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Influence of baking and *in vitro* digestion on steryl ferulates from wheat

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ABSTRACT

So far, data on absorption and metabolism of steryl ferulates from edible sources is scarce. Therefore, the impact of enzyme-aided baking and *in vitro* digestion was examined in this study. Wheat flours and wheat breads were subjected to a static *in vitro* digestion model and changes in the contents of steryl ferulates and free sterols (possible hydrolysis products of steryl ferulates) were monitored. Baking degraded steryl ferulates at a similar rate in all types of breads (43–47%) compared to the corresponding flours, while baking induced changes in free sterols showed no clear pattern. *In vitro* digestion provoked five folds lower content of steryl ferulates in flours than in breads and it also resulted in significant free sterol accumulation. Interestingly, bioaccessibility (0.01–0.25%) was not influenced by the cereal matrix. The four steryl ferulate species, which were detected in wheat, showed similar hydrolysis rates during digestion. As baking had a significant impact on the steryl ferulate content of wheat, we suggest that both raw and processed sources should be considered further *in vitro*, animal or human trials, when studying the metabolic fate of steryl ferulates.

1. Introduction

Steryl ferulates (phytosterols esterified to ferulic acid) are minor phytochemicals, occurring mainly in the outer layers of cereal grains (Fig. 1). They have been demonstrated to possess cholesterol lowering (Wilson et al., 2007) as well as antioxidative properties (Nyström et al., 2005). By lowering plasma cholesterol, steryl ferulates play an important role in the primary prevention against cardiovascular diseases, while their antioxidant character makes them potential anti-inflammatory (Akihisa et al., 2000), antiviral (Iwatsuki et al., 2003) and antitumor agents (Yasukawa et al., 1998). However, neither their exact mechanism of action, nor their full metabolic fate is clearly understood yet. Further, cereals are mostly consumed in a processed form, for instance as bread. However, no data is available on the effect of baking or on the combined effects of baking and digestion on steryl ferulates so far. Therefore, we focus our study on the influence of baking and digestion, in order to gain a more complete picture on the metabolic fate of these bioactive compounds from realistic, edible sources.

Data of Miller et al., 2004 suggest, that steryl ferulates are cleaved by intestinal cholesterol esterase, releasing free sterols in the gut. Free sterols are bioactive molecules, able to reduce plasma cholesterol levels. Suggested mechanisms of the cholesterol lowering activity of plant sterols have been reviewed in a recent publication (Smet et al., 2012). First, it was thought that they compete with cholesterol to be incorporated into dietary mixed micelles as well as into chylomicrons. Second, transporters localised in the brush border membrane were identified and it was supposed that they might play a role in the active transport of both plant sterols and cholesterol. Third, direct secretion of cholesterol from the systemic circulation into the intestinal lumen was shown and plant sterols have been found to play a role in activating this pathway. However, considering that none of the proposed mechanisms can fully explain all observations, the exact mechanism behind the cholesterol lowering activity of plant sterols remains most likely a complex interplay of multiple processes. In addition, the extent of steryl ferulate hydrolysis seems to depend upon the cereal source. In our recent *in vitro* study, we found a digestion induced decrease of 53% and 63% in steryl ferulates from maize bran and wheat bran respectively. Digestion induced decrease of steryl ferulates from polished rice, cargo rice, rice bran and wild rice was lower, namely 1–41% (Mandak and Nyström, 2012).

The few studies available on the absorption and metabolism of steryl ferulates suggest that these compounds are poorly absorbed.

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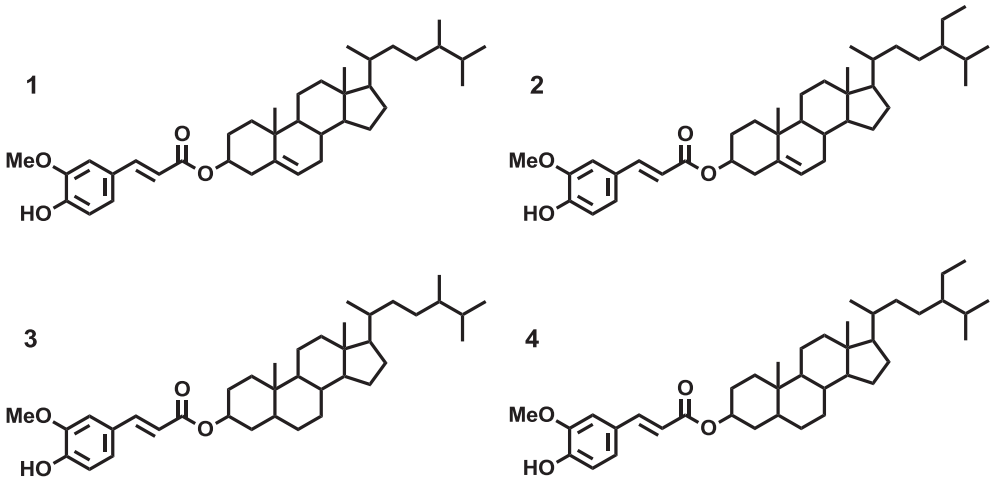

Fig. 1. Structural formulae of campesteryl ferulate (**1**), sitosteryl ferulate (**2**), campestanyl ferulate (**3**) and sitostanyl ferulate (**4**).

In their experiment of *in situ* absorption, Fujiwara et al., 1983 found that only 0.2% of ^{14}C-labelled γ-oryzanol was absorbed via the portal vein, while 89% of the dose remained in the luminal fluid with more than 95% staying intact. In a recent clinical trial, Lubinus et al., 2012 had similar findings, namely that nearly 80% of γ-oryzanol was recovered in intact form in the faeces. Huang (2003) reported that γ-oryzanol extracted from rice bran oil was poorly absorbed by intestinal cells *in vitro*. Additionally, in our recent *in vitro* study, we measured the availability of steryl ferulates for absorption from various raw cereals and we found it to be below 1.5% (Mandak and Nyström, 2012). Given that health promoting properties of steryl ferulates are well established, the above findings all indicate that absorption of steryl ferulates might not be necessary to achieve their health benefits.

Absorption has been demonstrated to heavily depend upon the matrix in which the compound in question is incorporated. Bioaccessibility of β-carotene for instance was found to be 14% from mixed vegetables (Hof et al., 2000), however mechanical homogenisation and heat treatment induced a twofold increase in its bioaccessibility (Rock et al., 1998). In the case of lutein, bioaccessibility was 67% (Hof et al., 2000) and it showed an additional 14% increase due to mechanical homogenisation (Hof et al., 1999). Further, ferulic acid bioaccessibility was found to be 0.6% from wheat bran, where it is bound to arabinoxylans and other indigestible polysaccharides, restricting its release in the small intestine (Mateo Anson et al., 2009a). However, when ferulic acid was added as a free compound to the flour, its bioaccessibility increased to almost 60% (Mateo Anson et al., 2009a). Ferulic acid bioaccessibility remained low due to physico-chemical processing, however a combination of fermentation and enzymatic treatment of wheat bran lead to a five-fold increase (Mateo Anson et al., 2009b). The enzyme preparations used for the treatment contained various cell-wall-degrading enzymes, namely xylanase and cellulase. These enzymes are commonly used in the procedure of enzyme-aided baking. Xylanases have been reported to improve bread volume and crumb structure, due to their capability to solubilise arabinoxylans by randomly cleaving the β-1,4 backbone between the xylose units. Further, a mixture of xylanase and cellulase was also found to improve texture properties and increase loaf volume of breads (Stojceska and Ainsworth, 2008).

The aim of the present study was to understand changes in the steryl ferulate content of wheat flour due to enzyme-aided baking and due to *in vitro* digestion. As far as we know, this study is the first to investigate the effect of baking on steryl ferulates and it is also the first to investigate the effect of *in vitro* digestion on steryl ferulates from baked samples. Results of this work would provide better insight into the metabolic fate of steryl ferulates from genuine sources. After preliminary screening of milling fractions, we baked breads from two types of wheat flour. Baking was performed either without enzyme addition or using cellulase or xylanase or a combination of these enzymes. The raw material and baked samples were all subjected to *in vitro* digestion. Their steryl ferulate and free sterol content were measured before and after digestion. Bioaccessibility of steryl ferulates was calculated as the percentage of steryl ferulates found in the supernatant compared to the total extractable amount (Stahl et al., 2002). The sterol composition of steryl ferulates was also analysed in both flours and breads, as well as before and after digestion.

2. Experimental

2.1. Chemicals

Calcium chloride dihydrate (≥99%), glucose (≥99.5%), potassium chloride (≥99%), potassium dihydrogen phosphate (≥99%), sodium bicarbonate (≥99%), sodium chloride (≥99%), sodium dihydrogen phosphate (≥99%) and uric acid (≥99%) were obtained from Fluka, Buchs, Switzerland. Bovine serum albumin (biochemical grade) was purchased from VWR, Lutterworth, UK. Acetic acid (Ph Eur), β-sitosterol (≥95%), lipase from porcine pancreas (biochemical grade), pancreatin (Ph Eur), pepsin (biochemical grade) and sodium hydroxide (Ph Eur) were from Merck, Darmstadt, Germany. Acetone (≥99.9%), acetonitrile (≥99.9%), α-amylase (biochemical grade), ammonium acetate (≥98%), ammonium chloride (≥99.5%), bile (biochemical grade), butanol (≥99.7%), disodium hydrogen phosphate (≥99%), glucosamine hydrochloride (≥99%), glucuronic acid (≥98%), magnesium chloride (≥98%), heptane (≥99%), hydrochloric acid (37%), isopropanol (≥99%), mucin (biochemical grade), potassium hydroxide (≥85%) and urea (biochemical grade) were obtained from Sigma–Aldrich, St. Louis, MO, USA. Cycloartenyl ferulate (≥99%) and γ-oryzanol (≥98%) were purchased from Wako, Osaka, Japan.

2.2. Samples

Whole grain wheat flour, wheat baking flour (type 550), flour fractions C10 I, C10 II, C13 I, C13 II from reduction rolls and two fractions from bran finisher (KLM I and KLM II) were provided by