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Isoflavones and soyasaponins in soy infant formulas in Brazil: Profile and estimated consumption

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ABSTRACT

In the present study we determine the contents of isoflavones and soyasaponins in seven soy-based infant formulas available in the Brazilian market to estimate the intake of these bioactive compounds by infants. The mean contents of isoflavones and soyasaponins were 65.9 mg/kg and 55.0 mg/100 g, respectively. β-Glycosylated isoflavones and soyasaponin B-I were the most abundant components in the analysed samples. The mean estimated intake of isoflavones by infants fed soy-based formulas was 0.8 mg/day/kg of body weight, which is twice that of Japanese adults. For soyasaponins, the mean estimated intake was 9.2 mg/day/kg of body weight, which is up to 6 times higher than the daily intake of saponins from beans by vegetarians. Considering the estimated intake of these bioactive compounds from soy-based formulas and the paucity of data regarding their bioavailability, the potential biological effects of isoflavones and soyasaponins in infants should not be overlooked and merits further investigation.

1. Introduction

Infant formulas are manufactured liquid or powder food products used to satisfy the nutritional needs of the infant if breastfeeding is impaired. When the infant presents conditions, such as galactosemia, lactose intolerance and Ig-E mediated cow milk allergy, or when the parents follow a vegetarian lifestyle, soy-based formulas are prescribed (Bhatia & Greer, 2008). In the USA, it is estimated that approximately 20% of infants fed with formulas use those that are soy-based (Bhatia & Greer, 2008). These products contain from 14% to 16% of soy protein isolate, together with other ingredients, such as corn syrup, vegetable oils, sucrose, vitamins and minerals (Bhatia & Greer, 2008; Genovese & Lajolo, 2002).

In the last decades, the implications of the consumption of soy-based foods on health have been extensively studied (Adlercreutz et al., 1991; Ganry, 2002; Reynolds et al., 2006). The interest in soybeans and soy-based foods is due to geographical epidemiology indicating that the high consumption of soy-based foods in Asian populations was associated with a reduced prevalence of various chronic diseases, such as breast and prostate cancers, cardiovascular diseases and osteoporosis (Adlercreutz et al., 1991; Ganry, 2002; Reynolds et al., 2006). These beneficial effects are related to the bioactive compounds naturally present in soybeans, mainly isoflavones and soyasaponins (Genovese & Lajolo, 2002). Since isoflavones have shown anti-rotavirus activity at the concentrations found in soy-based formulas (Andres, Donovan, Kuhlenschmidt, & Kuhlenschmidt, 2007), infants fed these formulas might be protected from viral infection and related acute gastroenteritis.

Although several studies measured isoflavones in infant formulas (Garrett, Lee, Friar, & Morgan, 1999; Genovese & Lajolo, 2002; Irvine, Shand, Fitzpatrick, & Alexander, 1998; Knight, Eden, Huang, & Waring, 1998; Kuo & Ding, 2004; Murphy, Song, Buseman, & Barua, 1997; Setchell, Zimmer-Nechemias, Cai, & Heubi, 1997), the only study investigating the contents of both isoflavones and soyasaponins in soy-based infant formulas was conducted in samples acquired in the US market (Murphy, Hu, Barua, & Hauck, 2008; Murphy et al., 1997). For soy infant formulas sold in the Brazilian market, only data regarding isoflavones contents are available (Genovese & Lajolo, 2002). Since these studies have shown that soy-based infant formulas are very rich in both classes of bioactive compounds, some concerns related to their potential biological effects on infants have been raised (Kang, Badger, Ronis, & Wu, 2010; Murphy et al., 1997). Even though soyasaponins are generally considered to have low bioavailability (Hu, Reddy, Hendrich, & Murphy, 2004), there is a need for a more comprehensive description of isoflavones and soyasaponins composition of infant formulas.

Considering the paucity of data on the composition of bioactive compounds in soy-based infant formulas, especially soyasaponins,

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the aim of this work was to determine the contents of isoflavones and soyasaponins in soy-based infant formulas available in the Brazilian market to estimate the intake of these bioactive compounds by infants.

2. Materials and methods

2.1. Standards and chemicals

Daidzin, glicitin, genistin, daidzein, glicitein, genistein, soyasapogenol B, soyasaponin B-I, soyasaponin B-II and soyasaponin B-III standards were purchased from Apin Chemicals Limited® (Abingdon, UK). All solvents were HPLC grade from Tedia (Fairfield, OH, USA). HPLC grade water was used throughout the experiments (Milli-Q system, Millipore, Bedford, MA, USA).

2.2. Samples

For the development and validation of the analytical method for the simultaneous analysis of isoflavones and soyasaponins in soy-based foods, a sample of soy fibre (Yoki®) was acquired in a local supermarket in Rio de Janeiro, Brazil. The seven soy-based infant formula samples available in the Brazilian market were purchased in drugstores located in Rio de Janeiro and São Paulo: AlergoMed (comidaMed, Germany), Nan® Soy (Nestlé Infant Nutrition, USA), Nursoy® (Wyeth Nutrition®, Ireland), Aptamil 1 and Aptamil 2 (Danone Nutrition Baby, Argentina) and Isomil® 1 and Isomil® 2 (Abbott Laboratories, Netherlands). Three lots of each brand were obtained and pooled for further analyses.

2.3. Analysis of protein content

The protein content of the soy-based infant formulas was determined in duplicate using Kjeldahl method for quantification of total organic nitrogen using the conversion factor of 6.25 (AOAC, 2000).

2.4. Analysis of isoflavones and soyasaponins by HPLC-DAD–MS

2.4.1. In-house validation

Linearity was evaluated using triplicates of six-points standard calibration curves with concentrations ranging between 0.1 to 5.0 μg/ml and 0.5 to 20.0 μg/ml for isoflavones and soyasaponins, respectively. In-house accuracy was assessed by a single-level recovery experiment. This test was performed in triplicate by analysing non-spiked and spiked (500 μg of soyasaponin B-I, 200 μg of genistin and 100 μg of genistein in 0.1 g of soy fibre) samples. The recovery for each analyte was calculated from the content found in the fortified sample in relation to the expected amount, subtracting the non-spiked sample content. Precision (repeatability) was determined for each analyte as the coefficient of variation from the three replicates analysed in the recovery experiment. For isoflavones, which were quantified using the diode array detector (DAD), the limits of detection (LOD) and of quantification (LOQ) were calculated using the following equations: LOD = 3.3 (σ/S); LOQ = 10 (σ/S), where σ is the standard deviation of the response of a blank (calculated from the linear coefficient of three calibration curves) and S is the mean angular coefficient of three calibration curves. For soyasaponins, which were quantified using the mass spectrometer (MS), LOD and LOQ were calculated as the concentrations equivalent to three and ten times the signal-to-noise ratio (S/N), respectively, of the lowest concentration calibration curve point. S/N ratios were calculated by LCMSolutions software, using a built-in tool. The employment of S/N ratio is preferable in comparison to calibration curves parameters for LOD and LOQ calculations, as the latter approach tends to underestimate these values.

2.4.2. Sample preparation

Samples were extracted in triplicate according to a modification of the methods of Genovese and Lajolo (2002), Fang, Yu, and Badger (2004) and Rostagno, Palma, and Barroso (2005). Briefly, 0.1 g of sample and 4 ml of aqueous methanol 80% was extracted in an Ultra-Turrax extractor (IKA®, T18 Basic) at 22,000 rpm for one min. The obtained extract was centrifuged for 10 min at 3000 rpm, the supernatant collected and the residue re-extracted twice following the same procedure. Next, supernatants were combined and placed in an ultrasound bath for 15 min. The organic solvent was removed with the aid of a rota-evaporator at 170 rpm (Büchi©, 131 EL, Switzerland). The concentrated extract was introduced into a Strata-X solid phase extraction (SPE) cartridge (3 ml, 200 mg, Phenomenex®, CA, USA), previously conditioned with 10 ml of methanol and 10 ml of water. The impurities contained in the extract were eluted with 10 ml of water and the cartridge was vacuum-dried for 15 min. The analytes were eluted with5 ml of methanol and the final extract was properly diluted with water prior to HPLC-DAD–MS analysis.

2.4.3. Chromatographic conditions

The LC system (Shimadzu, Kyoto, Japan) comprised a LC-10AD*vp* quaternary pump, a CTO-10AS*vp* column oven, an 8125 manual injector (Rheodyne) with a 20 μL loop and a SPD-M10A*vp* DAD. This LC system was coupled to a LC–MS 2010 MS (Shimadzu, Kyoto, Japan) equipped with an electrospray ion source. Chromatographic separations were achieved using a Kromasil® C18 column (150 × 2.1 mm, 5 μm, 100 Å, AkzoNobel, Bohus, Sweden) maintained at a constant temperature of 40 °C. The LC two-phase mobile system consisted of a gradient of water (eluent A) and acetonitrile (eluent B), both added with 0.3% formic acid, with a flow rate of 0.3 ml/min. Prior to injection, the column was equilibrated with 15% B. After injection of sample, this proportion was modified to 23% B in 1 min, kept constant until 23 min and increased to 50% B until the end of the 35 min run. Between injections, 20 min intervals were used to re-equilibrate the column with 15% B.

Isoflavones were monitored by DAD between 190 and 370 nm and soyasaponins were monitored by MS using positive ionisation, with a nebuliser gas (N_2) flow of 3.0 L/min, operated in the single ion monitoring (SIM) mode to detect pseudomolecular ions. Identification of compounds was performed by comparison with retention time and molecular weight of the respective standard. Malonylglycosilated and acetylglycosilated isoflavones, for which commercial standards were unavailable, were identified by their pseudomolecular ions in the MS.

Quantification was performed by external standardisation. Isoflavones were quantified by their DAD peak areas (250 nm). The contents of malonylglycosilated and acetylglycosilated isoflavones were determined from the calibration curve of the corresponding β-glycosylated isoflavone, correcting for differences in molecular weight. Soyasaponins (B-I, B-II and B-III) and soyasapogenol were quantified by their MS fragment ions, m/z 423 and m/z 223, respectively. Although soyasaponins B-II and B-III isolated standards were available, these compounds were quantified together, as it was not possible to chromatographically separate these compounds. Data were acquired by LCMSsolution software (Shimadzu Corp., version 2.00, 2000) for the mass spectrometer. Recovery values were taken into consideration for calculating the contents of these compounds in the samples.

2.5. Estimation of daily intake of isoflavones and soyasaponins

The daily intake of soy isoflavones and soyasaponins according to infant's age, expressed per kilogram of body weight, was estimated from the total content of these classes of bioactive