

## Research Article

# Dose effects of the food spice cardamom on aspects of hamster gut physiology

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The dose effects of pectic polysaccharide-rich extract from the food spice cardamom (*Amomum villosum* Lour.) on intestinal environment were investigated. The results showed that pectic polysaccharides and hemicellulose were the major polysaccharides in the cardamom extract. The administration of cardamom extract (0.5 and 1.5 g/100 g diet) effectively ( $p < 0.05$ ) shortened hamster gastrointestinal transit time by  $\approx 58\%$ , increased fecal moisture contents (148–174%), increased SCFA concentrations in hindgut (4.0- to 7.8-fold), decreased the activities of  $\beta$ -D-glucuronidase (by 71.4–85.7%),  $\beta$ -D-glucosidase (by 24.3–51.5%), mucinase (by 63.6–72.7%), and urease (by 88.8–90.4%) in feces, and reduced the production of toxic ammonia (by 16.1–64.5%). These findings suggested that the consumption of cardamom extract (at least 0.5 g/100 g diet or 40 mg/day) might exert a favorable effect on improving the gastrointestinal milieu, and also provide a clue to substantiate its traditional therapeutic uses and dosage for intestinal health improvement.

**Keywords:** *Amomum villosum* / Bacterial enzyme / Cardamom / Food spice / Intestinal environment

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## 1 Introduction

Normal intestinal health in general is closely related to the integrity and mechanical barrier function of intestinal wall as well as microbial growth in the intestine. Some studies have demonstrated that the consumption of certain non-starch polysaccharides might help support normal intestinal structure, influence the growth and enzyme activities of some intestinal microflora, and decrease the risk of gastrointestinal disease [1, 2]. Some end products (*i.e.* SCFAs) released from the fermentation of polysaccharides could serve as a source of energy for the host cell in colon and improve intestinal health and function, and therefore be used as an index to reflect the changes in intestinal environment and health [3]. Furthermore, the activities of several bacterial enzymes such as  $\beta$ -D-glucuronidase,  $\beta$ -D-glucosidase, mucinase, and urease in the colon and feces of rats and hamsters are commonly used as an index to access the changes in intestinal function, health, and integrity [4–6].

Accordingly, the determination of these parameters might provide useful clues to assess intestinal health and function.

The dried ripe fruit of *Amomum villosum* Lour. (Zingiberaceae), a member of grains-of-paradise, is a common food spice (cardamom) in Asia. Traditionally, the cardamom (ginger family) is added into tea, wine, or herbal drink for its flavor, and also used for traditional therapeutic purposes to improve appetite, check diarrhea, and promote intestinal health. It is considered to be one of the essential dietary components of general population. With respect to traditional Chinese practices, cardamom can be consumed for both the food and medicinal purposes. Our preliminary study has revealed that the dried extract obtained from cardamom seed ( $\sim 10.0$  g/100 g dried fruit, DW) was rich in water-soluble polysaccharide (63.0 g/100 g dried extract, DW). Depending on different recipes and dietary purposes (*e.g.* intestinal health enhancement), the general way of consumption is to cook  $\sim 30$ – $40$  g of the dried cardamom seeds in water. As there are still gaps in the literature about the beneficial effects of cardamom extract on the intestinal milieu and health, it is worth carrying out further studies to clarify the mechanistic relationship between this polysaccharide-rich extract and its physiological benefits.

The aim of this *in vivo* study was to investigate the dose effects of the food spice cardamom on the activities of some colonic bacterial enzymes, SCFA concentrations in the

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cecal content, and also certain biochemical parameters in the intestinal tract and feces. On the scientific basis, the potential effects of the polysaccharide-rich extract on maintaining normal, or improving, gastrointestinal milieu were discussed.

## 2 Materials and methods

### 2.1 Preparation of dried extract

The dried cardamom sample was obtained from Ko Da Pharmaceutical Co., Ltd. (Taiwan), and was finely ground to pass through a sieve (0.5 mm in diameter). The cardamom powder sample was mixed with distilled water (1:10 w/v) and boiled for 2 h with continuous stirring. After filtration, the filtrate was dried by lyophilization and then kept in a desiccator until use.

### 2.2 Proximate analysis

Moisture was determined by drying the sample to a constant weight at 105°C. Crude protein content was calculated by multiplying the nitrogen content obtained from a CHN-OS rapid element analyzer (Heraeus F002, Hanau, Germany) with a factor of 6.25. For the analysis of water-soluble polysaccharide, the polysaccharide was first separated according to the methods of Mondal *et al.* [7]. The polysaccharide fraction in the extract solution was precipitated with an addition of 4 volumes of 95% ethanol. After centrifugation at  $4024 \times g$  for 10 min, the precipitated polysaccharide fraction was separated, collected, and dried. The monomeric sugar components of the polysaccharide fraction were determined by the method as described by Chau and Huang [8]. Uronic acid was determined colorimetrically by AOAC method 45.4.11 [9] using D-galacturonic acid monohydrate as reference.

### 2.3 Diets and experimental design

Following the formulation of the AIN-93M diet [10], the control diet (basal formula) was composed of casein (14 g/100 g), cellulose (5 g/100 g), sucrose (10 g/100 g), corn starch (62.1 g/100 g), soybean oil (4 g/100 g), choline bitartrate (0.25 g/100 g), L-cystine (0.18 g/100 g), AIN-93M vitamin mix (1 g/100 g), and AIN-93M mineral mix (3.5 g/100 g). These ingredients were obtained from ICN Nutritional Biochemicals (Cleveland, OH, USA).

For pharmaceutical and intestinal health maintenance purposes of an adult (~60 kg), the traditional practice of Chinese medicine practitioners in using cardamom may be up to ~40 g of dried fruit (*i. e.*, less than 4 g of extract). The dried extract intake *per* day by hamsters was then estimated to be ~40 mg/day using a conversion factor of 0.01 between human and hamsters. As food intake of hamsters was about 8 g/day, the cardamom-containing diets were

prepared by adding different amount of dried extract (*i. e.* 170, 500, and 1500 mg extract/100 g diet) into the basal formula. Three test diets, namely low dose, medium dose, and high dose diets were then prepared by mixing 0.17, 0.5, and 1.5 g of the dried extract into 100 g of the basal formula, respectively.

The study protocol was approved by the Animal Care and Use Committee of National Chung Hsing University. Thirty-two male Golden Syrian hamsters (6 wk old) weighing  $105 \pm 2.0$  g were obtained from the National Laboratory Animal Center of Taiwan. After a seven-day acclimation period, animals were divided into eight weight classes of four each. The four diets were then randomly allocated to one of the two animals in each weight class. They were housed (in pairs) in a screen-bottomed, stainless steel cage in a room maintained at  $24 \pm 1^\circ\text{C}$ , with 12 h light-dark cycles, and had free access to food and water. For determining gastrointestinal transit time, animals were fed a colored diet, in which small amounts of carmine were added, after fasting for 14 h. The excretion of colored feces was observed at every 30 min intervals. The time for colored feces to be observed was taken as the gastrointestinal transit time. After that, the feeding experiment was carried out for 30 days using the four normal diets. The food intake and body weight were recorded daily. Feces were collected and weighed daily, and stored at  $-20^\circ\text{C}$  until analyzed. At the end of experiment, animals were anesthetized by diethyl ether after fasting for 16 h. After laparotomy, small intestine, cecum, cecal content, and large intestine were collected, weighed, and immediately frozen at  $-80^\circ\text{C}$  for analysis.

### 2.4 Analytical methods

#### 2.4.1 Determination of cecal ammonia

Cecal pH and ammonia were determined using the methods as described by Ebihara and Nakamoto [11]. Ammonia contents in the deproteinized solution of cecal content were determined spectrophotometrically at 630 nm.

#### 2.4.2 Determination of fecal moisture and ammonia

Fecal moisture content was determined by drying the fecal sample to constant weight in a  $105^\circ\text{C}$  air-oven. Following the method of Shiau and Chang [6], fecal sample was mixed in cold 0.01 M phosphate buffer (pH 7.2) (1:50 w/v) for 30 min. After homogenization (Glas-Col, Terre Haute, USA) and centrifugation at  $1006 \times g$  for 10 min, fecal ammonia content in the supernatant was then determined using the methods of Okuda and Fujii [12].

#### 2.4.3 Determination of bacterial enzyme activities in feces

Following the method of Shiau and Chang [6], fresh fecal samples collected in the last 3 days of the experimental per-

iod were homogenized (Glas-Col) in 0.1 M phosphate buffer (pH 7.2, 1:150 w/v). After being centrifuged at  $1006 \times g$  for 10 min, the bacterial enzyme activities in the supernatant were determined. Protein in the supernatant was determined using protein assay kit (Cat No 500-0006, BioRad, USA).

According to the method of Goldin and Gorbach [13], the  $\beta$ -D-glucuronidase activity, which was defined as  $\mu\text{mol}$  of phenolphthalein produced *per min per mg* of fecal protein, was determined by the amount of phenolphthalein released from 0.01 M phenolphthalein  $\beta$ -glucuronide (No. P0501, Sigma Chemical Co., USA). Moreover, the  $\beta$ -glucosidase activity, which was defined as  $\text{nmol}$  of nitrophenol produced *per min per mg* of fecal protein, was measured by the rate of release of nitrophenol from 1 mM 4-nitrophenyl  $\beta$ -D-glucopyranoside (No. N7006, Sigma.). Mucinase activity, which was expressed as  $\mu\text{mol}$  of reducing sugar released *per min per mg* of fecal protein, was determined by the amount of reducing sugar released from porcine gastric mucin (No. M1778, Sigma) using the method of Shiau and Chang [6]. Urease activity, which was defined as  $\text{nmol}$  of ammonia released *per min per mg* of fecal protein, was determined by the contents of ammonia released from 0.01 M urea (No. U0631, Sigma) using the methods of Okuda and Fujii [12] and Ling *et al.* [14].

#### 2.4.4 Determination of SCFA contents

The SCFA contents in the cecal content were determined by the method of Whitehead *et al.* [15] with slight modifications. Cecal content (0.3 g) was homogenized with cold saline (0.9% w/w) and centrifuged at  $1006 \times g$  for 10 min. The supernatant was mixed with a known amount of isocaproic acid as an internal standard. The SCFA was extracted with diethyl ether, and 1  $\mu\text{L}$  of the ether layer was assayed by a packed column (GP10% SP-1200/1% $\text{H}_3\text{PO}_4$  on 80/100 Chromosorb) using a gas chromatograph (Hitachi G-5000, Tokyo, Japan) fitted with a flame ionization detector. The conditions were as follows: oven temperature, initially held at  $80^\circ\text{C}$  for 3 min, then raised to  $130^\circ\text{C}$  at a rate of  $2^\circ\text{C}/\text{min}$ ; injector temperatures,  $200^\circ\text{C}$ ; detector temperatures,  $250^\circ\text{C}$ ; gas flow rate, 20 mL/min (carrier gas, nitrogen).

#### 2.5 Statistical analysis

All results expressed as mean  $\pm$  standard deviation were analyzed by one-way analysis of variance using the Statistical Analysis System (SAS). Values of  $p < 0.05$  were considered statistically significant.

### 3 Results and discussion

In this study, the amount of dried extract obtained from the cardamom sample after lyophilization was about 10.0 g/100 g dried fruit. The moisture content of the dried

**Table 1.** Proximate composition of the cardamom extract

Proximate composition	g/100 g of dried extract
Moisture <sup>a)</sup>	11.1 $\pm$ 0.26
Protein <sup>b)</sup>	6.8 $\pm$ 0.4
Polysaccharide fraction <sup>b)</sup>	63.0
Monosaccharide profiles <sup>b)</sup>	
Rhamnose	Tr <sup>c)</sup>
Arabinose	3.1 $\pm$ 0.1
Xylose	3.7 $\pm$ 0.3
Mannose	0.5 $\pm$ 0.1
Galactose	3.2 $\pm$ 0.3
Noncellulosic glucose	10 $\pm$ 1.4
Uronic acid	32 $\pm$ 2.1
Nonpolysaccharide fraction <sup>b),d)</sup>	30.2

a) Means  $\pm$  SD of triplicate.

b) Means  $\pm$  SD of duplicate. The contents of protein, polysaccharide fraction, and nonpolysaccharide fraction were expressed in dry weight.

c) Tr: trace amount ( $<0.1$ ).

d) Nonpolysaccharide fraction refers to the water-soluble components such as nonpolysaccharide carbohydrate, polyphenols, ash, and other bioactive compounds.

extract was 11.1 g/100 g dried extract. In Table 1, the proximate composition in dry weight revealed that the dried extract was rich in water-soluble polysaccharide fraction (63.0 g/100 g extract) and also possessed small amount of crude protein (6.8 g/100 g extract). Chemical analysis showed that the polysaccharides in the extract were composed of different monomeric sugars (up to 52.5 g/100 g extract), which accounted for  $\sim 83\%$  of the polysaccharide fraction. Table 1 shows that the monosaccharides such as uronic acid, noncellulosic glucose, xylose, galactose, and arabinose accounted for 61.0, 19.1, 7.1, 6.1, and 5.9% of the total sugars, respectively, with uronic acid to be the major monosaccharide. In general, the sugar components of pectic polysaccharides may include uronic acid, xylose, and arabinose while those of hemicellulose could be glucose, xylose, galactose, mannose, and arabinose [16]. As chemical analyses revealed that these monomeric sugars constituted almost all of the total sugars (up to 99%), the results suggested that the major polysaccharide ingredients in the cardamom extract were pectic polysaccharides, followed by hemicellulose. During the process of hot water extraction, cell-wall pectic polysaccharides are easily degradable by the solubilization of middle lamellae in plant tissue and breaking down of pectins through  $\beta$ -elimination [17, 18]. It was therefore inferred that significant amounts of pectic polysaccharides in the extract arose from the  $\beta$ -eliminative degradation of pectic substances-rich cardamom sample during the preparation of water extract.

During the entire duration of the experiment, all of the animals remained active and healthy. After 30 days of feeding, no significant differences in the body weight gain ( $0.6 \pm 0.1$  g/day) and food intake ( $7.73 \pm 0.05$  g/day) were

**Table 2.** Effect of cardamom extract on the gastrointestinal transit time, fecal moisture content, and fecal dry weight

Diet groups <sup>a)</sup>	Gastrointestinal transit time (h) <sup>b)</sup>	Fecal moisture content (g/100 g of feces) <sup>b)</sup>	Fecal dry weight (g/day) <sup>b)</sup>
Control	10.2 ± 0.07 <sup>w</sup>	27 ± 2.4 <sup>w</sup>	1.0 ± 0.1 <sup>w</sup>
Low dose	10.0 ± 0.05 <sup>w</sup>	37 ± 1.1 <sup>x</sup>	1.1 ± 0.1 <sup>w</sup>
Medium dose	4.22 ± 0.05 <sup>x</sup>	40 ± 3.8 <sup>x</sup>	1.1 ± 0.1 <sup>w</sup>
High dose	4.26 ± 0.01 <sup>x</sup>	47 ± 1.5 <sup>y</sup>	1.0 ± 0.0 <sup>w</sup>

a) The low, medium, and high dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of the dried extract into 100 g of the control diet (basal formula), respectively.

b) Values (means ± SD,  $n = 8$ ) in the same column with different superscripts are significantly different ( $p < 0.05$ ).

observed among the four diet groups, hence explaining the similar final body weight among the animals from all four diet groups. Although the inclusion of polysaccharide-rich extract among the three cardamom-containing diets varied from 0.17 to 1.5%, the comparable results in body weight gain suggested that the slight compositional differences among the diets did not result in any apparent change in total energy and nutrients intake.

In Table 2, the influences of the polysaccharide-rich cardamom extract on the gastrointestinal transit time, fecal moisture content, and fecal dry weight in hamsters were presented. It was revealed that the addition of cardamom extract into the control diet at 0.5 and 1.5% levels resulted in a significant ( $p < 0.05$ ) reduction in the gastrointestinal transit time by 58.6 and 58.2%, respectively, suggesting that the cardamom extract (at a 0.5% level or above) might stimulate the peristaltic rate of intestine. A reduction in gastrointestinal transit time was also reported in animals fed water extracts from some other herbs or medicinal plants such as *Rhazya stricta* and *Amomum xanthioides* [19, 20]. The decrease in transit time might protect the intestinal tract from bowel diseases by lowering its exposure to harmful substances [21]. As shown in Table 2, the inclusion of cardamom extract into the control diet effectively ( $p < 0.05$ ) increased the fecal moisture content (up to 136–174%) in a dose-dependent manner, while the fecal dry weight among all four diet groups (1.0–1.1 g/day) were comparable to each other. It was speculated that the reduced transit time with both the medium and high dose diets shortened the time for water re-absorption, hence resulting in higher moisture retention in feces. These findings suggested that the administration of cardamom extract at a 0.5% level or above could aid fecal excretion and help relieve constipation.

The effects of cardamom extract on both the cecal and fecal ammonia in hamsters are presented in Table 3. The results showed that the levels of cecal ammonia were significantly ( $p < 0.05$ ) reduced (–25.9 to –55.6%) by an increased consumption of cardamom extract in a dose-dependent response. Chemical analyses on the fecal sam-

**Table 3.** Effect of cardamom extract on the cecal ammonia, fecal ammonia, and daily fecal ammonia output

Diet groups <sup>a)</sup>	Cecal ammonia ( $\mu\text{mol/g}$ cecal content) <sup>b)</sup>	Fecal ammonia ( $\mu\text{mol/g}$ fresh feces) <sup>b)</sup>	Daily fecal ammonia output ( $\mu\text{mol/day}$ ) <sup>b),c)</sup>
Control	2.7 ± 0.5 <sup>w</sup>	44 ± 3.0 <sup>w</sup>	62 ± 4.5 <sup>w</sup>
Low dose	2.0 ± 0.1 <sup>x</sup>	35 ± 5.0 <sup>x</sup>	50 ± 2.9 <sup>x</sup>
Medium dose	1.3 ± 0.1 <sup>y</sup>	34 ± 1.9 <sup>x</sup>	52 ± 2.9 <sup>x</sup>
High dose	1.2 ± 0.0 <sup>z</sup>	11 ± 1.2 <sup>y</sup>	22 ± 2.4 <sup>y</sup>

a) The low, medium, and high dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of the dried extract into 100 g of the control diet (basal formula), respectively.

b) Values (means ± SD,  $n = 8$ ) in the same column with different superscripts are significantly different ( $p < 0.05$ ).

c) The fecal fresh weight *per* day with the low, medium, and high dose diets were 1.42, 1.68, and 1.96 g/day, respectively.

ples also revealed that the addition of cardamom extract into the control diet (0.17–1.5%) could significantly ( $p < 0.05$ ) reduce the concentration of ammonia in fresh feces (–20.5 to –75.0%). The production of fecal ammonia decreased with increasing amount of cardamom extract ingested. Respecting the daily fecal ammonia output, the amount of fecal ammonia excreted *per* day by hamsters fed the three test diets (22–52  $\mu\text{mol/day}$ ) were significantly ( $p < 0.05$ ) reduced by 16.1–64.5% relative to those having the control diet (62  $\mu\text{mol/day}$ ), hence confirming that the feeding of cardamom extract could decrease the daily fecal ammonia output in hamsters. As the growth of certain unfavorable intestinal bacteria was related to the content of ammonia produced in intestinal tract [22], it was inferred that the consumption of cardamom extract (at 0.17–1.5% levels) might lower the formation of ammonia in cecum and feces by inhibiting the growth of undesired microflora inside intestinal lumen.

Table 4 presents the effects of cardamom extract on the pH values and SCFA profiles of the cecal contents in hamsters. The results showed that the consumption of cardamom extract at 0.5 and 1.5% levels significantly ( $p < 0.05$ ) lowered the pH values of the cecal contents in hamsters. In this study, the cecal content of the control group (1.09 g/100 g body weight) was comparable with those of the low and medium dose groups (1.00 and 1.04 g/100 g body weight, respectively), whereas a significant ( $p < 0.05$ ) higher value was observed in the high dose group (1.61 g/100 g body weight). In Table 4, the total SCFA levels in the cecal content of the control diet fed hamsters (12  $\mu\text{mol/g}$  cecal content) was relatively low when compared with those reported for rats (–52.8–93.3  $\mu\text{mol/g}$  cecal content) in other literature [23]. It is inferred that the varied SCFA levels might be partly attributed to the differences in animal types and their gut physiology. As compared with the control group, significant ( $p < 0.05$ ) increases of SCFA pool in the cecum of

**Table 4.** Effect of cardamom extract on the cecal pH and the SCFA concentrations<sup>a)</sup> in cecal contents

Diet groups <sup>b)</sup>	Cecal pH	Acetate ( $\mu\text{mol/g}$ cecal content)	Propionate ( $\mu\text{mol/g}$ cecal content)	Butyrate ( $\mu\text{mol/g}$ cecal content)	Total SCFA ( $\mu\text{mol/g}$ cecal content)
Control	$8.1 \pm 0.3^w$	$6.4 \pm 1.4^w$	$3.1 \pm 0.8^w$	$2.8 \pm 1.1^w$	$12 \pm 0.6^w$
Low dose	$8.1 \pm 0.2^w$	$9.8 \pm 1.1^x$	$2.8 \pm 0.3^w$	$2.4 \pm 0.3^w$	$15 \pm 9.9^w$
Medium dose	$7.7 \pm 0.1^x$	$34 \pm 4.0^y$	$11 \pm 1.8^x$	$3.1 \pm 0.5^w$	$48 \pm 7.2^x$
High dose	$7.4 \pm 0.2^x$	$56 \pm 0.7^z$	$33 \pm 2.3^y$	$3.5 \pm 0.1^w$	$93 \pm 1.5^y$

a) Values (means  $\pm$  SD,  $n = 8$ ) in the same column with different superscripts are significantly different ( $p < 0.05$ ).

b) The low, medium, and high dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of the dried extract into 100 g of the control diet (basal formula), respectively.

**Table 5.** Effects of cardamom extract on the activities<sup>a)</sup> of fecal bacterial enzymes

Diet groups <sup>b)</sup>	$\beta$ -D-Glucuronidase <sup>c)</sup>	$\beta$ -D-Glucosidase <sup>c)</sup>	Mucinase <sup>c)</sup>	Urease <sup>c)</sup>
Control	$1.4 \pm 0.1^w$	$136 \pm 3^w$	$1.1 \pm 0.1^w$	$250 \pm 19^w$
Low dose	$0.4 \pm 0.1^x$	$115 \pm 3^x$	$1.1 \pm 0.1^w$	$100 \pm 4^x$
Medium dose	$0.4 \pm 0.2^x$	$103 \pm 9^x$	$0.4 \pm 0.1^x$	$24 \pm 1^y$
High dose	$0.2 \pm 0.0^y$	$66 \pm 7^y$	$0.3 \pm 0.0^x$	$28 \pm 4^y$

a) Values (means  $\pm$  SD,  $n = 8$ ) in the same column with different superscripts are significantly different ( $p < 0.05$ ).

b) The low, medium, and high dose diets were prepared by mixing 0.17, 0.5, and 1.5 g of the dried extract into 100 g of the control diet (basal formula), respectively.

c) Enzyme activities were as described in Section 2

hamsters were observed in both the medium and high dose groups (up to  $\sim 4.0$ - and 7.8-fold, respectively). The administration of various food plants such as lettuce, apple, and corn bran might also lead to an increase of SCFA pool in cecum at different extent [24–26]. The increase in the total SCFA concentrations by taking cardamom extract at a 0.5% level or above, at which more fermentable carbohydrate was consumed, might in part explain the significant reduction of the corresponding cecal pH values. Hogberg and Lindberg [27] have reported that an alternation in the SCFA concentrations in hindgut was generally related to dietary carbohydrate as well as the extent of microbial fermentation. In Table 4, the analyses of volatile fatty acids (*i. e.*, acetate and propionate) indicated that the concentrations of these components increased with an elevated consumption of cardamom extract, whereas no apparent changes in butyrate level were observed. As compared to the control group, the concentrations of acetate and propionate were significantly ( $p < 0.05$ ) increased while having cardamom extract at 0.5% level (5.3- and 3.6-fold, respectively) and 1.5% level (8.8- and 11-fold, respectively). It was reported that acetate could stimulate colonic mucin secretion [28] and propionate helped lower the plasma cholesterol and hepatic cholesterol concentration in rats [29, 30]. Therefore, the elevation in the concentrations of acetate and propionate by taking cardamom extract at 0.5 g/100 g diet or above were desirable. It was postulated that the consumption of cardamom extract, to a certain extent, might alter the composition of intestinal microbiota or the capacity of microbiota to produce different end products, subsequently leading to an apparent change in SCFA profiles [31]. The findings from Grinder

and Piland [32] demonstrated that chemical stimulation of mucosal cells by SCFAs (*i. e.*, acetate, butyrate, and propionate) may trigger a peristaltic reflex and increase gut motility. Therefore, the reduction in gastrointestinal transit time with the cardamom-containing diet (Table 2) might be in part attributed to the significant increase in SCFA levels (Table 4). Furthermore, SCFAs may also be recognized by some specific receptors of SCFAs in gastrointestinal tract, leading to an activation of leukocytes, particularly neutrophils, and promotion of leukocyte recruitment to the site of infection [33].

The effects of taking cardamom extract on the activities of different fecal bacterial enzymes such as  $\beta$ -D-glucuronidase,  $\beta$ -D-glucosidase, mucinase, and urease are shown in Table 5. As the activities of these enzymes were expressed *per* mg of fecal protein, the comparable contents of fecal protein excreted *per* day (3.7–4.2 mg/day) and fecal dry weight (Table 2) among the four diet groups supported that any changes in these fecal bacterial activities were probably attributed to the physiological effects of cardamom extract. Table 5 shows that the inclusion of cardamom into the control diet at different levels (0.17–1.5%) resulted in a significant ( $p < 0.05$ ) reduction in the activities of  $\beta$ -D-glucuronidase (by 71.4–85.7%) and  $\beta$ -D-glucosidase (by 15.4–51.5%). Some authors have reported that  $\beta$ -D-glucuronidase and  $\beta$ -D-glucosidase, produced by certain colonic bacteria, might hydrolyze the conjugated products of detoxification to liberate certain toxic metabolites and carcinogens, and hence being associated with increased risk of colorectal tumors [34, 35]. Therefore, the reduction in the activities of these two bacterial enzymes was desirable.

As shown in Table 5, the mucinase activities of hamsters fed the medium dose diet *versus* control diet were significantly ( $p < 0.05$ ) decreased by 63.6%. The increase in mucinase activity might alter the hydrolysis of protective mucin layer which functions as a major local defense barrier to prevent most bacterial invasion [19]. Therefore, the remarkable reduction in mucinase activity by feeding the cardamom-containing diet (at least 0.5 g extract/100 g diet) was desirable in maintaining a gel-like barrier protecting against bacteria and toxins in intestinal lumen. Furthermore, our results (Table 5) demonstrated that the inclusion of cardamom extract in the control diet (at 0.17% level) was effective ( $p < 0.05$ ) in decreasing the urease activity by 60.0% while higher consumption of the extract (0.5 g/100 g diet or above) could even reduce the activity by 88.8–90.4%, significantly. About 20–25% of urea produced from amino acid degradation is hydrolyzed into ammonia by microbial urease in gastrointestinal tract, and the ammonia formed in turn enters blood stream and causes harmful effects to animal health [22]. In Tables 3 and 5, there was an apparent correlation ( $r = 0.72$ ,  $p < 0.05$ ) between the urease activity and fecal ammonia content of hamsters fed different diets. These results suggested that the administration of cardamom extract might lower microbial urease activity and reduced the harmful ammonia formed in large intestine.

#### 4 Concluding remarks

In the present study, the consumption of pectic polysaccharide-rich cardamom extract (at least 0.5 g/100 g diet) effectively shortened the gastrointestinal transit time, increased the fecal moisture content and the contents of various SCFAs in hindgut, lowered the activities of colonic bacterial enzymes in feces, and also reduced the exposure of intestinal mucosa to toxic ammonia and other harmful components along the intestinal tract. This effective dosage for hamsters was equivalent to about 4 g of crude extract for a human adult. These findings suggested that the consumption of the cardamom extract might exert a favorable effect on maintaining normal, or improving, gastrointestinal milieu, and also provide a clue to substantiate its traditional therapeutic uses and dosage for intestinal health improvement.

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#### 5 References

- [1] Edwards, C. A., Rowland, I. R., Bacterial fermentation in the colon and its measurement, in: Schweizer, T. F., Edwards, C. A. (Eds.), *Dietary Fiber – A Component of Food Nutritional Function in Health and Disease*, Springer-Verlag, London 1992, pp. 119–136.
- [2] Spaeth, G., Berg, R. D., Specian, R. D., Deitch, E. A., Food without fiber promotes bacterial translocation from the gut, *Surgery* 1990, 108, 240–247.
- [3] Hoshi, S., Sakata, T., Mikuni, K., Hashimoto, H., Kimura, S., Galactosylsucrose and xylosylfructoside alter digestive tract size and concentrations of cecal organic acids in rats fed diets containing cholesterol and cholic acid, *J. Nutr.* 1994, 124, 52–60.
- [4] Chau, C. F., Chien, P. J., Chen, C. H., Influence of insoluble fiber fractions from carambola and carrot on intestinal enzymes and fecal bacterial enzymes in hamsters, *Nutr. Res.* 2005, 25, 947–957.
- [5] Chau, C. F., Huang, Y. L., Chang, F. Y., Effects of fibre derived from passion fruit seed on the activities of ileum mucosal enzymes and colonic bacterial enzymes in hamsters, *J. Sci. Food Agric.* 2005, 85, 2119–2124.
- [6] Shiau, S. Y., Chang, G. W., Effects of dietary fiber on fecal mucinase and  $\beta$ -glucuronidase activity in rats, *J. Nutr.* 1983, 113, 138–144.
- [7] Mondal, S. K., Ray, B., Thibault, J. F., Ghosal, P. K., Cell-wall polysaccharides from the fruits of *Limonia acidissima*, Isolation, purification and chemical investigation, *Carbohydr. Polym.* 2002, 48, 209–212.
- [8] Chau, C. F., Huang, Y. L., Characterization of the passion fruit seed fibres-A potential fibre source, *Food Chem.* 2004, 85, 189–194.
- [9] AOAC, *Official Methods of Analysis*, 16th Edn., Association of Official Analytical Chemists, Washington, DC 1995.
- [10] Reeves, P. G., Nielsen, F. H., Fahey, Jr., G. C., AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet, *J. Nutr.* 1993, 123, 1939–1951.
- [11] Ebihara, K., Nakamoto, Y., Comparative effect of water-soluble and -insoluble dietary fiber on bowel function in rats fed a liquid elemental diet, *Nutr. Res.* 1998, 18, 883–891.
- [12] Okuda, H., Fujii, S., Kettyuu ammonia tyokusetsu hisyoku triryohou, *Saishin-igaku* 1986, 21, 622–627.
- [13] Goldin, B. R., Gorbach, S. L., The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer, *J. Natl. Cancer Inst.* 1976, 57, 371–375.
- [14] Ling, W. H., Korpela, R., Mykkanen, H., Salminen, S., Hanninen, O., *Lactobacillus* strain GG supplementation decreases colonic hydrolytic and reductive enzyme activities in healthy female adults, *J. Nutr.* 1994, 124, 18–23.
- [15] Whitehead, J. S., Kim, Y. S., Prizont, R., A simple quantitative method to determine short chain fatty acid levels in biological fluids, *Clin. Chim. Acta* 1976, 72, 315–318.
- [16] Schneeman, B. O., Dietary fiber: Physical and chemical properties, methods of analysis, and physical effects, *Food Technol.* 1986, 2, 104–110.
- [17] McDougall, G. J., Morrison, I. M., Stewart, D., Hillman, J. R., Plant cell walls as dietary fiber: Range, structure, processing and function, *J. Sci. Food Agric.* 1996, 70, 133–150.
- [18] Femenia, A., Sanchez, E. S., Simal, S., Rossello, C., Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis* Miller) plant tissues, *Carbohydr. Polym.* 1999, 39, 109–117.
- [19] Tanira, M. O. M., Ali, B. H., Bashir, A. K., Chandranath, I., Some pharmacologic and toxicologic studies on *Rhazya Stricta* Decne in rats, mice and rabbits, *Gen. Pharmacol.* 1996, 27, 1261–1267.

- [20] Yamazaki, T., Matsushita, Y., Kawashima, K., Someya, M., *et al.*, Evaluation of the pharmacological activity of extracts from Amomi Semen on the gastrointestinal tracts, *J. Ethnopharmacol.* 2000, 71, 331–335.
- [21] Takahashi, T., Maeda, H., Aoyama, T., Yamamoto, T., Physiological effects of water-soluble soybean fiber in rats, *Biosci. Biotechnol. Biochem.* 1999, 63, 1340–1345.
- [22] Kim, K. I., Lee, W. S., Benevenga, N. J., Feeding diets containing high levels of milk products or cellulose decrease urease activity and ammonia production in rat intestine, *J. Nutr.* 1998, 128, 1186–1191.
- [23] Yonekura, L., Suzuki, H., Some polysaccharides improve zinc bioavailability in rats fed a phytic acid-containing diet, *Nutr. Res.* 2003, 23, 343–355.
- [24] Ebihara, K., Nakamoto, Y., Effects of the particle size of corn bran on the plasma cholesterol concentration, fecal output and cecal fermentation in rats, *Nutr. Res.* 2001, 21, 1509–1518.
- [25] Aprikian, O., Duclos, V., Guyot, S., Besson, C., *et al.*, Apple pectin and a polyphenol-rich apple concentration are more effective together than separately on cecal fermentation and plasma lipids in rats, *J. Nutr.* 2003, 133, 1860–1865.
- [26] Nicolle, C., Cardinault, N., Gueux, E., Jaffrelo, L., *et al.*, Health effect of vegetable-based diet: Lettuce consumption improves cholesterol metabolism and antioxidant status in the rat, *Clin. Nutr.* 2004, 23, 605–614.
- [27] Hogberg, A., Lindberg, J. E., Influence of cereal non-starch polysaccharides on digestion site and gut environment in growing pigs, *Livest. Prod. Sci.* 2004, 87, 121–130.
- [28] Barcelo, A., Claustre, J., Moro, F., Chayvialle, J. A., *et al.*, Mucin secretion is modulated by luminal factors in the isolated vascularly perfused rat colon, *Gut* 2004, 46, 218–224.
- [29] Chen, W. J. L., Anderson, J. W., Jennings, D., Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibers in cholesterol-fed rats, *Proc. Soc. Exp. Biol. Med.* 1984, 175, 215–218.
- [30] Costa Rosa, L. F. B. P., Curi, R., Bond, J. A., Newsholme, P., Newsholme, E. A., Propionate modifies lipid biosynthesis in rat peritoneal macrophages, *Gen. Pharmacol.* 1995, 26, 411–416.
- [31] Grasten, S. M., Pajari, A.-M., Liukkonen, K.-H., Karppinen, S., Mykkanen, H. M., Fibers with different solubility characteristics alter similarly the metabolic activity of intestinal microbiota in rats fed cereal brans and inulin, *Nutr. Res.* 2002, 22, 1435–1444.
- [32] Grider, J. R., Piland, B. E., The peristaltic reflex induced by short chain fatty acids is mediated by sequential release of 5-HT and neuronal CGRP but not BDNF, *Am. J. Physiol. Gastrointest. Liver Physiol.* 2007, 292, G429–G437.
- [33] Le Poul, E., Loison, C., Struyf, D., Springael, J.-Y., *et al.*, Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation, *J. Biol. Chem.* 2003, 278, 25481–25489.
- [34] Arakawa, S., Okumura, M., Yamada, S., Ito, M., Tejima, S., Enhancing effect of carrageenan on the induction of rat colonic tumors by 1,2-dimethylhydrazine and its relation to  $\beta$ -glucuronidase activities in feces and other tissues, *J. Nutr. Sci. Vitaminol.* 1986, 32, 481–485.
- [35] Gudiel-Urbano, M., Goni, I., Effect of edible seaweeds (*Undaria pinnatifida* and *Porphyra tenera*) on the metabolic activities of intestinal microflora in rats, *Nutr. Res.* 2002, 22, 323–331.