Journal of Functional Foods 22 (2016) 113–121

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Development of novel *in vitro* human digestion systems for screening the bioavailability and digestibility of foods

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ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 16 December 2015
Accepted 5 January 2016
Available online

Keywords:
In vitro human digestion
Enzymes
Gastrointestinal tract
Enterobacter bacteria
Bioavailability

ABSTRACT

The *in vitro* models employed have included all steps involved in digestion upon passage through the mouth, stomach, small intestine, and the colon. Importantly, enterobacter bacteria (*Escherichia coli* and *Lactobacillus casei*) are included to simulate the colon step. After *in vitro* human digestion in the small and large intestines, the antioxidant activity of rutin increased dramatically, whereas the antioxidant activity was not influenced by digestion in the mouth or the stomach. Before *in vitro* human digestion, the antioxidant activity of quercetin and chlorogenic acid was increased, and after *in vitro* human digestion in the small intestine and large intestines (with enterobacteria), the antioxidant activity of quercetin increased although enterobacteria did not influence the antioxidant activity. However, the antioxidant activity of chlorogenic acid was not influenced by *in vitro* human digestion in the small intestine and large intestines (with enterobacteria). This study provides data supporting an alternative to the use of animals and humans for rapid screening of food digestibility and bioavailability.

1. Introduction

In vivo studies are time consuming and costly. As a result, much effort has been devoted to the development of *in vitro* procedures (Boisen & Eggum, 1991). *In vitro* digestion methods are ethically superior, faster, and less expensive than *in vivo* techniques, and provide a useful alternative to animal and human models for rapidly screening food ingredients (Coles, Moughan, & Darragh, 2005). An ideal *in vitro* digestion model would provide highly accurate results in a short time (Coles et al., 2005) and could serve as a tool to study the digestibility or bioavailability of various foods (Hur, Lim, Decker, & McClements, 2011). However, any *in vitro* method is inevitably going to fail to match that achieved by studying food digestion *in vivo* (Coles et al., 2005). This is because the results of human digestion are dependent on many factors associated with food composition, structure, amount, and enzyme characteristics (Hur, Lim et al., 2011). In the past ten years, we have worked to develop a more realistic *in vitro* human digestion system for food applications. The purpose of the current study was to develop an *in vitro* model that included all steps of the human digestion system and to use this model to rapidly screen the digestibility and bioavailability of food materials.

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http://dx.doi.org/10.1016/j.jff.2016.01.005
1756-4646/

2. Materials and methods

2.1. Materials

Analytical grade potassium chloride, potassium hydroxide, potassium persulphate, sodium sulphate, sodium bicarbonate, hydrogen chloride, potassium phosphate monobasic, magnesium chloride, hexane, methanol, acetate, phosphoric acid, ferric chloride, hydrochloric acid, sulphuric acid, chloroform, ether, and ethanol were purchased from Fisher Scientific (Pittsburgh, PA, USA). Analytical grade bicarbonate, potassium thiocyanate, sodium phosphate dibasic, sodium phosphate monobasic, sodium chloride, calcium chloride, ammonium chloride, urea, glucose, glucuronic acid, glucosamine, α-amylase, uric acid, mucin, bovine serum albumin, pepsin, pancreatin, lipase, bile salt extract, phenolphthalein, L-ascorbic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), nile red, and rutin were purchased from Sigma-Aldrich (St Louis, MO, USA). Wheat flour, starch, and palm oil were purchased from general commercial sources. LB broth and MRS broth were purchased from Difco (Sparks, MD, USA).

2.2. Compartments of in vitro human digestion

A human gastrointestinal digestion model (for adults) that simulates the mouth, stomach, small intestine, and large intestine was used in this study. This was a modified version of that described previously (Hur, Lim et al., 2011; Oomen et al., 2003; Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips, 2005). For simulation of digestion by the large intestine, enterobacter bacteria such as *Escherichia coli* and *Lactobacillus casei* were applied to the sample after digestion by the small intestine.

2.3. Temperature of in vitro human digestion

Normal human body temperature is 37 °C and most metabolic processes and digestive enzymes are optimized to this temperature. Therefore, *in vitro* human digestion was carried out at 37 ± 0.3 °C.

2.4. Transit times of in vitro human digestion

Transit times were chosen on the basis of physiology as reported previously (Christensen et al., 1985; Degen & Phillips, 1996; Hur, Lim et al., 2011) although the transit times depend on the condition of each sample. In general, transit times were 5 min for the mouth step, 2 h for the stomach step, 2 h for the small intestine step, and 4 h for the large intestine step.

2.5. pH of in vitro human digestion

The pH values for the digestive juices and gastrointestinal tract were selected based on existing human anatomy and medical physiology literature (Evans et al., 1988; Guyton & Hall, 2006; Marieb & Hoehn, 2010) and a previous study (Hur, Lim et al., 2011). In general, pH values were 6.8 ± 0.2 for the mouth step, 1.5 ± 0.2 for the stomach step, 8.0 ± 0.2 for the small intestine step, and 7.0 ± 0.2 for the large intestine step.

2.6. Digestive enzymes, inorganic and organic solutions of in vitro human digestion

Digestive enzymes, and inorganic and organic solutions used in this study were from those described previously (Hur, Lim et al., 2011; Oomen et al., 2003; Versantvoort et al., 2005). The compositions of the simulated saliva, gastric, duodenal, and bile juices are listed in Table 1.

2.7. Enterobacter bacterial preparations used for large intestine digestion

During *in vitro* human digestion, enterobacter bacteria were applied to samples during the large intestine digestion step. This is the first *in vitro* method to be developed that includes this step of human digestion.

E. coli (American Type Culture Collection (ATCC) 25922, Manassas, VA, USA) liquid agar was prepared using 2.5 g Luria–Bertani (LB) Broth (Sparks, MD, USA) with 100 mL deionized–distilled water (DDW). *L. casei* (ATCC 393) liquid agar was prepared using 5.5 g Lactobacilli MRS Broth (Sparks, MD, USA) mixed with 100 mL DDW. Each agar preparation was sterilized by autoclave at 121 °C for 15 min and cooled in tap water. Frozen (–80 °C) stock *E. coli* and *L. casei* were melted at room temperature then warmed to 37 °C. One per cent of *E. coli* and *L. casei* stocks were added to 100 mL of the appropriate sterilized liquid agar. *E. coli* and *L. casei* agar solutions were incubated at 37 °C for 12 h for activation. The activated *E. coli* and *L. casei*

Table 1 – Constituents and concentrations of the various synthetic juices used in the *in vitro* human digestion model representing fed conditions.

	Saliva (mouth step)	Gastric juice (stomach step)	Duodenal juice (small intestine step)	Bile juice (small intestine step)
Organic and inorganic components	1.7 mL NaCl[a] (175.3 g/L)[b] 8 mL urea (25 g/L) 15 mg uric acid	6.5 mL HCl (37 g/L) 18 mL $CaCl_2$ $2H_2O$ (22.2 g/L) 1 g bovine serum albumin	6.3 mL KCl (89.6 g/L) 9 mL $CaCl_2$ $2H_2O$ (22.2 g/L) 1 g bovine serum albumin	68.3 mL $NaHCO_3$ (84.7 g/L) 10 mL $CaCl_2$ $2H_2O$ (22.2 g/L) 1.8 g bovine serum albumin 30 g bile
Enzymes	290 mg α-amylase 25 mg mucin	2.5 g pepsin 3 g mucin	9 g pancreatin 1.5 g lipase	
pH	6.8 ± 0.2	1.50 ± 0.02	8.0 ± 0.2	7.0 ± 0.2

[a] The numbers are the concentration of chemicals used to make digestive juices.
[b] The numbers in parentheses are the concentrations of inorganic or organic components per 1 L distilled water.
After mixing all ingredients (inorganic components, organic components, and enzymes), the volume was increased to 500 mL with distilled water. If necessary, the pH of the juices was adjusted to the appropriate value.