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Influence of different soluble dietary fibers on the bioaccessibility of the minor *Fusarium* mycotoxin beauvericin

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ABSTRACT

Beauvericin (BEA) is a bioactive compound produced by the secondary metabolism of several *Fusarium* strains and is known to have various biological activities.

This study investigated the bioaccessibility of the BEA tested in concentrations of 5 and 25 mg/L, in a model solution and in wheat crispy breads elaborated with different natural binding compounds as the soluble alimentary dietary fibers β-1,3 glucan, chitosan low molecular weight (L.M.W.), chitosan medium molecular weight (M.M.W.), fructooligosaccharides (FOS), galattomannan, inulin and pectin, added at concentrations of 1% and 5%. The bioaccessibility was determinated by employing a simulated gastrointestinal digestion that simulates the physiologic conditions of the digestive tract until the colonic compartment. The determination of BEA in the intestinal fluids was carried out by liquid chromatography–mass spectrometry detection (LC–MS). The mean BEA bioaccessibility data in the model solutions ranged from 31.8% of the samples treated with only the duodenal digestion until 54.0% of the samples processed including the colonic fermentation, whereas in the alimentary system composed by the wheat crispy breads produced with different fiber concentration the duodenal and the duodenal + colonic BEA bioaccessibility resulted in 1.9% and 27.0% respectively.

1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide consisting of an alternating sequence of three D-a-hydroxy-isovaleryl and three *N*-methyl-L-phenylalanyl groups. It was originally isolated from *Beauveria bassiana* (Hamill et al., 1969) and has been detected since then in various fungal species, including *Fusarium* spp., a common contaminant of cereals and products composed by cereals (Jestoi, 2008).

The potential toxic role of BEA is exemplified by results from *in vitro* studies using cell lines. For instance, BEA induces significant cell deaths in insect, murine, and human tumor cell lines (Caló et al., 2004; Jow et al., 2004; Fornelli et al., 2004; Ferrer et al., 2009; Dornetshuber et al., 2009). Furthermore, BEA is a potent and specific cholesterol acyltransferase inhibitor in rat liver microsomes (Tomoda et al., 1992). In mammalian cell lines, cell deaths caused by BEA have been suggested to involve a Ca^{2+} dependent pathway, in which BEA induces a significant increase in intracellular Ca^{2+} concentration that leads to cell death as a result of a combination of both apoptosis and necrosis (Jow et al., 2004; Lin et al., 2005).

BEA has also been found as a natural contaminant of maize from Poland, Italy, USA, South Africa, Switzerland and Slovakia; feed samples from USA; rye from Finland; and oats, wheat and barley from Norway and Finland (Jestoi, 2008). Logrieco et al. (1993) reported high levels of BEA up to 60 mg/kg in maize from Poland, while Ritieni et al. (1997) reported high levels of BEA up to 520 mg/kg in maize from Italy. Recently, Meca et al. (2010b) have reported the contamination of cereals available in the Spanish market with BEA and levels ranged from 0.51 to 11.78 mg/kg.

In the analysis of the risk evaluation related to the human health, food ingestion is considered to be one of the important routes of exposure of many contaminants (Carolien et al., 2005).

To achieve any effect in a specific tissue or organ, the mycotoxins must be available, which refers to the compound's tendency to be extracted from the food matrix, and they must then be absorbed from the gut via the intestinal cells (Fernández-García et al., 2009).

The term bioaccessibility has been defined as the fraction of a bioactive compound present in a food matrix that passes unmodified complex of the biochemical reactions relationated to the gastrointestinal digestion and thus becomes available for intestinal absorption (Fernández-García et al., 2009).

Studies on animals and humans show that oral bioaccessibility of some bioactive compounds present in food can be significatively modified depending on the food source. Nowadays, there is no data in the literature regarding the bioaccessibility of the minor *Fusarium* mycotoxin BEA, but the study of the bioaccessibility of other mycotoxins has been evaluated by many authors. In particular,

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Avantaggiato et al. (2003, 2004) studied the bioaccessibility of zearalenone (ZEA), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), ochratoxin A (OTA), deoxynivalenol (DON) and aflatoxin B_1 (AFB_1) present in feed enriched with adsorbent materials, as activated carbons and others, that have the properties to adsorb the mycotoxins, and so reduce the presence of these compounds in the gastrointestinal tract, utilizing a laboratory system that simulated the metabolic processes of the gastrointestinal tract of healthy pigs.

In addition, Avantaggiato et al. (2007) evaluated the influence of the carbon/aluminosilicate based product added in the production of feeds, on the reduction of the bioaccessibility of FB_1, FB_2, OTA, DON and ZEA, demonstrating that the employment of the adsorbent materials can prevent the individual and combined adverse effects of some *Fusarium* mycotoxins in animals.

Motta and Scott (2009) studied the percentage of total bound fumonisin B_1 (TB-FB_1), formed for the reaction of the FB_1 with some components present in food as amino acids or sugars, in corn flakes applying an *in vitro* gastro-digestion model.

As demonstrated by the studies previously mentioned, the employment of the adsorbent materials in the technology of the production of feeds has the property to reduce the exposure risk to the *Fusarium* mycotoxins in animals fed with these bioactive feeds. These typologies of capturing mycotoxin materials are thus applicable to the animal nutrition, but, in nature, there are products that are capable of capturing some toxic components present in food, like the alimentary fiber.

According to the American Association of Cereal Chemists (AACC), dietary fiber is defined as the edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine (Nair et al., 2010).

Dietary fibers may be classified as water-soluble fibers, that are represented principally by pectins, β-glucans, glucomannans, fructooligosaccharides (FOS), galactooligosaccharides (GOT), and inulin, and water insoluble fibers represented mainly by cellulose, lignin, and hemicelluloses present mainly in wheat, most grain products, and vegetables (Nair et al., 2010). In particular, the soluble fibers are important for several biological and technological activities such as: prebiotic, glycemic index reducers, fat, protein and carbohydrate replacers, reducer in cholesterol and triglycerides absorption, etc. (Min et al., 2010; Cugnet-Anceau et al., 2010;Pereira et al., 2010; Nair et al., 2010; Rodrigues et al., 2011). There are only two publications relating mycotoxins to the dietary fiber, which are the studies by Meissonnier et al. (2009) and Rabassa et al. (2010). The first one demonstrated that the supplementation of an animal diet with glucomannan protects the same against immunotoxicity caused by AFB_1, and T-2 toxin during a vaccinal protocol. Rabassa et al. (2010) reported that the dietary fiber glucomannan added as sorbent material in a ruminant's diet, reduced possible liver aggression caused by AFB_1.

Considering all these aspects, the aim of the study was to evaluate different soluble fibers such as β-1,3 glucan, chitosan low M.W., chitosan medium M.W., fructooligosaccharides (FOS), galattomannan, inulin and pectin used as sorbent material, that influence the duodenal and colonic bioaccessibility *in vitro* of the minor *Fusarium* mycotoxin BEA: (a) in model solutions composed by aqueous solutions of each fiber (1% and 5% (w/v)) contaminated with 5 and 25 mg/L of BEA, (b) in cooked crispy breads prepared with 1% and 5% (w/w) of each fiber and contaminated with 5 and 25 mg/kg of BEA.

2. Materials and methods

2.1. Materials

Potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulfate ($NaSO_4$), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, α-amylase, hydrochloric acid (HCl), sodium hydroxide (NaOH), formic acid, pepsin, pancreatin, bile salts, phosphate buffer saline (PBS, pH 7.5), β-1,3 glucan, chitosan low M.W., chitosan medium M.W., fructooligosaccharides (FOS), galattomannan, inulin and pectin were obtained from Sigma–Aldrich (Madrid, Spain). Acetonitrile, methanol and ethyl acetate were purchased from Fisher Scientific (Madrid, Spain). Deionized water (<18 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath.

The BEA employed/utilized in this study were produced and purified according to the method of Meca et al. (2010a).

2.2. Model solution preparation

The model solutions were prepared in 100 mL Erlenmeyers, suspending 1 and 5 g of each soluble alimentary fiber (β-1,3 glucan, chitosan low M.W., chitosan medium M.W., (FOS, galattomannan, inulin and pectin) in 100 mL of distilled water, to obtain solutions at 1% and 5% (w/v) of each fiber. The solutions were mixed using ultrasound bath (Lab Police, Barcelona, Spain) operating at a temperature of 30 °C, and then 10 mL from each solution was contaminated with 5 mg BEA/L, and other 10 mL with 25 mg BEA/L. The contamination of the solutions was carried out using a stock methanolic solution (1000 mg/L) of BEA.

The solutions were digested with a simulated gastrointestinal digestion to assess the bioaccessibility of the BEA.

2.3. Wheat crispy breads production

For the production of the wheat crispy breads with different fiber concentrations, 300 g of wheat flour, 3 g of sucrose, and 6 g of NaCl, were mixed with 3.0 and 15.4 g of each dietary fiber (β-1,3 glucan, chitosan low M.W., chitosan medium M.W., (FOS, galattomannan, inulin and pectin) to obtain dough with 1% and 5% (w/w) of each prebiotic compound employed. These mixtures were then mixed with 180 mL of water during 5 min. No fermentation was done. The dough, divided in the shape of small round breads, was treated at 220 °C during 20 min.

2.4. Bacterial strains and growth conditions

Thirteen commercial probiotic strains were obtained for the *in vitro* system that simulates the physiologic condition of the colonic intestinal compartment. In particular *Lactobacillus animalis* CECT 4060T, *L. casei* CECT 4180, *L. casei rhamnosus* CECT 278T, *L. plantarum* CECT 220, *L. rhuminis* CECT 4061T, *L. casei casei* CECT 277, *Bifidobacterium breve* CECT 4839T, *B. adolescentes* CECT 5781T and *B. bifidum* CECT 870T, *Corynebacterium vitaeruminis* CECT 537, *Streptococcus faecalis* CECT 407, *Eubacterium crispatus* CECT 4840, *Saccharomyces cerevisiae* CECT 1324 were obtained at the Spanish Type Culture Collection (CECT Valencia, Spain), in sterile 18% glycerol.

For longer survival and higher quantitative retrieval of the cultures, they were stored at −80 °C. When needed, recovery of strains was undertaken by two consecutive subcultures in appropriate media prior to use (Laparra and Sanz, 2009; Meca et al., 2012a).

2.5. In vitro digestion model

The procedure was adapted from the method outlined by Gil-Izquierdo et al. (2002), with slight modifications. The method consists of three sequential steps; an initial saliva/pepsin/HCl digestion for 2 h at 37 °C, to simulate the mouth and the gastric conditions, followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate duodenal digestion (Fig. 1). The colonic conditions were simulated adding to the duodenal simulated fluid some bacteria representative of the gastrointestinal tract.

For the saliva/pepsin/HCl digestion, 10 mL of the model solution or 10 g of the crispy bread contaminated with 5 and 25 mg/kg of BEA, were mixed with 6 mL of artificial saliva composed by: KCl 89.6 g/L, KSCN 20 g/L, NaH_2PO_4 88.8 g/L, $NaSO_4$ 57 g/L, NaCl 175.3 g/L, $NaHCO_3$ 84.7 g/L, urea 25 g/L, 290 mg of α-amylase. The pH of this solution was corrected at 6.8 with NaOH 0.1 N.

These mixtures composed by model solutions and by the artificial saliva were placed in plastic bags, containing 40 mL of water and homogenized by Stomacher IUL Instruments (Barcelona, Spain) during 30 s.

To this mixture, 0.5 g of pepsin (14,800 U) dissolved in 25 mL of HCl 0.1 N was added. The pH of the mixture was corrected to a value of 2 with HCl 6 N, and then incubated in a 37 °C orbital shaker (250 rpm) (Infors AG CH-4103, Bottmingen, Switzerland) for 2 h.

After the gastric digestion, the pancreatic digestion was simulated. The pH was increased to 6.5 with $NaHCO_3$ (0.5 N) and then 5 mL of (1:1; v/v) pancreatin (8 mg/mL):bile salts (50 mg/mL), dissolved in 20 mL of water, was added and incubated in a 37 °C orbital shaker (250 rpm) for 2 h. An aliquot of 5 mL of the duodenal simulated fluid was sampled for the extraction of the BEA and the determination of the duodenal bioaccessibility.