Effect of *Escherichia coli* and *Lactobacillus casei* on Luteolin Found in Simulated Human Digestion System

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Abstract This study was conducted to investigate the effects of *in vitro* human digestion and enterobacteria (*Escherichia coli* and *Lactobacillus casei*) on the digestibility and structure of luteolin. Luteolin was passed through an *in vitro* digestion system that simulates the composition of the human mouth, stomach, small intestine, and large intestine and contains enterobacteria. The luteolin content was not altered by mouth or stomach digestion, but it was decreased by small intestine digestion. Large intestine digestion by enterobacteria also decreased the luteolin content; *L. casei* reduced the luteolin content more than *E. coli*. Moreover, digestion in the large intestine by a combination of *E. coli* and *L. casei* reduced the luteolin content more than digestion by individual enterobacteria. This study will provide insight into how enterobacteria influence the digestibility and structure of phytochemicals.

Keywords: *In vitro* human digestion, luteolin, enterobacteria, escherichia coli, lactobacillus casei


1. Introduction

Phytochemicals are well known for their various pharmacological properties, such as antioxidant and disease preventive/protective effects. Polyphenol luteolin (a yellow flavone) is a common dietary form of luteolin, which is present in various fruits, herbs, and vegetables (Lin, et al., 2008; Mencherini, et al., 2007). Luteolin has been shown to have stronger antioxidant activity and lower potential prooxidant activity than many common flavonoids, such as quercetin and myricetin, and to show potential health benefits in humans (Seelinger, et al., 2008). Numerous studies have shown that luteolin exhibits a number of health-promoting functions, including anticancer (Lee, et al., 2001; Ong, et al., 2010), anti-inflammatory (Seelinger et al., 2008; Xagorari et al., 2001), and cardioprotective (Liao, et al., 2011) properties. Therefore, luteolin is regarded as one of the most important bioactive phytochemicals, with potential applications in a wide range of pharmaceutical, nutraceutical, and functional food products.

In nutritional studies in both animals and humans, *in vivo* feeding methods usually provide the most precise results. However, they are time-intensive and expensive, which is why much effort has been dedicated to the development of *in vitro* processes (Boisen & Eggum, 1991). *In vitro* human digestion provides a useful alternative to *in vivo* models for rapidly screening food materials. The ideal *in vitro* human digestion model would provide accurate results in a short time and could thus serve as a tool for rapid screening of foods and delivery systems with different compositions and structures (Coles, et al., 2005; Fuller, 1991). A review by Hur et al. (2011) (S. J. Hur, Lim, et al., 2011) reported that during the last few decades, researchers have utilized a number of *in vitro* human digestion models to test the structural and chemical variation in different foods under simulated gastrointestinal conditions, although the reliability of these models has not yet been widely accepted. However, the effect of enterobacteria on phytochemicals during *in vitro* human digestion has not yet been evaluated. Therefore, development of new technologies in search for form changes of phytochemical by another factor is opportunities for the research database.

2. Materials and Methods

2.1. Materials

Bicarbonate, potassium thiocyanate, sodium phosphate dibasic, sodium phosphate monobasic, sodium chloride, calcium chloride, ammonium chloride, urea, a-amylase, uric acid, mucin, bovine serum albumin, pepsin, pancreatin, lipase, bile salt extraction were purchased from Sigma Aldrich chemical company (St Louis, MO, USA). All other reagents were of the highest grade commercially available.

2.2. *In vitro* Human Digestion Model

A digestion model that simulates the mouth, stomach, and intestine was used in this research, modified from
those described by previous studies (Versantvoort, et al., 2005; Hur et al., 2009; Hur et al., 2011). To simulate digestion in the mouth, a 5 mL sample of luteolin in DMSO was mixed with 5 mL of saliva fluid (pH 6.8) and stirred for 5 min. Approximately 10 mL of simulated gastric fluid (pH 1.3) was added and the mixture was stirred with a magnetic stirrer for 2 h. Finally, 10 mL of duodenal juice (pH 8.1) and 5 mL of bile juice (pH 8.2) were added and the mixture was stirred for 2 h. All digestive juices were heated to 37°C. The composition of the simulated saliva, gastric, duodenal, and bile fluids are listed in Table 1. During in vitro simulation of human digestion, the large intestine step requires enterobacteria such as E. coli and L. casei. Following the small intestine step, 35 mL of E. coli and L. casei were applied to the sample and incubated for 4 h at 37°C. A schematic diagram of the in vitro model of human digestion of luteolin using enterobacteria is shown in Figure 1.

2.3. Preparation of Microorganisms

E. coli was obtained from the American Type Culture Collection (ATCC), and L. casei MCL was isolated from feces collected from healthy adults. The E. coli and L. casei were stored at -80°C until they were used. The E. coli was cultured in Luria-Bertani (LB) medium (Difco™ LB broth, Miller, MD, USA) and the L. casei was cultured in MRS medium (Difco™ Lactobacilli MRS Broth, Miller, MD, USA) in a shaker-incubator at 37°C and 150 rpm. After incubation, the final colony counts for E. coli and L. casei were in the range of log 10⁸–10¹⁰.

2.4. Measurement of Luteolin by HPLC

Luteolin, digested luteolin, and luteolin digested by intestinal microorganisms were analyzed using high-performance liquid chromatography (HPLC, HP Agilent 1100, Hewlett Packard Co) on a Fortis H₂O column (250 mm × 4.6 mm, 3 μm) using a water : tetrahydrofuran : trifluoroacetic acid gradient (97.9 : 2 : 0.1, v/v/v) and
acetonitrile at a flow rate of 1.2 mL/min. The volume of sample injected for analysis was 20 μL, and the detection wavelength was set at 350 nm. All solutions were passed through a 0.45-μm Whatman membrane filter before injection onto the HPLC column.

3. Results and Discussion

The results showed the changes of luteolin contents at different concentrations (0.5, 1.0, and 2.0 mg/mL) during in vitro human digestion (Table 2). Changes in luteolin content were not observed following mouth or stomach digestion (data not shown), but luteolin content was reduced after small intestine and large intestine digestion. Thus, luteolin is stable under the digestion conditions found in the mouth and stomach, but is unstable under the digestion conditions found in the small intestine. A possible mechanism for the changes in luteolin content observed during in vitro human digestion is pH fluctuation. Boyer et al. (2005) found that flavonoids are stable at lower storage pH and less stable at higher pH (Boyer, et al., 2005). Bermudez-Soto et al. (2007) also reported that polyphenols are largely stable during gastric digestion (under acidic conditions), but that they are quite sensitive to mildly alkaline conditions, as found in the small intestine. In the model of digestion used in this study, the pH shifts dramatically between the stomach and the small intestine, from pH 1.5 to pH 7.5, mainly because bile salt has a higher pH. This change in pH is the primary factor involved in the irreversible breakdown of luteolin. Therefore, we hypothesize that in vitro digestion in the small intestine of humans may affect the structure of luteolin.

Figure 2. Effect of in vitro human digestion and enterobacteria on the digestion/absorption rate of luteolin. A; 0.5, B; 1.0, C; 2.0 mg/mL of undigested luteolin. D; 0.5, E; 1.0, F; 2.0 mg/mL of digested luteolin. Luteolin following in vitro digestion with E. coli (G; 1.0, H; 2.0 mg/mL). Luteolin following in vitro digestion with L. casei (I; 1.0, J; 2.0 mg/mL). Luteolin following in vitro digestion with mixed E. coli and L. casei (K; 1.0, L; 2.0 mg/mL.)
Figure 3. Effect of in vitro human digestion and enterobacteria on the mass spectrum of luteolin. A: luteolin, B: luteolin following mouth digestion, C: luteolin following stomach digestion, D: luteolin following small intestine digestion, E: luteolin following large intestine digestion with *E. coli*, F: luteolin following large intestine digestion with *L. casei*, G: luteolin following large intestine digestion with *E. coli* and *L. casei*
may be due to antagonism between *E. coli* and *L. casei* during *in vitro* human digestion. The mechanism of the antagonism remains unclear, but we hypothesize that lactic acid production by *L. casei* and the consequent pH decline might inhibit the growth of *E. coli*. Lactic acid can act on the cell membrane of *E. coli*, changing the fatty acid composition and affecting the H⁺ and Na⁺ ion exchange or H⁺/ATPase activity of the plasma membrane. In this study, we found that the population of *E. coli* was smaller than that of *L. casei* during *in vitro* human digestion, although the initial populations were the same (data are not shown). In an earlier study, Reid et al. (1988) found that lactobacilli were coaggregated with *E. coli*.

These results indicate that *L. casei* are antagonistic to *E. coli* in the gastrointestinal tract; this antagonistic activity might have influenced the digestibility and structure of luteolin during *in vitro* human digestion.

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### References

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