Effect of Extraction Conditions on the Recovery and Potency of Antioxidants in Oat Fiber

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The inhibitory activities toward the lipoxygenase action on linoleic acid were quantified and compared from different fractions derived from oat fiber. Based on these findings, a suitable procedure that yields a fraction with the high inhibitory activity was developed. The unit operations of the procedure involve alkali aqueous extraction followed by pH adjustment and separations of insoluble fiber particles from soluble extracts. The inhibitory activity was most effectively extracted into the solution using an extraction media with pH 10. By relowering the pH of the alkali aqueous extract, the inhibitory activity could be collected as a precipitate. Such a fraction comprised 7.2% of the fiber dry matter. Compared to the untreated oat fiber, the inhibitory activity was purified 17-fold and the yield of the activity was 125%. Such a fraction had high protein (88% w/w) and lipid (12%) contents, whereas the carbohydrate content (3%) was low.

Keywords: *Oat fiber; dioxygenation; antioxidants; aqueous extract*

INTRODUCTION

There has been a great interest toward the possibility for using nonsynthetic antioxidants from plant origin to stabilize the easily oxidized compounds during food processing and preservation. The majority of both the synthetic and nonsynthetic antioxidants have a phenolic structure, and various phenolic compounds with antioxidative activity are present in the various plant tissues including cereal seeds. In oat seed, the presence of abundant amounts of tocopherols, sterols, and esters of phenolic acids has been established (Duve and White, 1991; Dimberg et al., 1993; White and Armstrong, 1986). These compounds are predominantly localized in both the endosperm cell walls and the aleuronic layer and are thus found mainly in the fiber fraction (Lempereur et al., 1997; Miller et al., 1995; Nordkvist et al., 1984). The phenolic compounds are effectively recovered from the plant material by extraction with solvents of low polarity, e.g., methanol. In the extraction process, the phenolic compounds become detached from cell residues into the solvent phase where they can be easily concentrated. However, there are a few problems in such a process: (1) a high amount of raw material is needed to prepare the desired antioxidative potential (Tian and White, 1994), (2) a solvent extraction is relatively expensive and requires a hermetic extraction vessel, and (3) organic solvents have toxicity and hazardous properties. Furthermore, when applied at effective concentrations it is very likely that these natural antioxidants pose a similar health threat, as do relevant synthetic compounds.

An alternate approach, reviewed by Matsuno and Adachi (1993), for the control of the oxidation in both aqueous systems (López-Nicolás et al., 1997; Lin et al., 1995; Iwami et al., 1987, 1988) and dehydrated systems (Moreau and Rosenberg, 1996; Iwami et al., 1988; Bishov and Henic, 1972) is to protect the labile molecules with various encapsulating compounds. Cereals are a potential source for the production of both phenolic antioxidants and various antioxidatively acting compounds capable of encapsulating lipids. In our previous study we compared the antioxidative capacity of different cereals, without any attempts to extract or identify the antioxidative compounds (Lehtinen and Laakso, 1997). A distinct difference between the different cereals was noticed, and of the major cereals, oat and further the oat fiber were most effective. Similarly, the bran fraction from durum wheat also has a more powerful antioxidative activity than other milling fractions (Onyeneho and Hettiarachchy, 1992).

The encapsulating fractions in cereal seeds, mainly hydrocolloidal material, hemicellulose, and protein, can be extracted with more polar solvents, e.g., with water, than phenolic compounds. The object of the present study was to study whether a fraction with concentrated encapsulating capabilities can be derived from oat fiber and to develop an industrially feasible procedure for producing such a material. We have extracted the oat fiber with various extraction schemes and studied the distribution of dioxygenation inhibitor activity. The extraction and precipitation processes used were constructed by a goal-oriented manner, i.e., fractions having strongest inhibition activity were selected for further processing.

MATERIALS AND METHODS

Oat Fiber. Oat fiber was manufactured by Primalco Ltd., Finland. Industrially made fiber was remilled (Fritsch, pulverisette 14, sieve ring 0.5 mm) to give a finer flour. The composition of the fiber as reported by Primalco Ltd. was as follows: dry substance (d.s.) 94.0%, protein 22.2% of d.s., starch 21.3% of d.s., fat 11.7% of d.s., mineral 5.2% of d.s., and β-glucan 15.02% of d.s.

Extraction and Precipitation of Soluble Oat Fiber with Aqueous Buffers. The oat fiber was distributed with

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Table 1. Distribution of Dry Matter and Total Dioxygenation Inhibitor Activity Derived from 1 g of Crude Fiber into the Particle-Free Extract and Remaining Fiber Particles after Different Extraction Procedures

extraction procedure	fiber dry matter in the extract (%)[a]	inhibitory units in the extract	inhibitory units in the extract residue
crude fiber			48
aqueous extractions:			
buffer of pH 4	15.1	*b*	49
buffer of pH 6	16.0	9	64
buffer of pH 8	15.5	10	66
buffer of pH 10	15.0	94	63
pH 10 extract of fiber washed at pH 4	10.0	75	49
methanol extract	6	60	48

[a] The nonvolatile salts from added buffers and pH adjustments are subtracted from the actual dry weight measurement. [b] A 50% inhibition was not established.

15 volumes of extraction media using an Ultra Turrax homogenizer and was thereafter extracted for 2 h at 40 °C. The following extraction media were used: 0.05 M sodium acetate (pH 4), 0.05 M sodium phosphate (pH 6, 7, and 8), 0.05 M sodium carbonate (pH 10), and anhydrous methanol. After the extraction, the fiber particles were separated from the soluble material by centrifuging the suspension at 10000*g* for 15 min. As the ionic strengths of the buffers were kept relatively low in order to avoid artifact effects of buffer salts, the pH was slightly altered during the extraction so that the pH values at the end of extractions were within 0.2 pH unit of the buffer pH.

To obtain an extract with concentrated inhibitor content, the following extraction scheme was also performed. The fiber was extracted consecutively with the 0.05 M sodium acetate (pH 4) after which the insoluble fiber particles were suspended in water and the pH was adjusted to pH 10.0 with 0.1 M NaOH. After reextraction the soluble fraction was separated from insoluble fiber particles by centrifuging the slurry at 10000*g* for 15 min. This extract was further fractionated by adjusting the pH to the pH values 4, 6, or 8. The precipitate formed was separated from the soluble fraction by centrifuging the suspension at 10000*g* for 15 min.

Prior to inhibitory activity measurements, all the extracts were neutralized with 0.1 M NaOH or HCl, while the particle fractions and precipitates were first suspended in water and the suspension was neutralized. The inhibitory activities of these neutralized suspensions were studied.

Measurement of the Dioxygenation Rate. Oxidation of linoleic acid was measured by using polarography (Clark electrode, YSI 5300) to follow the oxygen consumption of aqueous suspension. A varying amount of a sample, 0.86 mg of free linoleic acid (Sigma L-1376, St. Louis, MO) in 0.3 mL of Tween 20−NaOH (Axelrod et al., 1981), and 0.2 M sodium phosphate buffer (pH 7.0) to make the total volume 7.3 mL were mixed for 100 s in a reaction cuvette. After the mixing period, 8.8 nkat of soybean lipoxygenase (Sigma L-8383, St. Louis, MO) was introduced into the solution. The rate of oxygen consumption was recorded.

Quantifying the Dioxygenation Inhibitor. The inhibitor was quantified as inhibitory units (IU). The amount of fraction needed to cause the dioxygenation rate to diminish to one-half in a system consisting of 0.86 mg of linoleic acid and 8.8 nkat of lipoxygenase in the total volume of 7.3 mL was defined to contain 1 IU (Lehtinen and Laakso, 1997). The total IU contents of different fiber fractions derived from 1 g of crude fiber were evaluated.

Composition Analysis of Different Fractions. The selected fractions were analyzed for protein content by the modified ninhydrin method (Barbehenn, 1995), carbohydrate content by the phenol sulfuric acid method (Dubois, 1956), and total phenolics as gallic acid equivalents by Folin Ciocalteau method (Maillard et al., 1996). Fatty acids were converted to methyl esters by saponification and methylation essentially as described by Suutari et al. (1990). Absolute amounts of methyl esters were determined by adding heptadecanoic acid methyl ester as an internal standard prior to saponification. Methyl esters were identified and quantified by gas chromatography with a flame ionization detector. At least one set of duplicated analyses was performed.

RESULTS

Solubility of Inhibitor Using Various Extraction Media. The oat fiber was extracted with aqueous buffers of pH values varying from pH 4 to 10 or with anhydrous methanol. The inhibitory activities toward dioxygenation activity of lipoxygenase were evaluated in the particle-free extracts as well as in the suspension of the remaining insoluble fiber particles. The appearances of the inhibitory properties in different fractions were compared as the total inhibitory activities in the fractions derived from 1 g of untreated crude oat fiber. One gram of the crude fiber embodied an inhibitory activity of 48 inhibitory units (IU) (Table 1).

Upon extraction with aqueous buffers, the amount of the total inhibitory activity in the soluble extract was high at alkaline extraction (Table 1). The extract obtained at pH 10 carried twice the total inhibitory activity of the untreated oat fiber, while the yield of the dry matter in the extract was 15%. On the contrary, the soluble extracts possessed little inhibitory activity when the extraction was done in acid or near-neutral pH. The yields of dry matter were, however, not markedly affected by the extraction pH.

The observation that the dioxygenation inhibitory activity was efficiently extracted at high pH values but not in neutral or slightly acidic pH enabled the development of a more concentrated form of the inhibitor by consecutive extractions at varying pH values. The fiber was first washed with a buffer at pH 4 followed by reextraction of the fiber particles at higher pH. The yield of dry matter and the inhibitory content in the final extract were 66% and 80%, respectively, of the values obtained in the single alkali extract indicating that the dioxygenation inhibitor was purified 1.2-fold compared to the single alkaline extraction.

A slightly smaller amount of inhibitory activity was gained by methanol extraction than by the alkaline extraction (Table 1). Considering that the recovery of dry matter in the methanol extract was 6% compared to the 15% in aqueous extractions, the inhibitory capacity was evidently in a more concentrated form.

Unexpectedly, even though the alkali and methanol extracts appeared to carry inhibition activity superior to that of the crude fiber, the remaining insoluble particles still carried the inhibitory activity in similar amounts to that of the nonextracted crude fiber (Table 1).

Table 2. Distribution of Total Dioxygenation Inhibitor Activity Derived from 1 g of Crude Fiber and Dry Matter into the Precipitate and Soluble Fraction upon Altering the pH of the Alkali Extract

	precipitate		solution	
target pH	IU	recovery (%) of fiber dry matter	IU	recovery (%) of fiber dry matter[c]
pH 10[a]	*b*	1.3	76	8.4
pH 8	2	1.4	66	8.5
pH 6	60	7.2	4	2.3

[a] Precipitate resulting from recentrifuging with no pH adjustment. [b] A 50% inhibition was not established. [c] The nonvolatile salts from added buffers and pH adjustments are subtracted from the actual dry weight measurement.

Recovery of the Inhibitory Activity from the Extract by Precipitation. As the dioxygenation inhibitory activity was extracted from the oat fiber by alkali buffer, it was of interest to study whether it could be recovered from the dilute solution obtained by alkaline extraction into a more concentrated form by relowering the pH.

When the pH of the alkali extract was lowered to pH 6, the majority of the dry matter in the alkaline extract was precipitated and the remaining solution was optically clear. Simultaneously, the dioxygenation inhibitory activity vanished from the soluble fraction and appeared in the precipitate (Table 2). However, reduction of pH to a pH of 8 was not sufficient to cause the dioxygenation inhibitor to precipitate.

Composition Analysis of the Fractions. To reveal the major molecular species present in the fractions showing the greatest inhibition activity on dioxygenation reaction, the different fractions were analyzed for protein, carbohydrate, total fatty acid, and total phenol contents (Table 3).

Distribution of protein, between the soluble and insoluble fractions, which together with carbohydrate made up the majority of all studied fractions, was as expected strongly dependent on the extraction pH. Similar to whole meal oat flour (Wu et al., 1977) the protein and total fatty acid contents of the oat fiber extract were high, 24% and 6%, respectively, when the extraction pH was alkaline, while carbohydrate was prominent in the extract obtained at the lower pH. The amounts of salts introduced as buffers or during pH adjustments contributed between 30% and 50% to the total dry matter in aqueous extracts. However, salts could easily be removed by acid precipitation so that the final precipitate contained 89% protein and 13% fatty acids.

The methanol extract was composed of 57% fatty acids, whereas the total phenolic content was 2 mg/g. Compared to alkaline aqueous extracts the total phenolic content of the methanol extract was lower, whereas the amount of total phenolics in the acid precipitate was comparable to that in the methanol extract.

DISCUSSION

The solubility of dioxygenation inhibitory activity in the oat fiber was greatly affected by the pH and the polarity of the extraction media. Upon different fractionation procedures, a greater inhibitory activity was gained in the fractions with high protein and total fatty acid contents. Contradictory, the carbohydrate content correlated negatively with the inhibitory activity. However, previously it has been found that the protein fraction derived from the whole meal oat was a less potent inhibitor than the carbohydrate-rich oat fiber (Lehtinen and Laakso, 1997). The fact that the protein-rich fraction derived from fiber carries an inhibitor activity superior to the protein fraction derived from whole meal oat suggests that the inhibitory activity is carried by the protein fraction bound to fiber particles during the wet processing of oat to yield protein, starch, and fiber fractions. Even though the lipid content of the methanol extract was higher than in the alkaline extract, the inhibitory capacity of it was lower. It is plausible that alkaline extraction yields compounds that in the aqueous system have greater antioxidative activity than fractions obtained by the straightforward extraction of lipid-soluble antioxidants.

The dioxygenation inhibitory activity obtained by the alkali extraction of oat fiber was quantified to be 2-fold of that detected in crude untreated fiber. Furthermore, the activity once obtained by alkaline extraction was preserved even after precipitation by relowering the pH. This, together with the fact that after the extraction process the insoluble fiber particles maintained the inhibitory activity, suggests that the inhibitory effect is not limited by the total concentration of some specific fiber compound but more by a concentration of the favorable physical state of such a compound. This favorable physical state can be induced by an alkali treatment.

The interactions between the cereal protein and lipids in the aqueous suspensions have been widely studied. These interactions have been shown to alter the aggregation of proteins (Békés et al., 1983; Carcea and Schofield, 1996; Zawistowska et al., 1938). However, only little information is available showing the effect of these interactions on the oxidative stability of lipids (Pike and Pen, 1988). The protein-rich fraction derived from oat fiber could be used to stabilize the lipids in water-rich environments. The 1 g of untreated fiber was processed into the fraction that carried 72 mg of dry matter and embodied 60 IU (Table 2). This means that

Table 3. Composition of Oat Fiber Fractions at Different Stages of the Extraction Process[a]

	protein		total fatty acids		carbohydrates		added salts[b]	total phenolics
	mg/g	(%)	mg/g	(%)	mg/g	(%)	mg/g	mg/g
crude fiber	250	(100)	90	(100)	600	(100)		nd
pH 4 extract	93	(6)	11	(12)	579	(15)	307	1.2
pH 10 extract	241	(20)	63	(14)	340	(12)	343	nd
pH 10 extract of fiber washed at pH 4	252	(21)	49	(11)	155	(5)	496	4.1
acid precipitate	885	(19)	127	(7.4)	34	(0.3)		1.9[c]
methanol extract	137	(3.3)	575	(39)	76	(0.8)		2.0

[a] In the parentheses are presented the percentage recovery of protein, total fatty acids, and carbohydrates from the amounts of these compounds present in the untreated oat fiber. [b] Values obtained by adding the concentrations of nonvolatile buffer salts together with Na^+ and Cl^- ions used in the pH adjustments. [c] Value obtained by subtracting the phenolic content in the solution after the precipitation from the phenolic content in the alkali extract before the precipitation.

0.8 mg of the fraction contains 1 IU and can thus reduce the dioxygenation rate of the system, containing 0.86 mg of endogenous linoleic acid, to one-half of the control situation. It can consequently be estimated that to achieve a significant reduction in the dioxygenation rate, the amount of the fraction needs to be at least equal (w/w) to the amount of endogenous free fatty acid present in the system.

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