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Estimation of hydroxymethylfurfural availability in breakfast cereals. Studies in Caco-2 cells

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Abstract

The transport and availability of hydroxymethylfurfural (HMF), an intermediary product of the Maillard reaction, was investigated in the Caco-2 cell line after *in vitro* gastrointestinal digestion. The study was carried out at two levels; (a) an HMF-spiked culture medium, and (b) digested commercial breakfast cereals (BC). In both assays, the higher the amount of HMF offered to the cells, the higher the absolute value of transported HMF. However, HMF availability and transport are not directly proportional to the initial HMF content since HMF is partly retained in the non-soluble fraction after digestion. In addition, HMF is degraded to some extent during the gastrointestinal digestion of both HMF-spiked cell medium and BC. Average HMF availability from three commercial breakfast cereals was 9.1% (4.98–12.99%). Variations in HMF availability may be related to the particular composition of each BC, where fibre could play an important role. On the other hand, possible metabolization into the cell should also be considered.

Keywords: Hydroxymethylfurfural; Breakfast cereals; *In vitro* digestion; Caco-2 cells; Availability

1. Introduction

During the heat treatment of foods in home cooking or in industrial processing, the reaction between amino acids and reducing sugars, known as non-enzymatic browning or the Maillard reaction, takes place, causing changes in the colour, flavour, functional properties, and nutritional value of the food (Friedman, 1996). Hydroxymethylfurfural (HMF) is an intermediate in the Maillard reaction sequence (Berg and Van Boekel, 1994), also formed by the caramelisation of sugars at high temperature (Kroh, 1994). While the Maillard reaction is favoured in systems with intermediate moisture content, temperature over 50 °C and pH 4–7, caramelisation requires more vigorous conditions, such as temperatures higher than 120 °C, $3 < pH > 9$ and low water activity (Ramírez-Jiménez et al., 2001).

In an analytical screening of nearly 500 food samples, fairly high HMF concentrations, exceeding 1 g/kg, have been found in products such as juice, dried fruits or caramel (Bachmann et al., 1997). Thus, HMF, among other Maillard reaction products (MRP), is regularly consumed in our daily diet (milk, bakery products, caramel, breakfast cereals, coffee, beer, chocolate, etc.), however, very little has been elucidated about its metabolic transit and bioavailability (Faist and Erbersdobler, 2001). It is not clear whether human exposure to HMF represents a potential health risk, since *in vitro* studies on genotoxicity/mutagenicity have given controversial results. Experiences in rats at high HMF concentrations have shown cytotoxic effects, causing irritation to eyes, respiratory tract, skin and mucosas with an oral LD_{50} of 3.1 g/kg of rat body weight (Ulbricht et al., 1984). Tumorigenic activities has also been associated with this compound, which acts as an initiator and a promoter of preneoplasic lesions, such as the colonic aberrant crypt (Archer et al., 1992; Bruce et al., 1993) or skin papillomas (Surh et al., 1994).

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Although the mechanisms of the toxic activity of HMF remain unclear, it has been proposed that it is metabolically activated through the sulfonation of its allylic hydroxyl functional group, which can take place in the liver by hepatic sulfotransferase. The electrophilic intermediate obtained, 5-sulfooxymethylfurfural (SMF), can interact with critical cellular nucleophiles (e.g. DNA, RNA and proteins) resulting in structural damage which can lead to toxicity and mutagenicity (Surh et al., 1994; Surh and Tannenbaum, 1994). In support of this idea, Lee et al. (1995) have found a strong direct mutagenicity of chemically synthesized SMF in Salmonella typhimurium TA104. They also demonstrated that the mutagenicity of HMF was markedly decreased by sulfotransferase inhibitors.

HMF can also be transformed into a strong mutagen when it is metabolized via allylic chlorination, producing 5-chloromethylfurfural (CMF), which has been shown to be much more mutagenic in *S. typhimurium* than is SMF. However, there is no clear evidence of its formation in biological systems. The place where chlorination could occur is in the gastric juice, where the presence of chloride ion enables the formation of CMF as a product of the gastric digestion of foods (Surh and Tannenbaum, 1994).

Assays on the toxicological effects of HMF have been mainly carried out *in vitro* or in animals after topic or parenteral administration. However, although absorption through the intestinal epithelium after the digestive process is a prerequisite for any *in vivo* health effect, studies of HMF absorption are still lacking.

Thus, the aim of the present study was to examine HMF availability in biological systems using a human intestinal cell culture model, the Caco-2 cell line. Firstly, HMF transport across the cell monolayers was investigated, adding HMF to the culture medium in a range of concentrations commonly found in foodstuffs. After having established the absorption, HMF availability from *in vitro* digested breakfast cereals was studied.

2. Materials and methods

2.1. Chemicals

All chemicals for rp-HPLC determinations were from Merck (Darmstadt, Germany) unless mentioned otherwise. HMF was also from Merck (Darmstadt, Germany). All cell culture media, cell culture grade chemicals, and those for *in vitro* digestion were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Culture flasks were purchased from Corning Costar (Cambridge, MA).

2.2. Samples

HMF-spiked culture medium. Solutions of cell culture medium containing three levels of HMF were prepared (13, 65 and 130 mg l^{-1}) and used in the cell assay.

Breakfast cereals. Three different breakfast cereals (A, B and C), purchased from the supermarket, were selected on the basis of their different flour composition. The total content of each package was powdered in a grinder, homogenized and stored in polyethylene containers under vacuum at 4 °C until analyzed. The carbohydrate, fat and dietary fibre contents, as declared by the manufacturer, are stated in Table 1. *In vitro* gastrointestinal digestion (described below) was applied to the breakfast cereals before being used in the Caco-2 cell assays.

Table 1
Composition of breakfast cereals

Breakfast cereal	Raw material	Carbohydrates[a] (%)	Protein[b] (%)	Fat[a] (%)	Fibre[a] (%)
A	Rice	85.0	4.70	2.5	2.5
B	Maize	80.8	7.56	0.8	1.5
C	Wheat	46.0	14.5	3.5	28.0

[a] Values declared by manufacturer.
[b] Analyzed by the AOAC 992.15 (1995) procedure.

2.3. Protein determination

Protein was analyzed in a LECO model FP-2000 (Leco Instruments, Madrid, Spain) following the AOAC 992.15 procedure (AOAC, 1995). Data are expressed as g of protein/100 g of product and are shown in Table 1.

2.4. In vitro digestion of breakfast cereals

The technique of Miller et al. (Miller et al., 1981), modified to our requirements, was followed. It comprised two stages: gastric digestion and intestinal digestion. Shortly before use, 0.4 g of pepsin was dissolved in 2.5 ml of 0.1 M HCl. For intestinal digestion, 0.1 g of pancreatin and 0.125 g of bile salts were dissolved in 25 ml of 0.1 M $NaHCO_3$. One gram of each breakfast cereal was suspended in 10 ml of milli-Q water. pH was adjusted to 2 with HCl 6 N, a pepsin solution was added at a proportion of 0.05 g of pepsin/g of sample, and samples were incubated at 37 °C in a shaking water bath at 110 oscillations/min for 2 h for the gastric digestion. For the intestinal digestion, the pH of the digest was raised to pH 6 with 1 M $NaHCO_3$ dropwise, and 2.50 ml of pancreatin + bile salts mixture was added. The pH was then adjusted to pH 7.5 with1 M $NaHCO_3$, and samples were incubated at 37 °C at 110 oscillations/min for 2 h.

After gastrointestinal digestion, the digestive enzymes were inactivated by heat treatment for 4 min at 100 °C in a polyethylenglycol bath. The samples were then cooled by immersion in an ice bath and centrifuged at 3200*g* for 60 min at 4 °C (CS-6R centrifuge, Beckman) to separate soluble and non-soluble fractions. The supernatants were carefully separated and the percentages of HMF in the soluble fraction were calculated from the initial breakfast cereal concentration by rp-HPLC.

2.5. Cells

Cell culture. Caco-2 cells were purchased from the European Collection of Cell Cultures (ECACC) at passage 20 and used in experiments at passages 22–30. The cells were grown in 75-cm^2 plastic flasks containing high-glucose Dulbecco's modified minimal essential medium (DMEM), with heat-inactivated fetal bovine serum (15%), $NaHCO_3$ (3.7 g/L), non-essential amino acids (1%), HEPES (15 mM), bovine insulin (0.1 UI ml^{-1}), and 1% antibiotic–antimycotic solution. The cells were maintained at 37 °C in an incubator in an atmosphere of air/CO_2 (95:5) at 90% humidity, and the medium was changed every 2 days.

Trypsinisation and seeding of cells were performed as described elsewhere (Navarro et al., 2000). At 80% confluency, the cells were collected and seeded in the insert of bicameral chambers (Transwell, 24-mm diameter, 4.7-cm^2 area, 3-μm pore size, Costar) at a density of 90,000 cells/cm^2. They were maintained with 2.5 ml of medium in the well (basolateral cell side) and 1.5 ml of medium in the insert (apical cell side). The medium was changed every second day and the day before the cultures were used for the transport experiments. The development of functional tight junctions during differentiation of the Caco-2 cells was monitored by determining transepithelial electrical resistance (TEER) as described elsewhere