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Saponin content in different oat varieties and in different fractions of oat grain

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Using a high-performance liquid chromatography (HPLC) technique, the avenacoside A and B content was determined in 16 oat cultivars and in four fractions of oat kernel. The saponin content was significantly different ($P < 0.05$) between the oat cultivars and ranged from 0·020 to 0·050% with a mean of 0·040% (dry matter basis). There was no significant correlation between lipid and saponin content. Milled oat kernels were separated into fractions according to particle size. The fractions with the smallest particle sizes had the highest concentrations of saponins. Thus, the oat kernel saponins seem to be situated mainly in the endosperm.

INTRODUCTION

Oats have rapidly gained increasing popularity in recent years, as a result of their serum cholesterol lowering properties. These are mainly attributed to β-glucans (Anderson *et al.*, 1990) but may be partly caused by other components including saponins (Price *et al.*, 1987). Saponins contain a steroid or triterpenoid aglycone linked to one or more sugar chains (Price *et al.*, 1987). They occur in a wide variety of plants, where they are supposed to have antibiotic effects. Other important properties of saponins are bitterness, membranolytic activity and cholesterol binding capacity *in vitro* (Price *et al.*, 1987). The structures of two isolated oat saponins, avenacoside A and B, have been elucidated (Tschesche *et al.*, 1969; Tschesche & Lauren, 1971). They have a steroid aglycone, nuatigenin, and two sugar chains containing glucose and rhamnose. Avenacoside B contains one more glucose residue than avenacoside A. The amount of saponins is influenced by the plant species, the cultivar, the part of the plant examined and its physiological age (Price *et al.*, 1987). The aim of this investigation was to study the variation of saponin content among oat varieties and in different fractions of the oat grain.

MATERIALS AND METHODS

Materials

Various oat cultivars were grown in Sweden in 1989 (Magne, Vital 1 and Chihuahua) and in 1990 (Adamo, Magne 8II, Sang, Svea, Vital 2, Sv 842097, Sv 843675, Sv 90634, Sv 90677 and Å 83165). Two oat cultivars (NZ89-701 and NZ89-703) were grown in New Zealand in 1989. The oat samples were obtained dehusked from Svalöf AB, Svalöv, and stored in a freezer (−40°C). Before analysis the samples were milled to a particle size less than 0·25 mm (Cyclotec 1093 Sample Mill, Tecator).

Oat kernels from two oat varieties (Magne and Selma) were separated into four fractions according to particle size. For Magne the fractions were: >500 μm (percentage of total weight = 35), 250–500 μm (16), 150–250 μm (44) and <150 μm (5), and the Selma samples were separated into fractions >1050 μm (30), 650–1050 μm (21), 250–650 μm (18) and <250 μm (31). The oat fractions were ground to a particle size less than 0·25 mm before analysis.

An oat leaf extract was used as standard in the quantitative analysis. The standard was kindly donated by J. Kesselmeier, Max-Planck-Institute for Chemistry, Mainz.

Extraction

Fourteen grams of oatmeal were defatted with 150 ml of light petroleum (b.p. 60–80°C) for 16 h in a Soxhlet apparatus. The saponins were then extracted with 150 ml of methanol for 24 h. The extract was evaporated to dryness and dissolved in 5 ml of methanol.

Lipid

The light petroleum extract obtained was evaporated and weighed. In this estimate of lipid (mainly triglyceride), bound lipids are not included (Youngs, 1987).

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Saponin

A high-performance liquid chromatography (HPLC) method was used as described by Kesselmeier and Strack (1981) and modified by Önning and Asp (1993). Samples (20 μl) of the methanol extract were injected into a Varian 500 Liquid Chromatograph with UV detection at 200 nm via a Rheodyne injection valve. Commercially packed columns (Merck, Darmstadt, 125 mm × 4 mm) of octyl-silica (LiChrosorb 100 CH-8/2, 5 μm) were used. Gradient elution was performed with 25–40% acetonitrile in water for 15 min with a flow rate of 2 ml/min. The quantities of avenacoside A and B were estimated from peak areas by injecting known amounts of desglucoavenacosides.

Statistical analysis

All analyses were performed at least in duplicate and the mean values are presented on a dry matter basis. The pooled standard deviation for the method for analysis of total saponin content was determined to 0·0025% (n = 16). Analysis of variance (one-way) and mean separation (Duncan's multiple range test) sub-programs of SPSS/PC, were used to determine the significance of differences in the saponin content, with respect to oat cultivar. The SPSS/PC correlation sub-program was used to determine the correlation between lipid and saponin content.

RESULTS AND DISCUSSION

Variation of saponin content among oat cultivars

Lipid and avenacoside content in 16 oat cultivars is presented in Table 1. The oat cultivars had a wide range of lipid contents, ranging from 5·7 to 15·6% with a mean of 9·4%. The mean value is high because some new high-lipid oat varieties were included in the study (NZ89-701, NZ89-703, Sv 90634 and Sv 90677). Saponin (avenacoside A + B) content was significantly different ($P < 0·05$) for the 16 oat cultivars, and ranged from 0·020 to 0·050% with a mean value of 0·040%. The avenacoside A content was 2–4 times higher than the avenacoside B content in the oat cultivars.

Sang is the main variety used for oatmeal and rolled oats for human consumption in Sweden. The saponin content in Sang was determined to be 0·044%, which is close to the mean value. The New Zealand grown varieties had a high lipid and a low avenacoside content. There was, however, no significant correlation between lipid and saponin content in this study. The low saponin content in NZ89-701 and NZ89-703 may be related to the climate and soil in New Zealand, but genetic factors, are probably also important.

No other investigation has studied the variation of saponin content among oat cultivars. There are, however, some publications regarding saponin variation in alfalfa and legumes. A large variation in saponin content was found in alfalfa (Livingston *et al.*, 1984). Six alfalfa varieties grown in different parts of the USA had saponin contents ranging from 0·11 to 1·71%. For legumes, the variation seems to be smaller. The saponin content in eight varieties of chickpeas, determined by a spectrophotometric method, varied from 3·9 to 4·2% in one study (Jood *et al.*, 1986). For four varieties of black gram the range of saponin content was 2·7–2·8%. In four bean varieties (navy, dark red kidney, pinto and black turtle soup) the saponin content, analysed by thin-layer chromatography (TLC), varied from 4·0 to 4·3% (Drumm *et al.*, 1990). In another investigation (Khokhar & Chauhan, 1986), the saponin content was determined in four varieties of moth bean by a spectrophotometric method, and levels ranging from 2·8 to 3·3% were found. The saponin values in the above studies on legumes are probably too high as a result of unspecific methods (Price *et al.*, 1987). In studies where more specific methods have been used the values are much lower. Thus, the saponin levels ranged from 0 to 0·65% for defatted seeds of 13 varieties of legume (including chickpea and soyabean) when a gas–liquid chromatography (GLC) method was used (Price *et al.*, 1986). The saponin content in soyabean was 0·65%, i.e., much higher than in oats. Even the high-saponin oat varieties had less than a tenth of the saponin content of soyabean.

Table 1. Lipid and saponin content in 16 oat varieties (%, dry matter basis)

Oat variety	Lipid	Avenacoside		
		A	B	Sum
Svea	$8·6^{e}$	$0·037^{ab}$	$0·013^{b}$	$0·050^{a}$
Vital 2	$6·3^{h}$	$0·034^{bcd}$	$0·016^{a}$	$0·050^{a}$
Sv 90677	$14·6^{b}$	$0·039^{a}$	$0·011^{bcd}$	$0·050^{a}$
Sv 842097	$7·5^{f}$	$0·036^{abc}$	$0·013^{b}$	$0·049^{ab}$
Adamo	$8·6^{e}$	$0·036^{abc}$	$0·012^{bcd}$	$0·048^{ab}$
Magne	$8·2^{e}$	$0·031^{de}$	$0·013^{b}$	$0·044^{ab}$
Sang	$6·9^{g}$	$0·033^{cde}$	$0·011^{bcd}$	$0·044^{bc}$
Chihuahua	$8·5^{e}$	$0·031^{de}$	$0·012^{bc}$	$0·043^{bcd}$
Magne 8II	$9·5^{d}$	$0·034^{cd}$	$0·009^{d}$	$0·043^{bcd}$
Sv 90634	$15·6^{a}$	$0·029^{ef}$	$0·009^{d}$	$0·038^{cde}$
Sv 843675	$8·5^{e}$	$0·026^{fg}$	$0·011^{bcd}$	$0·037^{de}$
Selma	$7·6^{f}$	$0·026^{g}$	$0·010^{cd}$	$0·036^{ef}$
Vital 1	$5·7^{i}$	$0·024^{g}$	$0·011^{bcd}$	$0·035^{ef}$
Å 83165	$6·4^{h}$	$0·021^{h}$	$0·010^{d}$	$0·030^{f}$
NZ89-701	$12·6^{c}$	$0·015^{i}$	$0·009^{d}$	$0·024^{g}$
NZ89-703	$15·5^{a}$	$0·015^{i}$	$0·005^{e}$	$0·020^{g}$
Mean	9·4	0·029	0·011	0·040
CV (%)	35	26	21	23

[a–i] Mean of at least duplicate analyses; means within columns with the same superscript are not significantly different ($P < 0·05$).

Saponin content in different oat kernel fractions

Table 2 presents the saponin content of four fractions derived from oat kernels of the varieties Magne and Selma. Figure 1 shows the saponin content in the four fractions and the fraction sizes. For both oat varieties,

Table 2. Avenacoside A and B contents in various particle size fractions of milled oats (%, dry matter basis)

	Avenacoside A	Avenacoside B	Sum
Variety: Magne			
Oat kernel	0·031	0·013	0·045
>500 μm	0·009	0·006	0·015
250–500 μm	0·045	0·019	0·065
150–250 μm	0·052	0·015	0·067
<150 μm	0·031	0·012	0·052
Variety: Selma			
Oat kernel	0·026	0·010	0·036
>1050 μm	0·008	0·005	0·013
650–1050 μm	0·014	0·010	0·024
250–650 μm	0·035	0·017	0·052
<250 μm	0·054	0·014	0·068

the fractions with the smallest meal particle sizes, for Magne <500 μm and for Selma <650 μm, contained the highest concentration of saponin. Milling fractions generally do not correspond precisely to particular botanical structures of the kernel (Kent, 1983), but the analysis results indicate that oat saponins are situated mainly in the endosperm.

In quinoa, a plant grown in South America, the saponins are located in the outer layers of the seeds. The content of two quinoa saponins (A and B) was 0·91% in polished seeds and 2·3% in the bran (the material remaining after polishing) (Ruales, 1992). The saponins are supposed to have an antibiotic effect, and in that context it is surprising that the oat saponin content is lower in the outer parts (corresponding to the coarser meal fractions) of the oat kernel.

The distribution of saponins in the oat kernel was similar for the two varieties. Glycoalkaloids have a similar structure and share many physico-chemical properties with saponins (Price *et al.*, 1987). In potatoes, the glycoalkaloids are concentrated in the outer parts, but there are differences in distribution between potato varieties (Olsson & Jonasson, 1990).

Earlier studies have shown that the saponins in legumes are associated with proteins and therefore concentrated in protein-rich fractions (Fenwick & Oakenfull, 1983; Curl *et al.*, 1985). In our fractions from oat kernels of the variety Selma, the highest protein concentration was in the fraction >1050 μm (Frølich & Nyman, 1988). Thus, oat saponins do not seem to be associated with proteins in the same way as in legumes.

Preliminary studies of spray-dried oatmeal indicate that the amount of avenacosides is reduced by processing. Further studies are needed to elucidate whether the oat saponins have a cholesterol-lowering effect and in what way the saponins are influenced by processing.

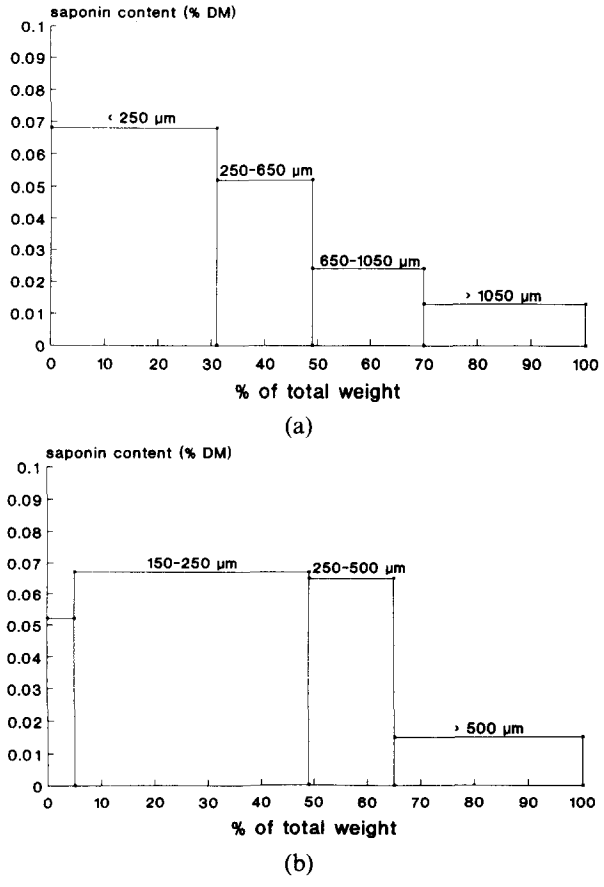

Fig. 1. Saponin content (avenacoside A+B) in various particle size fractions and the fraction sizes. (a) Selma; (b) Magne. The left column for Magne represents material with particle size <150 μm.

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