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Effect of maize genotype, developmental stage, and cooking process on the nutraceutical potential of huitlacoche (*Ustilago maydis*)

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ABSTRACT

Huitlacoche galls were produced in 15 creole maize genotypes harvested at two stages of development, 23 and 28 days after inoculation, and in a hybrid genotype harvested at six stages of development. Raw and cooked galls were evaluated for proximate constituents, carbohydrate composition and antimutagenic activity. Huitlacoche grown in creole maize exhibited differences in concentrations of some of the proximate and carbohydrate components, due to genotype and stage of development; some effects were also observed in hybrid maize, as a result of stage of development and cooking. Huitlacoche has a considerable amount of crude protein (9.8% average in creole maizes, and 11.3% in hybrid maize). Most of the values for total dietary fibre, β-glucans, and total free sugars were higher than those reported for other edible mushrooms. The high concentration of antimutagenic substances appears to be an asset of this culinary delicacy.

1. Introduction

Edible mushrooms have been part of the human diet in many cultures for many years. Although about 2000 species of edible fungi are known, only a few of them (approximately 22 species) are commercially cultivated (Paredes-López & Valverde, 1999).

Mushrooms contain 90.0% water, 1.0–4.0% protein, 0.2–0.8% fat, 0.3–2.8% carbohydrate, 0.3–7.0% fibre, and 0.6–1.0% ash; with potassium, calcium, phosphorus, magnesium, iron, zinc, and copper accounting for most of the mineral content (Barros, Baptista, Correia, Morais, & Ferreira, 2007; Vanegas, Valverde, Paredes-López, & Pataky, 1995). Mushrooms also contain essential amino acids, vitamins (thiamin, riboflavin, and niacin), and phenolic compounds (Valentão et al., 2005; Wasser & Weis, 1999). The search for medicinal substances from fungi has become a matter of great interest. It has been confirmed that higher Basidiomycetes contain bioactive substances that possess hyperlipidaemic, antitumoral, immunomodulating, anti-inflammatory, antimutagenic, antiatherogenic, hypoglycaemic, and other health-promoting properties (Barros, Baptista, Correia, et al., 2007; Valentão et al., 2005). It is estimated that approximately 50% of cultivated edible mushrooms possess nutraceutical or medicinal properties (Lull, Wichers, & Savelkoul, 2005; Wasser & Weis, 1999).

Among the health-promoting compounds found in edible and non-edible mushrooms, a large variety of biologically-active substances have been described, including triterpenes, proteins, peptides, lipids, cerebrosides, nucleotides, phenols, and an important number of biologically-active polysaccharides. These substances have been identified and characterised in medicinal mushrooms, such as *Ganoderma lucidum* and in several edible genera (Valentão et al., 2005; Wasser & Weis, 1999). These compounds have therapeutic health effects without any significant toxicity (Wasser & Weis, 1999).

The primary bioactive components in mushrooms are carbohydrates and glycoproteins, including two major types of mushroom polysaccharides: glucans and heteroglycans. Mushrooms contain dietary fibre, such as β-glucans, chitin, and heteropolysaccharides (pectinous substances, hemicelluloses, polyuronides, etc.). An adequate dietary fibre intake has benefits for fitness and disease prevention (Dikeman, Bauer, Flickinger, & Fahey, 2005). Dietary

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fibre adsorbs hazardous materials, such as carcinogenic substances, preventing their absorption in the intestine and hastening their excretion; thus, it may work effectively to prevent colon and rectal cancer (Wong et al., 2005). Not all types of fibre exert all the physiological effects to the same degree. Soluble fibres tend to have the largest impact on gastric emptying, small intestinal transit time, generation of short-chain fatty acids in the proximal colon, promotion of microbial growth, and stimulation of colonic epithelial proliferation. In contrast, insoluble fibres have their greatest effect on stool bulking, maintenance of the colonic muscle layer, prevention of bacterial translocation, and short-chain fatty acid production in the distal colon (Green, 2001).

Glucans are high molecular-weight substances containing glucose as the sole building block, and can vary in their glycosidic linkages and in their side-chain sugar constituents that may include arabinose, galactose, glucuronic acid, mannose, ribose, or xylose. Several reports indicate that β-glucans show antihyperglycaemic, antihypertriglyceridaemic, antihypercholesterolaemic, anti-arteriosclerosis, anti-tumour effects, and others (Kaneno et al., 2004; Shimizu et al., 2008). Heteroglycans, such as fucans, galactans, mannans, and xylans, have a backbone composed of sugars different to glucose, like arabinose, fucose, galactose, glucuronic acid, mannose, or xylose (Dikeman et al., 2005).

There are some reports about changes in the content of health-promoting compounds in several edible mushrooms. The studies include the effect of cooking process and maturity stage (Barros, Baptista, Correia, et al., 2007; Barros, Baptista, Estevinho, et al., 2007; Dikeman et al., 2005); cooking process resulted in a loss of moisture, and a subsequent concentration of nutrients and health-promoting compounds (Barros, Baptista, Correia, et al., 2007; Dikeman et al., 2005). Barros, Baptista, Estevinho, et al., 2007 reported that the stage of maturity affects the total phenols, flavonoids, and pro-vitamin content, and the antimicrobial activity of *Lactarius deliciosus* and *Lactarius piperatus* fruiting bodies. They found the lowest concentrations of these compounds in the last stage of maturity.

Huitlacoche is the edible young galls, growing on maize ears, due to Basidiomycete fungus *Ustilago maydis* infection; and it is consumed as the main component of a dish, or as a condiment. Galls, which form the huitlacoche product, usually consist of mycelia, spores, and modified tissue of cortex, xylem, phloem, parenchyma, and sclerenchyma strands. Galls may vary in size from minute pustules to several centimetres in diameter. Galls are at first covered with a glistening, greenish-white to silver-white tissue. The interior of the galls darkens and turns into masses of powdery, dark olive-brown to black teliospores; the mature gall is rather spongy. (Valverde, Paredes-López, Pataky, & Guevara-Lara, 1995). When huitlacoche is cooked it may become darker than the raw sample. It is a good source of protein (10–25%, dry matter basis), with a high content of lysine; also, it has a high content of fibre (10–30%, dry matter basis) and key fatty acids, such as linolenic, linoleic, and palmitic acids (Valverde et al., 1995; Vanegas et al., 1995). There are no reports about the toxicity or human health damage produced by huitlacoche. The extended popular consumption of huitlacoche for decades in Mexico and in the US has demonstrated the safety of this food (Valverde et al., 1995). No community has reported any toxicity whatsoever. In 1995 Vanegas et al. reported a preliminary study on the variation of huitlacoche proximate constituents and fatty acid composition due to hybrid maize genotypes.

The objective of this study was to analyse huitlacoche produced in creole maizes cultivated in El Bajío, Mexico, and in one hybrid maize cultivated in Urbana, IL. Huitlacoche was harvested at different stages of development and the effect of cooking on its chemical constituents, with emphasis on carbohydrate content, was assessed.

2. Materials and methods

2.1. Biological materials

Huitlacoche was produced in 16 different creole maize genotypes grown in El Bajío, Mexico, and the material produced in the hybrid genotype was grown in Urbana, IL. These genotypes were Criollo de Cañada de Flores (Number 1), Criollo del Norte (2), Criollo de Don Francisco (3), Criollo de Loma de Cabras (4), Criollo de Cruz del Palmar (5) Criollo del Norte de Guanajuato (6), Criollo del Tigre (7), Criollo de la Biznaga (8), Criollo de las Cruces (9), Criollo de San Juan Xidoo (10), Criollo de la Palmilla (11), Criollo de Pinalillo (12), Criollo de la Cuadrilla (13), Corina de Michoacán (14), Corina Cajete (15), Corina 85-2 (16), and MS 3877 (hybrid). Huitlacoche produced in creole maize was collected at two stages of development, 23 and 28 days after inoculation (dai), and galls produced on hybrid maize were collected at six different stages of development (11, 13, 15, 17, 20, and 32 dai). Visual characteristics of huitlacoche samples appeared to be the best between 17 and 28 days. Creole maizes are utilised by small growers for personal use and local marketing, in different communities of El Bajío. Genotypes were named according to their origin or growing place, and were selected by empirical experience of the farmers. The hybrid maize is a commercial material used to produce huitlacoche in Urbana, IL. In both cases, huitlacoche was produced in experimental fields during the summer. Ten infected ears from each treatment were collected, and galls were separated from the ears and mixed. All materials were freeze dried, ground to a powder (fine flour) in a lab mill, and stored at 4 °C until analysis. Raw and cooked galls produced in hybrid maize were analysed, and cooking was done by roasting the galls in a conventional skillet on a thermoblock (Corning, New York, NY), at 65 °C for 15 min approximately, without any other ingredients.

2.2. Chemical analyses

All samples were analysed for dry matter, ash, crude protein, and total fat by AOAC methodology (2002), and total carbohydrates were estimated by difference, adding the average values of the ash, crude protein and total fat components, and the sum was subtracted from 100%; these values were expressed on a dry matter basis. The total dietary fibre and insoluble fibre were measured by enzymatic hydrolysis, according to Prosky et al. (1992), using 0.5 g of each freeze-dried sample. Soluble dietary fibre was calculated as total dietary fibre minus insoluble dietary fibre.

The β-glucan concentration was quantified following enzymatic hydrolysis with lichenase (50 U/ml; Megazyme, Bray, County Wicklow, Ireland), and β-glucosidase (2 U/ml; Megazyme), using the AOAC methodology (2002) with the modifications reported by Manzi and Pizzoferrato (2000). Glucose released from β-glucan was quantified by glucose oxidase–peroxidase methodology according to AOAC (2002), and samples were read on a Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 450 nm. Oat and barley were used as positive controls (Dikeman et al., 2005). Free sugars were quantified using high-performance liquid chromatography (HPLC); 0.02 g samples were homogenised with hot water and extracted at 80 °C in a water bath for 1 h (without hydrolysis); then they were filtered through Whatman # 541 filter paper and transferred to a Centriprep-10 centrifuge filter (Amicon, Beverly, MA). Samples were centrifuged at 2790g and 25 °C for 90 min, and the supernatant was used for chromatographic analysis with a Dionex (Dionex, Sunnyvale, CA) DX500 HPLC system, consisting of an AS3500 autosampler, GP 50 gradient pump, and ED40 pulsed electrochemical detector equipped with a gold working electrode. A CarboPac PA-1 column

(Dionex, Sunnyvale, CA) and guard column were used. The mobile phase consisted of 40 mM sodium hydroxide at a flow rate of 1 ml/min, with a postcolumn addition of 300 mM sodium hydroxide at 0.5 ml/min (Dikeman et al., 2005). The injection volume was 25 μl, and the detection of components was compared to an external standard with a regression factor equal to, or greater than 0.9998. Standards for quantification included glucose, fructose, galactose, arabinose, xylose, mannose, sucrose, and fucose, all from Sigma (Sigma, St. Louis, MO).

2.3. Antimutagenic activity

Methanolic extracts were prepared to test antimutagenic activity. Each huitlacoche sample (1 g) was thoroughly mixed with methanol (100%) at room temperature. The methanolic extract was filtered, and concentrated to dryness under nitrogen flow; this procedure was repeated twice. The antimutagenic activity of methanolic gall extracts (20 μl) was measured with *Salmonella typhimurium*-microsuspension assay (TA100), according to Maron and Ames (1983) with modifications. A dose–response curve with different concentrations of methanol (0, 20, 40, 60, 80, or 100%) was generated. Sodium azide (mutagen) was used as a negative control. Percent inhibition (percentage of antimutagenic activity) was calculated as follows:

$$(\text{His}^{+}\ \text{revertants of control plate} - \text{His}^{+}\ \text{revertants of test plate})/(\text{His}^{+}\ \text{revertants of control plate}) * 100$$

2.4. Statistical analysis

All determinations were carried out in duplicate in two independent experiments, and the proximate composition was conducted in only one independent experiment. An entirely randomised 15 × 2 factorial design was used for all determinations in huitlacoche produced in creole maize, and an entirely randomised 6 × 2 factorial design for hybrid maize. Multifactor ANOVA was performed, and means were compared using Tukey's Multiple Range Test ($p = 0.05$). Statgraphics Centurion XV 2007 (StatPoint Inc.; Warrenton, VA) was used for statistical analyses. Correlation analysis between free sugar concentration and antimutagenic activity in huitlacoche produced in creole and hybrid maize, were performed using Excel programme (Microsoft® Excel 2002).

3. Results and discussion

3.1. Proximate analysis

Results of proximate analysis of huitlacoche produced in creole maize are presented in Fig. 1. Genotype 2 (Criollo del Norte) did not produced huitlacoche, thus, the corresponding analysis cannot be conducted. In general, maize genotype had an influence on the concentration of the proximate components, except for ash; stage of development only influenced fat concentrations. In most of the cases, the corresponding average values were very close or almost the same for all components (in relation to stage of development, in each genotype). Tukeýs test showed five statistical groups, and genotypes 9 and 10 showed the highest dry matter content, at both stages of development. In some genotypes, dry matter concentrations diminished at 28 dai (genotypes 4, 8, 11, and 16), and in others increased (genotypes 1, 3, 7, 9, 10, and 12). Despite there being no effect of maize genotype and stage of development over ash content, there was a wide range in this constituent in creole maize flour. Ash values were in the range, and sometimes significantly higher (as in the cases of huitlacoche produced in Criollo de las Cruces (23 dai) and Criollo de la Palmilla (28 dai) maizes), than those reported by Vanegas et al. (1995), for huitlacoche produced in hybrid maize, and those percentages reported for edible mushrooms by León-Guzmán, Silva, and López (1997), and Dikeman et al. (2005). Hence, huitlacoche produced in Criollo de las Cruces and Criollo de la Palmilla could be a potential source of minerals. For protein analyses, eight statistical groups were arranged. The highest protein content was found in genotype 6 (Criollo del Norte de Guanajuato), 1 (Criollo de Cañada de Flores), 4 (Criollo de Loma de Cabras), and 5 (Criollo de Cruz del Palmar) at both stages of development. In general, maize genotype and stage of development affected total fat content. Total fat concentration was low in all maize genotypes, and ranged from 2.0% to 6.3% at 23 and 28 dai, respectively. Genotype 11 (Criollo de la Palmilla) had the highest total fat content, and was statistically different from all genotypes. Total fat was more abundant at 23 dai than 28 dai. Total carbohydrate concentration (data not shown) ranged from 72.7% to 86.6% at 23 dai, and 74.0% to 86.8% at 28 dai.

Proximate composition of huitlacoche produced in hybrid maize is shown in Fig. 2. Cooking process modified slightly the appearance of galls. Developmental stage affected all proximate constituents, except ash, and cooking process affected dry matter only, resulting in an increase in the content of dry matter as expected (except at 32 dai where dry matter diminishes). At most times, ash content was lower in huitlacoche produced in hybrid maize than in creole (2.9–5.3% and 4.2–4.8% in cooked samples, respectively); these values are in the range as those previously reported (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004; Vanegas et al., 1995). Crude protein percentages ranged from 8.9% to 18.8% (11.3% on average) in raw samples, and from 8.8% to 19.3% (11.2% on average), in cooked huitlacoche. Protein was more abundant in the last stage of development (32 dai), followed by the first stage (11 dai) in raw and cooked samples; no statistical differences were observed in the other stages of development. It has been published that there are notable differences in protein expression during *U. maydis* spore germination, being more abundant in the teliospore state (Sacadura & Saville, 2003); at 32 dai, the tissue is almost completely constituted with teliospores. During plant infection by *U. maydis* some proteins are secreted and some are translocated into plant cells (Kämper et al., 2006). Proteins presented in maize grain could have been hydrolysed during *U. maydis* invasion, but further analysis must be conducted to demonstrate this effect. The protein average values for hybrid maize were higher than those for creole maize, but were in the range, and sometimes superior, to those previously reported for huitlacoche produced in other hybrid maizes (Valverde et al., 1995; Vanegas et al., 1995) and other edible mushrooms (León-Guzmán et al., 1997), and they were superior to the average maize protein content (10%) (Paredes-López, Guevara-Lara, & Bello-Pérez, 2006). Therefore, huitlacoche could be proposed as an alternative protein source for vegetarian diets as other edible mushrooms have been proposed (Paredes-López & Valverde, 1999). The highest concentration of total fat was found at 20 dai, and was statistically different from the other stages, followed by 32 dai; 15 and 17 dai was the statistical group with the lowest total fat content. It has been reported that lipids enclosed in lipid bodies in vacuoles, are involved in the development of spores from Basidiomycetes, and they are more abundant at the first and last stages of development (Oláh & Reisinger, 1981). Even when the values for total fat in huitlacoche produced in creole and hybrid maizes are relatively low, a good balance of essential fatty acids has been reported in huitlacoche (Valverde et al., 1995); these values were in the range reported for huitlacoche produced in hybrid maize (Vanegas et al., 1995) and other edible mushrooms, except for *A. rubescens* (León-Guzmán et al., 1997). Barros, Baptista, Correia, et al. (2007), Dikeman et al. (2005), and Manzi et al., 2004) found that cooking affected the chemical composition of other edible

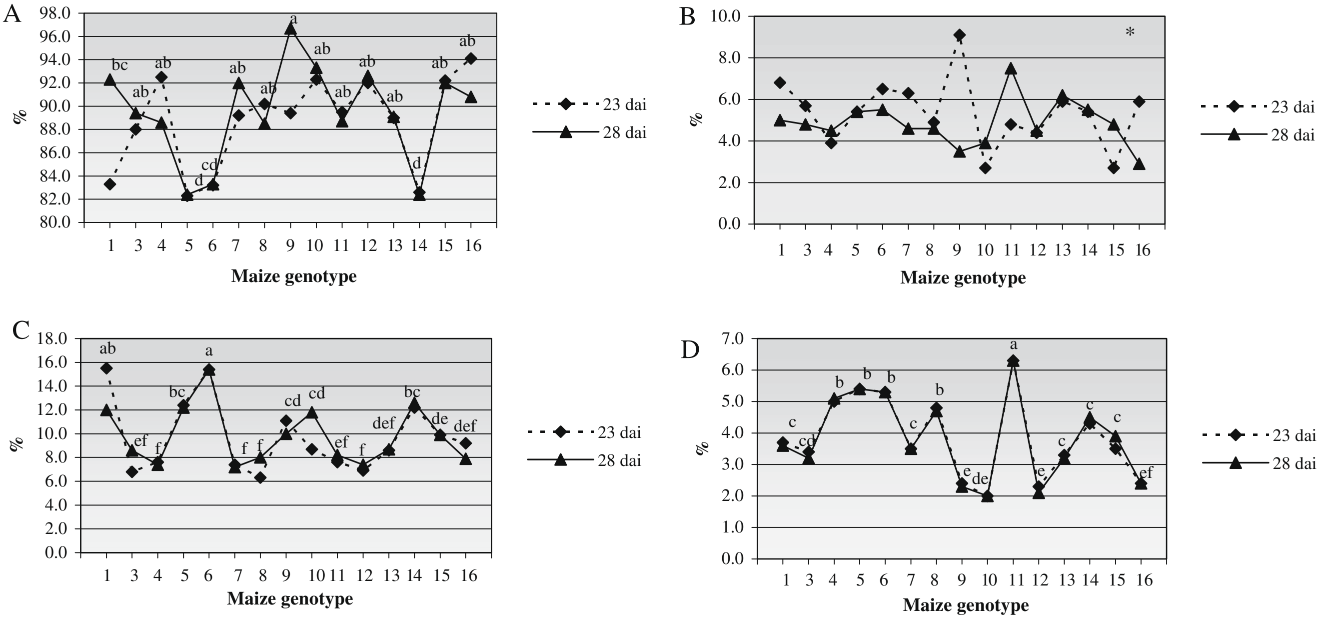

Fig. 1. Proximate constituents in lyophilised huitlacoche flour produced in creole maize harvested at 23 and 28 days after inoculation (dai). Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$). *There are no statistical significant differences between genotypes. A, Dry matter; B, ash; C, protein; D, total fat.

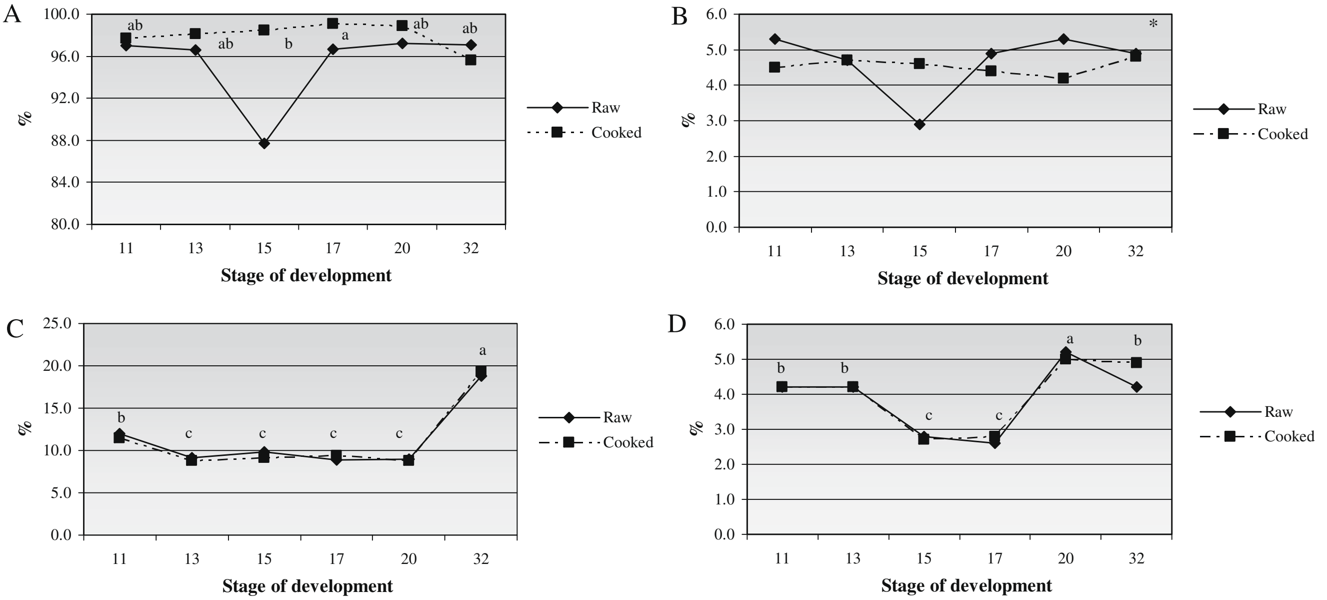

Fig. 2. Proximate constituents in raw and cooked samples of lyophilised huitlacoche flour cultivated in hybrid maize harvested at six stages of development. Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$). *There are no statistical significant differences between stages of development. A, Dry matter; B, ash; C, protein; D, total fat.

mushrooms, and that some nutrients were lost in the process. In this work, statistical analyses showed that only dry matter was affected by the cooking process.

3.2. Total dietary fibre, soluble fibre, insoluble fibre, and β-glucans

Results of total dietary fibre, soluble and insoluble fibres, and β-glucans for huitlacoche grown in creole genotypes are presented in Fig. 3. Total dietary fibre percentages varied from 39.4% to 60.4% at 23 dai (52.6% on average) and from 40.2% to 58.5% at 28 dai (49.7% on average). Although high quantities (90% on average) of total dietary fibre have been found in mushroom sclerotia (Wong & Cheung, 2005), our results were higher than those previously reported for huitlacoche produced in hybrid maizes (Vanegas et al., 1995), and in some cases higher than values reported for other edible mushrooms, such as white button, portabella, enoki, maitake, shiitake, and crimini (Dikeman et al., 2005). Maize genotype and stage of development affected all types of fibre and β-glucans. In relation to developmental stage, the average values for total dietary fibre and insoluble fibre were lower at 28 dai than at 23 dai; and higher for soluble fibre and β-glucans at 28 dai than at 23 dai. Concerning genotype effect, six, ten, eight and five statistical

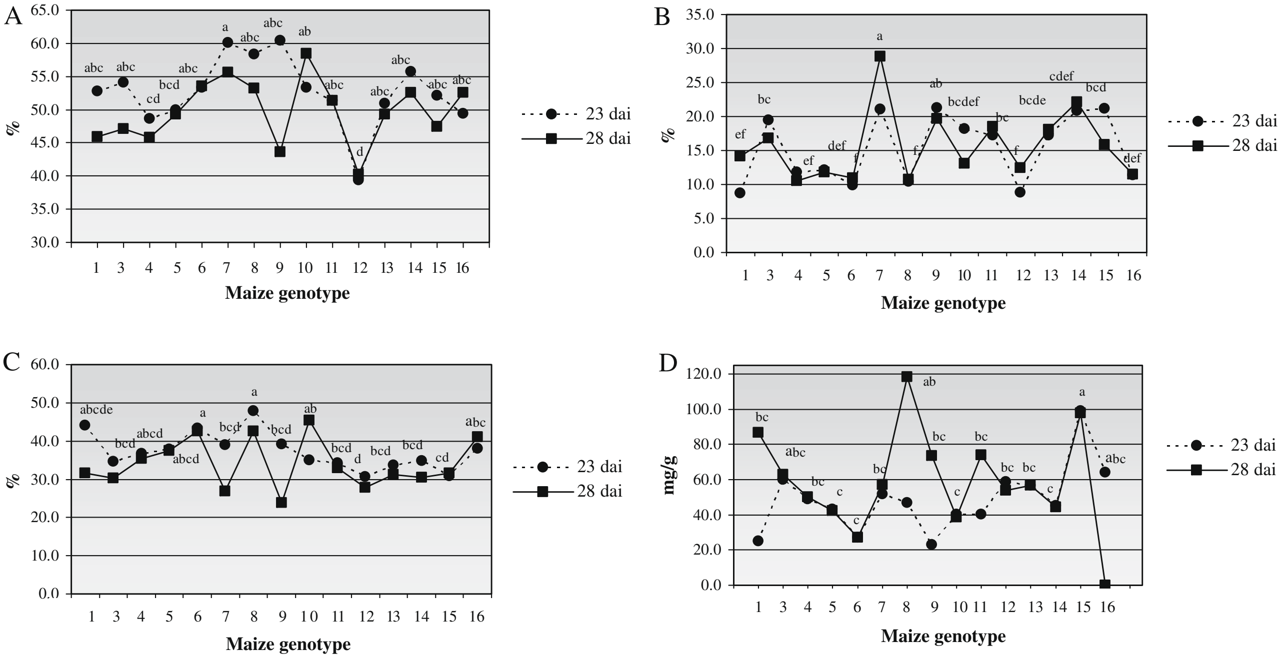

Fig. 3. Concentrations (dry matter basis) of total dietary fibre, soluble and insoluble fibres, and β-glucans in huitlacoche produced in creole maize harvested at 23 and 28 days after inoculation (dai). Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$). A, Total dietary fibre; B, soluble fibre; C, insoluble fibre; D, β-glucans.

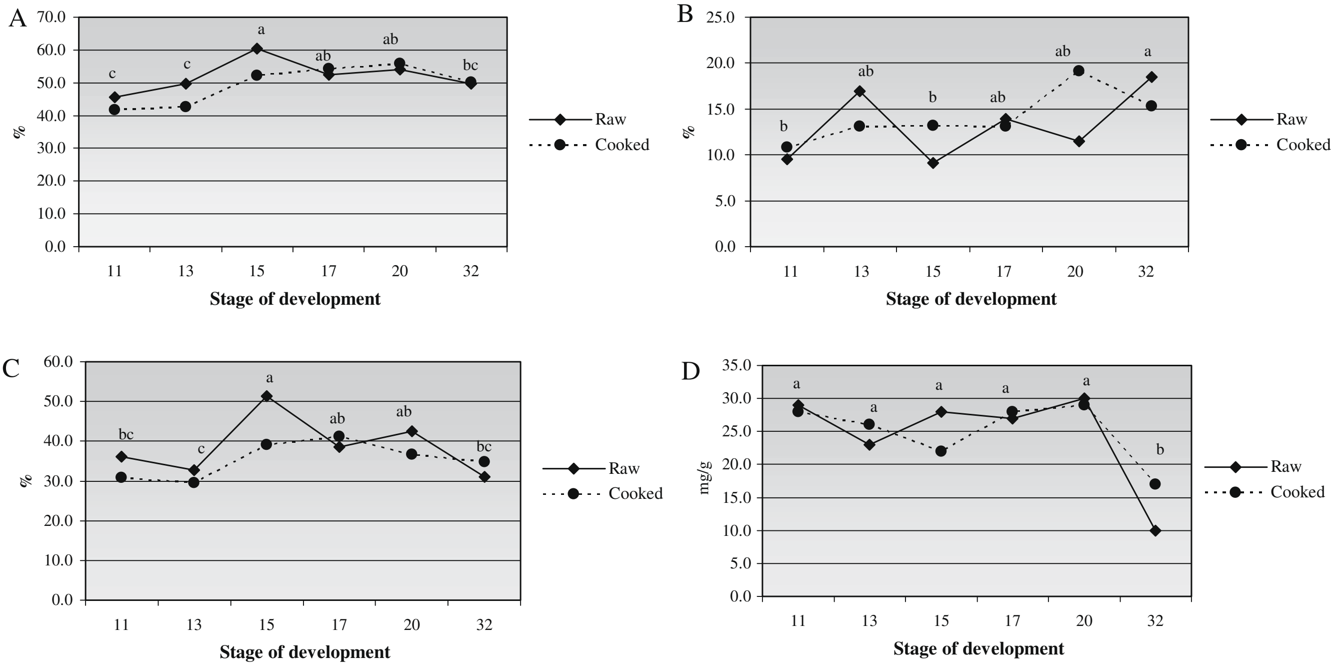

Fig. 4. Total dietary fibre, soluble and insoluble fibres, and β-glucan concentrations (dry matter basis) in raw and cooked samples of huitlacoche produced in hybrid maize harvested at six stages of development (days after inoculation). Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$). A, Total dietary fibre; B, soluble fibre; C, insoluble fibre; D, β-glucans.

groups were arranged for total dietary fibre, soluble and insoluble fibres, and β-glucans, respectively. High quantities of β-glucans were found in galls produced in creole maizes, genotypes 15 and 8 (Corina Cajete and Criollo de la Biznaga, respectively) had the highest concentrations. Our values were higher than those found by Dikeman et al. (2005) and Manzi and Pizzoferrato (2000) for *Pleurotus ostreatus* and *Lentinula edodes*, respectively, and in some cases too close to those previously reported for oat and barley (De Francisco, Franciele Rosa, & Sartori da Silva, 2006). We have assessed the β-glucan content in some creole genotypes and hybrid maize, and found concentrations ranging from 2.3 to 3.8 mg/g (flour, dry matter basis) in creole maize and 2.0 mg/g in hybrid maize. Hence, *U. maydis* development on the maize grain confers high levels of β-glucans in huitlacoche.

Total dietary fibre in hybrid maize ranged from 45.7% to 60.5% (52.0% on average) in flour of raw samples, and 41.7% to 55.8% (49.5% on average) in cooked samples (Fig. 4). Tukeýs test showed that stage of development affected all analysed compounds, and cooking process affected only total dietary fibre and insoluble fibre. Dikeman et al. (2005) also found changes in nutrient content as a result of the stage of maturity in other edible mushrooms. Very similar fibre percentages were found between huitlacoche produced in creole and hybrid maize. Fibre in hybrid maize was higher than that previously reported for huitlacoche (Vanegas et al., 1995) and other foods, such as common beans, whose total dietary fibre content ranges from 16% to 27% (Paredes-López et al., 2006) and similar, but in some cases higher than values reported for other edible mushrooms (Dikeman et al., 2005) and wheat bran (Saura-Calixto, 2006). β-Glucans in huitlacoche produced in hybrid maize were at higher concentrations than reported for other edible mushrooms (Dikeman et al., 2005; Manzi & Pizzoferrato, 2000) and slightly lower than reported for barley (de Francisco et al., 2006). No statistical difference was obtained between 11 and 20 dai in β-glucan content, but this parameter considerably diminished at 32 dai. Glucans that accumulate intracellularly during fungi development are used as reserve material and utilised at critical stages of development (Bowman & Free, 2006). It has been reported that the main compounds in edible mushrooms that confer anticarcinogenic activity are polysaccharides, such as β-glucans, and proteins (Guerra-Dore et al., 2007; Shimizu et al., 2008). β-Glucans have been tested in different *in vitro* and *in vivo* systems in the *Agaricus*, *Pleurotus*, and *Polyporus* genera for their antihyperglycaemic, cytotoxic, and other effects (Kaneno et al., 2004; Wong et al., 2005). Guerra-Dore et al. (2007) reported a 60.4% inhibition of ear oedema in mice with 10 mg/kg β-glucans injected intravenously. Huitlacoche produced in creole and hybrid maize possessed significant amounts of fibre; and some of these genotypes can be proposed as a special food with outstanding β-glucans content.

3.3. Free sugars

Fig. 5 presents the results for free sugars content of huitlacoche produced in creole maize. On average, and in decreasing order, the free sugars found were glucose, fructose, galactose, arabinose, xylose, and mannose. Fucose and sucrose were not found in any sample. Maize genotype had an influence on all free sugars concentration, and stage of development affected fructose, galactose, xylose and mannose concentration. Fructose, galactose and xylose were more abundant at 28 dai than at 23 dai, and mannose was higher at 23 dai than at 28 dai. Four, twelve, seven, four, five and four statistical groups were established for glucose, fructose, galactose, arabinose, xylose and mannose, respectively. In the case of fructose, a high number of statistical groups were determined, but none of them was totally different from the others; this means that fructose values were closer between genotypes than other free sugars. Sucrose has not been reported as a free sugar in edible

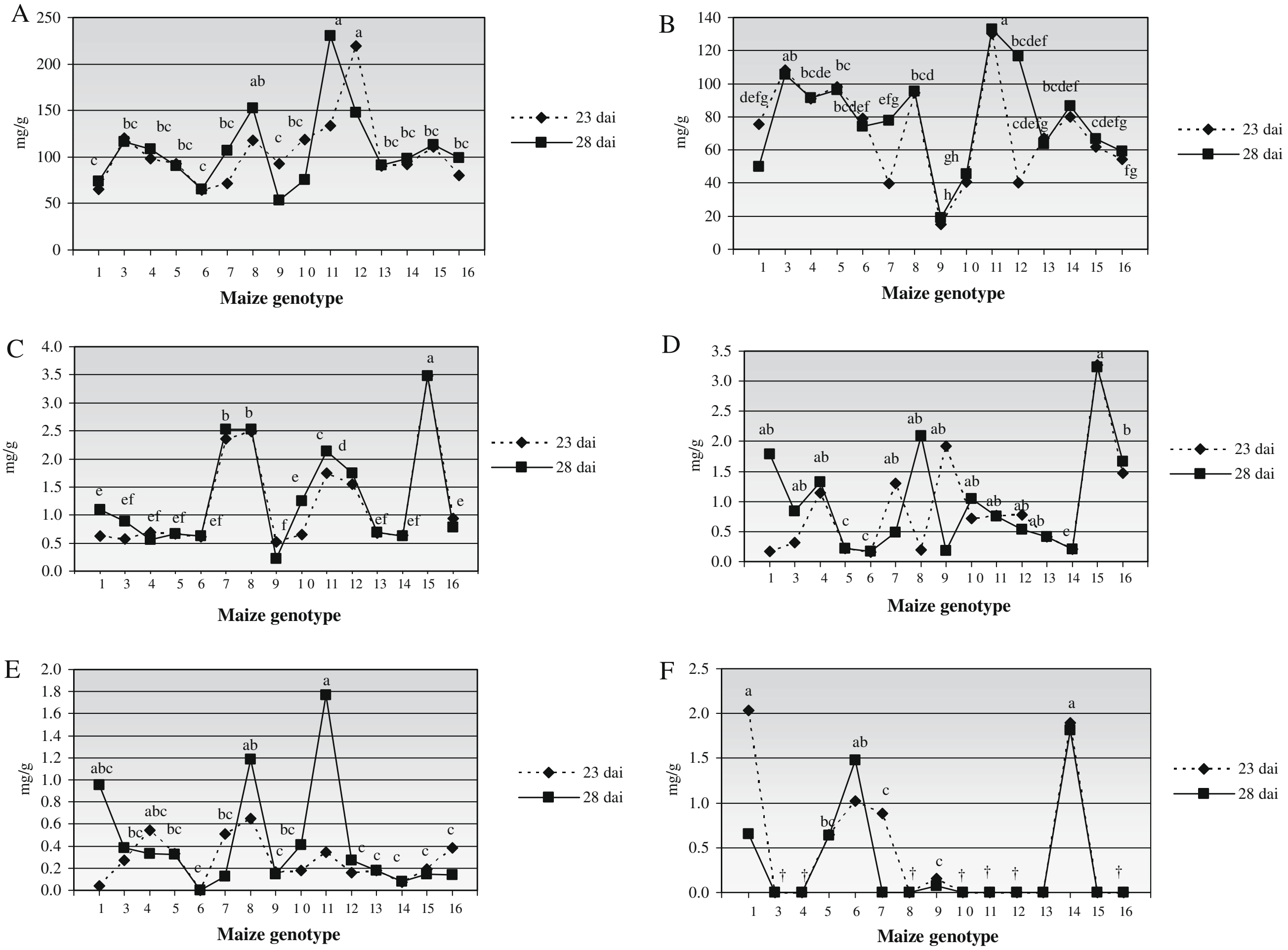

Fig. 5. Concentrations (dry matter basis) of free sugars in huitlacoche produced on creole maize harvested at 23 and 28 days after inoculation (dai). Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$). †None found. A, Glucose; B, fructose; C, galactose; D, arabinose; E, xylose; F, mannose.

mushrooms; it was reported in moderate concentrations in different maize genotypes (Alexander & Greech, 1976). Total content of free sugars ranged from 111.0 to 267.0 mg/g flour (179.0 mg/g on average) at 23 dai, and from 56.2 to 268.0 mg/g (182 mg/g on average) at 28 dai. Barros, Baptista, Correia, et al. (2007) reported a total free sugars concentration ranging from 34.9 to 303.0 mg/g (dry matter) for wild edible mushrooms. Our data were in this range, or slightly below. Glucose, arabinose, and mannose content obtained in all creole genotypes were higher when compared to values reported by Dikeman et al. (2005) for other edible mushrooms. Total free sugars were predominantly composed of free glucose (64.3–220.0 mg/g at 23 dai, and 53.7–231.0 mg/g at 28 dai). Glucose has been reported in grains of different white maize genotypes in concentrations ranging from 1.0 to 8.9 mg/g dry weight (Shaw & Dickinson, 1984), and in normal and extra sweet hybrid maizes in concentrations ranging from 15.7 to 45.7 mg/g and 23.4 to 49.8 mg/g (dry matter basis), respectively, depending on grain moisture content (Reyes, Varseveld, & Kuhn, 1982). The high levels of glucose in huitlacoche are conferred by *U. maydis*. In other foods, such as papaya and banana, glucose has been reported in concentrations of 44.3 and 43.7 mg/g dry weight, respectively; while fructose concentration was 35.9 and 41.7 mg/g dry weight in papaya and banana, respectively (Torija et al., 1998). Consequently, the concentration of these two monosaccharides is higher in huitlacoche produced in creole maize than reported for papaya and banana. Mannose and xylose levels were low in some genotypes, and were not found in others. Genotype 15 (Corina Cajete) showed the highest content of galactose and arabinose and was statistically different to the rest of all genotypes. The importance in studying some of these free sugars is due to their potential health benefits; galactose, arabinose, xylose, and mannose have nutraceutical properties (Iisakka, 2003). Galactose is a sugar substitute with functional applications and beneficial properties, such as rapid absorption potential, while maintaining more stable blood sugar concentrations than other sugars. Arabinose, commonly used in pharmaceutical applications, also has been used in weight control and diabetic dietary applications. Xylose is another sugar substitute, and when converted to xylitol has the property of reducing tooth decay and plaque formation. Mannose has extensive nutraceutical and pharmaceutical applications, and has been utilised as an agent against viruses and bacterial infections (Iisakka, 2003).

Free sugars found in huitlacoche produced in hybrid samples are presented in Fig. 6. The stage of development affected the content of all free sugars, and cooking process affected fructose, galactose and xylose content; these monosaccharides increased in the cooked samples. The generated heat from the cooking process

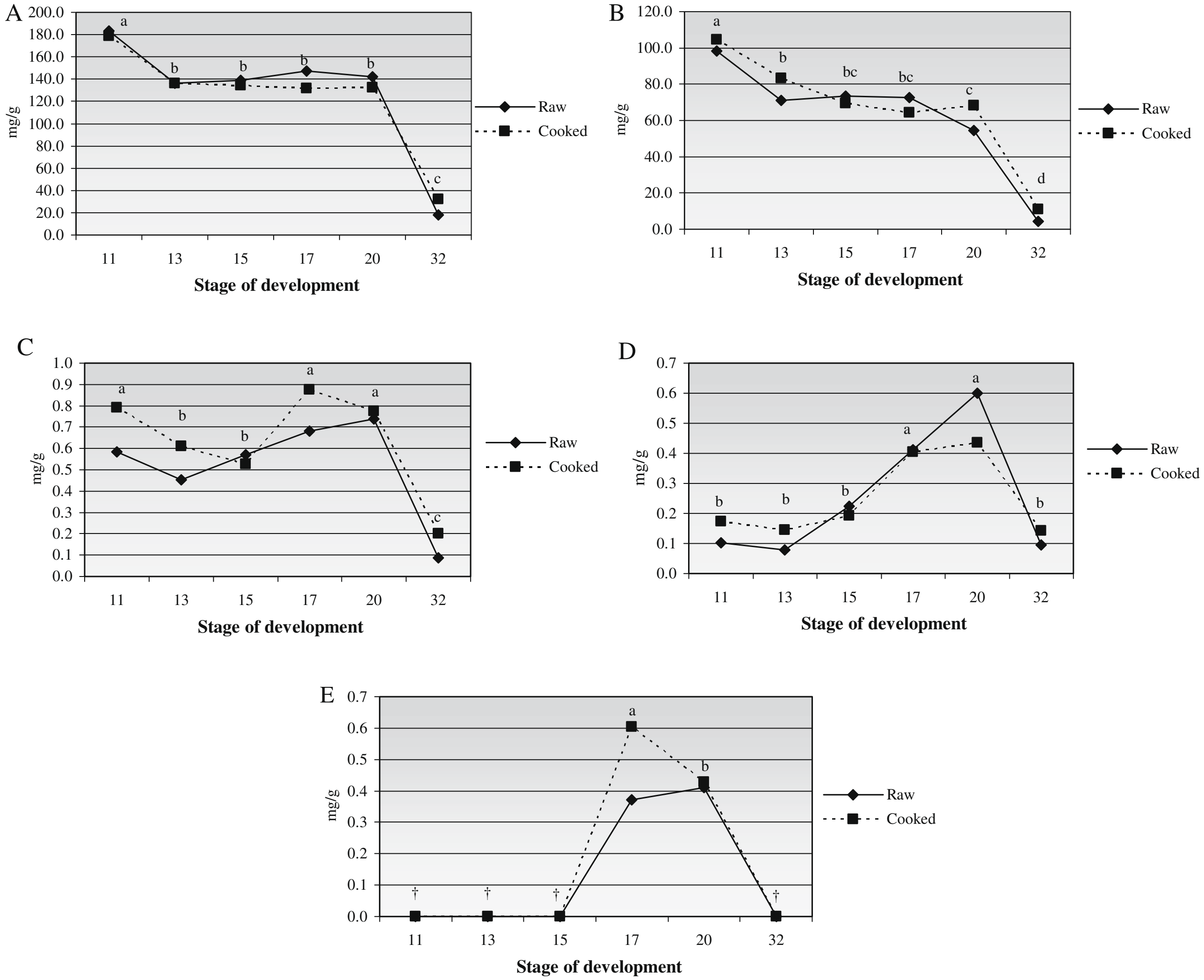

Fig. 6. Concentrations (dry matter basis) of free sugars in huitlacoche produced on creole maize harvested at six stages of development (days after inoculation). Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$). †None found at these stages of development. A, Glucose; B, fructose; C, galactose; D, arabinose; E, xylose.

could have solubilised some sugars bound to other molecules. Dikeman et al. (2005) and Barros, Baptista, Correia, et al. (2007) found in different edible mushrooms that cooking affected carbohydrate content, and most of the time the concentration decreased. In this work, glucose and fructose were the main free sugars quantified in hybrid maize, and galactose, arabinose, and xylose were found in low concentrations, but higher than concentrations reported by Dikeman et al. (2005). Xylose was found at 17 and 20 dai, and mannose was not found in huitlacoche produced in hybrid maize. The content of glucose, fructose and galactose decreased at intermediate stages of development (13–17 dai), with respect to 11 dai; this trend may be explained by their energetic role in fungal growth. Hammond and Nichols (1976) reported changes in the content of soluble carbohydrates during the growth of sporophores from *Agaricus bisporus*; they found that the sugar concentration sometimes increased and other times decreased.

3.4. Antimutagenic activity

Antimutagenic test is a short-term assay and is used to determine the mutagenic or antimutagenic effect of a compound, and to understand the mechanisms of action of potentially toxic compounds. The *S. typhimurium* histidine (his) reversion system is a microbial assay that measures his$^-$ → his$^+$ reversion induced by chemicals (mutagens). The system induces base changes or frameshift mutations in the genome of the microorganism (strain TA100 and TA98, respectively), and it may grow in a histidine-free culture medium. Fig. 7A presents the results of the antimutagenic activity assay for methanolic extracts of huitlacoche produced in creole genotypes. Inhibition percentages for these samples ranged from 49.0% to 69.0% at 23 dai, and from 53.0% to 76.0% at 28 dai. There was an influence of maize genotype on antimutagenic activity, but there was no effect of developmental stage on this criterion. Methanol, at the concentration tested (data not shown), did not exhibit an antimutagenic effect on *S. typhimurium*. Three colonies were observed growing in the culture medium which represents a mutational natural frequency of less than 1×10^{-8}. The Creole genotype with the highest antimutagenic activity was Criollo del Norte de Guanajuato (genotype number 6) (69.0% and 76.0% at 23 and 28 dai, respectively).

Results of antimutagenic activity in the hybrid genotype are shown in Fig. 7B. Antimutagenic capacity was from 41.0% to 68.0% in raw samples, and from 41.0% to 64.0% in the cooked material. Tukeýs test showed that developmental stage and cooking process affected antimutagenicity. Antimutagenic activity tended to diminish at 15 and 20 dai; this effect could be a result of the synthesis of some compounds by the maize plant, such as sesquiterpenes, phenolic compounds and indole to protect itself from pathogens damage. In preliminary studies, Basse (2005) observed that this mechanism is used by the plant to diminish the damage caused by *U. maydis* invasion.

Antimutagenic activity of huitlacoche produced in creole and hybrid maize appears to be acceptable compared with that reported by Grüter, Friederich, and Würgler (1990) for *Craterellus cornucopioides*, *Agaricus bisporus*, *Lactarius lilacinus,* and other edible mushrooms. They found an inhibition up to 97% against 2-nitrofluorene using *S. typhimurium* TM677. Bunkova, Marova, and Nemec (2005) noted that an inhibition from 60% to 80% represents a strong positive antimutagenic activity using *S. typhimurium* TA98.

Kaneno et al. (2004) reported the effect of methanolic extracts on the enhancement of the cellular-positive response of tumour-bearing mice during the initial phase; these extracts contained

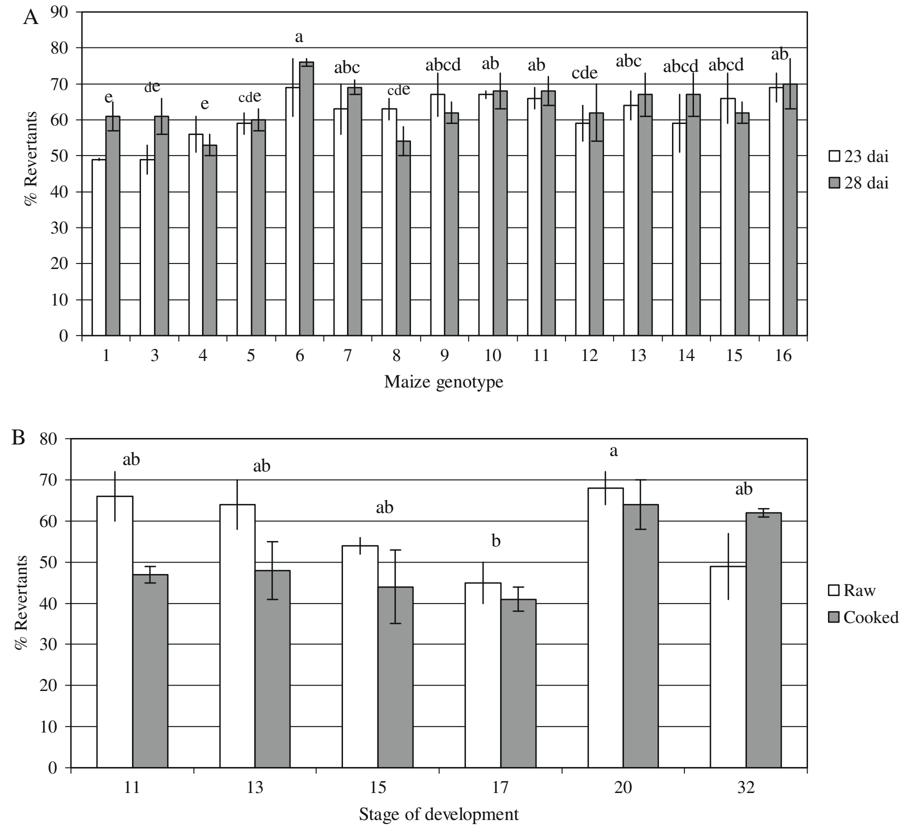

Fig. 7. Antimutagenic activity shown by methanolic extracts of huitlacoche. (a): Huitlacoche produced in creole maize harvested at 23 and 28 days after inoculation (dai). (B): Huitlacoche (raw and cooked) cultivated in hybrid maize harvested at six stages of development (days after inoculation). Different letters mean statistically significant differences between genotypes (Multiple range test; Tukey, $p = 0.05$).

some small sugars and amino acids. In our experiment, there was no correlation between free sugars concentration and antimutagenic activity. In order to determine the compounds that confer the antimutagenic activity observed in the huitlacoche methanolic extracts, further assays should be conducted.

4. Conclusion

In summary, mushrooms are valuable health foods with long recognised nutritional value. Huitlacoche produced under different conditions had high concentrations of selected nutrients and compounds with nutraceutical potential, which showed variations due to maize genotype, stage of development, and cooking process. Despite that 23 and 20 dai being the stages of development with the best visual characteristics (creole and hybrid maize, respectively), these did not always show the highest concentrations of the evaluated nutraceutical compounds.

Our work is the first report assessing concentrations of compounds with nutraceutical potential and antimutagenic activity of huitlacoche; and it is a preliminary study to assess the effect of maize genotypes and stages of development on huitlacoche quality. Huitlacoche must be incorporated into the daily diet because of its attractive characteristics, such as a unique flavour, and valuable compounds such as those found in this study.

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