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Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population

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ABSTRACT

5-Hydroxymethylfurfural (HMF) is naturally formed during food processing or cooking activities, giving its ubiquity in the Western diet. HMF could be metabolised to 5-sulfooxymethylfurfural making HMF potentially harmful in an extent unknown at present. Coffee is the main exposure source. Occurrence of HMF, 5-hydroxymethyl-2-furoic acid (HMFA) and 2-furoic acid (FA) were measured in commercial ground coffee and soluble coffee marketed in Spain. Levels of 110, 625, 1734, 2480 mg HMF/kg were obtained for natural, blend, torrefacto and soluble coffee, respectively, giving four classes significantly different. Soluble coffee showed the largest variability in HMF. Levels of HMFA and FA did not change significantly being about 600 mg/kg. Dietary exposure to HMF coffee to consumption in the total Spanish population was estimated to be 8.57 mg/day by using a deterministic approach. However, median level was recalculated to 5.26 mg HMF/day when specific contribution of each type of ground and soluble coffee in the consumption habits was considered. Resultant value is above of the threshold of concern (1600 μg HMF/day, mTAMDI). A level of 8.57 mg HMF/day in persons with high consumption habits (95th percentile) was calculated for risk assessment.

1. Introduction

During heat processing of foods, a complex set of chemical and biochemical reactions are taking place which will definitively affect the final properties of food, including organoleptical, textural, nutritional and health-related properties (O'Brien and Morrissey, 1989). It is known that potential toxic compounds are formed during the extent of the browning reactions in foods such as heterocyclic amines or acrylamide. But toxicology and genotoxicity of other substances like furfurals and related substances which are formed in large quantities from both sugar dehydratation and Maillard reaction are still under evaluation (EFSA, 2005; NTP, 2008).

Hydroxymethylfurfural (HMF) has been shown to be bioactivated in vitro to 5-sulfooxymethylfurfural (SMF), through sulfonation of its allylic hydroxyl functional group, catalysed by sulfotransferases (SULTs). In the resulting ester, the sulphate is a good leaving group, thus producing a highly electrophilic allyl carbocation, which could be stabilized by distribution of charges on the furan ring (Surh and Tannenbaum, 1994). SMF has been demonstrated to induce genotoxic and mutagenic effects to bacterial and mammalian cells (Glatt and Sommer, 2006). The subsequent interaction of this reactive intermediate with critical cellular nucleophiles (i.e., DNA, RNA, and proteins) may result in structural damages to these macromolecules, thereby causing toxicity and mutagenicity, although its occurrence in vivo has not been confirmed yet (Glatt and Sommer, 2006; Janzowski et al., 2000). HMF has been identified as initiator and promoter of colon cancer in rats (Zhang et al., 1993) and nephrotoxicity (Bakhiya et al., 2009). But extrapolation to humans could be more dramatic since humans express SULT in extrahepatic tissues more extensively than rats and may therefore be more sensitive to HMF (Teubner et al., 2007).

In foods, HMF and related substances formed from dehydratation reactions of reducing sugars are summarised in Fig. 1. In addition, HMF and furfural (FURF) can react further by decarboxylation (a), oxidation (b), dehydration (c), and reduction (d) reactions to form different intermediates, apart from polycondensation reactions to form final Maillard reaction products such as melanoidins (Antal et al., 1990; Feather and Harris, 1973; Theander, 1981; Kroh, 1994). Extensive dehydration of HMF will give levulinic acid and formic acid. HMFA and furan-2,5-dicarboxylic acid (FDCA) are formed from oxidation of HMF where furoic acid FA and hydroxyfuroic acid (HFA) are formed from oxidation of furfural (FURF).

HMF is widespread distributed in the western diets since it is largely formed in many processed foods and domestic cooking, see Morales (2009) for a review. In a recent study on dietary exposure to HMF from Norwegian population by a 24 h dietary recall, coffee was identified as the most important source of HMF (63%),

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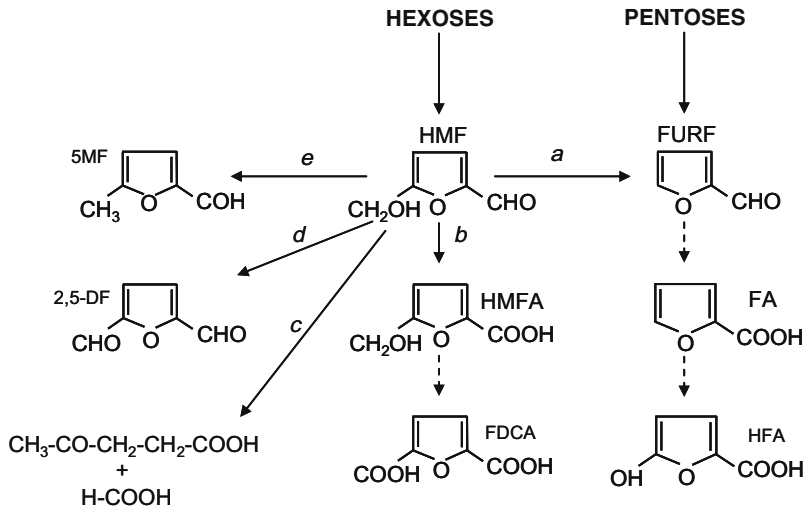

Fig. 1. Routes of formation and degradation of hydroxymethylfurfural (HMF) and related substances by decarboxylation (a), oxidation (b), dehydratation (c) and reduction (d). Hydroxymethylfuroic acid (HMFA), 2-furoic acid (FA), furfural (FURF), 2,5-dicarboxilic acid (2,5DF), 5-methylfurfural (5MF), hydroxyfuroic acid (HFA).

both because of the high levels of HMF in coffee and because the high consumption (Husøy et al., 2008). It was estimated the HMF intake at 5.56 mg/day. Then, given the popularity of coffee in western diet it is relevant to obtain a picture of the current situation in other EU countries with different habits of consumption such as Spain. In this sense, Rufián-Henares and de la Cueva (2008) published an assessment of HMF intake in the Spanish diet with a mean of 10 mg/day from total consumption databases and the contribution of coffee was 50.43% to the total dietary exposure. However, this study could be biased since HMF data from some food items were extrapolated from studies in other countries and did not match the situation in Spain. Previously, in a study on a cohort of Spanish adolescent it was estimated a mean HMF intake of 5.08 mg/day where cereals and related food commodities were the main contributors (Delgado-Andrade et al., 2007).

Besides the toxicological relevance of HMF and related substances, it is mandatory to evidence and to compare the intake with other EU state members before to further decision on risk management. In addition, it is necessary to supply the tools to assess the monitoring progresses which are necessary in minimisation strategies applied at industrial level. Since, coffee account significantly to the daily intake of HMF (Husøy et al., 2008) it is necessary to get more insight on the occurrence and sources of variability. This paper is aimed to contribute to a better understanding of sources and levels of HMF intake from coffee consumption in the Spanish population taking into account the partial contribution of ground and soluble coffee.

This investigation presents a survey on commercial roasted ground coffee (natural, torrefacto, and blend) and soluble coffee marketed in Spain and its contribution to daily intake of HMF based on a deterministic exposure model. At the same time this investigation will provide scientific data basis to afford a precise study in a future by Spanish Administration bodies.

2. Materials and methods

2.1. Chemicals

All chemical used were of an analytical grade and were obtained from Sigma–Aldrich (St. Louis, MO, USA), unless mentioned otherwise. 5-Hydroxymethyl-2-furoic acid (HMFA, CAS# 6338-41-6) was purchased from Matrix Scientific (Columbia, USA), HMF (5-hydroxymethylfurfural, CAS# 67-47-0), FURF (furfural, CAS# 98-01-1) and FA (2-furoic acid, CAS# 88-14-2) from Sigma–Aldrich (St. Louis, MO, USA). Tetramethyl-ammonium hydrogen sulphate (THAMS) was obtained form Fluka (Buchs, Switzerland).

2.2. Samples

Experiments were conducted with a series of commercial roasted coffee (35 brands from 21 producers) and soluble coffee (19 brands from 11 producers) randomly purchased on different supermarkets in June 2008. Samples were distributed according to their label, being natural, torrefacto and blend (natural with variable proportions of torrefacto roasted coffee). Samples (300 g) were grinded, if necessary, and a portion (100 g) was distributed in two containers and stored under vacuum and light protected at 4 °C until analysis.

2.3. RP-HPLC–UV determination of HMF and furfural

HMF and furfural determinations in coffee were determined by reversed-phase chromatography. Ground sample (500 mg) was suspended in 5 mL of 0.1% formic acid solution in a 10 mL centrifuge tube. The tube was shaken vigorously for 1 min and clarified with 250 μL each of potassium ferrocyanide (15% w/v) and zinc acetate (30% w/v) solutions. The resulting mixture was centrifuged at 4500*g* for 10 min at 4 °C. The supernatant was collected in a 10 mL volumetric flask and two further extractions were performed using 2 mL of 0.1% formic acid solution. The supernatants were pooled, filtered (0.45 μm) and diluted 10-fold for HPLC analysis. The quantification of HMF and FURF was conducted with a Shimadzu HPLC system (Kyoto, Japan) equipped with a LC-20AD pump, a SIL-10ADvp autosampler, a CTO-10ASVP oven, and a DAD (SPD-M20A). The chromatographic separations were performed on a Mediterranean-Sea ODS2 column (250 × 4.0 mm, 5 μm, Teknokroma, Barcelona, Spain) termostatised at 32 °C at a flow rate of 1 mL/min. The mobile phase was a mixture of acetonitrile in 0.1% formic acid (5% v/v). The UV detector was set at 280 nm and 10 μL of the extract was injected. HMF and FURF were quantified using the external standard method within the range 0.1–10 mg/L, and 1–10 mg/L for HMF and FURF respectively. Sample reporting levels of HMF or FURF outside the calibration range were additionally diluted 10-fold in mobile phase. LOQ for HMF and FURF were 4 mg/kg and 20 mg/kg respectively. Recovery of HMF and FURF in spiked (150 μg/kg) coffee samples was 98.6% and 92.1% for HMF and FURF, respectively.

2.4. IP-HPLC–UV determination of HMFA and FA

Procedure was adapted from Murkovic and Bornik (2007). Ground sample (500 mg) was suspended in 5 mL of 0.1% formic acid solution in a 10 mL centrifuge tube and was shaken vigorously for 1 min. Solution was clarified with 250 μL each of potassium ferrocyanide (15% w/v) and zinc acetate (30% w/v) solutions, and centrifuged at 4500*g* for 10 min at 4 °C. The supernatant was collected in a 10 mL volumetric flask and two further extractions were performed using 2 mL of 0.1% formic acid solution. After that, supernatants were pooled. Supernatant (0.5 mL) was loaded onto a pre-conditioned SPE cartridge (SEP-PAK plus C18, Waters, Milford, MA, USA) and eluent was discharged. Ten milliliter of a 10% methanol solution containing 5 mM of TMAHS was loaded and eluent was collected after discard the first eight drops. The analyses of HMFA and FA were carried out on a liquid chromatograph Shimadzu HPLC system (Kyoto, Japan) previously described. The separation was carried out on a Mediterranean-Sea ODS2 column (250 × 4.0 mm, 5 μm, Teknokroma, Barcelona, Spain). HMF and HMFA were eluted with a mixture of water (90%) and methanol (10%) containing 5 mM TMAHS as ion-pairing reagent for the carboxylic acid. The flow was 1 mL/min and the injection volume 20 μL. HMFA