials and Methods

3.1 Oat cultivars

Eight oat (*Avena sativa*) cultivars and two accessions (named SW151304 and SW151106) of oats were provided by Lantmännen Cerealia AB (Järna, Sweden). The two accession varieties are henceforth called cultivars too. Cultivars and some basic properties are displayed in Table 1. All cultivars were cultivated in the same field in the south of Sweden and each cultivar come from one single harvest.

Table 1. *Some properties of the analysed oat cultivars and accessions*

Sample nr	Cultivar	Cultivar properties[1]
1	Kerstin	Suitable for cultivation in all parts of Sweden and for grain processing. High yield and good cultivation properties. Low cadmium absorption.
2	Belinda	Well established forage cultivar with high yield and cultivation assuredness. High oil- and protein content.
3	Matilda	Forage cultivar with high oil content.
4	SW 151304	No available information.
5	SW 151106	No available information.
6	Galant	High yield cultivar suitable for grain processing.
7	Fatima	Forage cultivar with high oil content. Higher yield- and protein content compared to its predecessor cultivar Kerstin.
8	Avanti	High yield cultivar with relatively short straws and good straw strength.
9	Guld	High yield cultivar with good straw strength.
10	Nike	Forage cultivar which in many cases gives the highest yield of different oat cultivars. High cultivation assuredness.

1 Cultivar properties are compiled from Lantmännen, (n.d.), Roland, J. (2012) and Sixtensson,O. (2016)

3.2 Antioxidants

Avenanthramides (2c, 2f, 2p, 2fd, 3f) were provided and synthesized by Fagerlund *et. al.* (2009). Hydroxycinnamic acids (caffeic, *p*-coumaric, ferulic and sinapic acid) were bought from Aldrich-Chemie. Tocols (α-tocopherol, α-tocotrienol, β-tocopherol and β-tocotrienol) were bought from Calbiochem.

3.3 Processing of oats

3.3.1 Dehulling

A small scale dehulling equipment provided by Lantmännen Cerealia (Järna, Sweden) was used for dehulling of the oat kernels. About 250 g of kernels from each sample were dehulled and the groats were stored in a refrigerator (8 °C) for about eight hours followed by freezing until further analyses.

3.3.2 Malting

Groats were malted in duplicates (n=2) regarding each cultivar to see variances in results deriving from the malting process.

About 7 g groats of each sample were soaked in sterilized $CaCl_2$ in tap water (2%) followed by incubation for 22h at 37 °C. The groats were drained and sterilized by immersion in 60 mL of 1% natriumhypochlorite in tap water for 30 min. The solution was discarded and the groats were washed with 3*25 mL of sterilized tap water through a sieve. The groats were placed in petri dishes (90 mm diameter) containing filter papers soaked with 6 mL of sterilized tap water. Germination was preceded in an incubator at 37 °C for 96 h in darkness followed by drying with the lids of the petri dishes removed at 37 °C for 44 h.

3.3.3 Milling

About five g of dehulled raw- and malted groats were milled into flour using a small scale Retsch ZM-1 grinder mill to pass 5 mm particle size. The groats were milled in four rounds at different days; first raw sample 1-5 (see Table 1) followed by raw samples 6-10. Immediately after milling, the flours were extracted (see 3.4).

3.3.4 Dry matter

Quantification of antioxidants was based on dry matter (DM). Dry matter was calculated by weighing flour from each sample before and after drying. About 0.1 g of each flour was weight in and dried for 16 h in a heating cabinet at 105 °C followed by cooling to ambient temperature in a desiccator. Duplicates were carried out and mean values were used to calculate dry matter.

3.4 Extraction of antioxidants

The method used for extraction is a modified version described by Dimberg & Jastrebova (2009). Immediately after milling, 8 mL of MeOH (99.8%) was added to 1 g of each flour in duplicates and was suspended by vortexing in centrifuge tubes. Extraction was preceded by immersion of the tubes in a shaking water bath at 50 °C for 10 min followed by centrifugation (Multifuge 3s Heraeus) at 2500 rpm for 10 min. The extraction process was repeated two times and the supernatants were combined. The collected supernatants were vacuum evaporated using a Buchi Rotavapor R-210 for 1h and 20 min from atmospheric pressure down to about 25 mbar. Residuals were suspended in 2 mL of MeOH.

When possible during the extraction procedure, the samples were covered by aluminium foil to minimize light exposure. The extracts were stored in a refrigerator at 8 °C until further preparation.

3.4.1 Extract preparation

Extracts from the suspended residuals were transferred from the vacuum tubes to Eppendorf tubes. For tocol analysis, 1 mL of the suspended residuals of each sample was transferred to pre-weighed Eppendorf tubes and the solvent was evaporated using N_2. Oil content was calculated by dividing the weight of the residuals in the Eppendorf tubes by the weight of respective flour used. Oil residuals were resuspended in 200 µL hexane and 50 µL 2-propanol. The samples were centrifuged at 10000 rpm for 15 min (Eppendorf centrifuge 5417C) prior to tocol analysis.

The remaining suspensions were centrifuged at 10000 rpm for 15 min prior to analysis of avenanthramides, hydroxycinnamic acids and antioxidant activity.

3.5 Qualitative and quantitative analysis of antioxidants

3.5.1 HPLC (High Pressure Liquid Chromatography)

HPLC was used for separation, identification and quantification of avenanthramides, hydroxycinnamic acids and tocols (Table 2).

Table 2. *HPLC equipment and settings regarding avenanthramides, hydroxycinnamic acids and tocols*

	AVAs and hydroxycinnamic acids	Tocols
Instrument	Agilent 1100 series	Agilent 1100 series
Column	Reversed phase C18 HP ODS Hypersil, 5 µm, 125x4 column	Straight phase LiChroCART 250-4, 5 µm column
Mobile phase	A: 10 mM formic acid; 5% acetonitrile in dest. H_2O B: Acetonitrile	A: heptane:tertbutylmetyleether; tetrahydrofurane; methanol (79:20:0.98:0.02)
Gradient	Linear gradient from zero to 40% B in A within time interval	Isocratic A
Time interval	40 min	15 min
Detector	Diode array-detector at 340 nm	L4250 fluorescence detector at excitation 294 nm and emission 320 nm
Temperature	25 °C	22 °C
Injection	10 µL	20 µL
Flow rate	1mL/min	1mL/min
Integration	HP ChemStation Software Version 05.01	HP ChemStation Software Version 05.01

3.5.2 Identification

Chromatogram peaks derived from the extracts (see 3.3.1.) were identified by retention times and UV-spectra of external standards of avenanthramides (2c, 2f, 2p, 2fd, 3f), hydroxycinnamic acids (caffeic (c), *p*-coumaric (p), ferulic (f) and sinapic (s) acid) and tocols (α-Tocopherol, α-Tocotrienol and β- Tocopherol).

One peak (no. 4, Figure 3) was not identified but was still included and treated as a tocol during the analyses.

3.5.3 Quantification

Most results are based on mean area units (AU), but quantification of the most common avenanthramides (2c, 2p and 2f) and tocols was also performed. Duplicate

dilution series of the tocols and avenanthramides were used to create standard curves by using HPLC chromatogram results.

Standard curves for avenanthramides were based on 0,15 mg mL^{-1}, 0.10 mg mL^{-1}, 0.075 mg mL^{-1}, 0.05 mg mL^{-1} and 0.025 mg mL^{-1} in methanol and for tocols on 0.10 mg mL^{-1}, 0.075 mg mL^{-1}, 0.05 mg mL^{-1}, 0.025 mg mL^{-1}, 0.0125 mg mL^{-1} and 0.006125 mg mL^{-1} in hexane. There were linear relationships obtained within the concentrations used for both the avenanthramide- and the tocol series.

Concentrations were calculated based on chromatogram peak areas and equations corresponding to mean values of respective standard curve. Results were expressed as mean values of µg g^{-1} DM. Since some of the peak areas of avenanthramide 2c and 2f were too low for the dilutions series interval and as the series was considered as linear, it was decided to create equations cutting point zero in all of the avenanthramide standard curves.

3.5.4 Antioxidant activity by DPPH assay

A modified version described by Bratt *et al.* (2003) was used to measure reactivity toward DPPH (2,2-diphenyl-1-picrylhydrazyl) by hydrogen transfer from phenols to DPPH radicals. The assay measure antioxidant activity based on radical scavenging ability.

Extracts for antioxidant measurements (see 3.4.1.) were stored in a freezer until analysis. After incubation to room temperature, 100 µL of the extract was mixed with 1.9 mL of DPPH (0,076 mM in MeOH) in a cuvette. Spectrometric measurements (Shimadzu UV-spectrophotometer UV-1800) were initialized immediately. Absorbance was measured at 517 nm after 1 and 6 minutes. Duplicates were analysed and results were expressed as the mean absorbance decrease from the initial absorbance of the DPPH solution.

3.6 Statistical analysis

Students t-test (Microsoft Excel) and Pearson Correlation (MiniTab 15 Statistical Software) were used to express statistical results. Level of significance was set to $p<0.05$. Principal component analysis (PCA) was preceded (Unscrambler version 10.1 software). Statistical analyses were based on duplicates regarding raw flours (n=2) and duplicates regarding the two malted flours (n=2*2).

4 Results

4.1 Sample description after processing

No visible germination could be observed in any of the groats. The groats seemed to be oversteeped and had lost a lot of structure. Small pink spots, probably bacterial growth, and mold growth had appeared on some of the groats of the cultivar Kerstin. A deviant smell could be detected in some of the samples.

Some oat cultivars, e.g. Nike, SW151304, SW151106, Belinda and Matilda were sturdier than others to the dehulling process and visually, in these cultivars, the groats were not damaged. However, damages on groats from Galant, Avanti, Guld, Fatima and Kerstin could be observed after dehulling (Appendix 1, Figure 1&2).

Color of the dehulled raw groats varied between cultivars. Fatima, SW151304, SW151106, Nike and Matilda visually looked similar, close to the appearance of brown rice. Galant followed by Kerstin, Belinda, Avanti and Guld had a bright yellow-white color (Appendix 1, Figure 1&2).

After malting, color and dryness of the groats varied a bit. In some petri dishes, the groats were darker than in others and some even had a toasted look, particularly one of the samples of Belinda. Furthermore, from ocular inspection, the color of the flours from different cultivars varied. Some flours appeared white, e.g. Matilda, Galant and Guld, whereas some flours appeared more yellow, e.g. Belinda and Nike. In some cases, the color varied within duplicate flours, e.g. one sample of SW151106 and Avanti was a bit more yellow than the sample and the flour from the one sample of Belinda was browner than the duplicate sample.

4.2 Antioxidant amount

4.2.1 Avenanthramides

Over all, regardless of cultivar and petri-dish duplicates, the total amount of avenanthramides increased after malting of the groats by 238% (Table 3). Comparison of typical chromatograms of raw and malted groats is displayed in Figure 3.

Six different avenanthramides were identified from the HPLC chromatogram; 2c, 2p, 2f, 3f, 2pd and 2fd (Figure 3). The content of all individual avenanthramides increased, whereof 3f and 2fd increased the most (1056% and 825% respectively). Concentrations of 2fd, 2c, 2f and 2p increased by 360%, 168%, 108%, 100% respectively, but the increase of 2p was not statistic significant (Table 3).

Concentrations of 2c, 2p and 2f were calculated before and after malting based on standard curves. Concentration of 2c increased most after malting; from 7 mg kg^{-1} in raw groats to 19 mg kg^{-1} in malted groats. Concentration of 2f increased from 9 to 18 mg kg^{-1} whereas concentration of 2p increased from 6 to 12 mg kg^{-1} (Table 3).

There were other unidentified peaks in the chromatogram which might be avenanthramides (Dimberg, personal communication) but they are not included in further analysis. Most of them increased after malting.

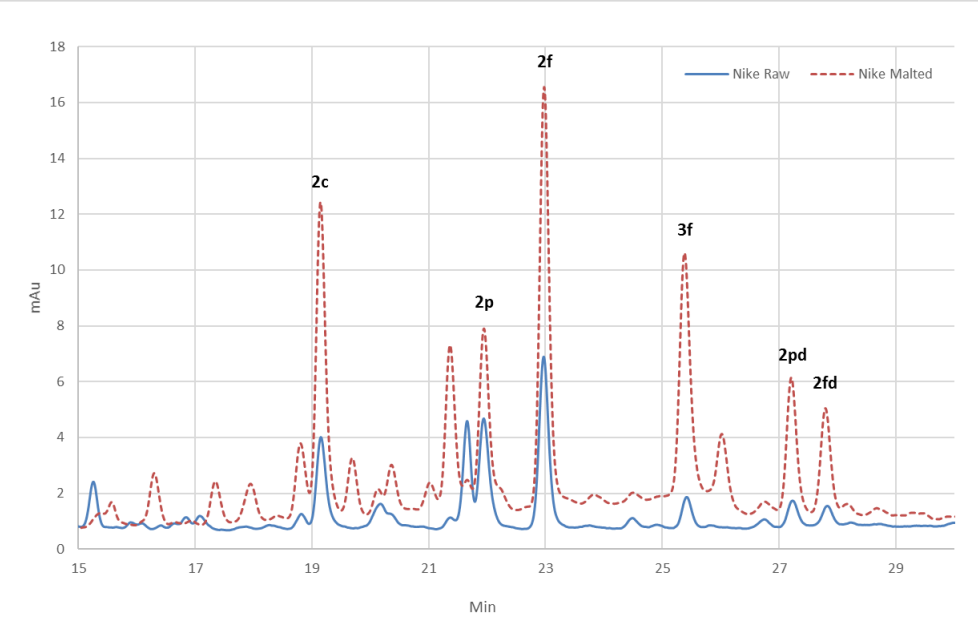


Figure 3. Typical avenanthramide HPLC chromatogram derived from raw and malted groats of the cultivar Nike. Identified avenanthramides are displayed.

Table 3 *Amount of identified avenanthramides and total concentration in raw and malted groats expressed as mean AU±SD g^{-1} DM regardless of cultivar and malting duplicates as well as calculated concentrations of each avenanthramide. Values derive from duplicate analyses of the raw flour and duplicate analyses of the two malted flours regarding each cultivar*

AVA	Raw mean AU g^{-1} DM (n=10)	Raw Conc. (mg kg^{-1})	Malted mean AU g^{-1} DM (n=20)	Malted Conc. (mg kg^{-1})	p*	Change (%) from raw groats
2c	31±17	7	83±69	19	*	+ 168
2p	40±25	6	80±81	12	ns	+ 100
2f	60±34	9	125±92	18	*	+ 108
3f	9±4	nd	104±118	nd	**	+ 1056
2pd	12±9	nd	111±138	nd	*	+ 825
2fd	10±6	nd	46±42	nd	**	+ 360
Total AVA	162±88	nd	549±739	nd	*	+ 238

* Levels of significance regarding raw and malted groats, set to * p<0,05, ** p<0,01, *** p<0,001. ns = no significance. nd = no data.

Variation between cultivars

Avenanthramide amount increased in most cultivars after malting. In average, amount increased most in Belinda, by 2943%, followed by SW151304, SW151106, Kerstin, Nike, Avanti, Guld and Galant which had increases of 454%, 400%, 320%, 297%, 134%, 50% and 13%, respectively. Groats of SW151106 contained the most avenanthramides both before and after malting followed by Guld, Fatima, Avanti, Galant, Nike, Kerstin, Matilda, SW151304 and Belinda (decreasing order) for raw groats (Figure 4; Appendix 1, Table 1).

In two of the cultivars, Matilda and Fatima, avenanthramide amount decreased after malting. Amount in Matilda decreased by -71% and in Fatima by -63%, although the content in raw groats of these cultivars was about average (Appendix 1, Table 1). However, no statistical evaluation of the change in individual cultivars was possible to perform, as only one sample (analytical duplicates) on the raw groats of each cultivar was analyzed.

Avenanthramide variations were small within duplicates derived from the same flour. However, it is important to notify that there were large variations regarding flours from the two malted samples in some cultivars. Standard deviations were in some cases high for this reason. Though, the same large variations were not observed for hydroxycinnamic acids or tocols. Variations of malting duplicates from avenanthramides, hydroxycinnamic acids and tocols are displayed in Table 4. Largest variation between malted duplicates could be observed for the cultivars Belinda and SW 151106.

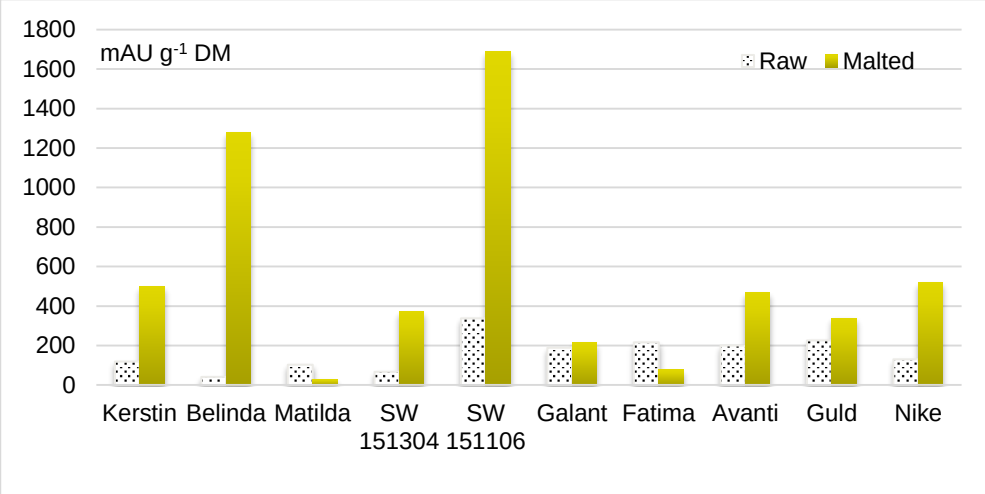


Figure 4. Total avenanthramide content of all oat cultivars before and after malting expressed as mean AU g^{-1} DM. Values derive from analytical duplicate analyses of the raw flour and duplicate analyses from the two malted flours regarding each cultivar.

Table 4. *Total amount of identified avenanthramides, hydroxycinnamic acids and tocols (Peak 4 included) in malting duplicates expressed as AU±SD g^{-1} DM. Numbers 1 and 2 represent malted duplicate samples within each cultivar*

Cultivar	n	AVA (n=1)	AVA change (%) from raw groats	HCA (n=1)	HCA change (%) from raw groats	Tocols (n=1)	Tocol change (%) from raw groats
Kerstin	1	747	+ 528	38	- 32	1557	- 11
Kerstin	2	252	+ 118	96	+ 71	2061	+ 18
Belinda	1	2200	+ 5138	22	- 69	1504	+ 14
Belinda	2	355	+ 745	33	- 54	1646	+ 25
Matilda	1	25	- 76	41	- 69	262	- 68
Matilda	2	35	- 67	28	- 79	276	- 67
SW 151304	1	382	+ 470	23	- 57	1745	+ 1
SW 151304	2	359	+ 436	15	- 72	1526	- 12
SW 151106	1	2961	+ 776	6	- 89	1106	- 15
SW 151106	2	419	+ 19	67	+ 18	1002	- 23
Galant	1	70	- 62	67	- 47	813	- 17
Galant	2	358	+ 89	33	- 74	1039	+ 6
Fatima	1	28	-87	75	- 9	1109	- 31
Fatima	2	128	-40	58	- 29	1492	- 7
Avanti	1	759	+ 280	29	- 57	1457	+ 9
Avanti	2	176	- 12	38	- 44	1379	+ 3
Guld	1	385	+ 70	47	+ 21	1277	+ 16
Guld	2	295	+ 31	45	+ 15	1177	+ 7
Nike	1	667	+ 409	41	0	1543	+14
Nike	2	372	+ 184	36	- 12	1727	+27

All identified avenanthramides decreased in the cultivars Matilda and Fatima and all identified avenanthramides increased in the rest of the cultivars (Appendix 1, Table 1).

In the cultivars Belinda and SW151106, where avenanthramide amount increased the most, the proportion of avenanthramides 3f and 2pd compared to 2c, 2p and 2f were higher than in the other cultivars (Appendix 1, Table 1).

4.2.2 Hydroxycinnamic acids

Over all, regardless of cultivar and petri-dish duplicates, the total amount of hydroxycinnamic acids decreased significantly by malting by -42% (Table 5). Amount of caffeic acid and ferulic acid decreased (-83% and -11% respectively) whereas amount of sinapic acid increased (+5%). However, only caffeic acid was affected significantly by malting (Table 5). Amounts of sinapic acid after malting were similar between cultivars (Appendix 1, Table 1). Generally, amount of *p*-coumaric acid was very low and was not included in the results.

The cultivars containing highest amount of the identified hydroxycinnamic acids (sinapic acid, ferulic acid and caffeic acid) were Matilda, Galant, and Fatima for raw groats and Kerstin, Fatima and Galant for malted groats (Appendix 1, Table 1). Total amount of the raw groats was highest in the cultivars Matilda, Galant and Fatima (decreasing order) and lowest for Guld, Nike and SW151304 (increasing order). Amount of hydroxycinnamic acids decreased the most after malting in the cultivar Matilda (-75%), followed by SW151304 (-65%), Belinda (-62%) and Avanti (-50%). In two of the cultivars the amount increased; Kerstin (+20%) and Guld (18%) (Appendix 1, Table 1).

Table 5. *Amount of identified phenolic acids caffeic, ferulic and sinapic acid and total concentration in raw and malted groats expressed as AU±SD g^{-1} DM regardless of cultivar and malting duplicates as well as calculated concentrations of each phenolic acid. Values derive from duplicate analyses of the raw flour and duplicate analyses of the two malted flours regarding each cultivar*

Phenolics	Raw mean AU g^{-1} DM (n=10)	Malted mean AU g^{-1} DM (n=20)	p*	Change (%) from raw groats
Caffeic acid	36±26	6±3	**	- 83
Ferulic acid	18±8	16±10	ns	- 11
Sinapic acid	19±5	20±14	ns	+ 5
Total	73±33	42±22	**	- 42

*Levels of significance regarding raw and malted groats, set to * p<0.05, ** p<0.01, *** p<0.001. ns = no significance.

4.2.3 Tocols

Out of four peaks in the chromatograms, three different tocols were identified; α-tocopherol (Peak 1), β-Tocopherol (Peak 2) and α-tocotrienol (Peak 3) (Figure 5). Since retention times of the chromatograms from the oat samples did not fully correspond with the references of β-Tocopherol, β-Tocotrienol and α-tocotrienol, absorbance UV-spectrum with wavelength 200-400 nm was used for final identification. Peak 4 was considered to be some kind of tocol, possibly β-tocotrienol, accordingly it was added to further analyses, although the UV-spectrum of peak 4 was not typical for that of tocols and was not similar to the other peaks.

α-Tocotrienol represented the major tocol in all samples followed by α-Tocopherol. Peak 4 represented the third largest peak and β-tocopherol the smallest peak in the chromatogram (Figure 3; Appendix 1, Table 2).

Over all, regardless of cultivar and petri-dish duplicates, the total amount of tocols decreased by -3% which was not a significant decrease (Table 6). Amount of α-Tocopherol and β-Tocopherol decreased significantly (-33% and -22% respectively) whereas amount of α-Tocotrienol and Peak 4 increased (+10% and +14% respectively).

Concentrations of α- and β-Tocopherol and α-Tocotrienol were calculated based on standard curves before and after malting. Concentration of α-Tocopherol changed the most after malting with a decrease from 13 to 9 mg kg^{-1} whereas amount of β-Tocopherol and α-Tocotrienol was similar before and after malting (Table 6).

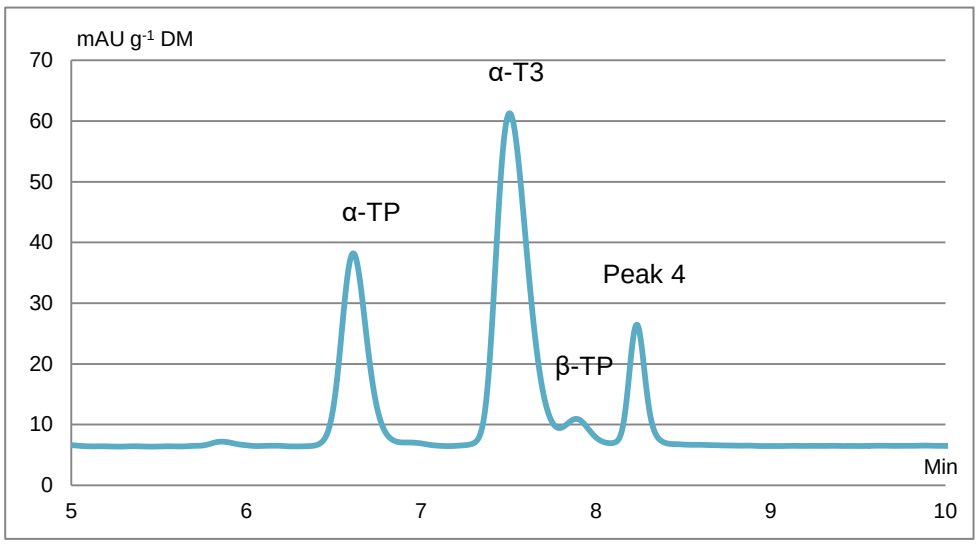


Figure 5. Typical HPLC tocol chromatogram derived from raw groats of the cultivar Nike. Identified tocols are displayed.

Table 6. *Amount of identified tocols and total concentration in raw and malted groats expressed as mean AU±SD g^{-1} DM regardless of cultivar and malting duplicates as well as calculated concentrations of each tocol. Values derive from duplicate analyses of the raw flour and duplicate analyses of the two malted groats regarding each cultivar*

Tocols	Raw mean AU g^{-1} DM (n=10)	Mean Conc. (mg kg^{-1})	Malted mean AU g^{-1} DM (n=20)	Mean Conc. (mg kg^{-1})	p*	Change (%) from raw groats
α-Tocopherol	382±99	13	257±103	9	**	- 33
α-Tocotrienol	731±169	41	804±305	45	ns	+ 10
β-Tocopherol	63±20	1	49±19	1	*	- 22
Peak 4	154±48	nd	175±68	nd	*	+ 14
Total (all peaks)	1330±303	nd	1285±457	nd	ns	- 3

*Levels of significance regarding raw and malted groats, set to * p<0.05, ** p<0.01, *** p<0.001. ns = no significance. nd = no data.

Variation between cultivars

Changes of the tocol amount after malting between cultivars did not vary as much as the avenanthramides did (Figure 6). Amount increased most in Nike (+20%), Belinda (+20%) and Guld (+12%) and decreased most in Matilda (-68%), Fatima (-20%) and SW151106 (-19%) (Appendix 1, Table 2).

Tocol amount of the raw groats was highest in the cultivars Kerstin, SW151304 and Fatima (decreasing order) and lowest for Matilda, Galant and Guld (increasing order) but as only one sample from each cultivar was analyzed, this could not be confirmed by statistical analyses. Amount of tocols in the malted groats was highest in the cultivars Kerstin, Nike and SW 151304 (decreasing order) and lowest in Matilda, Galant and SW 151106 (increasing order) (Figure 4; Appendix 1, Table 2). Large malting duplicate variations were not observed for tocol content (Table 4).

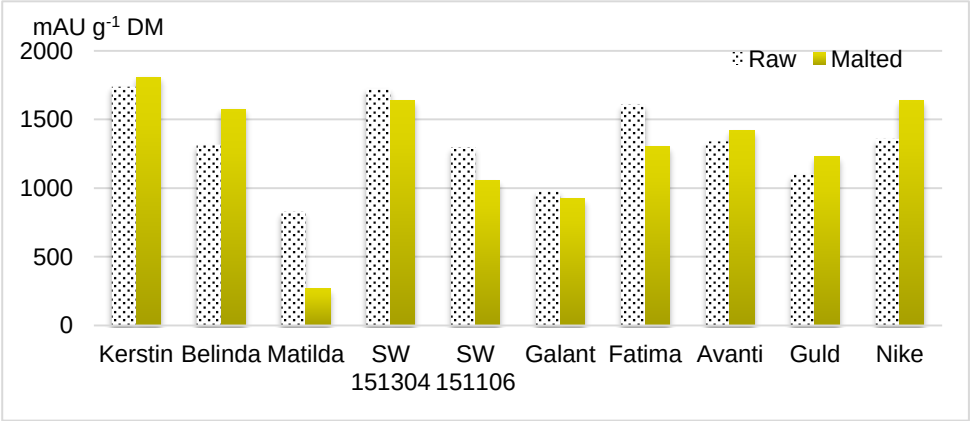


Figure 6. Total tocol content of all oat cultivars before and after malting expressed as mean AU g^{-1} DM. Values derive from analytical duplicate analyses of the raw flour and duplicate analyses from the two malted flours regarding each cultivar.

Oil content

Matilda was the cultivar with the highest oil content; 14%, followed by Belinda, SW151106 (12%), Fatima (11%), SW 151304 (8%), Avanti (8%), Nike (8%), Kerstin (7%), Galant (7%) and Guld (5%). The average oil content of all cultivars was 9%.

4.3 Antioxidant activity

Values derived from the DPPH-assay indicate significant higher antioxidant activity of 41% after six minutes of the malted samples compared to the raw samples. Antioxidant activity did increase by 19% after one minute, however not significantly (Table 7).

Table 7 *Antioxidant activity by DPPH assay of raw and malted groats expressed as mean Δ absorbance ±SD g^{-1} DM after 1 and 6 minutes, regardless of cultivar, malting duplicates and activity. Values derive from duplicate analyses of the raw flour and duplicate analyses from the two malted flours regarding each cultivar*

Time (min)	Raw ΔA g^{-1} DM (n=10)	Malted ΔA g^{-1} DM (n=20)	p*	Change (%) from raw groats
1	32±10	38±12	ns	+ 19
6	44±12	62±21	**	+ 41

*Levels of significance regarding raw and malted groats, set to * p<0.05, ** p<0.01, *** p<0.001. ns = no significance.

Variation between cultivars

Largest percental increase of activity after malting was observed in the cultivars Belinda, Nike, Kerstin, Guld and Avanti (+143%, +100%, +65%, +57% and +53% respectively). Lowest increase was found in SW151304 (+10%) and SW151106 (+23%) and in one of the cultivars, Matilda, amount decreased by -53% (Appendix 1, Table 3). Antioxidant activity of the malted samples was highest in the cultivars Belinda, SW151106 and Nike (decreasing order) after six minutes followed by SW151304, Kerstin and Avanti. Generally, raw samples responded to DPPH relatively quickly but activity did not increase as much after one minute as after six minutes (Figure 7). One clear example can be observed in Figure 7 for the raw sample of SW151106 which displays high activity after one minute whereas activity is only slightly higher after six minutes. Activity before malting was similar between cultivars, except for SW151106 and SW151304 which had a bit higher activity.

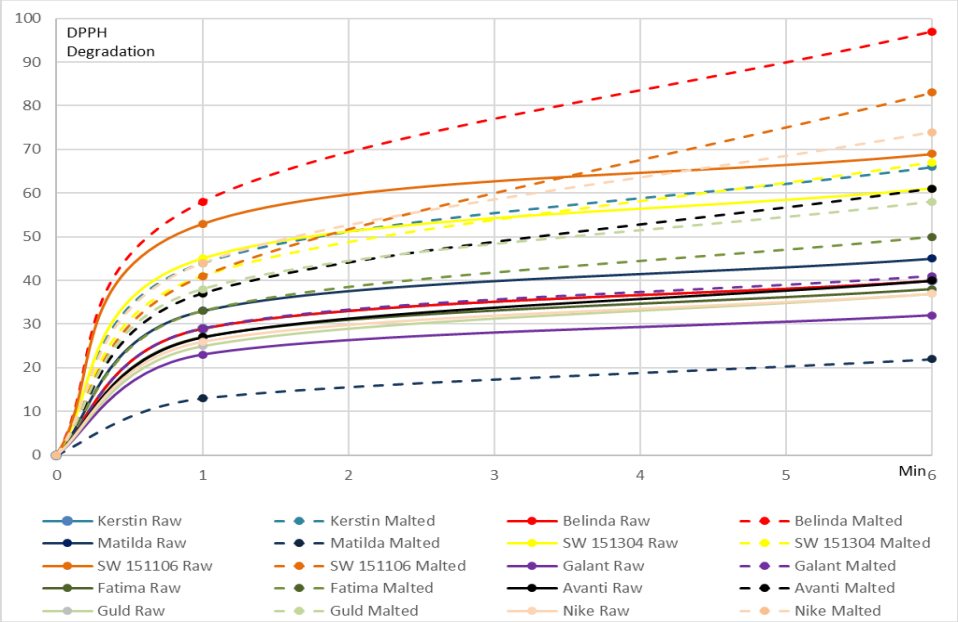


Figure 7. Radical scavenging activity by DPPH assay displaying antioxidant activity of all cultivars before and after malting. Means of duplicate analyses of the flours represents values after 1 and 6 min.

4.4 Correlations

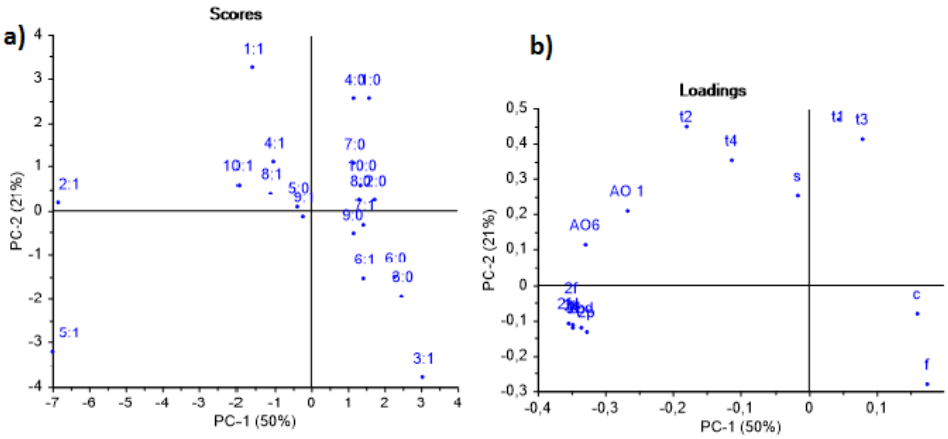


Figure 8. a) PCA of samples from analysed cultivars. 1=Kerstin, 2=Belinda, 3=Matilda, 4=SW151304, 5=SW151106, 6=Galant, 7=Fatima, 8=Avanti, 9=Guld, 10=Nike. 0 after cultivar number represents raw samples, 1 represents mean values of the malting duplicates.
b) Analysed parameters. Avenanthramide abbreviations can be observed as a cluster in the left corner. Identified hydroxycinnamic acids are displayed by letters (s,c and f) and tocopherols by t1 (α-tocopherol), t2 (α-tocotrienol), t3 (β-Tocopherol) and t4 (Peak 4). AO1 and AO6 represent antioxidant activity after one and after six minutes.

Figure 8 a) and b) from the PCA display analysed parameters expressed as loadings and corresponding samples expressed as scores. Scores include results from all cultivars from raw groats (0) and mean values of the malting duplicates (1). Clusters indicate correlations between groups of samples and parameters. The results indicates a correlation of all avenanthramides to each other as well as all tocols to each other, however to a lesser extent than the avenanthramides. Values of Belinda and SW151106 differed from the others with highest levels of avenanthramides from the malted flours (2:1 and 5:1 respectively) (Figure 8). Lowest amount of avenanthramides seemed to be present in malted groats of Matilda (3:1).
Antioxidant activity seemed to have a negative correlation to caffeic- and ferulic acid; most of the malted groats with high amount of avenanthramides, tocopherols and antioxidant activity contained lower amount of caffeic-, ferulic- and sinapic acid (Figure 8 a and b).

Table 8. *Pearson correlations from analyses of avenanthramides, caffeic, ferulic and sinapic acid as well as tocols and antioxidant activity measured by DPPH assay and expressed as significance*

	c	f	s	2c	2p	2f	3f	2pd	2fd	t1	t2	t3	t4	AO1
f	*													
s	ns	ns												
2c	ns	ns	ns											
2p	ns	ns	ns	***										
2f	ns	ns	ns	***	***									
3f	ns	ns	ns	***	***	***								
2pd	ns	ns	ns	***	***	***	***							
2fd	ns	ns	ns	***	***	***	***	***						
t1	ns	ns	ns	ns	ns	ns	ns	ns	ns					
t2	ns	**	ns	ns	ns	ns	ns	ns	ns	*				
t3	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	ns			
t4	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	***		
AO1	ns	ns	ns	**	*	**	*	*	**	ns	**	ns	ns	
AO6	ns	ns	ns	***	***	***	***	***	***	ns	**	ns	ns	***

*Levels of significance, set to * $p<0.05$, ** $p<0.01$, *** $p<0.001$. ns = no significance.

Correlations between avenanthramides, hydroxycinnamic acids, tocols and antioxidant activity are displayed in Table 8. All identified avenanthramides were significantly correlated to each other as well as with antioxidant activity; both after one and after six minutes. α-Tocotrienol (t2) was the only one of the tocols that correlated to the antioxidant activity. α-Tocopherol (t1) was correlated to α-Tocotrienol and β-Tocopherol (t3) but not to the fourth peak (t4). Peak 4, however, was correlated to β-Tocopherol (Table 8).

5 Discussion

5.1 Concentration of oat antioxidants

5.1.1 Avenanthramides

Regardless of cultivar and petri-dish variations, malting resulted in an avenanthramide increase, which is also according to results described by e.g. Bryngelsson *et al.* (2002), Skoglund *et al.* (2008) and Xu *et al.*, (2009). Generally, this result shows that malting is a method which can be used to increase avenanthramide content significantly.

Among all cultivars, the total amount of the most common avenanthramides, 2c, 2p and 2f (Boz, 2015), was 22 mg kg^{-1} for raw samples and 49 mg kg^{-1} for malted samples, which was significantly higher. However, it was lower than the amount reported by Tong *et al.* (2014). The amount was according to the lower parts of the avenanthramide interval reported by Li *et al.*, (2017). Although, it is important to note that these reports include other avenanthramides too which somehow makes them incomparable. Amount of 2c, 2p and 2f in the present study corresponds to the lower values recorded by the comparable studies of Dimberg & Jastrebova (2009), Li *et al.* (2016) and Xu *et al.* (2009), implying that generally, the results in the present study indicates quite low amounts of avenanthramides.

Amount of 2c increased most which was contrary to the results reported by Skoglund *et al.* (2008). Regarding antioxidant activity, high amounts of 2c is favourable since previous studies have found that 2c exert the highest antioxidant activity (Lee-Manion *et al.*, 2009; Yang *et al.*, 2014). Although, all common oat avenanthramides exert antioxidant activity to some extent (Bratt *et al.*, 2003).

The extraction methods used could have impacted the recorded content of avenanthramides. For example, other solvents like acidic ethanol has been found to enhance the extraction yield of avenanthramides (Dimberg, personal communication). However, it has other disadvantages. For example evaporation takes longer time. Also, since many of the oat components are bound to the grain matrix, extraction can be difficult and it can be hard to obtain equivalent yield of avenanthramides (Gangopadhyay *et al.*, 2015). Also, it was hard to dissolve the residuals from the glass tube in MeOH after vacuum evaporation. MeOH was flushed against the tube wall, but it was hard to dissolve the entire solid fraction. Some of the avenanthramides might have been lost during this step, even if they should be soluble in MeOH.

The chromatograms of the avenanthramides displayed many peaks. Some of the peaks were probably avenanthramides (Dimberg, personal communication) but were not identified or included in the results. Although these were minor peaks, they might have contributed to total avenanthramide content and antioxidant activity.

5.1.2 Hydroxycinnamic acids

The fact that avenanthramide amount increased significantly and amount of hydroxycinnamic acids decreased significantly after malting, as well as the negative correlations between hydroxycinnamic acids and avenanthramides, indicates that hydroxycinnamic acids are used in the synthesis of avenanthramides during malting. HHT is responsible for the synthesis and should also increase during malting, according to studies by Bryngelsson (2002) and Xu *et al.* (2009). However, only small amounts of hydroxycinnamic acids were recorded and not all of them could be responsible for the high increase of avenanthramides that were observed in some of the cultivars. Probably, *de novo* synthesis and the release of bound hydroxycinnamic acids into free ones was responsible for the increase (Xu *et al.*, 2009).

In some cultivars, however, it did not seem to be a clear correlation between the increase of avenanthramides and decrease of hydroxycinnamic acids. For example, amount in the cultivar Matilda decreased most of all cultivars regarding both compounds.

5.1.3 Tocols

Overall, tocol amount did not change significantly by malting which is beneficial for the purpose of the present study; detecting cultivars which responds to malting

with elevated levels of avenanthramides without reducing the amount of tocols significantly.

Tocol concentration was a bit higher than reported in previous studies (Gangopadhyay *et al.*, 2015; Bryngelsson, 2002; *Chu et al.* 2013). It is however important to notify that heat treatment was not applied in this method. Heat treatment is normally applied in oat processing which might also degrade some of the tocols.

According to previous studies (Bryngelsson, 2002; Skoglund *et al.*, 2008), the fourth peak of the tocol chromatogram should be β-Tocotrienol, by taking the retention order in consideration. It was also correlated to β-Tocopherol. However, Peak 4 was rejected as β-tocopherol since retention time was not in agreement with the reference and UV-spectra was not typical for tocols (Dimberg, personal communication). Although, Peak 4 was included as a tocol in the results. Tocols can become unstable during storage (Peterson, 1995) which also became clear during preparatory analyses. It was discovered by analysing the same tocol extract with one weak of interval. Degradation could be an explanation of the deviant external standard which could also result in a divergent peak in the chromatogram. In that case, Peak 4 could still be β-Tocotrienol, if the reference contained another compound. Also, if the mobile phase solvent was not mixed enough, it could lead to variations of the retention times (Dimberg, personal communication). However, since the UV-spectra did not look like the ones typical of tocols, Peak 4 was probably not a tocol after all and could have been removed from the results. This would have led to a slightly higher total decrease of tocols after malting, however not significant.

5.1.4 Cultivar effects

Belinda was clearly the cultivar with the greatest response by malting with elevated levels of avenanthramides. Although, by looking at avenanthramide amount both before and after malting, SW151106 was the superior cultivar. It also responded greatly by malting. Avenanthramide amount in SW151304, Kerstin and Nike (decreasing order) also increased a lot after malting, hence, they are also good cultivars for selection for malting purposes.

Kerstin and SW151304 contained higher tocol amount than the other cultivars. Tocol content of SW151106, which responded a lot to malting, decreased by 19%. Amounts only decreased more in the cultivars Matilda and Fatima. Matilda and Fatima had other things in common, like high oil content, which was not surprising since Fatima origins from Matilda and they have similar properties (Lantmännen, n.d.). As tocol content decreased in two of the cultivars with the highest oil content, it did not seem to be a correlation between oil content and tocols, which Bryngelsson (2002) previously has reported.

Colour of the groats and flours from each cultivar did not seem to correspond with the amount of antioxidants in the cultivars.

5.2 Antioxidant activity

Malted groats of Belinda had the highest antioxidant activity, implying correlations between antioxidant activity and avenanthramides, since Belinda contained the highest amount of avenanthramides after malting. The same applies for the other samples, e.g. high activity of SW151106 and low activity of Matilda. Correlations of the avenanthramides were also confirmed by the Pearson correlation analysis. However, some studies have not observed any correlation between antioxidant activity and avenanthramides (Chu *et al.*, 2013).

Although avenanthramides largely seemed to be responsible for the antioxidant activity, tocols probably are important since many of the spectrometric chromatograms showed patterns typical for tocols. Furthermore, α-Tocotrienol which was the largest recorded tocol was correlated to antioxidant activity. Bryngelsson (2002) and Fagerlund *et al.*, 2009 have found that the antioxidants which possess strong antioxidant capacity at the beginning, like tocols, loses their activity relatively fast whereas the ones which are initially less active, like the avenanthramides, possess activity for longer periods. α-Tocotrienol probably contributed a lot to the high increase of antioxidant activity initially whereas the avenanthramides largely were responsible for activity after one minute. This is also according to stronger correlation between avenanthramides and antioxidant activity after six minutes.

It is also important to notify that other compounds than avenanthramides and tocols are able to function as antioxidants (Bryngelsson, 2002). Furthermore, synergistic and antagonistic effects between compounds may also occur (Alfieri & Redaelli, 2015; Chu *et al.*, 2013; Tong *et al.*, 2014).

There are also other ways to measure antioxidant activity and different methods may provide differing results. The DPPH assay used in the present study measure the free radical scavenging capacity. Ferric Reducing Antioxidant Potential Assay is also a method that is similar to the one of DPPH assay which uses the reducing electron donating capacities of the compounds by certain reactions (Lee-Manion *et al.*, 2009; Xu *et al.*, 2009). One can also use the Comet Assay which measures the protective effects of the analysed compounds from genotoxic damage. Antioxidant activity can also be measured by determining the concentration of conjugated diene hydroperoxides formed by the activity of the analysed compounds, from oxidation of linoleic acid (Fagerlund *et al.*, 2009).

5.3 Processing factors

5.3.1 Dehulling

Processing of the oat grains in the present study was a bit different compared to the common methods used in the industry today, which could have affected the results. For example, the small scale dehulling equipment might have affected the groats more than the large-scale versions would have. Damages were obvious in groats of some cultivars after dehulling which might have affected the germination. However, the groats that visually looked intact did not germinate either. Bacterial- and/or fungal growth probably did not affect the germination rate either since only the cultivar Kerstin had visible growth of fungi and bacteria and this did not seem to affect the antioxidant amount in the cultivar as it was in line with the other cultivars. One interesting thing which was observed was that none of the cultivars with damaged groats had high levels of antioxidants or responded a lot to malting.

5.3.2 Milling

The small-scale sieve used for milling probably also differ a lot from the common equipment used in the industry. It sometimes became warm during milling, which could have affected heat sensible compounds like tocols. All kinds of harsh handling during the laborative work could result in degradation of tocols, e.g. during milling, during heat treatment and during handling of the samples in air and by light exposure (Gangopadhyay *et al.*, 2015; Peterson, 1995).

5.3.3 Malting

In the present study, uncontrolled malting was carried out, which makes the outcome more unpredictable compared to controlled malting. Unidentified errors might have occurred which may have played a part in the absent germination. After steeping, the samples looked oversteeped and disintegrated and germination was absent. The reason for this might have been too much water in the petri dishes during germination combined with damaged groat surface. Dehulling after germination could have been a way of protecting the groats from damages and from over steeping.

Probably the incubation step also affected the germination rate negatively. The thermostat in the incubator seemed unstable and sometimes the incubator felt

warmer than it should be. Incubation conditions could also be one of the reasons of the large petri dish duplicate variations that could be observed in some of the cultivars after malting. Petri dishes were arranged randomly in the incubator during germination and some of the dishes had to be placed on top of others in order to fit at the same time in the incubator. Probably, the ones placed on the floor of the incubator became warmer which could lead to variations in the antioxidant composition of the groats and contribute to the large variation observed in some of the duplicates.

5.4 Environmental factors

In this study, it was indicated that selection of cultivar is highly important regarding the impact on avenanthramides during malting, since the amount of some cultivars increased a lot whereas amount decreased in others. However, since only one single sample from one harvest of each cultivar was analysed, conclusions must be verified with larger sample sets. Environmental- and genotypic factors also have to be taken into consideration since these factors have been proven to contribute to significant variations in oat antioxidants, both regarding avenanthramides and tocols (Cui & Liu, 2013; Gangopadhyay *et al.*, 2015; Li *et al.*, 2017; Skoglund *et al.*, 2008). For example, avenanthramide content can be increased by plant infections and chemical treatment (Boz, 2015) and by increasing the nitrogen content of the soil (Bryngelsson, 2002). Germination methods also contributes significantly to the antioxidant amount of oats (Skoglund *et al.*, 2008).

5.5 Further studies

Approximate cultivation area, the same harvest and some basic properties of the cultivars are basically the only things that are known about the analysed cultivars in the present study. To be able to draw conclusions regarding antioxidant concentration in the cultivars, as well as the effect of malting, more oat analyses from each cultivar are required. Further studies could focus on collecting varying samples from different locations of growth, environmental conditions and year to analyse the general properties of specific cultivars. Another approach could be to cultivate the cultivars during similar and controlled conditions. This could also tell more about cultivars in relation to each other. It is also of interest in further studies to analyse β-glucan content of the cultivars and weather they are degraded by the malting, since many of the health benefits of oats are described to β-glucan and they are of importance to retain in the oat products.

5.6 Conclusion

Regardless of cultivar and petri-dish variations that were observed in some of the cultivars, malting resulted in a significant increase of avenanthramides without reducing the amount of tocols significantly. This suggests that food additives in oats could be avoided by malting, even though the groats did not germinate. At the same time, produced avenanthramides might provide with health beneficial effects in the final product. However, it is also important to analyse the antioxidant amount in the final product. Also, malting effects on β-glucans are needed to be investigated since they contribute a lot to the health beneficial effects ascribed to oats.

In the present study, it was indicated that selection of cultivar is highly important regarding malting impact on antioxidants. Although, environmental and genotypic factors cannot be ignored and more analyses have to be performed during more controlled conditions in order to be able to draw any conclusions regarding the cultivars. It is also of interest to test shelf life of products with antioxidants gained from malting instead of additions.

Acquired knowledge about oat antioxidants in the present study might be utilized in further analyses and practical applications.

References

Alfieri, M. & Redaelli, R. (2015). Oat phenolic content and total antioxidant capacity during grain development. *Journal of Cereal Science*, 65(1), pp 39–42.

Ames, N., Rhymer, C. & Storsley, J. (2013). Food oat quality throughout the value chain. *Oats Nutrition and Technology* [online]. Illinois: John Wiley & Sons, Ltd. Available from: https://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1495630. [Accessed 2017-05-17].

Berg, A., König, D., Deibert, P., Grathwohl, D., Berg, A., Baumstark, M. W. & Franz, I.-W. (2003). Effect of an oat bran enriched diet on the atherogenic lipid profile in patients with an increased coronary heart disease risk. A controlled randomized lifestyle intervention study. *Annals of Nutrition & Metabolism*, 47(6), pp 306–311.

Boz, H. (2015). Phenolic amides (Avenanthramides) in oats - A review. *Czech Journal of Food Sciences*, 33(5), pp 399–404.

Bratt, K., Sunnerheim, K., Bryngelsson, S., Fagerlund, A., Engman, L., Andersson, R. E. & Dimberg, L. H. (2003). Avenanthramides in oats (*Avena sativa L.*) and structure−antioxidant activity relationships. *Journal of Agricultural and Food Chemistry*, 51(3), pp 594–600.

Bryngelsson, S. (2002) Oat antioxidants- with emphasis on avenanthramides: Tissue distribution and effects of processing**.** *Acta Universitatis Agriculturae Sueciae Agraria. D*octoral thesis No 2002:347

Bryngelsson, S., Dimberg, L. H. & Kamal-Eldin, A. (2002). Effects of commercial processing on levels of antioxidants in oats (*Avena sativa L.*). *Journal of Agricultural and Food Chemistry*, 50(7), pp 1890–1896.

Chu, Y.-F., Wise, M. L., Gulvady, A. A., Chang, T., Kendra, D. F., van Klinken, B. J.-W., Shi, Y. & O'Shea, M. (2013). In vitro antioxidant capacity and anti-inflammatory activity of seven common oats. *Food Chemistry*, 139(1–4), pp 426–431.

Cui, S. & Liu, R. H. (2013). Health benefits of oat phytochemicals. *Oats Nutrition and Technology* [online]. Illinois: John Wiley & Sons, Ltd. Available from: https://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1495630. [Accessed 2017-05-17].

Delcour, J. A. & Hoseney, R. C. (2010a). Malting and brewing. *Principles of Cereal Science and Technology*. 3. ed. St. Paul: AACC International, Inc.

Delcour, J. A. & Hoseney, R. C. (2010b). Rice and oat processing. *Principles of Cereal Science and Technology*. 3. ed. St. Paul: AACC International, Inc.

Delcour, J. A. & Hoseney, R. C. (2010c). Structure of cereals. *Principles of Cereal Science and Technology*. 3. ed. St. Paul: AACC International, Inc.

Dimberg, L. H. & Jastrebova, J. (2009). Quantitative analysis of oat avenanthramides. *Healthgrain methods: analysis of bioactive components in small grain cereals.* pp 113–127. St Paul: AACC International, Inc.

European Commission. *Nutrition and Health Claims - European Commission*. [online] (2016-10-20). Available from: http://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/. [Accessed 2017-01-30].

Fagerlund, A., Sunnerheim, K. & Dimberg, L. H. (2009). Radical-scavenging and antioxidant activity of avenanthramides. *Food Chemistry*, 113(2), pp 550–556.

FAS (Foreign Agricultural Service) in USDA (United States Department of Agriculture). (2017). *Grain: World Markets and Trade.* https://apps.fas.usda.gov/psdonline/circulars/grain.pdf (Accessed 2017-01-18)

Gangopadhyay, N., Hossain, M. B., Rai, D. K. & Brunton, N. P. (2015). A review of extraction and analysis of bioactives in oat and barley and scope for use of novel food processing technologies. *Molecules*, 20(6), pp 10884–10909.

Gani, A. (2012). Whole-grain cereal bioactive compounds and their health benefits: A Review. *Food Processing & Technology* [online], 146 (3).

Gulvady, A. A., Brown, R. C. & Bell, J. A. (2013). Nutritional comparison of oats and other commonly consumed whole grains. *Oats Nutrition and Technology* [online]. Illinois: John Wiley & Sons, Ltd. Available from: https://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1495630. [Accessed 2017-05-17].

Guo, W., Nie, L., Wu, D., Wise, M. L., Collins, F. W., Meydani, S. N. & Meydani, M. (2010). Avenanthramides inhibit proliferation of human colon cancer cell lines *in vitro*. *Nutrition and Cancer*, 62(8), pp 1007–1016.

Guo, W., Wise, M. L., Collins, F. W. & Meydani, M. (2008). Avenanthramides, polyphenols from oats, inhibit IL-1beta-induced NF-kappaB activation in endothelial cells. *Free Radical Biology & Medicine*, 44(3), pp 415–429.

Head, D., Cenkowski, S., Arntfield, S. & Henderson, K. (2011). Storage stability of oat groats processed commercially and with superheated steam. *LWT - Food Science and Technology*, 44(1), pp 261–268.

Kris-Etherton, P., Khoo, C. S. & Chu, Y. (2013). Introduction: Oat nutrition, health, and the potential threat of a declining production on consumption. *Oats Nutrition and Technology* [online]. Illinois: John Wiley & Sons, Ltd. Available from: https://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1495630. [Accessed 2017-05-17].

Lantmännen. *Havre*. [online] (n.d.) (Lantmännen lantbruk). Available from: http://www.lantmannen-lantbruk.se/sv/produktkatalog/vaxtodling/varutsade/havre/. [Accessed 2017-05-09].

Lee-Manion, A. M., Price, R. K., Strain, J. J., Dimberg, L. H., Sunnerheim, K. & Welch, R. W. (2009). In vitro antioxidant activity and antigenotoxic effects of avenanthramides and related compounds. *Journal of Agricultural and Food Chemistry*, 57(22), pp 10619–10624.

Li, M., Koecher, K., Hansen, L., Ferruzzi, M.G. (2016). Phenolic recovery and bioaccessibility from milled and finished whole grain oat products. *Food and Function*. 7(8), 3370–3381.

Li, X., Li, M., Ling, A., Hu, X., Ma, Z., Liu, L. & Li, Y. (2017). Effects of genotype and environment on avenanthramides and antioxidant activity of oats grown in northwestern China. *Journal of Cereal Science*, 73(1), pp 130–137.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechanism of action. *The Journal of Nutrition*, 134(12 Suppl), pp 3479–3485.

Liu, R. H. (2007). Whole grain phytochemicals and health. *Journal of Cereal Science*, 46(3), pp 207–219.

Peterson, D.M. (1995). Oat tocols: concentration and stability in oat products and distribution within the kernel. *Cereal Chemistry*, 72(1), pp 21–24.

Peterson, D. M. & Dimberg, L. H. (2008). Avenanthramide concentrations and hydroxycinnamoyl-CoA:hydroxyanthranilate N-hydroxycinnamoyltransferase activities in developing oats. *Journal of Cereal Science*, 47(1), pp 101–108.

Roland, J. (2012). *Havre* [online]. Länna försöksstation: SLU. *Sverigeförsöken 2012*. (Sorter och odlingsteknik; L7-501). Available from: http://www.ffe.slu.se/Web-data/$serie/07F5R2012Havre.pdf [Accessed 2017-05-23].

Sixtensson, O. (2016). Sortförsök i havre [online]. Skåneförsök: Hushållningssällskapet Skåne. *Sverigeförsöken 2016*. (Sorter och odlingsteknik; L7-501). Available from: http://www.skaneforsoken.nu/skriftpdf/2017/skaneforsok/162-165.pdf [Accessed 2017-05-23].

Skoglund, M. (2008) Phenolic Compounds in Oats. Effects of Steeping, Germination and Related Enzymes. *Faculty of Natural Resources and Agricultural Sciences. D*octoral thesis No 2008:2.

Skoglund, M., Peterson, D. M., Andersson, R., Nilsson, J. & Dimberg, L. H. (2008). Avenanthramide content and related enzyme activities in oats as affected by steeping and germination. *Journal of Cereal Science*, 48(2), pp 294–303.

Slavin, J. (2003). Why whole grains are protective: biological mechanisms. *The Proceedings of the Nutrition Society*, 62(1), pp 129–134.

Sur, R., Nigam, A., Grote, D., Liebel, F. & Southall, M. D. (2008). Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. *Archives of Dermatological Research*, 300(10), pp 569–574.

Tiwari, U. & Cummins, E. (2009). Simulation of the factors affecting β-glucan levels during the cultivation of oats. *Journal of Cereal Science*, 50(2), pp 175–183.

Tong, L., Liu, L., Zhong, K., Wang, Y., Guo, L. & Zhou, S. (2014). Effects of cultivar on phenolic content and antioxidant activity of naked oat in china. *Journal of Integrative Agriculture*, 13(8), pp 1809–1816.

Trombino, S., Serini, S., Di Nicuolo, F., Celleno, L., Andò, S., Picci, N., Calviello, G. & Palozza, P. (2004). Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta-carotene, and ascorbic acid. *Journal of Agricultural and Food Chemistry*, 52(8), pp 2411–2420.

Wang, D., Wise, M. L., Li, F. & Dey, M. (2012). Phytochemicals attenuating aberrant activation of β-catenin in cancer cells. *PLoS ONE*, 7(12), p e50508.

Wise, M. L., Vinje, M. A. & Conley, S. P. (2016). Field application of benzothiadiazole (BTH) to oats (*Avena sativa*): Effects on crown rust resistance and avenanthramide production. *Crop Science*, 56(4), pp 1904–1913.

Xu, J. G., Tian, C. R., Hu, Q. P., Luo, J. Y., Wang, X. D. & Tian, X. D. (2009). Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda L*.) during steeping and germination. *Journal of Agricultural and Food Chemistry*, 57(21), pp 10392–10398.

Yan, W., Frégeau-Reid, J. & Fetch, J. M. (2013). Breeding for ideal milling oat: Challenges and strategies. *Oats Nutrition and Technology* [online]. Illinois: John Wiley & Sons, Ltd. Available from: https://ebookcentral.proquest.com/lib/slub-ebooks/detail.action?docID=1495630. [Accessed 2017-05-17].

Yang, J., Ou, B., Wise, M. L. & Chu, Y. (2014). In vitro total antioxidant capacity and anti-inflammatory activity of three common oat-derived avenanthramides. *Food Chemistry*, 160(1), pp 338–345.

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Appendix 1: Additional figures and tables

Figure 1. Appearance of groats of the cultivar Nike after dehulling displaying intact groats.

Figure 2. Appearance of groats of the cultivar Galant after dehulling displaying damaged groats.

Table 1. *Amount of identified avenanthramides and hydroxycinnamic acids and total and percental change of total content from raw to malted groats expressed as mean AU±SD g^{-1} DM regardless of malting duplicates. Values derive from duplicate analyses from the raw flour and duplicate analyses from the two malted flours*

AVAs and HCAs	n		c	f	s	Total c,f,s	Change (%) of total content from raw groats	2c	2p	2f	3f	2pd	2fd	Total AVA	Change (%) of total content from raw groats
Kerstin	1	Raw	23±1	14±0	19±0	56	+ 20	18±1	29±0	53±2	8±1	7±0	4±2	119	+ 320
	2	Malted	4±2	10±6	53±33	67		58±32	80±64	105±61	75±40	143±131	39±21	500	
Belinda	1	Raw	31±2	14±1	26±2	71	- 62	9±0	10±1	13±1	4±2	3±0	3±0	42	+ 2943
	2	Malted	0±0	3±1	24±8	27		170±169	152±145	269±262	278±320	289±292	120±117	1278	
Matilda	1	Raw	80±3	37±1	17±2	134	- 75	26±3	28±3	29±2	4±2	5±0	11±1	103	- 71
	2	Malted	4±2	20±6	9±1	34		9±4	4±1	8±2	3±0	3±0	3±0	30	
SW 151304	1	Raw	25±1	15±0	13±0	54	- 65	13±0	14±0	31±2	3±0	3±0	3±0	67	+ 454
	2	Malted	8±1	5±3	5±3	19		84±9	48±4	95±10	76±8	36±7	32±1	371	
SW 151106	1	Raw	27±2	21±5	9±2	57	- 35	55±6	97±9	130±15	14±1	22±3	20±2	338	+ 400
	2	Malted	8±12	17±20	11±11	37		225±213	278±304	288±260	353±395	421±499	125±127	1690	
Galant	1	Raw	78±4	28±2	21±1	127	- 61	30±3	42±5	79±8	13±3	13±2	12±2	189	+ 13
	2	Malted	8±1	31±30	11±7	50		26±32	31±31	73±60	19±22	41±40	24±20	214	
Fatima	1	Raw	44±1	18±1	20±1	82	- 18	37±2	42±2	70±2	16±2	33±2	18±1	216	- 63
	2	Malted	8±0	33±21	26±10	67		16±15	8±7	21±13	14±15	11±11	9±8	79	
Avanti	1	Raw	37±1	15±1	16±0	68	- 50	32±1	50±0	87±0	10±0	12±0	9±0	200	+ 134
	2	Malted	4±6	11±2	18±1	34		61±51	69±73	134±103	91±89	70±67	43±29	468	
Guld	1	Raw	9±0	11±0	20±2	39	+ 18	62±6	60±6	66±8	10±1	17±1	11±0	226	+ 50
	2	Malted	7±0	14±3	25±0	46		70±17	60±17	107±12	33±8	42±8	27±2	339	
Nike	1	Raw	3±0	11±2	26±1	41	- 7	32±1	30±0	45±2	9±2	8±1	7±0	131	+ 297
	2	Malted	9±2	15±3	14±1	38		111±53	73±31	148±55	97±39	49±17	42±14	520	

Table 2. *Amount of identified tocopherols and tocotrienols, total concentration and percental change of total content from raw to malted groats expressed as mean AU±SD g^{-1} DM regardless of cultivar and malting duplicates. Values derive from duplicate analyses from the raw flour and duplicate analyses from the two malted flours. Peak 4 is included in calculations*

	n		α-TP	α-T3	β-TP	Peak 4	Total	Change (%) of total content from raw groats
Kerstin	1	Raw	542±21	884±36	99±2	215±8	1740	+ 4
	2	Malted	401±126	1073±179	87±19	248±32	1809	
Belinda	1	Raw	290±46	792±101	36±2	199±0	1317	+ 20
	2	Malted	215±22	1036±32	25±7	299±97	1575	
Matilda	1	Raw	257±7	405±45	48±2	119±16	830	- 68
	2	Malted	42±16	108±10	24±6	95±1	269	
SW 151304	1	Raw	540±24	924±33	94±4	167±6	1725	- 5
	2	Malted	359±36	1040±88	64±5	174±17	1635	
SW 151106	1	Raw	402±5	732±11	62±0	100±1	1296	- 19
	2	Malted	210±17	709±66	41±1	95±25	1055	
Galant	1	Raw	280±84	537±150	52±12	114±31	983	- 6
	2	Malted	202±98	527±51	61±13	136±2	926	
Fatima	1	Raw	418±33	901±80	65±5	224±19	1608	- 20
	2	Malted	206±85	842±176	39±3	214±6	1300	
Avanti	1	Raw	362±90	756±195	64±15	159±39	1341	+ 6
	2	Malted	297±5	879±46	56±6	186±10	1418	
Guld	1	Raw	356±14	600±21	54±1	90±5	1100	+ 12
	2	Malted	328±4	745±57	49±3	105±7	1227	

Table 3. *Antioxidant activity by DPPH and percental change from raw to malted groats expressed as mean Δ absorbance g^{-1} DM± SD after 1 and 6 minutes, regardless of malting duplicates. Values derive from duplicate analyses from the raw flour and duplicate analyses from the two malted flours*

		1 min ΔA g^{-1} DM	Change (%) from raw groats 1 min	6 min ΔA g^{-1} DM	Change (%) from raw groats 6 min
Kerstin	Raw	29±2	+ 52	40±2	+ 65
	Malted	44±6		66±10	
Belinda	Raw	29±1	+ 100	40±1	+ 143
	Malted	58±8		97±13	
Matilda	Raw	33±3	-61	45±4	- 53
	Malted	13±1		21±1	
SW 151304	Raw	45±3	-9	61±5	+ 10
	Malted	41±1		67±2	
SW 151106	Raw	53±6	-23	69±8	+ 23
	Malted	41±14		83±34	
Galant	Raw	23±1	+ 26	32±2	+ 28
	Malted	29±5		41±13	
Fatima	Raw	27±2	+ 22	38±2	+ 32
	Malted	33±6		50±11	
Avanti	Raw	27±1	+ 37	40±2	+ 53
	Malted	37±7		61±18	
Guld	Raw	25±1	+ 52	37±3	+ 57
	Malted	38±1		58±1	
Nike	Raw	26±1	+ 69	37±2	+ 100
	Malted	44±3		74±5	

Appendix 2: Popular summary

Does malting affect the amount of oat antioxidants? If so, could it be applied in food processing?

Oats are a cereal crop used mainly as forage. However, interests in oats as foodstuff and pharmaceutics have gained interest during recent years. Oats contain several health beneficial compounds, like the β-glucans, Vitamin E and various phytochemicals, for example the avenanthramides which are oat specific antioxidants. Health claims of oats have mainly been ascribed to the β-glucans and Vitamin E, but several studies have found that phytochemicals like the avenanthramides possess health beneficial properties.

Background

Oats, compared to other cereals, contain high levels of unsaturated fatty acids. This can lead to problems during processing, since the unsaturated fatty acids become prone to oxidize when they are exposed to air, e.g. during milling or extrusion. Antioxidants are currently added to oat products for this purpose. Malting of oat grains prior to processing has in some studies been found to increase the amount of avenanthramides. If avenanthramides increase substantially by malting, it could be a way of replacing the added antioxidants. It could also prevent consumer concerns regarding food additives. However, the malting process may decrease sensitive health beneficial compounds like the tocols (Vitamin E) and β-glucans.

Results and conclusions

In the present study, quantitative and qualitative properties of avenanthramides and tocols of 10 different oat cultivars were investigated, both before and after malting. Some of the oat groats (grains without hulls) were malted before extraction but none of them germinated. Yet, malting resulted in a significant avenanthramide increase by 238%. Tocol amount, however, did not change significantly. This suggests that food additives could be avoided by malting.

Belinda was clearly the cultivar that responded most to malting, by +2943% followed by SW151304, by +454%. However, avenanthramide concentration varied a lot between cultivars. Concentration of two of the cultivars decreased; Matilda by -71% and Fatima by -63%. Belinda and SW151106 contained highest total levels of

avenanthramides after malting. Concentration of the hydroxycinnamic acids called caffeic-, ferulic- and sinapic acid decreased significantly by malting.

Large variations of avenanthramides were observed for the malting duplicates of some of the cultivars, which gave these malted samples high standard deviations.

Generally, avenanthramide concentration was a bit lower than recorded by previous studies whereas tocol concentration was a bit higher. Antioxidant activity was higher after malting and largely seemed to be attributed to the avenanthramides.

In further studies, it is of interest to analyse more samples of each cultivar as well as to take environmental and genotypic factors in consideration in order to assure cultivar variations. It would also be of interest to analyse malting effects on β-glucan, since many of the health claims of oats are ascribed to the β-glucans.