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Bioaccessibility and antioxidant activity of free phenolic compounds and oligosaccharides from corn (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) chips during *in vitro* gastrointestinal digestion and simulated colonic fermentation

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ARTICLE INFO

Chemical compounds studied in this article:
(+)-Catechin (PubChem CID: 9064)
Gallic acid (PubChem CID: 370)
Caffeic acid (PubChem CID: 689043)
Ferulic acid (PubChem CID: 445858)
Chlorogenic acid (PubChem CID: 1794427)
1,1-Diphenil-2-picrylhydrazil (DPPH) (PubChem CID: 2735032)
2,2-Azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) (PubChem CID: 9570474)
Raffinose (PubChem CID: 439242)
Stachyose (PubChem CID: 429531)
Verbascose (PubChem CID: 441434)

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ABSTRACT

Corn (*Zea mays* L.) and common beans (*Phaseolus vulgaris* L.) are alternative suitable ingredients for snacks, because of their content of bioactive compounds such as phenolic compounds (PC) and oligosaccharides (OS). However, there is no information about the transformation of these compounds associated with food matrix during gastrointestinal digestion. Therefore, the objective of this work was to simulate the whole digestion process (mouth to colon) to estimate bioaccessibility and small intestine permeability of free PC and OS, and the antioxidant capacity of free PC. Digested nixtamalized corn-cooked common bean chips exhibited significant different quantities of free PC and OS, and higher antioxidant activity compared to methanolic extract. The free PC showed high values of apparent permeability coefficients ($0.023–0.729 \times 10^{-3}$), related with their absorption in the small intestine. Both free PC and OS were retained in the non-digestible fraction of chips (10.24–64.4%) and were able to reach the colon. Our results suggest the digestion potential to increase chip bioactive compounds and antioxidant activity. Additional studies are required to evaluate their *in vivo* effects.

1. Introduction

Snack food products can be defined as those that are consumed between larger meals or the traditional three meals each day, being popular because of their convenience and the satiety effect on consumers, characterizing a significant element of recent eating behavior studies (Forbes, Kahiya, & Balderstone, 2016). Snack consumption has increased over the last few decades, despite their traditional reputation of being unhealthy products with an excess of calories. Most of them are subjected to frying processes and have little contribution of nutrients, leading to food-related health issues such as obesity, among others (Forbes et al., 2016; Story, Kaphingst, Robinson-O'Brien, & Glanz, 2008).

Public health agencies pointed out that low-energy density foods could be an effective strategy to counteract the effects of high calorie-snack consumption (Ello-Martin, Ledikwe, & Rolls, 2005). Hence, food snack industry has been aimed to develop healthy snack options, especially oriented to healthier child and youth (Story et al., 2008). On

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the other hand, common beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) have gained a reputation for being foods of Mexican origin with a high variety of bioactive compounds, such as dietary fiber (oligosaccharides) and polyphenols (Campos-Vega, Oomah, Loarca-Piña, & Vergara-Castañeda, 2013; Luo & Wang, 2012). For their antioxidant and anti-inflammatory potential, these compounds are associated to health benefits, for instance, the reduction non-transmissible chronic diseases (overweight, obesity and cardiovascular diseases, among others) (Ozcan, Akpinar-Bayizit, Yilmaz-Ersan, & Delikanli, 2014). Taking advantage of the biological potential of such foods, the development of healthy snacks suing mixtures of corn and common beans have been reported previously in tortillas (Treviño-Mejía, Luna-Vital, Gaytán-Martínez, Mendoza, & Loarca-Piña, 2016) and chips (Ochoa-Martínez, Castillo-Vázquez, Figueroa-Cárdenas, Morales-Castro, & Gallegos-Infante, 2016). The health functionality of bioactive compounds depends on the manner in which they are metabolized, and bioaccessibility studies are critical to validate functional foods health claims (Rein et al., 2013). *In vitro* gastrointestinal models may be a useful approach to estimate the capacity of bioactive compounds to produce an effect, simulating *in vivo* conditions. Thus, the aim of this work was to perform an *in vitro* gastrointestinal digestion and simulate a colonic fermentation of chips made from corn (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.), evaluating both *in vitro* bioaccessibility and intestinal permeability of polyphenols and oligosaccharides found in small and large intestine, as well their antioxidant activity.

2. Materials and methods

2.1. Materials

Northwest White Population (NWP) corn (*Zea mays* L.) kernels and Bayo Madero (BM) seeds from common beans (*Phaseolus vulgaris* L.) were provided by "Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Bajío" (INIFAP), located in Celaya (Guanajuato, Mexico). The damaged seeds were removed and samples were stored at 4 °C until the analysis was carried out. The chemicals were purchased from Sigma Chemical Co. and J. T. Baker (Mexico City, Mexico). The animals (14 Wistar male rats) were provided by "Instituto Nacional de Neurobiologia" (UNAM, Campus Juriquilla, Mexico). Both nixtamalized corn and cooked common bean flours were stored at 4 °C before their corresponding use.

2.2. Nixtamalization process

Nixtamalized corn flour was prepared with commercial lime (Ca $(OH)_2$) acquired in the local market, commonly used in the tortilla industry and prepared according to the procedure described by Serna-Saldivar, Gomez, and Rooney (1990). The resulted nixtamalized corn was milled (FUMASA 100, Mexico) and dehydrated using a flash dryer (CINVESTAV-GAV M2000, Mexico). Before storage, the nixtamalized corn was ground using a hammer mill (PULVEX 200, Mexico) equipped with a 0.85 mm screen. At the time of preparation, the nixtamalized corn flour was screened through a 250 μm mesh, where at least 50% of the flour passed through the mesh.

2.3. Bean flour preparation

Bayo Madero (BM) raw beans were cleaned and cooked accordingly to the procedure described by Aparicio-Fernández, Manzo-Bonilla, & Loarca-Piña(2005). Dehydrated beans were ground using a coffee grinder (KRUPS GX4100) and screened with a 425 μm mesh, where at least 50% of the flour was filtered through the mesh.

2.4. Chips preparation

Corn–bean chips were produced with 70% NWP nixtamalized corn flour and 30% BM cooked bean flour (70C/30B). These proportions of corn and beans were selected according to daily *per capita* consumption of tortillas and beans in Mexico reported by Mora-Avilés et al. (2007). For preparing chips dough, water was added to the flour in a 1:1 proportion and shaped into chips (3 ± 0.5 g weight) using a commercial tortilla roll machine (Villamex V-25, Monterrey, Mexico). The dough was baked at 180 ± 2 °C in a convection oven for 10 min, the conditions were adjusted after the preliminary tests were done to find the best method to produce chips.

2.5. Free phenolic compounds extraction and quantification

Methanolic extract was obtained according to the procedure of Cardador-Martínez, Loarca-Piña, and Oomah (2002).

The phenolics were analyzed according to Ramírez-Jiménez, Reynoso-Camacho, Mendoza-Díaz, and Loarca-Piña (2014). A High-performance liquid chromatography-diode array detection (HPLC-DAD) analysis was conducted on an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) using a Zorbax Eclipse XDB-C18 column (Agilent Technologies, 4.6 250 mm). The column was thermostatically controlled at 35 ± 0.6 °C and the flow rate was set to 1 mL/min. The mobile phase consisted of two solvents: solvent A was water adjusted with 1% acetic acid and solvent B was acetonitrile. A linear gradient was used as follows: 80–83% of solvent A was held for 7 min, 83–60% for 5 min, 60–50% for 1 min and 50–85% for 2 min. A detection was conducted at 280 nm with an acquisition speed of 1 s. A volume of 20 μL was injected and the samples were analyzed in duplicate. A quantification was carried out using the external standard method with commercial standards of (+)-catechin, quercetin, vanillin and ellagic, caffeic, ferulic, gallic, chlorogenic, and sinapic acids.

2.6. Antioxidant activity by DPPH/ABTS methods

Antioxidant activity was estimated using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the modified method reported by Cardador-Martínez et al. (2002). The ABTS method was done using the 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay with the modified method as reported by Loarca-Piña, Mendoza, Ramos-Gómez, and Reynoso (2010). The trolox equivalent antioxidant capacity (TEAC) value was calculated using Trolox as the standard for the calibration curve and expressed as μmol of Trolox equivalents per gram of the sample (μmol Trolox/g sample).

2.7. Oligosaccharides extraction and quantification

Oligosaccharides were extracted from chips according to the procedure reported by Díaz-Batalla, Widholm, Fahey, Castaño-Tostado, and Paredes-López (2006).

A high-performance liquid chromatography-refractive index detector (HPLC-RID) analysis was done with an Agilent 1100 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) using a Zorbax Eclipse XDB-C18 column (Agilent Technologies, 4.6 250 mm). The column was thermostatically controlled at 35 ± 0.6 °C and the flow rate was set to 1 mL/min. Water adjusted with 50% acetonitrile was used as mobile phase at 1.00 mL/min (Muzquiz, Burbano, Pedrosa, Folkman, & Gulewicz, 1999). The standard curves were determined with the use of raffinose, stachyose and verbascose (Sigma-Aldrich, St. Louis, MO, U.S.A.).

2.8. In vitro gastrointestinal digestion

The human physiological conditions were simulated following the procedure described by Campos-Vega, Vázquez-Sánchez, López-Barrera, and Loarca-Piña (2015) with slight modifications. All human subjects provided written consent before participating in the study. Chips (1 g) were chewed 15 times for ~15 s. After chewing, the product