Journal of Cereal Science 48 (2008) 294–303

Journal of CEREAL SCIENCE

www.elsevier.com/locate/jcs

Avenanthramide content and related enzyme activities in oats as affected by steeping and germination

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Received 6 March 2007; received in revised form 18 September 2007; accepted 19 September 2007

Abstract

Samples from three different oat (*Avena sativa* L.) cultivars, Vista, Gem and Dane were steeped and germinated in a pilot plant malting system. The content of avenanthramides, antioxidant compounds unique to oats, and some unknown compounds as well as the activities of the avenanthramide-synthesising enzyme hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase (HHT) and the avenanthramide-oxidizing enzyme phenoloxidase (PO) were measured. An increase in avenanthramide content of germinated seeds, as compared to raw grains, was observed for Dane (125%, $p < 0.001$) and for Vista (29%, $p = 0.007$). The HHT activity increased 62% ($p = 0.014$) in Dane, whereas no change was detected in Vista and Gem. The PO activity decreased slightly during the germination process for Gem ($p < 0.001$) and Vista ($p = 0.005$). Many of the unknown compounds increased significantly during germination in all three cultivars, and one of them was identified to be the avenanthramide *N*-(4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid. This study indicates that a highly controlled steeping and germination process can be a valuable method to increase the content of endogenous avenanthramides in oats.

Keywords: Avenanthramides; Germination; Hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase (HHT); Oats; Phenoloxidase

1. Introduction

Oat is a cereal that is known to provide healthy nutrients to consumers, either humans or animals. Health promoting effects reported for whole grain cereal products have been suggested to be related to the content of dietary antioxidants, which are often enriched in the outer part of the cereal grain (Miquel, 2001; Visioli and Galli, 2001). Oats contain high levels of antioxidants which contribute towards protecting foods from rancidity and may also help to preserve their colour and taste. Lately, consumer concern about chemical food additives has been a stimulus for researchers to focus on the potential for providing antioxidants obtained from natural sources. The food industry aims to produce food products that contain a maximum of endogenous antioxidants, by using mild processes to avoid damage to the nutrients.

One group of antioxidants that has been reported only in oats among the cereals are the avenanthramides, substituted *N*-cinnamoylanthranilic acids (Collins, 1989; Dimberg et al., 1993). The most abundant avenanthramides in this group, which are constitutive in oat seeds, are **2p**, **2c** and **2f** (**2** indicating 5-hydroxyanthranilic acid and **p**, **c** and **f** indicating the hydroxycinnamic acids *p*-coumaric, caffeic or ferulic acids,

Abbreviations: ANOVA, analysis of variance; DM, dry matter; HHT, hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase; HPLC, high performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; NMR, nuclear magnetic resonance; PC, principal component; PCA, principal component analysis; PO, phenoloxidase; **2c**, *N*-(3′,4′-dihydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid; **2f**, *N*-(4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid; **2p**, *N*-(4′-hydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid; **3f**, *N*-(4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid.

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0733-5210/$ - see front matter
doi:10.1016/j.jcs.2007.09.010

respectively). These avenanthramides have been reported to be related to the fresh taste of oat products (Molteberg et al., 1996). Avenanthramides can exert antioxidative activity both *in vitro* (Bratt et al., 2003; Dimberg et al., 1993; Peterson et al., 2002) and *in vivo* (Chen et al., 2007; Ji et al., 2003). Avenanthramide **2c** has been shown to attenuate reactive oxygen species production in tissues of exercised rats and to enhance activities of antioxidative enzymes in various tissues of rats (Ji et al., 2003). Also, the avenanthramides in an avenanthramide-enriched mixture have been suggested to act as antioxidants and to interact synergistically with vitamin C to protect LDL-oxidation in hamsters (Chen et al., 2004, 2007). Avenanthramides are especially interesting to study because of their similarity in structure to the commercial drug Tranilast. Tranilast has well studied physiological and pharmacological properties and is intended to be used to treat certain types of allergies (Isaji et al., 1998). Recent preliminary studies indicate that the avenanthramides might possess anti-inflammatory and antiatherogenic properties, since they can inhibit release of proinflammatory compounds from macrophages and also inhibit monocyte adhesion to human aortic endothelial cells (Liu et al., 2004). They have also been found to enhance the production of nitric oxide and might thereby contribute to a decreased blood pressure through dilation of blood vessels (Nie et al., 2006). Additionally, the avenanthramides might influence the production of proinflammatory compounds, as they can exert inhibitory activities towards lipoxygenase (Wakabayashi et al., 1986), which is a key enzyme in the biosynthesis of leukotrienes that participate in allergic and inflammatory responses (Celotti and Durand, 2003). So, a low but continuous intake of avenanthramides in the daily diet may prevent the development of disease in humans.

The enzyme hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxycinnamoyl transferase (HHT) catalyses the final condensation between anthranilic acids and hydroxycinnamoyl-CoA esters to form avenanthramides (Ishihara et al., 1999). HHT activity has been detected in the endosperm and embryo of oat seeds (Matsukawa et al., 2000).

Cereal seeds have been germinated for centuries to soften the kernel structure, to increase nutrient compounds, and to decrease antinutritional compounds (Kaukovirta-Norja et al., 2004). Germination is initiated by steeping, when dry grains absorb water to a moisture content of 43–45%, and the metabolic activity resumes (Bewley and Black, 1994). During the subsequent germination process, enzyme synthesis and kernel modification take place (Kunze, 1999). Little attention has been given to the effects of germination on phenolic compounds of oats, but studies have shown that the content of the avenanthramides, in some cases along with the activity of HHT, increases during germination (Bryngelsson et al., 2003a; Matsukawa et al., 2000; Pihlava and Oksman-Caldentey, 2001).

When oat flour is steeped in water, the content of the avenanthramides decreases (Bryngelsson et al., 2003b). This can probably be explained by the presence of the enzyme phenoloxidase (PO) that might oxidize avenanthramides (Skoglund, unpublished data). Oxidation of avenanthramides has been detected in both bran and endosperm flour from oats of several different cultivars (Bryngelsson et al., 2003b).

The aim of the present study was to investigate the effect of a highly controlled steeping and germination process, using a pilot plant malting system, on the content of avenanthramides and other phenolic compounds, as well as activities of HHT and PO in oats. This was performed to gain a more collective and comparative picture of what happens to the phenolic compounds in the oat kernel during germination.

2. Experimental

2.1. Oat material

Three different closely related North American covered oat cultivars (*Avena sativa* L.), Vista, Gem and Dane, were used in the study. The cultivars were all grown in 2001 in Madison, Wisconsin, USA. These cultivars were all developed in the University of Wisconsin oat breeding project, and are adapted to the upper Midwest. The harvested grains were dried to 10% moisture and then stored at room temperature until time of steeping and germination, which was performed in the autumn of 2003. For the isolation and identification of the avenanthramide **3f** the commercial product SPC-flakes (AS-Faktor, Lantmännen, Sweden), purchased in a health food shop, was used.

2.2. Chemicals

Synthetic avenanthramides *N*-(4′-hydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (**2p**), *N*-(3′,4′-dihydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (**2c**), *N*-(4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (**2f**) and *N*-(4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl)-5-hydroxy-4-methoxyanthranilic acid (**3f**) were provided by Dr. K. Sunnerheim, Department of Chemistry, Uppsala University, Sweden. *p*-Coumaroyl-CoA was synthesized according to Peterson and Dimberg (2008). Hydroxycinnamic acids (caffeic, *p*-coumaric and ferulic acids) were purchased from Sigma (Steinheim, Germany). 5-Hydroxyanthranilic acid was purchased from Alfa Aesar (Ward Hill, MA, USA), dithiothreitol, 4-morpholinepropanesulfonic acid, $MgCl_2$, glycerol, adenosine triphosphate, Co-enzyme A, catalase and protease inhibitor cocktail from Sigma and acetonitrile, ethanol, methanol, hexane and chloroform from Merck (Darmstadt, Germany). Commercial sodium hypochlorite (Chlorox) was purchased in a grocery store. All solvents were of analytical grade and were used without further purification.

2.3. Steeping and germination

Oat grains were surface sterilized using a 1% solution of sodium hypochlorite for 30 s before steeping. Both steeping and germination took place under controlled conditions in a malting system (Joe White, Melbourne, Australia). Two replicates of each cultivar were steeped and germinated. The whole process (steeping and germination) was performed at two different temperatures, 16 and 20 °C. The three cultivars