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The digestibility of mulberry fruit polysaccharides and its impact on lipolysis under simulated saliva, gastric and intestinal conditions

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ABSTRACT

The digestibility of mulberry fruit polysaccharides (MFPs) and its effect on lipid digestion were investigated in the simulated saliva, gastric and intestinal model. Results showed that the saliva, especially the salivary amylase had no effect on MFPs, while the gastric juice had greater effects on the MFPs digestion than intestinal juice, which could be evidenced from the decrease in molecular weight (Mw) from 128.7 ± 6.1, 13.6 ± 1.4 and 2.9 ± 0.1 to 84.3 ± 4.7, 5.2 ± 0.1 and 1.2 ± 0.1 KDa, respectively. It was observed that the amount of reducing ends were increased from 0.051 ± 0.003 to 0.451 ± 0.011 mM and no monosaccharide was released from the polysaccharide during the whole digestion period, indicating that gastrointestinal digestion cleaves the glycosidic bonds and produces no free monosaccharide. In addition, under the simulated saliva, gastric and intestinal digestion conditions, MFPs could reduce the rate and extent of lipid digestion in a concentration-dependent manner. Our findings provide information on the digestibility of polysaccharides from *Morus nigra* L. *in vitro*.

1. Introduction

Mulberry (*Morus nigra* L.), a member of the Moraceae family, is widely distributed in Europe, America, Africa and Asia (Nomura & Fukai, 1981). Mulberry fruit has been widely accepted for its good taste and nutrition (Abbasi, Khan, Khan, & Shah, 2013), and are mainly made into juice, wine or freshly consumed. In China, mulberry fruit is also used as a traditional medicine for treating fever, sore throat, hypertension, anemia, liver or kidney damage (Butt, Nazir, Sultan, & Schroën, 2008; Yildiz, 2013). This is because it has various bioactive compounds, including anthocyanin, polyphenols, polysaccharides, flavonoids etc., which have anti-inflammatory, antioxidant, antibiotic and hepatoprotective functions (Yoshinaga et al., 2007).

Mulberry fruit polysaccharides (MFPs) are one type of bioactive macromolecules. It has been proved to have functions in preventing chronic diseases such as hyperglycemia and hyperlipidemia, enhancing immunity, anti-aging, and anti-inflammatory *in vitro* and *in vivo* (rat tests) (Liu & Lin, 2012; Tian, Bo, & Li, 2011). Lipid, as one kind of important nutrients, has been reported to associate with some chronic diseases (Hu et al., 2013). Introducing non-starch polysaccharides into human diets could inhibit lipid digestion and glucose release, and thus could reduce the risk of obesity and some metabolic syndromes (Galisteo, Duarte, & Zarzuelo, 2008; Gunness & Gidley, 2010).

The physico–chemical properties of polysaccharides may change a lot during the gastrointestinal digestion course, because it has been widely acknowledged that bioactivities of polysaccharides can be greatly affected by its molecular mass, chemical components, structure and conformation (Edwards et al., 2014; Francisco, Franco, Wagner, & Jacob-Lopes, 2014). However, there is no report on the physico–chemical properties of the mulberry fruit polysaccharides after digestion, and the impact of MFPs on lipolysis also has not been investigated under the gastrointestinal condition.

In this study, the digestibility of MFPs and its impact on lipolysis were investigated using a digestion model *in vitro*. The results will provide insights into the MFPs degradation and its inhibitory effect on lipid digestion in the simulated gastrointestinal tract.

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2. Materials and methods

2.1. Materials

The mulberry fruits were purchased from a local fruit market (Xinjiang, China). Corn oil, containing approximately 15, 32, and 53% (w/w) of saturated, monounsaturated, and polyunsaturated fatty acids respectively was purchased from a commercial food supplier (Yihai Kerry Group, Singapore) and was stored at 4 °C before use. Monosaccharide standards, including fucose, arabinose, rhamnose, mannose, xylose, galactose, and glucose, were purchased from the Aladdin Chemistry Company (Shanghai, China). Gastric lipase (1.0×10^5 units/g), pepsin (3000 units/g), trypsin (300 units/mg), pancreatin and bile salts were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents used were reagent grade.

2.2. MFPs extraction

MFPs was extracted with hot water at 90 °C for 2 h with a weight ratio of solid to liquid of 1:40. The extract aliquot was collected by centrifugation at 5000 rpm for 10 min, de-proteinized by dissolved in sevag reagent (chloroform: n-butyl alco-hol = 4:1, v/v) and decolored using macro-porous resin (AB-8). Then the extracts were precipitated by adding absolute ethanol to a final ethanol concentration of 80% (v/v) and the mixture was kept at 4 °C for 24 h. After centrifugation (5000 rpm, 5 min), the supernatant was discarded, and the precipitate was re-dissolved in ultrapure water and then dialyzed using dialysis tubes (molecular weight cut off: 500 Da, Mym Biological Technology Co., Ltd. USA) in deionized water for 72 h. The content in dialysis tubes was freeze-dried to obtain MFPs.

2.3. Determination of the chemical properties of MFPs

The total carbohydrate content was determined by phenol–sulfuric acid method with D-glucose as the standard (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The uronic acid content was estimated using the m-hydroxybiphenyl method with D-galacturonic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973). The monosaccharide composition was determined using the dionex ion chromatography ICS equipped with Carbopac PA1 analytic column (Li et al., 2015). The MFPs should be hydrolysed thoroughly, and monosaccharide standards were used as standards.

2.4. Human saliva digestion

2.4.1. The collection of human saliva

The human saliva sample was collected from four healthy donors who had not been treated with antibiotics for at least 3 months prior to saliva collection. The saliva collection procedure was carried out as follows (Navazesh, 1993; Saari, Halinen, Ganlöv, Sorsa, & Konttinen, 1997; Stokes & Davies, 2006): (a) each donor rinsed the mouth with distilled water to obtain a neutral mouth state for 45 s; (b) then spitted the saliva into tube every 30 s. The saliva of the initial 30 s was discarded, and the saliva of the following 2 min was collected; (c) mix the saliva of the four donors; (d) the collected saliva was centrifuged at 5000 rpm for 10 min, and the supernatant was collected and stored at −20 °C for further use.

2.4.2. Amylase activity

Amylase activity of the human saliva was determined following a previously reported method (Van Ruth & Roozen, 2000). Potato starch suspension (20 ml, 2%, w/v) was cooked at a boiling water bath for 5 min, and then diluted by adding 80 mL of distilled water. The temperature for heat treatment was adjusted to 37 °C, and then 2 mL of human saliva was added and mixed thoroughly. Hydrolysates (0.3 mL) were taken at every 10 s intervals, and mixed with 0.3 mL of iodine solution (1%, w/v) until the blue color completely disappeared. The activity of amylase (D) was defined as following

$$D = \frac{10}{V} \times \frac{5}{n} \quad (1)$$

where v was the saliva volume (mL), and n was the time to reach the achromic point (min).

2.4.3. Simulated saliva digestion

The saliva digestion was performed following a previous described method with modifications (Asano, Hamaguchi, Fujii, & IINO, 2003; Yoon, Thompson, & Jenkins, 1983). MFPs were dissolved in water and make a solution with 2 mg/mL concentration. Tube A was the mixture of saliva (2 mL) and MFPs solution (2 mL), tube B was saliva (2 mL) mixed with water (2 mL), and tube C was the mixture of MFPs (2 mL) and water (2 mL). The test tubes were kept in a 37 °C water bath for 5 min, and then were put into a boiling water bath for 5 min to deactivate salivary amylase. After digestion, the molecular weight distribution of remaining MFPs was measured using a high-performance gel permeation chromatography (HPGPC). Each sample was replicated three times.

2.5. In vitro gastric digestion

The gastric medium was prepared according to the method reported previously (Hur, Lim, Decker, & McClements, 2011; Tedeschi, Clement, Rouvet, & Valles-Pamies, 2009). The gastric electrolyte solution (2 L) was composed of KCl (2.2 g), NaCl (6.2 g), $CaCl_2 \cdot 2H_2O$ (0.3 g) and $NaHCO_3$ (1.2 g). The pH value of the solution was adjusted to 3 using HCl (0.1 M). Then gastric lipase (350 mg), gastric pepsin (350 mg) and 1 M CH_3COONa (3 mL, pH 5) were added to 1500 g of gastric electrolyte solution. Finally, the solution was stirred thoroughly and the pH was adjusted to 3 using HCl (0.1 M). According to the previous method (Blanquet et al., 2004), 100 mL of MFPs solution (2 mg/mL) was kept at 37 °C water bath and simultaneously stirred gently using a magnetic stirrer (DF-101S, Yuhua Instrument Co., Ltd, Henan, China). The gastric medium was pumped into the MFPs solution at a constant flow of 0.56 mL/min (HL-2B, Jingke Co., Ltd, Shanghai, China). The pH of the reaction solution was controlled at 3 with HCl solution (1 M). The high-performance gel permeation chromatography (HPGPC) analysis of each sample was performed after 0, 1, 3, 5 and 7 h. Each sample was replicated three times.

2.6. In vitro intestinal digestion

The intestinal medium was prepared referring to the recorded method (Tedeschi et al., 2009). Briefly, 1 L of intestinal electrolyte solution was composed of KCl (0.65 g), NaCl (5.4 g), and $CaCl_2 \cdot 2H_2O$ (0.33 g). The pH of the solution was adjusted to 7 with NaOH (0.1 M). Then, 500 g of pancreatin solution (7%, w/w), 1000 g of bile salt solution (4%, w/w) and 0.13 g of trypsin were added to 500 g of intestinal electrolyte solution. And the solution was stirred thoroughly and pH was adjusted to 7 by NaOH (0.1 M). The intestinal digestion test was carried out by the method of Blanquet et al (Blanquet et al., 2004), the predigested MFPs under *in vitro* gastric condition was pumped into intestinal medium at a constant flow of 0.56 mL/min. The temperature was maintained at 37 °C, and the pH value of the reaction solution was controlled by adding $NaHCO_3$ (1 M). The high-performance gel permeation chromatography (HPGPC) analysis of each sample was performed after 0.5, 3, and 7 h. Each sample was replicated three times.