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Passion fruit (*Passiflora edulis*) peel increases colonic production of short-chain fatty acids in Wistar rats

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ABSTRACT

Passion fruit (*Passiflora edulis*) peel, a waste from food industrial processing, is still little explored. It contains fibers, including soluble fibers, which may be a substrate for enteric bacteria and contribute to maintaining bowel health. The purpose of this work was to investigate the action of *P. edulis* peel in the microbiota, production of short-chain fatty acids (SCFAs), and antioxidant potential in the colon of Wistar rats. Seventy-seven-day-old male Wistar rats were divided into two groups: Peel and Control ($n = 4$). Both groups were fed with standard diet (AIN-93M); however, 50% of the cellulose content in the Peel group diet was replaced by fiber from *P. edulis* peel flour (PPF). After 15 days, the animals were anesthetized and sacrificed. The results showed that PPF intake positively affected the SCFAs intestinal production. The Peel group had greater butyrate and acetate concentration in cecal content than the Control group ($P < 0.05$), without alteration in microbiota (counts of *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae*, and Total aerobic). Butyrate is a main substrate of the colonocytes and may improve mucus production, vascular flow, and mucosa barrier function. Changes in the antioxidant enzymes activity (GR, GPx, and SOD) and thiol groups (GSH) were not observed in the Peel group, which suggests that the period of PPF intake was insufficient to affect the oxidative status in the colon. These findings suggest that the *Passiflora* peel flour may improve bowel health by increasing SCFAs production, although more investigations are necessary about the effect of PPF on the colonic fermentation and the antioxidant status of the colon.

1. Introduction

The intake of fruits has been considered important for health maintenance and the prevention of chronic diseases since fruits are great sources of micronutrients, fiber and other bioactive compounds (Jaime & Monteiro, 2005; Ramalho, Dalamaria, & de Souza, 2012; Record, Dreosti, & McInerney, 2001). However, the processing of fruit leads the production of huge amounts of byproducts that may have functional properties.

Passiflora (passion fruit) peel accounts for 50% of weight of the fruit and it is wasted in the juice production, which is the main destination of the harvested fruits (Bocco, Cuvelier, Richard, & Berset, 1998; Senevirathne, Jeon, Ha, & Kim, 2009). Brazil is the largest producer of passion fruit in the world, producing primarily yellow passion fruit, and it was responsible for 85% of the marketed passion fruit in 2012 worldwide (Embrapa, 2012; Meletti, 2011; Zeraik, Pereira, Zuin, & Yariwake, 2010; Zeraik & Yariwake, 2010).

The passion fruit pericarp (epicarp, white part, and mesocarp, yellow part) is rich in soluble and insoluble fibers (Cazarin et al., 2011). Dietary fibers increase fecal bulking and viscosity, reducing the contact time between potential pathogens and mucosal cells, and acting in glycemic control, they are able to regulate energy intake, thus, increasing weight loss or maintaining healthy bodyweight (Lattimer & Haub, 2010). The ideal consumption of dietary

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fiber is considered to be approximately 25–35 g day^{-1}, of which 6 g should be soluble fiber (Lattimer & Haub, 2010).

Insoluble fiber is reported to reduce bowel transit time, while soluble fibers are involved in reducing both blood cholesterol and intestinal glucose absorption (Yapo & Koffi, 2008). Passion fruit contains large amounts of pectin, a complex carbohydrate from plants with technological and physiological functions (Yapo & Koffi, 2008). Pectin is the major substrate for fermentation by enteric bacteria, and then it is involved in the production of the short-chain fatty acids (SCFAs) butyrate, propionate, and acetate (products from bacterial fermentation) (Braga, Medeiros, & Araujo, 2010; Mortensen & Clausen, 1996; Thibault et al., 2010). Microorganisms present in the intestinal lumen play an important role in general health (Tuohy, Conterno, Gasperotti, & Viola, 2012) and some studies have shown that the microbiota can be altered by diet (Sekirov, Russell, Antunes, & Finlay, 2010; Turnbaugh, Bäckhed, Fulton, & Gordon, 2008). The SCFAs formed during the fermentation have beneficial actions in the intestine and could have a role in inhibition of cholesterol synthesis, cancer prevention, and anti-inflammatory activities among other properties (Lattimer & Haub, 2010).

The SCFAs butyrate is the preferred oxidative fuel of the colonocyte and stimulates mucosal proliferation, mucus secretion, vascular flow, barrier function, motility, water, and electrolyte absorption (Mortensen & Clausen, 1996; Thibault et al., 2010).

Reactive species of oxygen and nitrogen are produced during the normal metabolism or by factors such as pollution, sunlight, cigarette smoke, and emotional stress (Durackova, 2010; Kaliora, Dedoussis, & Schmidt, 2006). Oxidative stress may cause loss of function and cellular death (Habib & Ibrahim, 2011; Kaliora et al., 2006; Libby, 2007), and has been linked to many diseases (Elleuch et al., 2011). In this way, the interest in fruits has increased, primarily due to the presence of potential antioxidants that may prevent cellular oxidative stress (Beecher, 2003; Cerqueira, Medeiros, & Augusto, 2007; Habib & Ibrahim, 2011; Holt et al., 2009; Scalbert, Johnson, & Saltmarsh, 2005). Phenolic compounds from passion fruit could also improve the antioxidant status in the intestine (Durackova, 2010; Kaliora et al., 2006).

Therefore, this investigation reports the impact of passion fruit peel intake in the rat gut health by regarding colonic production of SCFAs, modulation of microbiota, and effect on antioxidant potential in the colon.

2. Materials and methods

2.1. Passiflora edulis peel flour (PPF)

Organic *P. edulis* harvested in June 2010 in Torre de Pedra, São Paulo, Brazil, was used to produce the peel flour. The fruits were cleaned and separated into pulp and peel (epicarp + mesocarp). The peels were cut into small pieces and dried in an oven with air circulation at 50 °C (Marconi, Piracicaba/SP, Brazil) until approximately 10% moisture. Dried samples were ground into a fine powder using a hammer mill (20 mesh). The water activity of *P. edulis* peel flour (PPF) was evaluated using AquaLab equipment (Pullman, WA, USA) at 24.7 °C, and PPF was stored in an amber flask at room temperature (24 °C) until further analysis.

2.2. Fiber content of PPF

Total dietary fiber and insoluble fractions were determined in the peel powder using an enzymatic method (Prosky, Asp, Schweizer, DeVries, & Furda, 1988). The samples were prepared to total (TF) and insoluble (IF) fiber determination using amylase termamyl 120 L, protease Alcalase 0.6 L and amyloglucosidase AMG 200 enzymes. The results of TF and IF were obtained by subtraction from ash and protein values (AOAC, 1995). The soluble fraction was calculated by determining the difference between total dietary fiber and insoluble fiber.

2.3. Total phenolic content

One gram of PPF was extracted with 25 mL of boiling water. After 35 min the extract was filtered under vacuum and stored under refrigeration (2–8 °C) in amber glass bottles. The total phenolic content was determined according to Folin-Ciocalteu's method (Swain & Hillis, 1959), with some modifications. The absorbance was read at 725 nm in a microplate reader Synergy HT, Biotek (Winooski, USA) spectrophotometer with Gen5™ 2.0 data analysis software. Gallic acid was used in a standard curve and the results were expressed in terms of gallic acid equivalent (mg GAE g^{-1}).

Vitexin, isovitexin, orientin, and apigenin, previously described in *P. edulis*, were analyzed by chromatographic analysis in the aqueous extract, using the methodology described by Da Silva et al. (2013).

2.4. In vivo experimental design

The study was approved by the Institutional Animal Care and Use Committee (protocol #2385-1, CEUA, UNICAMP, Brazil). All the procedures followed the institutional ethical guidelines. Male Wistar rats were maintained under controlled conditions of temperature (22 ± 2 °C), humidity (60–70%), and a light–dark cycle (12/12 h). They were fed with a commercial diet (Nuvilab®) for rodents until they reached adult age. Seventy-seven-day-old rats were randomized into two groups ($n = 4$): Peel and Control. The Control group was fed with standard diet (AIN-93M) (Reeves, Nielsen, & Fahey, 1993) and Peel group was fed with the same diet (AIN-93M) with one modification: 50% of the cellulose content was replaced by fiber from PPF. After 15 days of consuming the experimental diets, the animals were anesthetized with ketamine and xylazine (40 and 5 mg kg^{-1}, respectively) and euthanized by exsanguination by cardiac puncture.

2.5. Biochemical analyses

All the absorbance and fluorescence readings from biochemical analyses were measured in a microplate reader Synergy HT, Biotek (Winooski, USA) with Gen5™ 2.0 data analysis software. All analyses were carried out in triplicate.

2.5.1. Serum albumin and total protein levels

Albumin (#03900) and total protein (#03800) levels were determined using a commercial kit from LABORLAB (São Paulo, Brazil).

2.5.2. Colon antioxidant status

Colon was removed and homogenates were prepared in 50 mmol phosphate buffer (pH 7.4) using a Polytron homogenizer (MA102/Mini, Marconi, Piracicaba, SP, Brazil). Homogenates were kept at −80 °C until analyses. The protein concentration of all tissue homogenates was determined according to the Bradford method (Bradford, 1976). The methodologies used in the determination of thiol group content (GSH) (Ellman, 1959), glutathione peroxidase (GPx) (Flohe & Gunzler, 1984), glutathione reductase (GR) (Carlberg & Mannervik, 1985), and superoxide dismutase (SOD) (Winterbourn, Hawkins, Brian, & Carrell, 1975) were previously described by Da Silva et al. (2013).