In vitro digestion enhances anti-adhesion effect of tempe and tofu against Escherichia coli

Letters in Applied Microbiology

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https://doi.org/10.1111/j.1472-765X.2011.03189.x

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Letters in Applied Microbiology ISSN 0266-8254

NOTE TO THE EDITOR

In vitro digestion enhances anti-adhesion effect of tempe and tofu against *Escherichia coli*

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Keywords
antimicrobials, bioprocessing, *E. coli* (all potentially pathogenic types), fermentation, fermented foods.

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2011/1786: received 18 October 2011, revised 16 November 2011 and accepted 25 November 2011

doi:10.1111/j.1472-765X.2011.03189.x

Abstract

Aims: Enterotoxigenic *Escherichia coli* is one of the main pathogenic bacteria causing diarrhoea. Earlier studies have shown that tempe—a fungal fermented soya food—has anti-adhesive activity against *E. coli in vitro*. Our aims were to challenge the anti-adhesive activity under gastro-intestinal conditions and to assess the activity of the nonfermented soya product tofu.
Methods and results: In this study, we compared the anti-adhesive activity of two major soya bean products, tempe and tofu, and their ileum efflux after transit through a dynamic gastrointestinal system simulating digestion in the human stomach and small intestine. The results showed that both tempe and tofu have an anti-adhesive activity against *E. coli in vitro*. Tempe and tofu, after digestion through the stomach and small intestine, have even higher anti-*E. coli* adhesive activity.
Conclusions: In addition to the proven *in-vivo* activity of tempe, this confirms the potential antidiarrhoeal effect of both the soya products tempe and tofu.
Significance and Impact of the Study: As tofu has a much greater circle of consumers, this finding is relevant for the health of a large part of the world's population.

Diarrhoea is a widespread disease in developing countries, where 4·9 per 1000 children under the age of 5 years die from diarrhoea every year (Kosek *et al.* 2003). Enterotoxigenic *Escherichia coli* is one of the major pathogens associated with infant diarrhoea (Roubos-van den Hil *et al.* 2009). Antisecretory agents to treat diarrhoea have serious side effects and can impair the central nervous system. Therefore, natural compounds that provide substantial benefit combined with a safety and selectivity of action should be considered as a part of the diarrhoea treatment (Bhan 2000).

Extracts from tempe, an indigenously fermented soya bean product in some oriental countries, show a strong bioactivity *in vitro* by reducing the adhesion of enterotoxigenic diarrhoea-causing *E. coli* to animal and human intestinal cells (Kiers *et al.* 2002; Roubos-van den Hil *et al.* 2009).

Extracts of raw, soaked and cooked soya beans can reduce *E. coli* adhesion *in vitro*, although the effect was lower than that of tempe extracts (Roubos-van den Hil *et al.* 2009). Therefore, we assume that other soya products such as tofu could have a similar function. Tofu is one of the most popular soya bean products consumed in Asia (Quak and Tan 1998).

However, any bioactive components, when administrated orally, must withstand the harsh pH conditions in the stomach and hydrolytic enzymes and bile in the small intestine.

We used a dynamic computer-controlled gastrointestinal system that simulates the human digestion tract to test whether tempe and tofu retain their anti-adhesion effect against *E. coli* after transit through the digestion tract. In addition, we compared the *in vitro* anti-adhesion ability of tempe and tofu that had been prepared under

different soaking conditions and with different fermentation starter cultures to study the effect of processing methods.

Tempe was prepared according to the procedure described previously (Roubos-van den Hil *et al.* 2009). Beans were soaked in 30°C tap water or acidified water for 24 h and then cooked for 20 min. To evaluate the effects of processing parameters on *E. coli* adhesion, we selected two different soaking conditions: soaked in (i) tap water at neutral pH and (ii) in acidified water with lactic acid bacteria *Lactobacillus plantarum* (LU 852) at an inoculation level of 10^6 CFU ml^{-1}. After cooling, the beans were inoculated with the starter culture and incubated at 30°C for 48 h. Two starter cultures were used for the fungal fermentation of tempe, viz. a commercial mixed starter (Raprima, Semarang, Indonesia) and a pure culture of *Rhizopus microsporus* var. *microsporus* (LU573) provided by the Laboratory of Food Microbiology, Wageningen University. Fresh tempe was cooked in a microwave for 5 min before the digestion experiment.

Soya beans were soaked in tap water at 20°C overnight, milled with water to a milky slurry (ratio of bean dry weight to water = 1 : 8). The slurry was heated to 95–100°C for 4 min and filtered through a cheese cloth to separate the soya milk. Coagulation was done at 70–80°C by adding calcium sulfate (3·0% of the soya bean dry weight) under agitation. Then, the mixture was left for 10–15 min to complete the coagulation. The coagulated curd was pressed to remove excess water. Finally, a soft, cake-like tofu was formed. The fresh tofu was cooked in a microwave for 5 min before the digestion experiment.

The dynamic computer-controlled gastrointestinal system simulating the human stomach and small intestine has been described elsewhere (Minekus *et al.* 1995). Tempe or tofu was put into the stomach compartment with artificial saliva, amylase and water. In the jejunum and ileum compartments, bioaccessible nutrients went through the prefilter and semi-permeable membrane and were collected. The nondigested compounds were collected as ileum efflux at the end of the ileum compartment. The ileum efflux samples were similar to the chyme entering the colon, and we used this efflux to test the *in vitro* anti-adhesion activity.

All tempe and tofu products, as well as tempe and tofu ileum efflux, were freeze-dried (GR Instruments; Wijk bij Duurstede, Netherlands), then ground (Ultra Centrifugal Mill ZM 200; Retsch GmbH, Haan, Germany), sieved through a 0·5-mm sieve and stored at –20°C.

An accurately weighed 1 g of dried powder was dissolved in 30 ml distilled water and stirred for 1 h at room temperature. After centrifugation (2600 ***g*** at 4°C for 15 min), the supernatant was collected and the pellets were re-suspended with 10 ml distilled water and centrifuged again. This re-extraction step was repeated twice. All supernatants were collected and freeze-dried for the anti-adhesion experiments.

Enterotoxigenic *E. coli* (O149: $K88^{ac}$) strain ID1000 (ETEC K88; source: Animal Science Group, Wageningen UR, Lelystad, Netherlands) was used as the experimental strain and nonadhering *E. coli* (O149:K91) strain ID1084 (source: Animal Science Group, Wageningen UR, Lelystad, Netherlands) as a negative control. Effect of soya bean extracts and ileum efflux on the adhesive ability of *E. coli* to piglet intestinal brush border cells was measured according to the method described previously (Roubos-van den Hil *et al.* 2009). To detect concentration effects, bean, tempe or tofu extracts and ileum efflux were added at 1, 2·5 and 10 g l^{-1} concentrations in phosphate-buffered saline. These concentrations were physiologically relevant, considering a serving size of 100 g of soya bean product of which at least 30% of dry matter is solubilized by digestion; in the intestinal tract, this would be diluted to concentrations of at least 10 g l^{-1} (Kiers *et al.* 2000).

Figure 1 shows the anti-adhesion effect of cooked soya bean, tempe (raw or cooked), tofu (raw or cooked) and tempe and tofu after the transit through the *in vitro* gastrointestinal system (ileum effluent samples) on *E. coli* K88 to piglet intestinal brush border cells.

Tofu also had anti-adhesive activity, although lower than that of tempe. After the tempe and tofu transit through the gastrointestinal system, the anti-adhesion activity of ileum efflux did not only exist, but was even higher than undigested tempe or tofu. At the lowest level of 1 g l^{-1}, tempe and tofu ileum efflux still had significant inhibition against *E. coli* adhesion. The anti-adhesion effect is dose dependent, that is, the higher the concentration, the higher the anti-adhesion effect.

Tempe, either raw or cooked, had anti-adhesive activity against *E. coli* K88 to piglet intestinal brush border cells. After cooking, the activity decreased. Small differences in anti-adhesion activity were observed when tempe was produced under different soaking conditions and fermented with different starter cultures. Therefore, we chose tempe made by acidified soaking and inoculated with pure *Rhizopus microsporus* (LU573) (highest folate and vitamin B12 content, data not shown here) to compare the anti-adhesive activity with tofu to study the anti-adhesion activity after *in vitro* digestion.

Adhesion is expressed relative to the control as 100% without addition of the testing extract. The negative control represents the adhesion of a nonadhering strain *E. coli* K91. Tempe made by different processing methods did not have significantly different effects on *E. coli* adhesion. The anti-adhesion effect increased when the concentration of the extract increased. The anti-adhesive effect was dose dependent ($P < 0·05$).

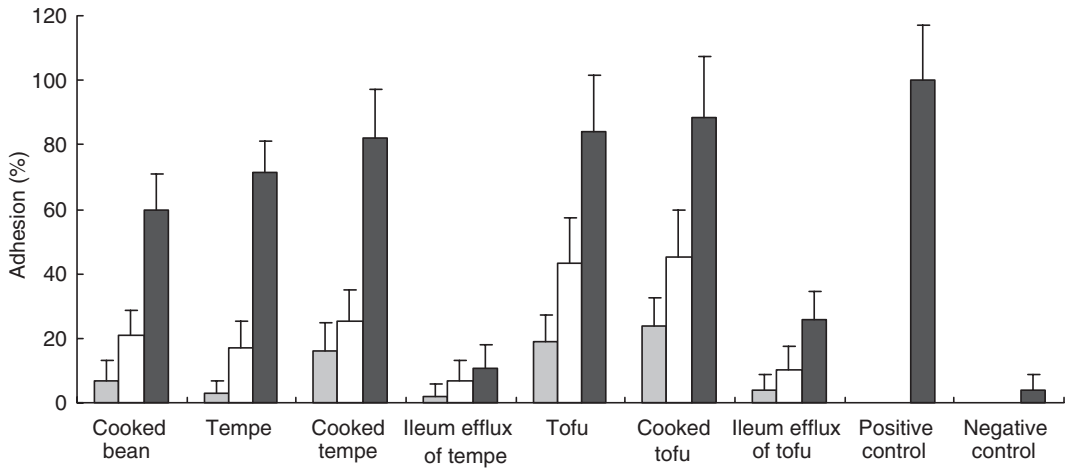

Figure 1 Adhesion of *Escherichia coli* K88 to piglet intestinal brush border cells. Extracts were added at three concentrations 1 g l^{-1} (black bar), 2·5 g l^{-1} (white bar) and 10 g l^{-1} (grey bar). Positive control was adhesion of *E. coli* K88 to piglet intestinal brush border cells without soya bean extract. Negative control used no-adhesive *E. coli* K91 to piglet intestinal brush border cells.

We suggest that the enhanced anti-adhesion effect of tempe and tofu after the *in vitro* digestion was caused by the absorption of low-molecular size digestion products in the small intestine. Consequently, it caused the accumulation of larger molecules in the efflux, among which the anti-adhesion bioactive component (Roubos-van den Hil *et al.* 2010) was present.

Soya bean and its products tempe and tofu all were shown to exhibit anti-adhesive activity against *E. coli in vitro*. Tempe made with different processing methods did not differ in anti-adhesion activity. The anti-adhesion activity is retained and even enhanced after the tempe and tofu have passed through the gastrointestinal system where they could withstand the harsh pH in the stomach and pancreatic secretions and bile in the small intestine. This underpins *in-vivo* observations (Kiers *et al.* 2003) showing that tempe has a protective function against diarrhoea-causing *E. coli*. Based on the *in vitro* data presented in this note, we hypothesize that tofu has a similar effect *in vivo*.

Acknowledgements

We thank Graduate School VLAG of Wageningen University for the research grant to H.M., and Dr Petra Roubos of Nutreco Boxmeer for providing the experimental materials and protocols.

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