Original Research Article

Influence of pro- and prebiotics on gastric, duodenal and colonic bioaccessibility of the mycotoxin beauvericin

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A B S T R A C T

Beauvericin (BEA) is a bioactive compound produced by the secondary metabolism of several Fusarium strains and known to have various biological activities. This study investigates the influence of several dietary fibers (galactomannan, glucomannan, citrus fiber, bamboo fiber, carrot fiber, potato fiber, β-glucan, xylan, and cellulose) and probiotic strains (Lactobacillus animalis, Lb. casei, Lb. casei, Lb. plantarum, Lb. rhuminis, L. casei, Bifidobacterium breve, Bf. Adolescens, Bf. bifidum, Corynebacterium vitaeruminis, Streptococcus faecalis, Eubacterium crispatus, and Saccharomyces cerevisiae) on the minor Fusarium mycotoxin BEA bioaccessibility employing a model solution. The bioaccessibility was determined using a simulated gastrointestinal digestion that mimics the physiological 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 reduction of BEA bioaccessibility in the experiments carried out using the prebiotic compounds ranged from 60 to 80%, whereas in the trials carried out using the probiotic strains the bioaccessibility observed ranged from 30 to 85%. A BEA degradation product produced by colonic fermentation was identified using the technique of LC–MS–LTQ.

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1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide consisting of alternating D-/D-hydroxy-isovaleryl and aromatic N-methyl-phenylalanine. This toxin is produced by various Fusarium species such as Fusarium avencum, F. poae, F. oxysporum and F. proliferatum, and naturally occurs on maize, wheat, barley, rice and oat (Logrieco et al., 1998; Uhlig et al., 2006; Jestoi, 2008; Sorensen et al., 2008; Kokkonen et al., 2010; Waskiewicz et al., 2010). BEA has been detected in grains throughout the world under different climates (South Africa, Poland, Norway, Spain, Croatia), with concentrations ranging from trace level up to 520 mg/kg in maize in Italy (Ritieni et al., 1997). Meca et al. (2010) have shown that BEA was present in cereals (barley, corn and rice) purchased in Spanish markets, with levels ranging from 0.51 to 11.78 mg/kg.

An in vivo study has shown that mice orally exposed to BEA presented an increase of mortality with a Lethal Dose 50 (LDSO) superior to 100 mg/kg bw (Jestoi, 2008). The cytotoxicity of BEA has been demonstrated in vitro in several cell line models, including human leukemia cells CCRF-CEM, human monocytic lymphoma cells U-937 and promyelocytic leukemia cells HL-60, monkey kidney epithelial cells Vero, Chinese hamster ovary cells CHO-K1 and murine macrophage J774 (Tomoda et al., 1992; Calo et al., 2004; Jow et al., 2004; Ruiz et al., 2011a,b).

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

To achieve any effects 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 is not modified structurally through the reactions related to the gastrointestinal digestion and thus become available for intestinal absorption (Fernández-García et al., 2009).

Probiotics are defined as ‘live microorganisms which when administered in adequate amount confer health benefits to the host’ (FAO/WHO, 2002). Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect...
the host animal by improving its intestinal microbial balance. Probiotics were originally used to improve the health of both animals and humans through the modulation of the intestinal microbiota. At present, several well-characterized strains of Lactobacilli and Bifidobacteria are available for human use to reduce the risk of gastrointestinal (GI) infections or treat such infections (Salminen et al., 2005). Some of the beneficial effects of probiotic consumption include improvement of intestinal health by the regulation of microbiota, and stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, and reducing the risk of certain other diseases (Kumar et al., 2010, 2011; Nagpal et al., 2007, 2010; Yadav et al., 2008).

The concept and understanding of probiotics have been evolving over time as new information emerges. ‘Prebiotic’ was first defined as a non-digestible food ingredient that beneficially affected the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Recent literature, however, does not restrict the colon as the only action site and defines a prebiotic as a selectively fermented ingredient that allows specific changes in the composition and/or activity of the gastrointestinal microbiota that confer benefits upon health and wellbeing of the host (Figuerola-Gonzalez et al., 2011). Thus not only are probiotics being examined for antipathogenic effects (such as inhibiting adhesion of pathogenic organisms to the gut mucosa), but they are also being developed to decrease fecal transit time, lower cholesterol and the glycemic response, improve bone health, lower daily energy (fat) intake, relieve symptoms of inflammatory bowel disease, and attempt to lower colon cancer rates (Pineiro et al., 2008).

In the scientific literature, only a few articles are available on the influence of prebiotics on the bioaccessibility of the minor Fusarium mycotoxins (Meca et al., 2012a,b), whereas the influence of the probiotics on the stability of this bioactive compound during gastrointestinal digestion has never been studied. For these reasons the aims of this study were (a) to evaluate the influence of several soluble and insoluble prebiotics on BEA bioaccessibility, (b) to evaluate how different probiotic strains can influence BEA’s bioaccessibility and (c) to determine the possible adduct with BEA and fibers or the degradation products produced by bacteria by LC–MS–LIT.

2. Materials and methods

2.1. Materials

Potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH₂PO₄), sodium sulfate (Na₂SO₄), sodium chloride (NaCl), sodium bicarbonate (NaHCO₃), urea, α-amylase, hydrochloric acid (HCl), sodium hydroxide (NaOH), formic acid, pepsin, pancreatin, bile salts, phosphate buffer saline (PBS, pH 7.5), galactomannan, β-glucan, xylan, cellulose high molecular weight (HMW), and cellulose medium molecular weight (MMW) were obtained from Sigma–Aldrich (Madrid, Spain). Glucomannan high molecular weight (HMW), glucomannan fine powder, citrus fiber, bamboo fiber, carrot fiber, and pie fiber were generously provided by Prof. Alberto Ritianni of the University of Naples “Federico II”.

Acetonitrile, methanol and ethyl acetate were purchased from Fisher Scientific (Madrid, Spain). Deionized water (<18 Ωcm 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 9200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath. The BEA used in this study were produced and purified according to the method of Meca et al. (2010).

2.2. Bacterial strains and growth conditions

Thirteen commercial probiotic strains were obtained for the in vitro system that simulates the physiologic condition of the colonic compartment. In particular Lactobacillus paracasei CECT 277, Lb. casei CECT 4180, Lb. rhamnosus CECT 278T, Lb. plantarum CECT 220, Lb. ruminis CECT 4061T, Lb. casei casei CECT 277, Bifidobacterium breve CECT 4839T, Bf. adolescentes CECT 5781T and Bf. bifidum CECT 870T, Bf. Longum CECT 4551, Corynebacterium vitaerumin CECT 537, 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 (Meca et al., 2012a).

2.3. Model solution preparation

The stock model solution used in this study to reproduce the food model was composed of water, glucose (1%), starch (5%), albumin (3%), sodium chloride (NaCl) (0.1%), and triolein (2%). The model solutions (with fibers) were prepared in 100 mL Erlenmeyer flasks and spiked with 1 and 5 g of each dietary fiber. In the study of the probiotic strains influence on the BEA bioaccessibility, the model solution was inoculated with 2 × 10⁸ of each bacterial strain tested. Solutions were mixed using ultrasound bath (Lab Police, Barcelona, Spain) operating at a temperature of 30 °C, and then 10 mL of each solution were contaminated with 10 mg BEA/L. Contamination of the solutions was carried out using a BEA solution stock (1 g/L) in methanol.

2.4. 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 by 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: 10 mL of KCl 89.6 g/L, 10 mL of KSCN 20 g/L, 10 mL of NaH₂PO₄ 88.8 g/L, 10 mL of Na₂SO₄ 57 g/L, 1.7 mL of NaCl 175.3 g/L, 20 mL of NaHCO₃ 84.7 g/L, 8 mL of urea 25 g/L, and 290 mg of a-amylase. The pH of this solution was corrected to 6.8 with NaOH 0.1 N. These mixtures composed of model solutions and the artificial saliva were placed in plastic bags, containing 40 mL of water and homogenized using a 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 6 N HCl and then incubated in a 37 °C orbital shaker (250 rpm) (Infors AG CH–4103, Bottmingen, Switzerland) for 2 h.

After gastric digestion, pancreatic digestion was simulated. The pH was increased to 6.5 with NaHCO₃ (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 fluid was sampled for the extraction of the BEA and the determination of the duodenal bioaccessibility.

To mimic the colonic compartment bacterial strains (previously described) were grown in a sterile plastic centrifuge tube overnight at 37 °C in MRS broth (Oxoid, Madrid, Spain) under anaerobic conditions.
conditions (5% CO₂/95% air). Then, the tubes were centrifuged at 4000 rpm for 5 min at 23 °C and bacteria were resuspended in sterile PBS. A 500 µL aliquot of a mixture of the bacterial suspensions at concentrations of 10¹⁴ CFU/mL was added to duodenal simulate intestinal fluid and incubated at 37 °C in 5% CO₂/95% air for 48 h. After this last digestion, 5 mL of the mixture was centrifuged at 4000 rpm for 10 min at 4 °C, and extracted for BEA determination and for duodenal + colonic bioavailability estimation.

2.5. BEA extraction from the simulated intestinal fluids

BEA contained in gastric, gastric + duodenal, and gastric + duodenal + colonic fluids were extracted as follows. Then 5 mL of each mixture previously described were placed in a 20 mL test tube, and extracted three times with 5 mL of ethyl acetate using a vortex VWR international (Barcelona, Spain) for 1 min. Then, the mixtures were centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 4000 rpm and at 4 °C for 10 min. The organic phases were completely evaporated by a rotary evaporator (Buchi, Switzerland) operating at 30 °C and 30 mbar pressure, resuspended in 1 mL of methanol and filtered with a 0.22 µM filter (Phenomenex, Madrid, Spain) before being analyzed by LC–MS/MS.

2.6. BEA analysis

BEA separation was achieved by an Agilent 1100 (Agilent Technologies, Santa Clara, California) LC coupled to an Applied Biosystems/MDS SCIEX Q TRAP TM linear ion trap mass spectrometer (Concord, Ontario, Canada). A Gemini (150 × 2.0 mm, 5 lm) Phenomenex (Torrance, California) column was used. LC conditions were set up using a constant flow at 0.80 ml/min and acetonitrile/water (80:30, v/v, with 0.1% of HCOOH) as mobile phases in isocratic condition were used. The instrument was configured in the positive ion electrospray mode using the following parameters: cone voltage 40 V, capillary voltage 3.80 kV, source temperature 350 °C, desolvation temperature 270 °C and collision gas energy 5 eV. The analyses of the ENs degradation products employing the liquid chromatography technique coupled to the ion trap was performed using the following procedure:

1. Characterization of the compound isolated with Enhanced Resolution (ER) scan modality, using the m/z range from 200 to 900 Da to obtain the general spectra of the degradation compound;
2. Characterization of the fragments obtained in the ER scan with the enhanced product ion (EPI) scan modality to obtain a MS² scan of the degradation product fragment.
3. Study of the fragments obtained in the EPI modality employing the modality MS/MS/MS that permits us to know the MS³ of a fragment selected in the ER scan.

The utilization of the mass spectrometry associated at the detection with the linear ion trap, used in these two modalities, permitted us to obtain a total characterization of the isolated compound (Meca et al., 2012a).

3. Results and discussion

3.1. Influence of the dietary fiber on the bioaccessibility of the mycotoxin BEA

Table 1 shows the influence of different types of soluble and insoluble dietary fiber on BEA bioaccessibility under simulated gastrointestinal tract condition. The mean bioaccessibility of BEA under simulated stomach conditions for all the treatments with fiber was of 38.8% which was 2.3 fold lower than the control. As shown in Table 1, the highest BEA bioaccessibility data under stomach condition (60.5%) was obtained in the experiment performed with 1% carrot fiber. The lowest BEA bioaccessibility of 15.7% was obtained with 5% HMW cellulose.

In the experiments carried out with the prebiotic compounds using the combination of the gastric and duodenal digestion the mean BEA bioaccessibility was 26.8%, 3.3 fold lower than the value
detected in the control experiment. The highest and the lowest bioaccessibility data of the bioactive compound studied using the combination of gastric, duodenal digestion were demonstrated in the experiments using carrot fiber (1%) and cellulose (HMW) with 38.6 and 11.2%, respectively. A considerable reduction of BEA bioaccessibility was also detected in the model solution enriched with cellulose medium molecular weight (MMW), with a 19.3%. The mean data of BEA bioaccessibility after the combination of gastric, duodenal and colonic digestion was of 32.9%, 2.54 fold lower than the value present in the control solutions. The highest bioaccessibility data recorded were detected in the experiments carried out with the fibers glucomannan HMW (1%) and carrot fiber (1%) with 44.1 and 44.6%, respectively, whereas the lowest data was determined in the model solution enriched with cellulose HMW (5%) with 14.1%.

Observing the data in Table 1, there are several important details that should be emphasized:

(a) The prebiotic compounds used in this study, soluble and insoluble, reduce the risk associated to minor *Fusarium* mycotoxin BEA intake, with a retention mechanism of this bioactive compound similar to the mechanism observed for polyphenols, proteins and minerals.

(b) The dietary fibers binding capacity observed for minor *Fusarium* mycotoxin BEA is dose dependent. In particular, when 5% concentration of prebiotic compounds was used instead of 1%, BEA bioaccessibility decreased from 1.06 to 1.92 fold. This phenomenon can be related to the presence of more active binding sites in the fibers structure of compounds used at 5% concentration, which helped to reduce BEA bioaccessibility.

(c) Insoluble fibers as cellulose exert an important effect on BEA bioaccessibility reduction, probably due to the high difficulty to ferment the fiber shown by the colonic model's bacteria and reduce in this way its bioaccessibility.

**Awad et al. (2009)** studied the influence in feed for chicken nutrition, of a probiotic strain as *Eubacterium sp.*, and of a prebiotic as the inulin, on the injuries reduction of the gastrointestinal tract induced by mycotoxin deoxynivalenol (DON). The authors demonstrated that the alteration caused by DON was reduced by supplementing the DON containing diets with probiotic feed additive. In the presence of DON acute toxicity, the dietary inulin supplementation may be useful in reducing the toxic effects of DON on intestinal glucose transport.

**Baines et al. (2011)** studied the influence of a mannose oligosaccharide (MOS) and yeast metabolites prebiotic complex (Celamax) on the bioaccessibility of the mycotoxins nivalenol (NIV), zearalenone (ZEA), deoxinivalinol (DON), 15-Acetyl-DON, 3-Acetyl-DON, neosolaniol (NEO), diacetoxyscirpenol (DAS), HT-2 and T-2 toxins, using dairy cattle as biological model. The mycotoxins were produced by several types of mycotoxicogenic fungi including *Fusarium culmorum*, *F. poae*, *F. verticillioides*, *F. sporotrichioides*, *Aspergillus flavus*, *Penicillium roqueforti*, *P. crustosum*, *P. paneum* and *P. citrinum* directly on feed. The application in feed of only 0.1% of Celamax reduced the bioaccessibility of the mycotoxins studied on a 90%, 2.0 fold lower than the BEA bioaccessibility reduction achieved in this study.

**Meca et al. (2012a)** evaluated the bioaccessibility of the mycotoxins 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 b-1,3 glucon, chitosan low molecular weight (L.M.W.), chitosan medium molecular weight (M.M.W.), fructooligosaccharides (FOS), galactomannan, inulin and pectin, added at concentrations of 1% and 5%. The bioaccessibility was determined using a simulated gastrointestinal digestion model that mimics the physiologic conditions of the digestive tract until the colonic compartment. The BEA bioaccessibility data mean 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. The data obtained in model solution by the authors are comparable with the data produced in our study.

**Table 1** Influence of different types of soluble and insoluble dietary fibers on bioaccessibility of BEA under simulated gastrointestinal tract conditions. Values are presented as means ± SD of 3 samples (n=3).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bioaccessibility (%)</th>
<th>Stomach</th>
<th>Stomach + duodenum</th>
<th>Stomach + duodenum + Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>93.2 ± 2.1</td>
<td>88.7 ± 1.5</td>
<td>83.8 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Galactomannan 1%</td>
<td>31.3 ± 1.1</td>
<td>24.9 ± 2.4</td>
<td>30.3 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>Galactomannan 5%</td>
<td>28.7 ± 1.4</td>
<td>24.3 ± 0.7</td>
<td>27.2 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Glucomannan HMW 1%</td>
<td>50.9 ± 1.8</td>
<td>30.6 ± 2.2</td>
<td>44.1 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Glucomannan HMW 5%</td>
<td>44.9 ± 3.4</td>
<td>31.9 ± 1.5</td>
<td>35.5 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Glucomannan fine powder 1%</td>
<td>35.0 ± 4.3</td>
<td>27.7 ± 2.4</td>
<td>36.3 ± 3.1</td>
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</tr>
<tr>
<td>Glucomannan fine powder 5%</td>
<td>33.9 ± 1.7</td>
<td>24.2 ± 1.7</td>
<td>30.0 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Citrus fiber 1%</td>
<td>31.1 ± 1.9</td>
<td>26.4 ± 2.2</td>
<td>32.3 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Citrus fiber 5%</td>
<td>37.0 ± 1.6</td>
<td>24.7 ± 1.3</td>
<td>30.1 ± 1.6</td>
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</tr>
<tr>
<td>Bamboo fiber 1%</td>
<td>49.7 ± 1.1</td>
<td>35.0 ± 2.3</td>
<td>37.5 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>Bamboo fiber 5%</td>
<td>40.1 ± 1.8</td>
<td>34.6 ± 2.5</td>
<td>34.8 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Carrot fiber 1%</td>
<td>60.5 ± 2.1</td>
<td>38.6 ± 2.3</td>
<td>44.6 ± 3.1</td>
<td></td>
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<tr>
<td>Carrot fiber 5%</td>
<td>35.5 ± 2.4</td>
<td>23.2 ± 2.0</td>
<td>30.0 ± 2.3</td>
<td></td>
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<tr>
<td>Pie fiber 1%</td>
<td>52.1 ± 1.8</td>
<td>34.6 ± 1.5</td>
<td>40.6 ± 1.9</td>
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</tr>
<tr>
<td>Pie fiber 1%</td>
<td>52.4 ± 2.3</td>
<td>23.5 ± 0.9</td>
<td>35.5 ± 0.9</td>
<td></td>
</tr>
<tr>
<td>β-Glucan 1%</td>
<td>42.0 ± 2.1</td>
<td>25.8 ± 1.7</td>
<td>31.9 ± 1.7</td>
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<tr>
<td>β-Glucan 5%</td>
<td>45.1 ± 2.3</td>
<td>33.2 ± 2.1</td>
<td>36.4 ± 3.2</td>
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</tr>
<tr>
<td>Xylan 1%</td>
<td>40.5 ± 2.6</td>
<td>22.3 ± 2.0</td>
<td>40.0 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Xilan 5%</td>
<td>35.2 ± 1.5</td>
<td>25.6 ± 1.5</td>
<td>32.4 ± 1.7</td>
<td></td>
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<tr>
<td>Cellulose HMW 1%</td>
<td>33.1 ± 2.8</td>
<td>25.8 ± 1.9</td>
<td>27.1 ± 15</td>
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<td>Cellulose HMW 5%</td>
<td>15.7 ± 1.5</td>
<td>11.2 ± 1.1</td>
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<tr>
<td>Cellulose MMW 1%</td>
<td>32.7 ± 2.4</td>
<td>23.0 ± 1.9</td>
<td>30.0 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Cellulose MMW 5%</td>
<td>25.8 ± 2.5</td>
<td>19.3 ± 1.9</td>
<td>22.7 ± 1.8</td>
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</tr>
</tbody>
</table>
3.2. Influence of probiotic bacteria on BEA bioaccessibility

All the probiotic strains tested in this study showed an influence on the reduction of the bioaccessibility of the minor *Fusarium* mycotoxins BEA present in the model solutions, during the different steps of the gastrointestinal digestion, as is possible to observe in Fig. 1.

In Fig. 2 the LC–MS/MS chromatogram of the BEA present in the simulated duodenal intestinal fluid related to the model solution spiked with the strain of *Lb. rhamnosus* is shown. During the gastric digestion the mean bioaccessibility data of the minor *Fusarium* mycotoxins BEA was of 46.2%, 2.0 fold lower than the data that can be seen in the control solution. The highest BEA bioaccessibility data was seen in the experiment carried out with the strain of *Bf. adolescentis* with 65.3% (Fig. 3), whereas the lowest was detected in the model solution spiked with *Bf. breve* with 36.1%. During the gastric more duodenal digestion is possible to observe a decrease of the BEA bioaccessibility probably due to the degradation of the bioactive compound BEA by the bacterial strains used in this study. In particular the mean bioaccessibility data observed was of 35.4%, 2.5 fold lower than the data obtained in the control solution. The highest reduction of BEA bioaccessibility was achieved in the experiment carried out with the strain of *Bf. Longum* evidencing a 45.4%, whereas the lowest bioaccessibility data was detected in the model solution digested with the strain of *Lb. rhamnosus*, with a 27.5%. Using the complete simulated gastrointestinal digestion model (gastric + duodenal + colonic digestion) the mean bioaccessibility data related to the bioactive compound BEA was of 26.4%. This data was 3.1 fold lower than the data obtained in the control and also 1.5 fold lower than the data collected in the experiments using only the duodenal model. This phenomenon can be related to the synergistic effect between the strains introduced in each trial and the mix of bacteria used to simulate the colonic conditions. The highest and lowest BEA bioaccessibility data were demonstrated in the model solution spiked with *C. vitaeniniis* and *Lb. rhamnosus*, with 33.7 and 13.9%, respectively.

The influence of the probiotic bacteria during the gastrointestinal digestion of the mycotoxins was studied by different authors. In particular Kabak et al. (2009) determined the release of aflatoxin B$_1$ (AFB$_1$) and ochratoxin A (OTA) from different food products in the gastro-intestinal tract in the absence and presence of probiotics, a possible adsorbent. The average bioaccessibility of AFB$_1$ and OTA without probiotics was about 90%, and 30%, respectively, depending on several factors, such as food product, contamination level, compound and type of contamination (spiked versus naturally contaminated). The six probiotic bacteria showed varying binding capacity to AFB$_1$ and OTA depending on the bacterial strain, toxin studied, type of food and contamination level. A reduction to a maximum of 37% and 73% as observed for the bioaccessibility of AFB$_1$ and OTA in the presence of probiotic bacteria, respectively. The range of bioaccessibility reduction shown by the authors was comparable with the reduction shown for the mycotoxin BEA. This article was the first report on the effect of probiotic bacteria on reducing the fraction of mycotoxins available for absorption in the gastrointestinal tract from different food products.

The interaction between the minor *Fusarium* mycotoxins BEA and bacterial strains characteristic of the gastrointestinal tract as
Bifidobacteria, Lactobacillus and Streptococcus was studied by Meca et al. (2012b). Levels of BEA in the fermentation liquid, on the cell walls and on the internal part of the cells were determined using liquid chromatography coupled to the mass spectrometry detector (LC–MS/MS). Results showed that the bacteria reduced the concentration of the BEA present in the medium, part of the mycotoxin was adsorbed by cell wall and part internalized by the bacteria. All the bacteria analyzed in this study showed a significant BEA reduction during the fermentation process, in particular the mean reduction ranged from 66 to 83%.

Kabak and Ozbey (2012), studied the aflatoxins (AFs) bioaccessibility from various spiked food matrices (peanut, pistachio, hazelnut, dried figs, paprika, wheat and maize) using an in vitro digestion model under fed conditions that employed six probiotic bacteria in reducing AF bioaccessibility. The AFs bioaccessibility from seven food matrices ranged from 85.1 to 98.1 and the inclusion of probiotic bacteria showed significant reduction in AFs bioaccessibility. The results discussed in this study are in agreement with the data shown in our study.

**Fig. 4.** (a) LC–MS-LIT chromatogram obtained in ER scan (200–1000 m/z) of the BEA present in the model solution fermented by Lb. rhamnosus and (b) ER-MS spectrum of the BEA degradation product.
3.3. LC–MS-LIT identification of the BEA degradation products

The samples of the simulated gastrointestinal fluids positive to BEA degradation produced by microbial fermentation, were injected in the LC–MS-LIT in the modality ER scan (m/z = 200–900) to determine the BEA degradation products. Fig. 4a presents the LC–MS-LIT chromatogram obtained in the modality of Enhanced Resolution (ER), of the BEA presents in the simulated gastrointestinal fluid fermented by Lb. rhamnosus. In the chromatogram is possible to evidence the presence of the BEA with a retention time (RT) of 5.31 min and also of another compound (RT = 6.51 min) close to the BEA peak corresponding to a BEA degradation product. In Fig. 4b is shown the mass spectrum related to the BEA degradation product, identified as the BEA with the loss of the hydroxyvaleric acid (HyLv), with the presence of several diagnostic signals that confirm the structure of this degradation compound. In particular the origin of the degradation compound from the mycotoxin BEA was confirmed by the presence of the ion with a m/z of 805.2 represented by the sodium adduct of the mycotoxin. Other important diagnostic fragments are represented by the ion with a m/z of 701.3, identified as the BEA sodium adduct with the loss of a HyLv, the ion with a m/z of 527.2, identified as the BEA sodium adduct with the loss of a HyLv and also of another important BEA structural component represented by the phenylalanine (Phe). The ion with a m/z of 306.3 represents the BEA sodium adduct with the loss of 2Phe and of 1HyLv unit. To confirm the structure of the BEA degradation product, produced by microbial fermentation of the simulated matrix by Lb. rhamnosus, the sample was injected in the modality of EPI scan to determine the MS2 of the neo formed compound. Fig. 5a shows the EPI-scan chromatogram of the BEA degradation product obtained using as reference ion the fragment with a m/z of 701.3, whereas in Fig. 5b the EPI scan spectrum of the neoformed compound. In the
spectrum can be seen some important fragments that confirm the structure of the BEA degradation compound. The presence of the HyLv and Phe in the neoformed compound structure is evident in the signals with a m/z of 683.3 and 603.1, and also in the fragment with a m/z of 503.2 where the loss of the Phe and HyLv units is simultaneously. The last confirmation that the degradation product is originated by BEA was carried out using the technique of the MS/MS/MS that permits to obtain the MS³ of a product identified in the ER scan. In Fig. 6, the MS³ of the BEA degradation product spectra obtained using as reference signals the ions with a m/z of 701.1 and 503.2 is shown. In the spectra a diagnostic signal with a m/z of 416.7 corresponding to two structural BEA components as the HyLv and the Phe is evident. In particular, the fragment corresponds to 2Phe and 1HyLv units present in the BEA structure.

4. Conclusions

The soluble and insoluble dietary fibers reduced significantly BEA bioaccessibility considering the three gastrointestinal compartments analyzed. In particular, the highest BEA bioaccessibility reduction was observed in the model solution treated with the insoluble fiber cellulose HMW (5%) with 14.1%. Also the probiotic bacteria tested in the model solution produced an important reduction of the BEA bioaccessibility, confirming the capacity of the lactic acid bacteria to reduce/bind toxic compounds present in food as the mycotoxins. The best performance in the reduction of the BEA bioaccessibility was provided by Lb. rhamnosus. Employing the LC–MS-LIT technique, a BEA degradation product produced by colonic microflora fermentation was identified as the mycotoxin BEA with the loss of the HyLv acid. Further investigations will be focused on the development of a symbiotic food with high capacity to reduce the bioaccessibility of minor Fusarium mycotoxins as the BEA.

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References


Fig. 6. MS/MS/MS spectrum of the BEA degradation product.


