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Tracking isoflavones in whole soy flour, soy muffins and the plasma of hypercholesterolaemic adults

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ABSTRACT

Information on changes in soy isoflavones during processing is needed to better understand the relationship between isoforms, food matrix, bioavailability and health. The abundance and transformation of isoflavones were tracked in soy flour, a baked soy muffin and plasma of hypercholesterolaemic adults (n = 142) who consumed muffins daily for 6 weeks. Isoflavones were identified and quantified in soy flour and muffins by HPLC-UV and in plasma by LC-MS/MS. Equol status was determined and used to assess the relationship between plasma isoflavones and dietary factors. Baking soy flour altered the relative proportion of isoflavone isoforms by promoting a conversion of malonyl- to β-glucosides in soy muffins ($P < 0.001$). Daily soy consumption resulted in >3-fold increase in plasma isoflavones with a doubling of dose ($P < 0.001$). Dietary factors did not correlate with plasma isoflavones. These findings may be used to guide the development of soy-based functional foods.

1. Introduction

Habitual soy consumers appear to benefit from lower coronary heart disease (CHD) risk, as indicated by healthy plasma lipid profiles and lower incidence of CHD (Zhang et al., 2003; Zhang et al., 2008). Soybeans contain many bioactive components, such as isoflavones, soluble fibre and short peptide sequences from the 7S globulin, which modulate serum lipids and have antioxidant and anti-inflammatory properties (Clair & Anthony, 2005). However, the cardiovascular benefits of soy are not consistently achieved in soy human intervention studies, particularly when isoflavones are extracted from the soy food matrix (Qin et al., 2013; Taku, Umegaki, Ishimi, & Watanabe, 2008). It is possible that these inconsistencies may be related to the heterogeneity of the soy products tested. Food processing alters the relative proportion of individual isoflavone isoforms found in different soy food products (Coward, Smith, Kirk, & Barnes, 1998), which influences the bioavailability and possibly the bio-efficacy of isoflavones (Cassidy et al., 2006).

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Abbreviations: CHD, coronary heart disease; CON, control; DWSF, de-fatted whole soy flour; EFSA, European Food Safety Authority; HDS, high-dose soy; LDS, low-dose soy; LPH, lactase-phlorizin hydrolase; NIH, National Institutes of Health; ODMA, O-desmethylangolensin

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Consequently, the available literature on soy and cardiovascular health is difficult to interpret; this presents a challenge for manufacturers seeking to develop soy-based functional foods.

The 3 principal isoflavones (daidzein, genistein, glycitein) exist in 4 chemical forms (aglycones, β-glucosides, acetylglucosides, malonylglucosides), giving rise to 12 isoforms (Villares, Rostagno, García-Lafuente, Guillamón, & Martínez, 2011). Soybeans contain predominantly malonylglucosides, but isoflavones in soy foods are typically a mixture of all 4 chemical forms, depending on the processing technique used. For example, during soymilk and tofu production, hot water extraction results in the hydrolysis of malonylglucosides, yielding β-glucoside isoflavones (Barnes et al., 2011). By contrast, toasting soy flour increases the proportion of acetylglucosides (Ghafoor, Al-Juhaimi, & Park, 2013) and fermentation, which promotes endogenous β-glucosidase activity, increases the proportion of isoflavone aglycones in soy foods (Inbaraj & Chen, 2013). Indeed, although soy isoflavones may exist as any of the 12 native isoforms, the dominant form in food depends on the food processing technique used.

In humans, isoflavones are extensively metabolized as they pass through the gastrointestinal tract, appearing in plasma and urine primarily as conjugated derivatives, isoflavone metabolites (equol and O-desmethylangolensin – ODMA) and to a lesser extent as free aglycones (Barnes et al., 2011). There is evidence to suggest that the bioavailability of isoflavones may be related to glucoside composition. An early study by Setchell et al. (2001) demonstrated the rapid absorption of daidzein and genistein compared with their β-glucoside isoforms, and more recently, Yerramsetty, Gallaher, and Ismail (2014) showed that β-glucosides appeared in plasma more rapidly and attained higher maximal plasma concentrations than malonylglucosides. Given these findings, it is plausible that discrepancies in soy clinical trial outcomes could be related to differences in isoform distribution among intervention products. The National Institutes of Health (NIH) and the European Food Safety Authority (EFSA) emphasize adequate characterization of soy intervention products as a key priority for the advancement of soy research (Klein et al., 2010; EFSA, 2010). Thus, it is important to obtain a complete description of isoflavones through food processing to guide development of soy products that are optimized for health.

The effect of processing on soy isoflavone distribution has been explored in fermented soymilk (Hati, Vij, Singh, & Mandal, 2015), fermented raw soy flour (Handa, Couto, Vicensoti, Georgetti, & Ida, 2014), germinated soy germ (Kim et al., 2013), steamed black soybean (Huang & Chou, 2008) and soy breads (Ahn-Jarvis, Riedl, Schwartz, & Vodovotz, 2013; Shao et al., 2011). However, the isoflavone distribution and bioavailability of isoflavones from a baked soy flour muffin has not been assessed. Further, it is unclear whether isoflavone bioavailability changes during prolonged consumption, which may impact long-term bioactivity (Williamson & Manach, 2005).

De-fatted whole soy flour (DWSF) is a by-product of soy processing and represents a potentially important ingredient for soy-based functional foods. The low density cholesterol (LDL)-lowering effect of a soy flour muffin was explored in a recent clinical trial, which showed no intervention effect (Padhi et al., 2015). Therefore, the aims of the present study were to track the abundance and transformation of soy isoflavones in DWSF and a baked soy muffin, and to determine whether there is a dose-dependent increase in plasma isoflavones in humans. The batch-to-batch variability in total isoflavone concentration from a sampling of soy muffins was also determined, and the relationship between plasma isoflavones and age, gender, BMI and diet was explored.

2. Materials and methods

2.1. Chemicals and standards

Isoflavone standards for aglycones (daidzein, genistein, glycitein) and β-glucosides (daidzin, genistin, glycitin) with >99% purity, and aqueous *Helix pomatia* digestive juice containing β-glucuronidase activity (EC 3.2.1.31; ≥100,000 U/mL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (Nanopure, Dubuque, IN, USA) was used to prepare all solutions. All solvents and other chemicals were of HPLC grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) or Caledon Laboratories Ltd. (Georgetown, ON, Canada). Aglycone stock solutions (200 μg/mL) were prepared by dissolving weighed portions of standards in HPLC-grade acetonitrile.

2.2. Sample preparation

2.2.1. Soy flour

Prolia® 200/70 DWSF (Cargill Ltd., Winnipeg, MB, Canada) was provided by Soy 20/20 (Guelph, ON, Canada). De-fatting was achieved by hexane-based solvent. Two batches of DWSF, each comprising a shipment of 4–6 bags (22.7 kg/bag), were used over the duration of the production schedule, as needed and came from the same cultivar of soybeans. Soy flour samples (~500 g) were stored in freezer-safe re-sealable Ziploc® plastic bags (Brantford, ON, Canada) that were covered in aluminium foil and stored at 4 °C until use. Of this, approximately 50 g was reserved for isoflavone analysis. A 15 g portion was transferred to a Nasco Whirl-Pak™ 2-oz sample bag (Fort Atkinson, WI, USA), dried using a FreeZone® Plus 12 L Cascade freeze dry system (Kansas City, MO, USA) and stored until use at –22 °C.

2.2.2. Soy muffins

Soy muffins were produced by the Department of Food Science at the University of Guelph (Guelph, ON, Canada) twice weekly between March 2012 and July 2013 in batches of 48, totalling 400–600 muffins per production day. After baking, muffins were cooled at room temperature, stored at –22 °C until completely frozen, then packaged and stored at –22 °C until use. In order of ingredient abundance, soy muffins contained: DWSF (24%), soft-wheat flour (9.4%), sugar (12.4%), canola oil (12.0%), Novation®4600 corn starch (4.3%), artificial vanilla and banana bread or lemon flavouring (1.4%), double-acting baking powder (1.1%), citric acid (0.2%), and salt (0.1%). A random sample of 2–3 muffins was reserved each production week and kept at –22 °C until analysis, from which 3 muffins from 7 production days were selected for isoflavone analysis. Muffins (n = 21) were defrosted and half discarded; the remaining halves were combined and crumbled by hand. Homogenized muffins were