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Beauvericin degradation during bread and beer making

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ABSTRACT

Beauvericin (BEA) is a bioactive compound produced by the secondary metabolism of several *Fusarium* species and known to have various biological activities. This study investigated the degradation of the minor *Fusarium* mycotoxin BEA present at the concentration of 5 mg/kg in barley and wheat flour during beer and bread making. The influence of the making processes and of the formation of degradation products of BEA were evaluated during the beer and bread making. The concentration of BEA and its evolution during the production processes were determined with the technique of the liquid chromatography tandem mass spectrometry in tandem (LC-MS/MS), whereas the formation of the BEA degradation products was determined with the technique of the LC-MS coupled to a linear ion trap (LC-MS-LIT). The degradation of BEA during beer making ranged from 23 to 82%. During bread making, BEA reduction ranged from 75 to 95%. The highest degradation activity of BEA for both beer and bread was evidenced during the heat and fermentation processes. Also two degradation products formed during these processes were identified.

1. Introduction

Beauvericin (BEA) (Fig. 1) is a depsipeptide with antibiotic and insecticidal effects belonging to the enniatin family (Hamill, Higgens, Boaz, & Gorman, 1969). It was initially isolated from the fungus *Beauveria bassiana*, but it can also be produced by other fungi, including several *Fusarium* species (Logrieco et al., 1998); it may therefore occur in grains (such as corn, wheat, and barley) contaminated with these fungi (Jestoi et al., 2004; Logrieco et al., 1998). BEA is active against gram-positive bacteria and mycobacteria, and it is also capable of inducing programmed cell death in mammals (Meca, Font, & Ruiz, 2011).

BEA has 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; Ritieni et al. (1997) reported high levels of BEA up to 520 mg/kg in maize from Italy. Recently, Meca, Zinedine, Blesa, Font, and Manes (2010) have reported the contamination of cereals available in the Spanish market with BEA and the levels ranged from 0.51 to 11.78 mg/kg.

Related to the methodologies employed for BEA reduction in food, only a US patent (Duvick & Rod, 1998) and a scientific article (Meca, Ritieni, & Mañes, 2012) are available in the scientific literature. In particular Duvick & Rod (1998), employing as detoxification agent, a strain of *Norocardia glubera*, reduced the BEA contamination in wheat kernels by 50% with an initial contamination of the mycotoxin at1000 mg/kg; Meca, Ritieni et al. (2012) published a study on the degradation of the mycotoxin BEA during heat treatments related to crispy bread production.

The degradation of several *Fusarium* mycotoxins by a UDP-glucosyltransferase isolated from plant *Arabidopsis thaliana* was studied by Poppenberger et al. (2003). The authors demonstrated that the enzymes purified from the plant detoxified completely the *Fusarium* mycotoxins deoxynivalenol (DON) and 15-acetyl-deoxynivalenol (15-Ac-DON).

The degradation of fumonisin B_1 (FB_1) by microbial enzymes was studied by Heinl et al. (2010). The authors isolated two enzymes from liquid culture of *Sphingopyxis* sp. MTA144, the bacterial strain capable of detoxifying FB_1. The enzymatic degradation of FB_1 by the bacterial strain was also studied by Hartinger et al. (2011). Cramer, Keonigs, and Humpf (2008) evaluated the degradation of mycotoxin ochratoxin A (OTA) during the coffee roasting, evidencing a reduction variable from 69 to 96%, correlating the degradation with the roasting time employed.

The degradation of *Fusarium* toxin nivalenol (NIV) during the baking and cooking processes was studied by Bretz, Knecht, Geockler, and Humpf (2005). It was found that under all the

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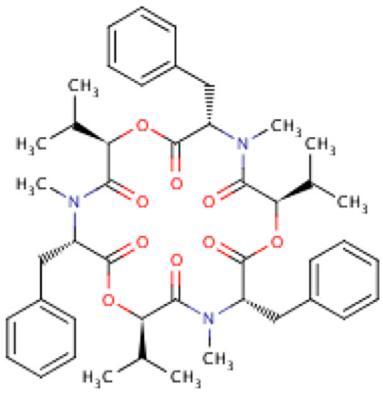

Fig. 1. BEA chemical structure.

conditions employed the degradation of NIV was accelerated with the increase of the temperatures used during the heat treatments.

Bretz, Beyer, Cramer, Knecht, and Humpf (2006) studied the degradation products of DON using model heating experiments with compounds that mimicked the typical food constituents. The model solutions were heated at different temperatures (150–200 °C) for different time periods.

The results showed that the degradation of DON was correlated to the increase of the temperature.

The aims of this study were to evaluate: a) the degradation of mycotoxin BEA during bread and beer production and b) the formation of BEA degradation products during the heat and fermentation processes in the bread and beer production.

2. Materials and methods

2.1. Materials

Sodium chloride (NaCl), sucrose ($C_{12}H_{22}O_{11}$) and formic acid (HCOOH) 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, utilized in this study was purchased from Sigma–Aldrich (Madrid, Spain).

2.2. Yeast strains and methodology

The study was carried out using three strains of *Saccharomyces cerevisiae* named LO9 (for Bavarian Weizen beer), A34 (for scotch Ale beer), and QRI (commercial bread instant yeast, Fleischmann's Quick-Rise Instant Yeast, www.breadworld.com). The strains were obtained by the personal collection of Dr. Ting Zhou of the Guelph Food Research Centre, Agriculture and Agri-Food Canada in PDA slant at 4 °C.

For longer survival and higher quantitative retrieval of the cultures, they were stored in sterile 18% glycerol at −80 °C. When needed, recovery of strains was undertaken by two consecutive subcultures in appropriate media prior to use.

The microorganisms were cultured in 50 mL sterile plastic centrifuge tubes with 20 mL Potato Dextrose Broth (PDB) as growth medium. They were incubated at 25 °C in aerobic conditions with 150 rpm shaking for 48 h.

2.3. Beer making

Beer making was carried out with a homemade process based on the following steps: 10 g of malt were contaminated with 25 mg/kg of BEA. The malt was toasted (T) at two different toasting conditions to simulate the low (40 °C for 2 days) and medium (100 °C for 1 day) toasting processes normally used in the brewery industry. The malt was finely grounded to produce malt flour that was boiled (B) with 30 mL of water for 30 min to permit the macronutrient dissolution necessary for the yeast fermentation. After that, α-amylase was added to promote starch hydrolysis (E) at 63 °C for 30 min. The mixture was centrifuged at 4000 rpm for 5 min. The supernatant was boiled at 100 °C for 5 min, and the hoops were added (H). The fermentation (F) of the malt extract was carried out with the three yeast strains, A34, LO9 and QRI individually for 4 days at 23 °C under anaerobic conditions. After the fermentation, the mixtures were centrifuged and the resulting beers were analyzed for BEA levels as described below. The BEA level was also determined at the end of each step during the beer making (Fig. 2).

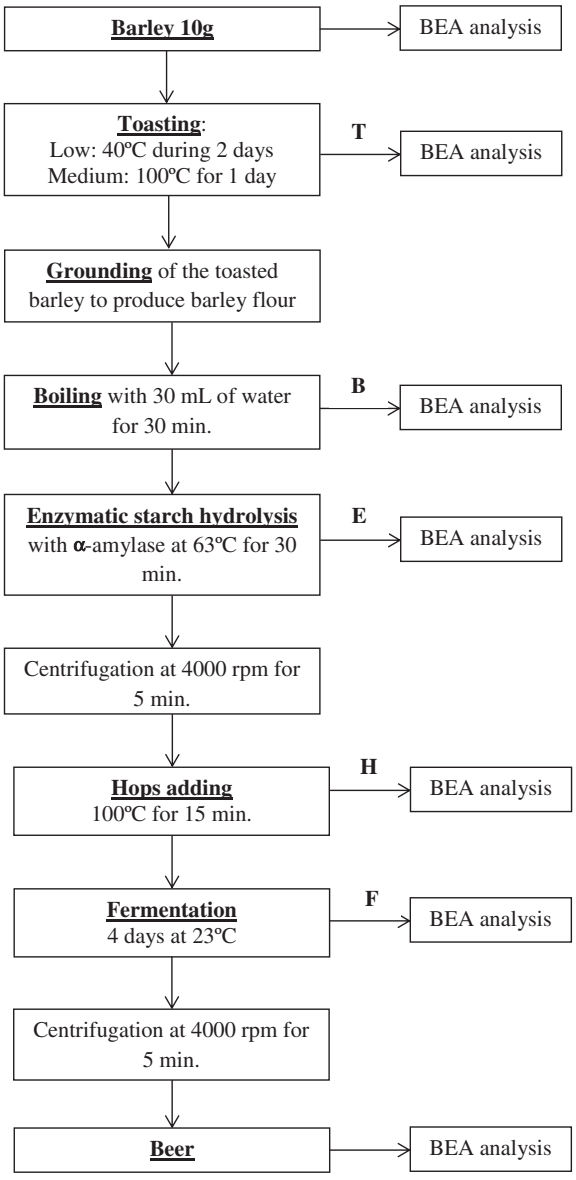

Fig. 2. Experimental plan used for the production of beer.