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Phenolic glucosides in bread containing flaxseed

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ABSTRACT

Three phenolic glucosides, secoisolariciresinol diglucoside, *p*-coumaric acid glucoside and ferulic acid glucoside, were analyzed in commercial breads containing flaxseed. The total phenolic glucoside content ranged from 15 to 157 mg/100 g dry bread. Secoisolariciresinol diglucoside was the dominating phenolic glucoside with an average relative content of 62%, followed by *p*-coumaric acid glucoside (20%) and ferulic acid glucoside (18%). Strong positive correlations between the phenolic glucosides were found, indicating no major effect of raw material or bread-making process on the relative content of the phenolic glucosides in flaxseed.

1. Introduction

Flaxseed (*Linum usitatissimum* L.) is today commonly used as an ingredient in breads for its high content of the omega-3 fatty acid, α-linolenic acid. Flaxseed is also a rich source of an oligomeric complex (Klosterman & Smith, 1954) containing the lignan secoisolariciresinol diglucoside (SDG) (Ford, Huang, Wang, Davin, & Lewis, 2001; Kamal-Eldin et al., 2001). SDG is a precursor of the mammalian lignans that are recognized to protect against hormonal-dependent breast cancer (Chen & Thompson, 2003; Saarinen et al., 2002; Thompson, Chen, Strasser-Weippl, & Goss, 2005). Other phenolic compounds in the complex are two hydroxycinnamic acid derivatives, *p*-coumaric acid-4-*O*-glucoside and ferulic acid-4-*O*-glucoside (Johnsson et al., 2002), and the flavonoid herbacetin diglucoside (Struijs et al., 2007). The limiting factors for flaxseed consumption are the high content of cyanogenic glucosides (100–300 mg hydrogen cyanide/kg seed) and cadmium (294–1543 μg/kg) (Rosling, 1993; Oomah et al., 2007). In absence of qualitative toxicological data, the provisional tolerable daily intake has been set at 12 μg cyanide/kg body weight (WHO, 1996) and 1 μg cadmium/kg of body weight (JECFA, 1993). With regard to the levels of cyanogenic glucosides, the intake of flaxseed should be limited to 10–20 g whole flaxseed per day and the National Food Administration in Sweden advises against usage of crushed or milled flaxseeds since the bioavailability of cyanides increases with disintegration. Thus, although flaxseed is the richest source of lignans, its contribution to intake is limited.

In this study, different types of bread products containing flaxseed were obtained from major food outlets. Seventeen breads were analysed for their contents of the phenolic glucosides, SDG, *p*-coumaric acid glucoside and ferulic acid glucoside.

2. Materials and methods

2.1. Samples

Different major food outlets were searched for food products containing flaxseed and 17 different soft and crisp breads were selected. Thirteen out of the 17 products had information of the content of flaxseed on the product label. Representative parts of breads were cut into smaller pieces and placed on aluminium trays for freeze-drying. After freeze-drying, the dry weight of each bread was calculated. Dried samples were disintegrated in a kitchen mixer and milled (0.5 mm sieve, Retsch type ZM 1, Haan, Germany).

2.2. High-performance liquid chromatography analysis of phenolic glucosides

Samples (0.5 g) were mixed with internal standard *o*-coumaric acid (0.06 mg in 0.2 ml methanol) and extracted with dioxane/ethanol for 48 h, essentially as described by Johnsson, Kamal-Eldin, Lundgren, and Åman (2000). In this study, dried dioxane/ethanol extracts were hydrolyzed to break ester bonds using 1 ml of aqueous sodium hydroxide (1 M) for 1 h at room temperature under

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constant rotation. Hydrolysates were acidified to pH 3 using 2 M sulphuric acid, and the volume was made to 10 ml using methanol. The extracts were left at room temperature for at least 10 min and centrifuged (900 g, 10 min) to precipitate and remove water-soluble polysaccharides and proteins. The extracts were evaporated to dryness at 40 °C and dissolved in 500 μl of methanol and filtered through a GHP Acrodisc® 13 mm syringe filter with 0.45 μm GHP membrane and transferred to vials. All breads were extracted in duplicate.

The contents of the phenolic glucosides, SDG, *p*-coumaric acid glucoside and ferulic acid glucoside, were determined by HPLC on a Dionex PDA-100 (Dionex, Sunnyvale, CD, USA) system with a UV–Vis diode array detector and Chromelion software. All bread samples, except three, were analysed according to Eliasson, Kamal-Eldin, Andersson, and Åman (2003). Due to interfering peaks, three bread samples were analysed using different gradients of mobile phase, (A) 5% acetonitrile in phosphate buffer (0.01 M, pH 2.8) and (B) acetonitrile; A:B (v/v): 0 min (100:0), 23 min (77:23), 30 min (77:23) and 40 min (70:30) at a flow rate of 1 ml min^{-1}.

3. Results and discussion

The flaxseed content in the seventeen breads ranged from 1.5 to 9 g/100 g of fresh bread (Table 1). In an average consumption of 150 g bread per day and person, this flaxseed content is within the advised intake of flaxseed by the National Food Administration in Sweden. In flaxseed, the phenolic glucosides, SDG, *p*-coumaric acid glucoside and ferulic acid glucoside, can be analysed by direct alkaline hydrolysis (Eliasson et al., 2003). However, mixing alkali with bread samples containing high amounts of starch increases the viscosity of the samples and complicates further sample treatment. By using extraction solvents such as dioxane/ethanol, the starch-free extract was hydrolyzed with alkali and no viscosity was obtained. All bread products had comparable HPLC chromatograms, similar to those obtained for flaxseed (Eliasson et al., 2003), except for three bread samples (2, 5 and 6) in which some peaks interfered with the internal standard, *o*-coumaric acid. These interfering peaks have not been observed in UV chromatograms of flaxseed. The most probable source of these peaks must be other ingredients mixed into these breads. By including a short isocratic step in the gradient of the mobile-phase, the internal standard and the interfering peaks were separated (Fig. 1).

The 17 breads showed considerable variation in the total content of the phenolic glucosides in soft bread (15–157 mg/100 g dry bread, $n = 12$) and crisp bread (18–55 mg/100 g dry bread, $n = 5$) (Table 1). The major phenolic glucoside was SDG, ranging from 7.6 to 105 mg/100 g of dry bread. In a database of nine phytoestrogens in foods, two breads with flaxseed contained an amount of SDG equivalent to 16 and 24 mg SDG/100 g of dry bread (Thompson, Boucher, Liu, Cotterchio, & Kreiger, 2006). Muir and Westcott (2000) found that the content of SDG in 10 commercially produced flaxseed soft breads ranged from about 4.1 to 136 mg/100 g of dry bread which is comparable to the amount of SDG in the breads from this study. Less abundant phenolic glucosides in our breads were *p*-coumaric acid glucoside (3.3–33 mg/100 g dry bread) and ferulic acid glucoside (3.3–18 mg/100 g of dry bread).

In different breeding lines of flaxseed grown in Sweden, the content of SDG ranged from 6.1 to 25.9 mg/g of whole flaxseed (Eliasson et al., 2003; Johnsson et al., 2000). Considering an average

Table 1
The content (mg/100 g dry bread) and the relative composition (%) of the phenolic glucosides SDG, *p*-coumaric acid glucoside and ferulic acid glucoside in breads

Breads	Flaxseed content (g/100 g FW)	Phenolic glucoside content			
		p-Coumaric acid glucoside	Ferulic acid glucoside	SDG	Total
Soft bread[a]					
1	6.5	33 (21%)	18 (12%)	105 (67%)	157
2	n.g.[c]	21 (18%)	13 (11%)	81 (70%)	114
3	9	27 (23%)	15 (13%)	74 (64%)	116
4	3	21 (22%)	17 (18%)	56 (60%)	93
5	3.9	11 (16%)	12 (18%)	44 (65%)	67
6	n.g.	12 (21%)	8.3 (15%)	35 (64%)	55
7	2.5	7.2 (16%)	3.7 (8%)	35 (76%)	46
8	3	11 (25%)	7.7 (18%)	24 (56%)	42
9	n.g.	4.7 (18%)	4.4 (16%)	17 (66%)	27
10	2	3.9 (15%)	4.6 (18%)	17 (67%)	26
11	1.5	3.5 (15%)	5.3 (22%)	15 (63%)	24
12	n.g.	3.8 (26%)	3.3 (22%)	7.6 (52%)	15
Crisp bread[b]					
13	2.5	8.2 (15%)	5.4 (10%)	42 (75%)	55
14	2.2	8.2 (21%)	5.2 (14%)	25 (65%)	38
15	3	4.1 (17%)	5.2 (22%)	15 (62%)	24
16	5	9.4 (29%)	7.7 (25%)	14 (46%)	31
17	4	3.3 (18%)	7.2 (39%)	7.9 (43%)	18

The flaxseed content (g/100 g fresh weight) in the bread was obtained from the product label.
[a] The soft breads had dry weight content ranging from 57% to 69%.
[b] The crisp breads had dry weight content ranging from 90% to 94%.
[c] Flaxseed content was not given (n.g.) on the product label.

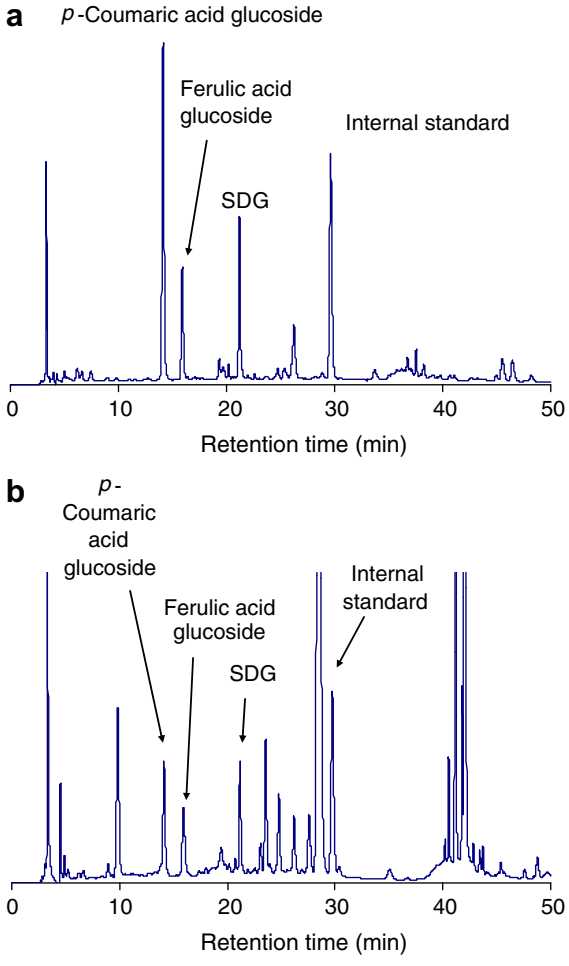

Fig. 1. Examples of HPLC chromatograms (UV 280 nm) of the phenolic glucosides, SDG, *p*-coumaric acid glucoside and ferulic acid glucoside in the oligomeric complex extracted with dioxane/ethanol and hydrolysed with alkali; (a) bread sample analysed with the standard method; (b) bread sample analysed with a modified gradient due to peaks interfering with the internal standard (*o*-coumaric acid). For gradients and other conditions, see Section 2.

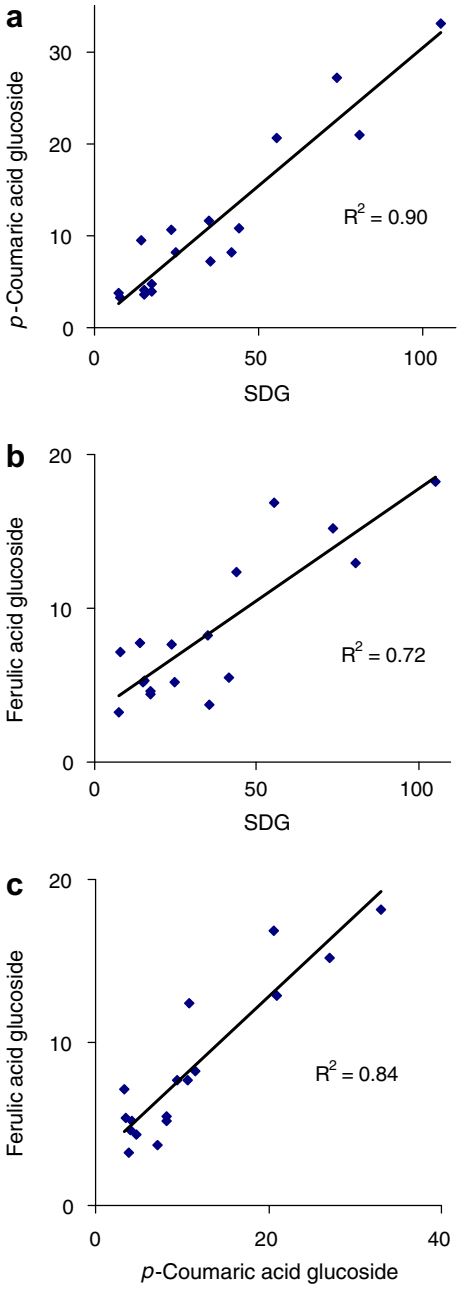

Fig. 2. Correlation between the phenolic glucosides (mg/100 g dry bread); (a) *p*-coumaric acid glucoside and SDG, (b) ferulic acid glucoside and SDG, and (c) ferulic acid glucoside and *p*-coumaric acid glucoside. R^2 is the correlation coefficient (n = 17).

content of 16 mg SDG/g of whole flaxseed, the contents of SDG in the breads are in the expected range with the exception for breads 1 and 3. The relative content of SDG in the crisp breads 16 and 17 was lower than those in the other breads (Table 1), and lower than the relative content in different flaxseeds analyzed previously (Eliasson et al., 2003). These differences in content and relative proportions of the phenolic glucosides might be due to different flaxseed varieties included in the breads and/or to unequal distributions of flaxseed in the breads.

Strong positive correlations were obtained between SDG and the hydroxycinnamic acid derivatives, and between *p*-coumaric acid glucoside and ferulic acid glucoside (Fig. 2). In different flaxseed breeding lines grown in two different locations in Sweden, positive correlations were obtained between SDG and the hydroxycinnamic acid derivatives (Eliasson et al., 2003). These correlations indicate that the bread ingredients and the bread-making conditions have only a small impact on the proportion of the phenolic glucosides. In the future, more attention should be paid to the contribution of flaxseed in bread to the intake of flaxseed lignans.

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