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Microbial metabolites profile during *in vitro* human colonic fermentation of breakfast menus consumed by Mexican school children

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ABSTRACT

The nutrition transition promotes the development of childhood obesity. Currently, Mexico is affected by this serious public health problem. The nutritional and functional characterization of a whole menu has a number of advantages over the study of single nutrients. Since breakfast is considered the most important meal of the day, this study aimed to evaluate the metabolite profile produced by *in vitro* human colonic fermentation of the isolated indigestible fraction (IF) from three different Mexican breakfast (M-B) menus (Modified "MM-B", traditional "TM-B", and alternative "AM-B"), previously identified as commonly consumed by Mexican schoolchildren in Nayarit State, Mexico. The M-B's consist of egg, corn tortilla, beans (higher in TM-B), sugar and chocolate powder (higher in AM-B) and milk, combined in different proportions. The IF in all breakfasts was about 4.7–5.6 g/100 g FW, with a relatively high content of protein (≈21%), which might have negative physiological implications. Fermentation of IF from TM-B resulted in the largest pH decrease after 72 h (pH = 6.07), with a low short chain fatty acid (SCFA) production (0.75 to 47.23 mmol/L), but greater relative concentration of other fatty acids (FA) (C7, C8, C9). Besides, 55 volatile compounds were detected in the fermentation media by SPME-GC-MS and three principal components (PC) were identified. PC1 was influenced by SCFA production, low FA esters production (<8C), and low volatile organic acids production. PC2 was influenced by the decrease in pH and an increase in antioxidant capacity ($p < 0.0001$). These results suggest that the production of different metabolites in the luminal medium may affect the pH and antioxidant status in the colon. Fermentation of IF from TM-M, assessed after 48 and 72 h, showed the highest correlation for PC2; the metabolic pattern registered for this IF maybe considered beneficial.

1. Introduction

Childhood obesity has been associated with numerous negative health and psychological outcomes. It has been explained as consequence of globalization-related changes in diet and lifestyle that promote positive energy balance (Malik, Willett, & Hu, 2013). Most dietary guidelines agree on the importance of a correct distribution of calories and nutrients ingested throughout the day, which is achieved principally by a regular meal consumption of breakfast, lunch, and dinner (US-DHHS and USDA, 2015). In Mexico, obesity epidemic among schoolchildren has resulted in federal and state policies that aim to improve school food environments (Levy et al., 2012). It is often stated that breakfast is the most important meal of the day, and the association between breakfast consumption and its nutritional quality is related with a reduced risk to develop metabolic syndrome (Odegaard et al., 2013). However, taking into account that nutrients or foods are rarely eaten isolated, studies of whole menus or dietary patterns have advantages over the single-nutrient or single-food approach, as they take into account the occurrence of synergistic or antagonistic biochemical interactions among nutrients, as well as the existence of different food sources of the same nutrient (Barbaresko, Koch, Schulze, & Nöthlings, 2013).

Abbreviations: AM-B, alternative Mexican breakfast; AOX, antioxidant capacity; CGC/MS, gas chromatography–mass spectrometry; DP, dietary pattern; FA, fatty acid; FW, fresh weight; HCA, Hierarchical cluster analysis; HS-SPME, headspace solid-phase microextraction; IF, Indigestible fraction; MM-B, modified Mexican breakfast; PCA, Principal component analysis; SCFA, short chain fatty acid; TM-B, traditional Mexican breakfast.

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Most available information on breakfast dietary patterns (DP) and health in schoolchildren is derived principally from epidemiological studies (Karatzi et al., 2014). However, information is scare about the composition of whole menus identified in different DP. Probably, the combination of epidemiological and *in vitro* analysis of meal menus may contribute to a better understanding of the effects that food can exert on health. The fermentative processes that take place in the human large intestine have raised much interest in the last decades. Different fermentation-derived metabolites have important physiological activity at both colonic and peripheral levels (Flint, 2016). In this work, we evaluated the metabolites profile obtained after *in vitro* colonic fermentation of the indigestible fraction (IF) isolated from three breakfast menus consumed by Mexican schoolchildren in Nayarit State, Mexico. Particular attention was paid to metabolites associated with changes in pH and antioxidant capacity (AOX). Chemical composition, extractable and non-extractable polyphenols content, and AOX of the isolated IFs were also evaluated.

2. Material and methods

2.1. Preparation of breakfast menus

Data sources on DP and food frequency consumption at breakfast were obtained from a nutritional survey carried out on eleven public schools in Tepic (Nayarit State, Mexico). The study, being reported elsewhere, revealed no clear-cut relationship between overweight-obesity status of school children and diet types, although the macronutrient intake profile was dependent on the type of diet consumed (Zamora-Gasga et al., in press). For this reason, frequently consumed foods in each DP were used to create three breakfast menus, which comprised the following foods: a) Modified Mexican breakfast (MM-B) (≈394 g total weight, 295 Kcal): one scrambled egg, three corn tortillas (90 g), chocolate milk (264 mL whole milk, 7 g sugar cane, 13 g chocolate powder); b) Traditional Mexican breakfast (TM-B) (≈478 g total weight, 377 Kcal): one scrambled egg, three corn tortillas (90 g), refried beans (83 g), chocolate milk (264 mL whole milk, 6 g sugar cane, 11 g chocolate powder); c) Alternative Mexican breakfast (AM-B) (≈370 g total weight, 308 Kcal): one scrambled egg, two corn tortillas (60 g), chocolate milk (256 mL whole milk, 10 g sugar cane, 18 g chocolate powder). Individual ingredients were purchased from the local market and menus were prepared in the laboratory kitchen according to traditional regional customs. Freshly prepared menus were homogenized in a food processor (NB-101B, Nutribullet, China), frozen (−80 °C), freeze-dried (FreeZone 6, Labconco, USA), ground, sieved through a mesh size of 500 μm, and stored at −20 °C until analysis. Each menu was prepared by triplicate.

2.2. Quantification and isolation of indigestible fraction (IF) in menus

IF was evaluated according to Saura-Calixto, García-Alonso, Goni, and Bravo (2000), a method that simulates the physiological conditions in the upper gastrointestinal tract. The method was run at preparative scale, according to the modifications proposed by Tabernero, Venema, Maathuis, and Saura-Calixto (2011). Insoluble (IIF) was considered as the digestion residues pelleted by centrifugation, while those retained by dialysis represented soluble IF (SIF); the sum of both fractions equals total IF (TIF). TIF was collected, freeze-dried, milled (IKA M20, USA), sieved (500-μm mesh), and stored in seal bags at −20 °C.

2.2.1. Chemical composition of indigestible fraction (IF)

Moisture content, ash, protein, and fat in IF were analyzed according to AOAC (1990) 925.10, 923.03, 920.87 and 920.39 methods, respectively. Indigestible carbohydrates were estimated by Dubois, Gilles, Hamilton, Rebers, and Smith (1956) method; briefly, 250 mg of IF isolated from breakfast menus were suspended with 25 mL of water in constant stirring, 1 mL of this suspension was mixed with 0.5 mL of phenol (5% w/v) and 2.5 mL of concentrated sulfuric acid (36% v/v). The absorbance measured at 480 nm was compared to a standard glucose curve in the range 10–100 μg/mL. Resistant Starch (RS) was evaluated with the methodology proposed by Goñi, García-Diz, Mañas, and Saura-Calixto (1996). Briefly, 25 mg IF were solubilized in 4 M KOH, neutralized with HCl and hydrolyzed with amyloglucosidase (A-9913, Sigma Aldrich, USA, pH 4.75, 60° C). RS was quantified measuring released glucose, using the GOD-POD enzymatic/colorimetric kit (Spinreact, Spain).

2.2.2. Antioxidant compounds and capacity (AOX) analysis in the indigestible fraction

TIF samples (250 mg) from the different menus were extracted with aqueous-organic solution according to the methodology proposed by Pérez-Jiménez, Arranz, and Saura-Calixto (2009). Total soluble polyphenols (TSP) in extractable fraction were determined with the Folin–Ciocalteu's reagent (Montreau, 1972) using a 96-well microplate reader (Biotek, Synergy HT, Winooski VT, USA) with Gen5 software, and the results were expressed as gallic acid equivalents (g GAE/100 g menu FW). Supernatants were evaluated for AOX by1,1-Diphenyl-2-picryl hydrazyl (DPPH) antiradical activity assay was performed according to the method of Prior, Wu, and Schaich (2005). Ferric reducing antioxidant power (FRAP) assay was performed as described by Benzie and Strain (1996). AOX were expressed as mmol of Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic) equivalent (TE; mmol/g IF, dry weight, DW).

The residues of the extraction were treated for non-extractable polyphenols quantification, which includes condensed tannins (CT) and hydrolyzable polyphenols (HP). CT were assessed by the method proposed by Reed, McDowell, Van Soest, and Horvath (1982). The residues were treated with 10 mL butanol/HCl (97.5:2.5 v/v) and 0.7 g $FeCl_3$ (3 h, 100 °C) for proanthocyanidin hydrolysis. The absorbance was determined at 555 nm and results were expressed as CT equivalents g/100 g menu FW, using a carob pod (*Ceratonia siliqua*) proanthocyanidin standard. HP were evaluated by Hartzfeld, Forkner, Hunter, and Hagerman (2002) method. The HP values were determined by methanol/H_2SO_4 90:10 (v v-1) hydrolysis of the residues at 85 °C for 20 h. The absorbance was determined at 750 nm and results were expressed as g GAE/100 g menu FW.

2.3. In vitro colonic fermentation by human microflora

The assays were preformed using a pool of fresh fecal samples collected from five healthy schoolchildren (9–12 years), three male (Nutritional status based on body mass index: normal-weight, overweight and obese, respectively) and two female (Nutritional status based on body mass index: normal weight and overweight, respectively), apparently free from gastrointestinal diseases and who did not receive antibiotic treatment during the previous 3 months. Parents signed an assent approving the donation of fecal samples.

The fermentation process was developed according to Campos-Vega et al. (2009). TIF of breakfast menus was fermented at 37 °C under anaerobic conditions. Two different controls were also conducted in parallel: a) raffinose, used as a fermentable sugar reference that produce SCFA, was incubated in the medium with faeces inoculum, and b) the fecal suspension was incubated without addition of substrate, serving as negative control. All incubations were performed in triplicate; samples were collected at 12, 24, 48 and 72 h, and centrifuged (Hermle Z 323 K; Wehingen, Germany) (3500 ×*g*, 15 min, 4 °C). Supernatants were placed into a 20 mL vial sealed with a magnetic cap with a polytetra-fluoro-ethylene (PTFE)/silicon septum. The vials were immediately stored at −80 °C in order to minimize any deteriorating changes in the volatile components of the samples until they were processed.