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Enterolignans enterolactone and enterodiol formation from their precursors by the action of intestinal microflora and their relationship with non-starch polysaccharides in various berries and vegetables

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ABSTRACT

The aim of this work was to investigate the production of enterolactone (ENL) and enterodiol (END) both enterolignans, from their precursors by the action of intestinal microflora and their relationship with non-starch polysaccharides (NSP) in common plant foods such as berries and vegetables. For the investigation of the bioconversion of plant lignans the technique of *in vitro* fermentation was used and the quantitative analysis of their metabolites ENL and END was performed by HPLC with coulometric electrode array detection. The enterolignan production from various berries ranged from 7.8 to 382.8 nmol/g as well as from vegetables – from 10.5 till 91.2 nmol/g. By comparing different kind of berries, the cloudberry, raspberry, and strawberry were the best enterolignan producers. Considering vegetables, potatoes produced the highest quantity of total enterolignans. Garlic, zucchini and broccoli were the other good producers of enterolignans in this product group. The quantitative relationship between NSP components and their associated lignan metabolites were determined. The results showed that there is a correlation between the particularities of fermented food matrices and the production of enterolignans. For berries, an intermediate correlation was found between the total NSP and ENL values. For vegetables, higher correlations between NSP and END were found.

1. Introduction

The International Agency on Research on Cancer (IARC) and the International Union of Nutritional Sciences (IUNS) noted that dietary fibre plays a very important role in human nutrition. Numerous epidemiological studies have shown that diets low in fat and rich in complex carbohydrates from vegetables, fruits and grains are associated with a decreased risk of chronic diseases (American Institute for Cancer Research, 2007: p. 506). International epidemiological comparisons have linked the semi-vegetarian diet in some Asian countries with reduced incidence of these diseases (i.e. the major hormone-dependent cancers, colon cancer, and coronary heart disease), indicating that some non-nutrient compounds in this diet may contribute to homeostasis and thus have a role in the maintenance of health. One of these non-nutrient groups of compounds are lignans, detected and identified in human body fluids. Lignans are diphenolic compounds in plant foods, which belong to the group of phytoestrogens. Their molecular weights and structures are similar to those of steroids, implying that they could be important dietary modulators of the human hormonal system (Adlercreutz, 1990). Plant lignans can be converted by intestinal bacteria into hormone-like compounds so-called enterolignans: enterodiol (END) and enterolactone (ENL). Lignans possess several biological activities, such as antioxidative and (anti)estrogenic activities, and may thus reduce the risk of certain cancers as well as cardiovascular diseases (Adlercreutz & Mazur, 1997; Adlercreutz et al., 1992; Arts & Hollman, 2005; Raffaelli, Hoikkala, Leppala, & Wähälä, 2002; Vanharanta, Voutilainen, Rissanen, Adlercreutz, & Salonen, 2003). Protective associations were reported for breast (McCann, Moysich, Freudenheim, Ambrosone, & Shields, 2002),

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ovarian (Mc Cann, Freudenheim, Marshall, & Graham, 2003), endometrial (Horn-Ross, John, Canchola, Stewart, & Lee, 2003) and thyroid (Horn-Ross, Hoggatt, & Lee, 2002) cancer.

The health effects of lignans depend on both the amount consumed and the bioavailability. Plant lignans have been detected and quantified in a large number of plant foods. Flaxseed and sesame seed are the richest known sources of these lignans, however this food is generally consumed in limited amounts. Other sources are whole-grain cereals, beans, berries, nuts and various seeds (especially sesam seed), vegetables, fruits and beverages (Horn-Ross et al., 2000; Mazur, 1998; Mazur & Adlercreutz, 1998; Meagher & Beecher, 2000). These data have been incorporated into several phytoestrogen databases (Horn-Ross et al., 2000; Milder, Arts, van de Putte, Venema, & Hollman, 2005; Valsta et al., 2003). Until now, data on the lignan content in berries, except lingonberries and red fruits such as strawberries, were not available (Mazur, Uehara, Wähälä, & Adlercreutz, 2000; Milder et al., 2005). Therefore, special attention has been paid in this study to less investigated plant products which could be rich sources of lignans.

Although numerous of lignans have been identified but it is not known which of them are converted to enterolignans (Smeds et al., 2006; Smeds et al., 2004). For about two decades only secoisolariciresinol and matairesinol were known to be precursors of enterolignans. Recently however it was shown that also pinoresinol and lariciresinol are efficient converted into enterolignans (Heinonen et al., 2001). Lignins which are structurally related to lignans may also be converted to enterolignans in rats (Begum et al., 2004). Thus enterolignans have several possible precursors. The metabolism of intestinal bacteria have also a profound effect on the conversion, enterohepatic circulation and bioavailability of lignans (Kilkkinen et al., 2001; Kleessen, Bezirtzoglou, & Mättö, 2000). Therefore to evaluate the health effects of the intake of lignans in a *in vitro* fermentation model using human fecal microbiota has a position in studies of the release and bioconversion of plant lignans (Aura et al., 2006).

Juntunen et al. (2000), and Jacobs, Pereira, Stumpf, Pins, and Adlercreutz (2002) have observed a positive correlation between total dietary fibre intake and lignan excretion. Because lignans are closely associated with the dietary fibre matrix of plants food it is possible that their composition might influence lignan availability; however there are no data available in this field. Furthermore, the composition of dietary fibre has been suspected to influence the growth of certain species of intestinal bacteria and thus may affect the enterolignan production. In view of this deficiency in the field of dietary fibre and lignan research, it is of importance to know more about the possible correlations between lignan bioconversion and the dietary fibre composition in plant products.

The aim of this work was to investigate enterolignan (ENL and END) production from their precursors by using *in vitro* fermentation with human fecal microbiota and their relationship with dietary fibre components in common plant products such as berries and vegetables.

2. Materials and methods

2.1. Materials

Because of scarce comprehensive data in the literature of dietary fibre and their associated bioactive compounds are available, the various plant foods common used in the human diet in Europe such as berries and vegetables were chosen for the experiments.

The quantities of the constituent sugars of non-starch polysaccharides (NSP), such as arabinose, xylose, mannose, glucose and galactose, and enterodiol (END) and enterolactone (ENL) production from their precursors were analyzed in berries – blackcurrant, blueberry, buckthorn, chokeberry, cloudberry, cranberry, crowberry, lingonberry, strawberry; vegetables – potato, garlic, zucchini broccoli, red paprika, red cabbage, pumpkin, onion, carrot, rape. Additionally flaxseed was investigated which according to literature, contains the highest quantity of lignans.

For analysis, samples with higher water content (berries and vegetables) have been freeze-dried and milled, and those with low water content (flaxseed) were milled as such.

2.2. In vitro fermentation of berries and vegetables

In vitro fermentation was performed according to a modified incubation method of Karppinen, Liukkonen, Aura, Forssell, and Poutanen (2000). The *in vitro* colon model assists in elucidation of the role of microbiota in the metabolic network of human digestive system and it helps in the identification of the crucial reactions.

A carbonate–phosphate buffer solution with trace elements was held in an anaerobic chamber for 2 days prior to fermentation. Faeces were collected from three healthy human volunteers, who suffered no digestive disease, and had not received antibiotics for at least 3 month. Freshly passed faeces were immediately taken in an anaerobic chamber, pooled, and homogenized with an equal weight of culture medium using a Warring blender. The slurry was diluted to 16.7% (v/v) with the culture medium, filtered through a 1 mm sieve, and used immediately as inoculums. 0.1 g of an analyzed food sample was weighed into 50 ml glass vials, and 10 ml of the inoculum was added and stored in a 30 °C anaerobic chamber. The vials were sealed with rubber stoppers and shaken in a 37 °C water bath for 24 h. The fermentation was stopped by plunging the vials into ice water, after which the vial contents were freeze-dried. Duplicate incubations were carried out for each sample. Also, duplicate blanks, containing only culture medium and inoculum, were incubated for 0 and 24 h.

2.3. Determination of enterolignans by HPLC with coulometric electrode array detection

Quantitative analyses of END and ENL were conducted using HPLC with the coulometric electrode array detection (CEAD) according to Heinonen et al. (2001). The HPLC system consisted of a pump model 580 (ESA, Chelmsford, USA) and an automatic injector model 540 (ESA, Chelmsford, USA). An intersil ODS-3 (GL Science Inc., Japan) column (3.0 × 150 mm, 3.3 μm, 9LI 500 10) in combination with precolumn Quick Relate C_{18} (Upchurch Scientific Inc., WA) and for detection a Coulochem Electrode Array Detector (ESA, Chelmsford, USA) equipped with an eight electrode cell was used.

The freeze-dried incubated samples were weighed (approximately 20 mg) and 500 μl of water and 10 μl of 6 M HCl was added. The samples were extracted twice with 5 ml of diethyl ether. The extracts were combined and evaporated to dryness under N_2 flow. The samples were dissolved in 500 μl MeOH and subsequent diluted with the mobile phase.

Pure standards of END and ENL used for quantitative analyses were obtained from Fluka Chemie (Buchs, Switzerland). Quantification was performed with the standard solutions of END (7.0–350 μg/l) and ENL (10.834–541.7 μg/l) dissolved in MeOH and diluted with mobile phase prior to HPLC analysis. The mobile phase consisted of 20% solution B (50 mM NaAc, pH 5/MeOH/ACN, 40/40/20, v/v/v) and 80% solution A (50 mM NaAc, pH 5/MeOH, 80/20, v/v).

Prepared sample extracts or standard solutions were injected, the compounds were separated (flow rate of 1.2 ml/min) on the reversed phase column and were detected at +180 mV (channel 1) till +720 mV (channel 8). The END and ENL were quantified using calibration curves and by evaluation of their quantities determined