Food Research International 84 (2016) 160–169

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

An exploratory study on the influence of orange juice on gut microbiota using a dynamic colonic model

Ana Luiza Rocha Faria Duque [a], Magali Monteiro [a], Maria Angela Tallarico Adorno [b], Isabel Kimiko Sakamoto [b], Katia Sivieri [a,*]

[a] Department of Food and Nutrition, School of Pharmaceutical Sciences, São Paulo State University-UNESP, Araraquara, SP, Brazil
[b] Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo-USP, São Carlos, SP, Brazil

ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form 18 March 2016
Accepted 24 March 2016
Available online 26 March 2016

Keywords:
Citrus juice
SHIME®
Intestinal microbiota
Colonic fermentation

ABSTRACT

The aim of this research was to evaluate the influence of fresh orange juice (FOJ) and pasteurized orange juice (POJ) on gut microbiota using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®) in a long-term experiment. SHIME® vessels were used to investigate orange juice fermentation throughout the colon and to assess changes in microbial composition and fermentation metabolites (short-chain fatty acids, or — SCFA, and ammonium). Antioxidant activity of the SHIME® vessels and juice was also evaluated. The FOJ increased ($p \leq 0.05$) *Lactobacillus* spp., *Enterococcus* spp., *Bifidobacterium* spp., and *Clostridium* spp. and reduced ($p \leq 0.05$) enterobacteria. The POJ increased ($p \leq 0.05$) *Lactobacillus* spp. and reduced ($p \leq 0.05$) enterobacteria. The PCR-DGGE analysis showed a reduction in total bacteria population richness values. The FOJ and POJ increased ($p \leq 0.05$) butyric, acetic, and propionic acid concentrations, whereas ammonium production was reduced. High values of antioxidant activity were observed as a result of the FOJ and POJ treatments. Principal component analysis indicated that both POJ and FOJ juices had a positive influence on gut microbiota. The FOJ and POJ were found to exhibit selective prebiotic activity, particularly in terms of gut microbiota. This finding is in agreement with increases in both SCFAs and commensal bacteria, as well as with decreases in ammonium levels, though total bacteria richness values were reduced.

1. Introduction

Gut microbiota represents a complex and diversified system of microorganisms that colonize the gastrointestinal (GI) tract, particularly in the colon region within the large intestine. The favorable conditions of this part of the GI tract include slow gut transit time, the availability of nutrients, and favorable pH (Frick & Autenrieth, 2013; Payne, Zihler, Chassard, & Lacroix, 2012). There are approximately 10^{12} bacterial cells per gram of luminal content inhabiting the colon. Estimates of the number of bacterial species present in the gut microbiota vary from 500 to 1150 (Frick & Autenrieth, 2013).

The gut microbiota has a direct impact on host's health, since this system plays an important role in immunological, physiological, and metabolic processes in the human body (Gerritsen, Smidt, Rijkers, & De Vos, 2011). This microbiota is involved in the synthesis of vitamins B and K, in the resistance of the colonization of pathogenic microorganisms, and in the synthesis of fermentation products that supply energy to the epithelium of the colon, such as short-chain fatty acids, or SCFAs (Davila et al., 2013; Gerritsen et al., 2011; Sekirov, Russell, Antunes, & Finlay, 2010). The gut microbiota is also responsible for regulating the immune system by promoting the maturation of immune cells and by maintaining the motor functions of the gastrointestinal tract (Clemente, Ursell, Parfrey, & Knight, 2012; Round & Mazmanian, 2009).

Both the composition and the metabolism of the gut microbiota are strongly influenced by diet. Many studies indicate that nutritional intervention may selectively modify certain groups of bacteria (Russell et al., 2011; Scott, Gratz, Sheridan, Flint, & Duncan, 2013; Walker et al., 2011). Gut microbiota can be modulated using probiotic microorganisms, prebiotic fibers, or symbiotic combinations (Bianchi et al., 2014; Chaikham & Apichartsrangkoon, 2014; Costabile et al., 2015; Pereira-Caro, Oliver, et al., 2015). However, few studies have been performed to evaluate the impact of fruits or fruit juices on the modulation of gut microbiota (Mosele et al., 2015; Sánchez-Patán et al., 2015).

The importance of orange juice consumption has long been established. It is a source of vitamin C, flavonoids, and carotenoids and also contains folic acid, potassium, and fibers (Stella, Ferrarezi, Santos, & Monteiro, 2011; Stinco et al., 2012). Orange juice consumption has been associated with a reduced risk of chronic diseases, largely because of the presence of bioactive compounds such as ascorbic acid, carotenoids, and flavonoids (Aptekmann & Cesar, 2013; Ghanim et al., 2010;

* Corresponding author at: Department of Food and Nutrition, School of Pharmaceutical Science, São Paulo State University-UNESP, Rodovia Araraquara-Jaú Km 1, 14801-902, Araraquara, SP, Brazil.
E-mail address: sivierik@fcfar.unesp.br (K. Sivieri).

http://dx.doi.org/10.1016/j.foodres.2016.03.028
0963-9969/

Morand et al., 2011). It also contributes to vascular health and to reduce in atherogenesis. Ascorbic acid is considered the main antioxidant compound of orange juice (Ness, Khaw, Bingham, & Day, 1996; Simon, 1992). Flavonoids — particularly hesperidin and narirutin — also exhibit antioxidant activity (Tripoli, La Guardia, Giammanco, Di Majo, & Giammanco, 2007), anti-inflammatory properties (Milenkovic, Deval, Dubray, Mazur, & Morand, 2011), lipid-lowering properties (Monforte et al., 1995), and anticarcinogenic properties (Birt, Hendrich, & Wang, 2001; Yang, Landau, Huang, & Newmark, 2001). Carotenoids exhibit provitamin A activity and also reduce the risk of developing macular degeneration (Krinsky & Johnson, 2005).

The bioactive compounds present in orange juice — particularly polyphenols — may also be associated with the metabolism of gut microbiota (Laparra & Sanz, 2010; Pereira-Caro, Oliver, et al., 2015; Pereira-Caro, Borges, et al., 2015). Some studies have shown that gut microbiota transforms phenolic compounds into bioactive metabolites. It therefore contributes to intestinal homeostasis, stimulates the growth of beneficial bacteria (*Lactobacillus* spp. and *Bifidobacterium* spp.), and inhibits pathogenic bacteria. In these ways, it has a prebiotic effect (Dueñas et al., 2015; Guglielmetti et al., 2013; Parkar, Trower, & Stevenson, 2013).

The objective of this study was to evaluate the influence of fresh orange juice (FOJ) and pasteurized orange juice (POJ) on gut microbiota using the Simulator of the Human Intestinal Microbial Ecosystem (SHIME®). The SHIME® is an *in vitro* model that dynamically simulates the human gastrointestinal tract and has proven useful for nutrition studies that analyze the composition and activity of the gut microbial community (De Boever, Deplancke, & Verstraete, 2000; Possemiers, Marzorati, Verstraete, & Van de Wiele, 2010; Sivieri et al., 2013; Van de Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2007).

2. Material and methods

2.1. Orange juice

The FOJ and POJ were prepared from Pêra-Rio orange, a Brazilian variety, responsible for most of the exportation. The FOJ was extracted using a JBT 391B extractor at the JBT FoodTech pilot plant in Araraquara, SP, Brazil. The POJ was supplied by a citrus industry from Araraquara region, SP, Brazil. This juice was pasteurized at 95 °C for 30 s (Bisconsin-Junior, Rosenthal, & Monteiro, 2014). The juices were frozen and kept at −18 °C until use.

2.2. Physicochemical characteristics of the orange juice

Soluble solids, pH, titratable acidity, ascorbic acid, reducing sugars, and total sugars were determined following the methods described in the AOAC (2012). All analyses were performed in triplicate.

2.3. Simulator of Human Intestinal Microbial Ecosystem (SHIME®)

The SHIME® (registered trademark from Ghent University and ProDigest) is a simulator of the human intestinal microbial ecosystem in which environmental conditions (pH, retention time, and temperature) are controlled. The SHIME® consists of five double-jacketed vessels representing the stomach, small intestine and ascending colon (AC), the transverse colon (TC) and the descending colon (DC) of the human gastrointestinal tract (Molly, Woestyne, De Smet, & Verstraete, 1994). The overall retention time of the last three vessels, simulating the large intestine, was 76 h. The pH, retention time, and volumetric capacity corresponding to each vessel are based on the methodology described by Possemiers, Verthé, Uyttendaele, and Verstraete (2004).

The vessels operated at 37 °C and were stirred continuously using a magnetic stirrer. The inside of each vessel was kept in anaerobiosis through the daily injection of N_2 for 30 min, and the pH of each portion of the tract was automatically adjusted with the addition of 0.5 N NaOH or 0.5 N HCl (Molly et al., 1994; Possemiers et al., 2004).

2.3.1. Carbohydrate-based medium composition

The carbohydrate-based medium used in the SHIME® was prepared in distilled water. It was made of 3 g/L of starch (Unilever, Brazil), 2 g/L of pectin (Sigma, USA), 4 g/L of type III mucin from porcine stomach (Sigma, USA), 1 g/L of xylan (Sigma, USA), 1 g/L of peptone (Acumedia, USA), 1 g/L of arabinogalactan (Sigma, USA), 0.4 g/L of glucose (Synth, Brazil), 3 g/L of yeast extract (Acumedia, USA), and 0.5 g/L of L-cysteine (Sigma, USA) following Possemiers et al. (2004).

2.3.2. Fecal inoculum

The fecal inoculum was prepared using a fecal sample from an adult volunteer who had not taken antibiotics for 2 years prior to the experiment. A 20-gram fecal sample was collected. The sample was diluted in 200 mL of phosphate buffer containing 0.05 mol/L of Na_2HPO_4, 0.05 mol/L of NaH_2PO_4, and 0.1% sodium thioglycolate ($C_2H_3NaO_2S$); pH was 6.5. The diluted sample was homogenized in a sample homogenizer (Model No. 130, Nova Ética, Brazil) for 10 min and centrifuged for 5 min at 3000 rpm. Next, 40 mL of supernatant were added to each of the three simulated colon vessels (AC, TC, and DC) and each vessel was then filled with the carbohydrate-based medium (Possemiers et al., 2010).

2.3.3. Experimental protocol

The SHIME® experiment was performed continually for 8 weeks. During the control period, the carbohydrate-based medium (210 mL) was added to the system twice a day for 14 days so that the microbial community could be adapted to the physicochemical and nutritional conditions that dominate the different parts of the colon, and also so that a stable microbial community could be formed (Van de Wiele et al., 2007). After two weeks of adaptation, the treatment periods were initiated (FOJ and POJ). The microbial communities present in the last three vessels of the culture system were fed, twice a day, with the carbohydrate-based medium (105 mL) and juices (105 mL). The treatments lasted for 14 days. At the end of each treatment, a seven-day washout period was initiated. During this period, the carbohydrate-based medium (210 mL) was added into the SHIME®. As shown in Fig. 1, the experimental conditions used in the SHIME® were consistent with the methodology described by Chaikham and Apichartsrangkoon (2014).

2.4. Microbiological analysis

The number of colony forming units log CFU/mL SHIME® fluid was determined by plating serial dilutions of samples of sterile 0.1% peptone water on selective culture media according previously described by Bianchi et al. (2014). Numbers of total aerobic bacteria and facultative anaerobic bacteria were determined by plating on Standard Methods agar and incubation at 37 °C/48 h aerobically or anaerobically, respectively. MRS agar with incubation at 37 °C/48 h, anaerobically, was used to determine the number of lactobacilli. BIM-25 agar with incubation at 37 °C/72 h, anaerobically, was used to determine the number of *Bifidobacterium* spp. *Clostridium* spp. was enumerated anaerobically using Reinforced Clostridial agar at 37 °C/48 h. *Enterococcus* spp. was enumerated aerobically using KF Streptococcus agar at 37 °C/48 h. MacConkey agar was used as selective media for enterobacteria with incubation at 37 °C/48 h, anaerobically.

2.4.1. Analysis using Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE)

The PCR-DGGE of the total bacteria was performed in order to determine the effect of the treatments on the microbial community of the colon. The DNA was extracted from the simulated colon vessels samples (AC, TC, and DC) using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, though with changes to the initial sample quantity (200 mg per 2 mL) and an ATE