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ScienceDirect
Food Chemistry 104 (2007) 390–397
Food Chemistry
www.elsevier.com/locate/foodchem

Interactions between dietary fibre-rich preparations and glycoconjugated bile acids *in vitro*

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Received 6 July 2006; received in revised form 4 October 2006; accepted 22 November 2006

Abstract

Binding in small intestine and excretion of bile acids constitute a major hypocholesterolemic pathway. Interactions between different types of commercial and laboratory-made dietary fibres and glycoconjugated bile acids were investigated *in vitro* at pH 5.0 and 6.5. The interactions were greater at the lower pH and with dihydroxy-bile acids. Digested cereal products (barley, oat, rye and wheat flour; oat bran), alcohol-insoluble substances from apples, strawberries, rowan berries, carrots, white cabbage, red beets and sugar beet pulp, as well as arabinoxylan, bound 1.21–1.77 μmol bile acids/100 mg of preparation at pH 5.0. Novelose bound approximately 0.65 μmol bile acids/100 mg. Carob fibre had the highest binding capacity (1.83–1.96 μmol bile acids/100 mg) whereas cellulose had no effect. Besides the source and chemical composition, the bile acid binding correlated especially well with the presence of three-dimensional cell wall structures of the tested preparations but less well with the proportions of soluble and insoluble dietary fibre.

Keywords: Dietary fibre; Cereals; Fruits; Vegetables; Bile acid binding; *In vitro*

1. Introduction

Dietary fibres consist of a large group of substances (mainly of plant origin) that are not hydrolysed by enzymes of the human small intestine. The main sources of dietary fibre in human nutrition are cereals, fruits and vegetables. Dietary fibres have several preventive medical and nutritional effects in the intestinal tract, depending on their structure and molecular weight, as well as on their solubility and on their physicochemical properties (water binding, viscosity). They occur in isolated, more or less soluble form (e.g., pectin, β-glucan, carrageenan, guaran) in the diet or as a part of the more or less intact complex cell wall architecture in plant materials. Food processing may also influence the properties of dietary fibres (van der Kamp, Asp, Miller Jones, & Schaafsma, 2004). Therefore, it is often difficult to find the mechanisms behind the physiological dietary fibre effects.

It has been shown, in several studies, that dietary fibre may influence bile acid and cholesterol metabolism. Bile acids are necessary for the digestion of lipids in the small intestine. Normally, they are practically reabsorbed completely in the ileum and then transported to the liver via the enterohepatic circulation by different mechanisms (Hofmann, 1994). Several dietary fibres are able to interact with bile acids in the small intestine, resulting in a lower reabsorption, in an increased transport toward the large intestine and, finally, in a higher excretion of bile acids (Dongowski, Huth, & Gebhardt, 2003; Marlett et al., 1994). Because the bile acid pool is limited, a higher excretion of bile acids requires an increased hepatic synthesis of bile acids from blood cholesterol. This is probably the

Abbreviations: AIS, alcohol-insoluble substance; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; TCA, taurocholic acid.

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doi:10.1016/j.foodchem.2006.11.053

major hypocholesterolemic pathway, occurring especially in hypercholesterolemic individuals or animals (Braaten et al., 1994; Garcia-Diez, Garcia-Mediavilla, Bayon, & Gonzalez-Gallego, 1996).

In most studies, only one type of dietary fibre has been investigated *in vitro* in relation to bile acid-binding properties, for example isolated macromolecular dietary fibre components, such as pectin, β-glucan, galactomannans and psyllium (Dongowski, 1995; Kritchevsky, 1996; Lee et al., 2003), preparations with more or less intact botanically-grown cell wall structures (Dongowski & Ehwald, 1999; Kotcharian, Kunzek, & Dongowski, 2004; Pickard, Dongowski, & Kunzek, 2004) or complex dietary fibre-containing food products (Camire & Dougherty, 2003; Camire, Zhao, & Violette, 1993; Goel et al., 1998; Górecka, Korczak, Konieczny, Hęś, & Flaczyk, 2005; Hoagland & Pfeffer, 1987; Huth, Dongowski, Gebhardt, & Flamme, 2000; Kahlon & Woodruff, 2003a, 2003b; Sayar, Jannink, & White, 2005). Furthermore, proteins or protein-rich foods were also able to interact with bile acids (Kahlon & Woodruff, 2002; Yoshie-Stark & Wasche, 2004).

In this study, different types of commercial and laboratory-made dietary fibre preparations, with different structures and from different sources, were allowed to interact with glycoconjugated bile acids *in vitro* under the pH conditions of the small intestine.

2. Materials and methods

2.1. Dietary fibre products

Wheat fibre Vitacel® type WF 101 (Rettenmaier & Söhne, Holzmühle, Germany) was prepared from straw by pre-grinding, mechanical removing of fines and foreign materials, aqueous extraction of carbohydrates, disintegration of cellulose and hemicelluloses (NaOH, >100 °C; overpressure), filtration and washing, followed by drying, grinding and sieving. The microcrystalline cellulose preparation, Vivapur® type 101 (Rettenmaier & Söhne), was prepared from cellulose pulp by acidic hydrolysis, followed by filtration, drying and classifying. The arabinoxylan was prepared, at the Technical University Berlin, from process water of a wheat starch plant in a pilot scale process consisting of enzymatic, fermentative and mechanical treatments, ultrafiltration and spray-drying (Zunft, Lueder, Koebnick, Imhof, & Meuser, 2004). The carob fibre preparation Caromax® (Nutrinova GmbH, Frankfurt/M., Germany) was prepared from carob pulp by water extraction of deseeded locust bean husk. The commercial res7istant starch preparation, Novelose 330®, was obtained from National Starch & Chemical (Bridgewater, HJ, USA).

Commercially-available barley, rye and wheat flours were used. Oat flour and bran were obtained from Peter Kölln Köllnflockenwerke (Elmshorn, Germany).

To prepare the alcohol-insoluble substances (AIS), ripe fruits and vegetables, as well as fresh sugar beet pulp, were cut into small pieces in ethanol (end concentration 65%; w/v), using a mixer, and then boiled under reflux, first for 30 min and then twice for 15 min. The liquid phase was removed after each heating step. The AIS was intensively washed with 65% and 96% (v/v) ethanol, as well as with acetone, and dried in air and in vacuum.

2.2. Analytical methods

Insoluble and soluble dietary fibre were analysed by the enzymatic-gravimetric AOAC method (Prosky et al., 1988). Pectin (galacturonan) was determined by the *m*-hydroxydiphenyl method and the degree of esterification of pectin with methanol was analysed by the chromotropic acid method (Dongowski, 1995). Resistant starch was measured by a modified Englyst method (Englyst, Klingman, & Cummings, 1992). First, the digestible starch was hydrolysed by incubation with pancreatin (Merck, Darmstadt, Germany) and amyloglucosidase (Sigma, St. Louis, MO, USA) in acetate buffer (pH 5.2) for 2 h at 37 °C, simulating starch hydrolysis in the small intestine. After addition of the fourfold amount of 96% EtOH and centrifugation (10 min at 4 °C and 2800*g*), the hydrolysed starch products were extracted twice (with 80% EtOH). The freeze-dried resistant starch-containing residue was dissolved in 1 M NaOH. The diluted solution was hydrolysed with amyloglucosidase at pH 4.6, and the released glucose was determined enzymatically using a hexokinase and glucose-6-phosphate dehydrogenase kit (Boehringer, Mannheim, Germany). β-Glucan was determined as described previously (Drzikova, Dongowski, Gebhardt, & Habel, 2005).

2.3. In vitro digestion of the cereal products

The cereal flours and oat bran were digested enzymatically to remove digestible starch, proteins and lipids, simulating physiological conditions by treatment with a mixture of pancreatin (Merck) and amyloglucosidase (Sigma), at pH 5.2 and 37 °C for 2 h. Then, a fourfold amount of 96% EtOH was added and the mixture was centrifuged (10 min at 4 °C and 2800*g*). The residue was extracted twice (with 80% EtOH), heated under reflux in 96% EtOH for 15 min (enzyme inactivation), centrifuged and then dried.

2.4. Binding of bile acids by the cereal products and dietary fibre preparations

The binding experiments were performed using the conjugated bile acids, glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA) and glycodeoxycholic acid (GDCA), as sodium salts, from Sigma. These bile acids are predominant in human bile.

In most of the experiments, the dietary fibre-containing samples (100 mg dry matter) were suspended in 4 ml of Sörensen buffer (pH 5.0 or 6.5) containing 0.5 mM bile acid and treated for 2 h at 37 °C under shaking. After centrifugation (10 min at 4 °C and 3000*g*), 0.5 ml of the supernatant (containing the un-bound bile acids) was purified by