Influences of exogenous probiotics and tea polyphenols on the production of three acids during the simulated colonic fermentation of maize resistant starch

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Abstract In the present study, influences of nine probiotics and tea polyphenols on the production of acetic, butyric, and lactic acid from simulated colonic fermentation of maize resistant starch (RS) were investigated. RS was fermented by fecal extracts of healthy adults at 12 g L⁻¹ and 37 °C for 18–48 h in the presence and absence of exogenous probiotics and tea polyphenols. The added probiotics increased acetic and butyric acid production by 25–216 %. *Eubacterium faecalis*, *Lactobacillus acidophilus*, *L. casei*, and *L. helveticus* increased lactic acid production by 7–58 %; however, other probiotics decreased lactic acid production. Tea polyphenols facilitated lactic acid production but inhibited acetic and butyric acid production clearly. More importantly, the added probiotics weakened the inhibitory effects of tea polyphenols on the two acids, enhancing acetic and butyric acid production by 152–641 and 825–1,777 %, respectively. It is concluded that both probiotics and tea polyphenols have different impacts on the production of acetic, butyric and lactic acid during the colonic fermentation of RS. The impacts of other probiotics and food components on the colonic fermentation of RS and other dietary fibers should be investigated in future study to clarify their possible interactions.

Keywords Maize resistant starch · Colonic fermentation · Fecal extract · Acid production · Probiotics · Tea polyphenols

Introduction

Resistant starch (RS) is considered to be a dietary fiber because it cannot be digested and is instead fermented in the large intestine (Brites et al. 2011; Devi et al. 2014). RS has received special attention in the past years due to its potential health benefits including anti-obesity, anti-diabetic, and cholesterol-reducing properties (Dona et al. 2010). Furthermore, RS increases fecal content, maintains colonic microbiota populations, and contributes to the production of short-chain fatty acids (SCFAs) (Liu and Xu 2008), especially of butyric acid (Ahmed et al. 2000). Butyric acid is protective against colitis and colorectal cancers, maintains healthy colonocytes, and reduces tumor formation *in vitro* (Bird et al. 2006; Scharlau et al. 2009; Fuentes-Zaragoza et al. 2010). RS and other dietary fibers are prebiotics (Stewart et al. 2008; Rodríguez-Cabezas et al. 2010; Williams et al. 2011; Sreenivas and Lele 2013), and are widely studied for their health benefits.

The colonic fermentation of inulin, oat fiber, guar gum, and RS depends on the fiber types (Bourquin et al. 1996; Souza da Silva et al. 2013; Yang et al. 2013) and on the colonic microbiota (Zhao and Lin 2009; Jonathan et al. 2012). Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit on the host (Araya et al. 2002). Common probiotics include strains of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Streptococcus*, and *Leuconostoc* (Ashraf and Shah 2011; Butel 2014).

RS is a fermentable substrate for probiotics (Purwani et al. 2012). It has been reported that some microorganisms enhance SCFAs and especially butyric acid production during colonic

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fermentation of maize RS and retrograded maize starch by fecal human extracts (Zhao and Zhu 2013; Zhu and Zhao 2013). These results suggest that certain RS-microorganism combinations could contribute to high acid production levels in the large intestine.

Plant foods are rich in phenolic compounds with antioxidant and anti-cancer activities (Kanwar et al. 2012; Vijayalaxmi et al. 2014). Phenolic compounds can be utilized by intestinal microbiota (Jaquet et al. 2009); therefore, they are also considered to be prebiotics (Parkar et al. 2008; Bauer et al. 2012; Ajila and Prasada Rao 2013). The impacts of phenolic compounds on acid production during the colonic fermentation of RS thus should be investigated. In this study, we assessed the effects of exogenous probiotics and tea polyphenols on acid production during a simulated colonic fermentation of maize RS. RS was fermented by fecal extracts of healthy adults in the presence and absence of exogenous probiotics and tea polyphenols. Acetic, butyric, and lactic acid levels were quantified by chromatographic and spectrophotometric methods. The objective of this study was to assess the impacts of probiotics and tea polyphenols on acid production during colonic fermentation of RS and to clarify any possible interactions between probiotics and tea polyphenols.

Materials and methods

Materials

High-amylose maize starch (Hylon VII) and tea polyphenols were obtained from National Starch & Chemical Ltd. (Bridgewater, NJ, USA) and Xiya Reagent Research Center (Shandong, China), respectively. The compound 4-phenylphenol used in the lactic acid assay was purchased from Aladdin Chemistry Co. Ltd. (Shanghai, China). Hexane (HPLC grade) was purchased from Tianjing Kermel Co. Ltd. (Tianjing, China). Peptone, tryptone and beef extract were obtained from Beijing Aoboxing Bio-Tech Co. Ltd. (Beijing, China), while yeast extract was purchased from Oxoid Ltd. (Basingstoke, England). All other chemicals and solvents used in this study were of analytical grade. Distilled water was used throughout the experiments.

Probiotics and culture conditions

The probiotics studied were *Bifidobacterium infantis*, *Eubacterium faecalis*, *Lactobacillus acidophilus*, *L. delbrueckii* subsp. *bulgaricus* (*L. bulgaricus*), *L. brevis*, *L. casei*, *L. helveticus*, *L. plantarum*, and *Streptococcus thermophilus*. These probiotics were maintained at the Key Laboratory of Dairy Science (Northeast Agricultural University), Ministry of Education.

The probiotics were revived and maintained in a deMan-Rogosa-Sharpe (MRS) medium (Atlas 2004), which consisted of the following compounds (g L⁻¹), tryptone, 10; peptone, 5; beef extract, 5; yeast extract powder, 5; glucose, 20; Tween-80, 1; ammonium citrate, 2; sodium acetate, 5; K₂HPO₄, 2; MgSO₄·7H₂O, 0.58; and MnSO₄·4H₂O, 0.25. To the *Bifidobacterium* sp. medium, L-cysteine hydrochloride was added. The final pH values of the media were adjusted to 6.2–6.4, 5.8–6.0, and 7.2 for *Lactococcus* sp., *Lactobacillus* sp., and *Bifidobacterium* sp., respectively.

Preparation of maize RS and adult fecal extracts

The preparation of maize RS was performed as described elsewhere.¹⁵ Fecal samples were collected from eight healthy adult volunteers (4 males and 4 females, 22–25 years of age) with no history of antibiotics, laxatives, or gastrointestinal infections in the previous 3 months. Fecal samples were collected, processed within 1 h of passage,¹⁵ and stored at –20 °C.

Simulated colonic fermentation of RS

Aliquots (2.5 mL) of fecal extracts were mixed with 0.5 mL of sterilized water, 2.5 mL of a buffer (Table 1) and RS (12 g L⁻¹). The chemical composition of the buffer was slightly different to that reported by Lebet et al. (1998). Sterilized water was replaced by one of the cultured probiotics (0.5 mL), which was added to the test tubes containing the buffer and RS. All test tubes were capped with glass stoppers, mixed, incubated at 37 °C in anaerobic conditions for 6, 12, 18, 24, 36, and 48 h, respectively, mixed, and centrifuged. The resulting supernatant (2 mL) was used for the determination of acetic, butyric, and lactic acid levels.

Aliquots (2.5 mL) of the fecal extracts, cultured probiotics (0.5 mL), and tea polyphenols (0.5 mL of 1.0 g L⁻¹) were transferred to test tubes containing the buffer and RS. The test tubes were subjected to the same fermentation conditions described above.

Table 1 Chemical composition of the buffer solution used

Trace element solution (per liter)		Buffer solution (per liter)	
FeSO ₄ ·7H ₂ O	3.68 g	NaHCO ₃	9.24 g
MnSO ₄ ·H ₂ O	1.159 g	Na ₂ HPO ₄ ·12H ₂ O	7.125 g
ZnSO ₄ ·7H ₂ O	0.44 g	NaCl	0.47 g
CoCl ₂ ·6H ₂ O	0.12 g	KCl	0.45 g
NiCl ₂	0.1 g	Urea	0.4 g
CuSO ₄ ·5H ₂ O	0.098 g	CaCl ₂ ·6H ₂ O	0.108 g
		Na ₂ SO ₄ (anhydrous)	0.1 g
		MgCl ₂ ·6H ₂ O	0.1 g
		Trace element solution	10 mL

Acid analyses

In this experiment, 1 mL of the collected supernatants or standard acid solutions ($10\text{--}60\text{ mmol L}^{-1}$) was added into test tubes. Absolute ethanol (2.4 mL) was added into the tubes and mixed. The solutions were transferred to low borosilicate glass ampoules and mixed with concentrated sulfuric acid (0.1 mL) and hexane (1.0 mL). The ampoules were quickly sealed by a blast burner, heated in a water bath at $60\text{ }^{\circ}\text{C}$ for 1 h, and shaken to ensure effective esterification. Subsequently, the ampoules were left at room temperature and mixed vigorously for 5 min. After phase separation, the acetic and butyric acid levels in the upper phase ($1.0\text{ }\mu\text{L}$; hexane phase) were measured by chromatography (Fenster et al. 2003) using a gas chromatograph (7890 Agilent, Agilent Technologies, Santa Clara, USA) coupled to a flame ionization detector, split injector, and a HP-5 capillary column ($30\text{ m}\times 0.32\text{ mm}$; Agilent Technologies, Santa Clara, USA). The hexane phase was injected in the split mode at a 40:1 ratio. The preset temperatures of the injector and detector were 200 and $220\text{ }^{\circ}\text{C}$, respectively. The initial column temperature was $30\text{ }^{\circ}\text{C}$, which was held for 3.5 min and subsequently increased to $40\text{ }^{\circ}\text{C}$ at $5\text{ }^{\circ}\text{C min}^{-1}$. The column temperature was increased to $150\text{ }^{\circ}\text{C}$ at $15\text{ }^{\circ}\text{C min}^{-1}$. The carrier gas was nitrogen gas at 1.0 mL min^{-1} .

Lactic acid in the collected supernatants was assessed by a spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) as per the method (Barker and Summerson 1941). The production levels of the three acids were corrected relative to those obtained at baseline (0 h).

Statistical analyses

All experiments and analyses were performed in triplicate. Data were analyzed by SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA) and expressed as means $\pm$ standard deviations.

Results and discussion

Acid analyses

Figure 1 shows the GC analysis results of a standard solution and a sample. During GC analysis, the retention times of acetic and butyric acid were approximately 3 and 8 min, respectively. The calculated detection limits of acetic, butyric, and lactic acid were 0.59, 0.08, and 0.06 mmol L^{-1} , respectively. The average addition recoveries of acetic, butyric, and lactic acid were approximately 98.2, 102.4, and 97.4 %, respectively, when the addition levels were set at $1.0\text{--}30\text{ mmol L}^{-1}$. The results revealed that the analytical method

Fig. 1 GC profiles of acetic and butyric acid from a standard solution (**a**) and sample supernatant (**b**). Peaks 1 and 2 represent acetic and butyric acid, respectively. The standard solution and sample supernatant were esterified by sulfuric acid-ethanol, extracted by cold hexane, and analyzed by GC

used in this study was applicable for the measurement of the three acids.

Impacts of probiotics on acid production

The 48-h fermentation of fecal extracts in the absence of RS and probiotics generated acetic (1.98 mmol L^{-1}), butyric (0.62 mmol L^{-1}), and lactic acid (0.45 mmol L^{-1} ; Tables 2, 3 and 4). When RS was added, the production of acetic, butyric, and lactic acid increased to 10.3, 4.46, and 5.19 mmol L^{-1} , respectively (Tables 2, 3 and 4). Therefore, RS was metabolized by the colonic microorganisms in the fecal extracts, resulting in higher acid production levels. When both RS and selected probiotics were added, acid production (Tables 2, 3 and 4), especially of acetic and butyric acid, increased. The addition of *B. infantis* resulted in a maximum acetic acid production of 30.7 mmol L^{-1} , while the addition of other probiotics contributed to acetic acid levels of 12.9--

Table 2 Acetic acid (mmol L^{-1}) produced by *in vitro* fermentation of resistant maize starch under different conditions

Substrates ^a	Probiotics ^b	Acetic acid production at different fermentation times (h) ^c					
		6	12	18	24	36	48
FE	No	NP	1.98±0.27	1.28±0.13	1.46±0.04	1.50±0.11	1.49±0.03
FE + RS	No	1.33±0.40	4.24±0.36	7.51±0.36	4.85±0.78	6.28±0.42	10.3±0.77
FE + RS	BI	5.70±1.19	9.41±1.04	14.7±0.63	16.9±0.43	30.3±2.62	30.7±1.63
FE + RS	EF	0.83±0.38	4.11±0.92	7.02±0.77	9.18±0.57	10.7±0.96	14.8±1.34
FE + RS	LA	1.44±0.60	3.47±0.38	5.11±0.36	6.11±0.44	9.20±0.97	17.2±0.18
FE + RS	LB	2.95±0.78	5.08±0.78	7.17±0.85	12.7±1.22	19.1±2.05	0.74±0.39
FE + RS	LBr	0.83±0.48	1.53±0.54	1.62±0.62	3.17±0.71	5.38±0.93	12.9±0.30
FE + RS	LC	3.16±0.08	4.40±0.23	5.48±0.49	11.6±0.48	11.8±0.63	13.1±1.03
FE + RS	LH	1.45±0.27	1.15±0.84	0.31±0.05	1.36±0.87	6.91±0.06	14.2±0.97
FE + RS	LP	1.30±0.06	2.59±0.66	3.89±0.31	4.13±0.69	4.37±0.72	13.3±1.01
FE + RS	ST	2.08±0.13	1.46±0.51	1.03±0.61	3.66±0.39	6.17±0.83	14.0±1.27
TPP + FE	No	0.84±0.04	0.97±0.03	0.50±0.11	0.41±0.06	NP	NP
TPP + FE + RS	No	1.64±0.05	1.97±0.24	0.54±0.07	0.34±0.15	NP	NP
TPP + FE + RS	BI	4.82±0.86	4.53±0.53	1.35±0.30	0.48±0.29	3.89±0.86	6.90±0.85
TPP + FE + RS	LA	3.07±0.52	3.38±1.42	4.58±0.51	5.56±0.81	10.1±0.53	14.6±0.52
TPP + FE + RS	LB	0.86±0.21	3.07±0.30	5.41±0.84	7.09±0.09	0.37±0.05	2.03±0.26
TPP + FE + RS	LBr	0.19±0.09	0.93±0.60	2.31±0.83	4.96±0.53	NP	NP
TPP + FE + RS	LH	3.46±0.49	5.07±0.92	1.89±0.91	0.30±0.24	2.37±0.30	2.54±0.56
TPP + FE + RS	ST	4.70±1.06	6.26±0.44	4.83±0.99	3.98±0.37	3.44±0.14	1.93±0.12

^a FE, fecal extracts; RS, resistant starch; TPP, tea polyphenols^b LP, *L. plantarum*; LB, *L. bulgaricus*; LA, *L. acidophilus*; LBr, *L. brevis*; LH, *L. helveticus*; LC, *L. casei*; EF, *E. faecalis*; ST, *S. thermophilus*; BI, *B. infantis*; No, without probiotics addition^c NP, not produced

19.1 mmol L^{-1} (Table 2). The addition of *B. infantis* and *E. faecalis* led to 14.0–14.1 mmol L^{-1} butyric acid levels, while other probiotics resulted in butyric acid levels of 8.02–13.5 mmol L^{-1} (Table 3). Therefore, the addition of these probiotics enhanced the production of acetic and butyric acid by 25–198 and 80–216 %, respectively. Lactic acid production was also affected by both fermentation time and probiotics (Table 4). Lactic acid was mainly generated during the initial fermentation stage (6–12 h); in most cases, prolonged fermentation periods (18–48 h) contributed to lower lactic acid production levels. Fermentation by *E. faecalis*, *L. acidophilus*, *L. casei*, and *L. helveticus* contributed to the highest lactic acid levels (5.56–8.22 mmol L^{-1}), whereas other probiotics contributed to lower lactic acid production levels (2.21–5.20 mmol L^{-1} ; Table 4). These results revealed that the four probiotics enhanced lactic acid production by 7–58 %, while other probiotics decreased lactic acid production by 0–57 %. Of the probiotics investigated, *E. faecalis*, *L. acidophilus*, *S. thermophilus*, and *B. infantis* contributed to the highest production levels of the three acids.

The *Bifidobacterium* and *Lactobacillus* genera belong to dominant and sub-dominant colonic microbiota, respectively (Butel 2014). Certain *Lactobacillus* strains are capable of

utilizing non-digestible oligosaccharides (Crittenden et al. 2002). Kaplan and Hutkins (Kaplan and Hutkins 2000), who focused on lactic acid bacteria and bifidobacteria, reported that 12 of 16 *Lactobacillus* strains and seven out of eight *Bifidobacterium* strains are capable of fermenting fructooligosaccharides. Compared to other probiotics, *B. bifidum* contributed to higher butyric acid production level from the fermentation of sago RS (Siew-Wai et al. 2010). These results indicate that certain microorganisms have superior fermentative capabilities. Zhou et al. (2013) reported that the production of SCFAs (especially of butyric acid) was greatly enhanced by fermentation time. When retrograded maize starch and maize RS were fermented by human intestinal microflora, certain exogenous microorganisms had beneficial effects on acid production, especially butyric acid (Zhao and Zhu 2013; Zhu and Zhao 2013). The results obtained in this study were consistent with those reported by other researchers; *i.e.*, probiotics enhanced the production of acetic and butyric acid during the colonic fermentation of RS.

The results of this study revealed that the colonic fermentation of RS contributes to lactic acid production during the early fermentation stage; however, longer fermentation times contribute to less lactic acid production (Table 4). Certain

Table 3 Butyric acid (mmol L⁻¹) produced by *in vitro* fermentation of resistant maize starch under different conditions

Substrates ^{a)}	Probiotics ^{b)}	Butyric acid production at different fermentation times (h) ^c					
		6	12	18	24	36	48
FE	No	0.06±0.03	0.23±0.07	0.55±0.06	0.62±0.12	0.46±0.05	0.30±0.17
FE + RS	No	0.09±0.07	1.13±0.20	1.61±0.25	1.85±0.22	1.94±0.34	4.46±0.32
FE + RS	BI	0.57±0.10	4.70±0.21	6.46±0.16	12.2±0.31	13.1±0.25	14.0±1.22
FE + RS	EF	0.08±0.03	1.31±0.14	2.07±0.21	4.98±0.61	7.93±0.53	14.1±1.31
FE + RS	LA	NP	1.28±0.19	2.53±0.35	4.38±0.37	8.86±0.25	9.28±0.31
FE + RS	LB	1.03±0.20	2.34±0.18	3.20±0.34	5.17±0.69	7.75±0.78	8.02±0.36
FE + RS	LBr	0.23±0.12	4.14±0.17	4.63±0.51	6.10±0.38	8.33±0.88	11.2±1.03
FE + RS	LC	0.51±0.17	1.63±0.21	2.55±0.15	7.03±0.14	7.14±0.37	12.5±0.16
FE + RS	LH	0.26±0.04	4.58±0.65	4.80±0.17	5.76±0.46	8.89±0.74	11.5±0.48
FE + RS	LP	0.05±0.04	3.20±0.50	4.40±0.43	5.79±0.13	5.91±0.43	9.73±0.22
FE + RS	ST	0.11±0.07	3.59±0.11	3.88±0.18	5.29±0.32	6.78±0.36	13.5±0.73
TPP + FE	No	0.19±0.06	0.37±0.08	0.42±0.05	NP	NP	NP
TPP + FE + RS	No	0.10±0.04	0.39±0.06	0.20±0.06	0.11±0.03	0.11±0.04	0.65±0.06
TPP + FE + RS	BI	0.12±0.08	0.43±0.09	4.00±0.35	6.92±0.49	10.3±0.59	12.2±0.14
TPP + FE + RS	LA	0.49±0.10	0.76±0.06	3.12±0.30	5.99±0.14	7.96±0.32	9.29±0.36
TPP + FE + RS	LB	0.09±0.05	0.41±0.11	3.03±0.02	4.43±0.11	5.90±0.17	6.01±0.22
TPP + FE + RS	LBr	0.20±0.07	2.20±0.09	4.16±0.37	5.86±0.16	7.52±0.42	7.60±0.46
TPP + FE + RS	LH	0.93±0.25	1.46±0.16	2.53±0.27	3.90±0.46	5.66±0.37	6.37±0.33
TPP + FE + RS	ST	0.71±0.03	2.16±0.13	3.61±0.61	5.44±0.65	6.35±0.86	9.46±0.56

^a FE, fecal extracts; RS, resistant starch; TPP, tea polyphenols

^b LP, *L. plantarum*; LB, *L. bulgaricus*; LA, *L. acidophilus*; LBr, *L. brevis*; LH, *L. helveticus*; LC, *L. casei*; EF, *E. faecalis*; ST, *S. thermophilus*; BI, *B. Infantis*; No, without probiotics addition

^c NP, not produced

bacteria are capable of converting lactic acid into acetic acid and other compounds (Lopez et al. 2001). Bourriaud et al. (2005) reported that lactic acid and lactate could be utilized by human intestinal microflora as precursors for butyrate synthesis. Some researchers have found that certain microorganisms can convert lactate into butyric acid (Duncan et al. 2004; Morrison et al. 2006). Lactic acid generated during the early stage of the colonic fermentation was metabolized by the fecal microorganisms during the later stages of the colonic fermentation.

Impacts of tea polyphenols on acid production

Tea polyphenols were added into the fermentation system to assess their influences on acid production; the results are shown in Tables 2, 3 and 4. Fermentation of the fecal extracts in the presence of tea polyphenols but in the absence of RS resulted in acetic, butyric, and lactic acid levels of 0.97, 0.42 and 2.67 mmol L⁻¹, respectively. This result indicated that the addition of tea polyphenols inhibited acetic and butyric acid production because in the absence of tea polyphenols the production levels of acetic and butyric acid were higher (1.98 and 0.62 mmol L⁻¹, respectively; Tables 2 and 3).

However, lactic acid production was enhanced by the addition of tea polyphenols (*i.e.*, lactic acid levels increased from 0.45 to 2.67 mmol L⁻¹; Table 4). Therefore, tea polyphenols increased lactic acid production but inhibited acetic and butyric acid production. This result suggests that tea polyphenols had different impacts on acid production during the colonic fermentation of RS.

When RS was fermented by the fecal extracts in the presence of tea polyphenols, the production levels of acetic, butyric, and lactic acid were 1.97, 0.65 and 8.92 mmol L⁻¹, respectively (Tables 2, 3 and 4). Acetic and butyric acid production was inhibited while lactic acid production was enhanced because in the absence of tea polyphenols the production levels of the acids were 10.3, 4.46, and 5.19 mmol L⁻¹, respectively. If the probiotics were inoculated into the fermentation system, an interesting phenomenon was observed. Firstly, acetic and butyric acid production were significantly enhanced (4.96–14.6 and 6.01–12.2 mmol L⁻¹, respectively; Tables 2 and 3); *i.e.*, acetic and butyric acid production increased by 152–641 % and 825–1,777 %, respectively. These values revealed that the inoculated probiotics contributed to an increase in two acid production, especially butyric acid production; therefore, acetic and

Table 4 Lactic acid (mmol L⁻¹) produced by *in vitro* fermentation of resistant maize starch under different conditions

Substrates ^{a)}	Probiotics ^{b)}	Lactic acid production at different fermentation times (h) ^{c)}		
		6	12	18
FE	No	0.45±0.09	NP	NP
FE + RS	No	5.19±0.45	4.48±0.24	2.92±0.99
FE + RS	BI	3.45±0.90	1.80±0.42	NP
FE + RS	EF	8.22±1.36	2.25±1.55	NP
FE + RS	LA	6.80±2.08	NP	NP
FE + RS	LB	5.20±1.58	2.20±1.64	NP
FE + RS	LBr	3.08±1.09	2.93±1.52	2.01±0.97
FE + RS	LC	5.82±1.72	6.69±1.16	2.79±1.57
FE + RS	LH	5.56±2.40	3.81±1.63	1.43±1.27
FE + RS	LP	2.21±0.34	1.80±1.45	NP
FE + RS	ST	3.94±1.17	2.58±1.81	1.58±1.34
TPP + FE	No	2.67±0.35	1.05±0.34	0.46±0.23
TPP + FE + RS	No	8.90±1.85	8.92±1.06	0.15±0.07
TPP + FE + RS	BI	5.66±1.44	1.83±0.92	NP
TPP + FE + RS	LA	11.7±1.37	4.84±1.78	1.66±1.45
TPP + FE + RS	LB	6.47±0.70	NP	NP
TPP + FE + RS	LBr	3.85±1.21	NP	NP
TPP + FE + RS	LH	6.26±0.60	3.85±0.54	3.29±1.54
TPP + FE + RS	ST	8.30±0.36	7.85±0.49	5.29±0.47

^a FE, fecal extracts; RS, resistant starch; TPP, tea polyphenols

^b LP, *L. plantarum*; LB, *L. bulgaricus*; LA, *L. acidophilus*; LBr, *L. brevis*; LH, *L. helveticus*; LC, *L. casei*; EF, *E. faecalis*; ST, *S. thermophilus*; BI, *B. Infantis*; No, without probiotics addition

^c NP, not produced

butyric acid production increased. Secondly, lactic acid production was less than 8.92 mmol L⁻¹ except in the presence of *L. acidophilus*. This result reveals that lactic acid production was mostly inhibited by the inoculated probiotics. Whereas tea polyphenols inhibited RS fermentation, the inoculated probiotics weakened the inhibition and improved acetic and butyric acid production. This result reveals the interaction between the probiotics and tea polyphenols during the colonic fermentation of RS, and indicates another important role of the probiotics: they decrease the adverse impacts of certain food components on the colonic fermentation of RS or dietary fibers.

Phenolic compounds including polyphenols are widely disturbed functional molecules in plant foods (Abuajah et al. 2014), and can be metabolized by colonic microbiota (Grun et al. 2008). Consumption of fruit polyphenols can effectively affect intestinal microbes and improve disbiosis (Parker et al. 2008). For example, Lee et al. (2006) reported that tea polyphenols had significant impacts on the colonic environment and could regulate intestinal bacterial populations. Epidemiological researches have suggested that

polyphenols-rich foods are protective against colorectal cancer. However, a recent study also observed that dietary intake of tea polyphenols had no protective effect against colorectal cancer (Wang et al. 2013). This finding might be partly supported by the present result, as tea polyphenols could inhibit butyric acid production during the colonic fermentation of RS whereas butyric acid is considered to be protective against colorectal cancer. It is thus suggested that potential effects of tea polyphenols on colonic fermentation of other dietary fibers should also be thoroughly investigated.

The formation of acetic, butyric, and lactic acid should be a favorable trait of colon-targeted functional foods. The present study evidenced that both tea polyphenols and exogenous probiotics either increase or decrease acid production during the colonic fermentation of RS. Future studies should assess the impacts of other probiotics and food components on acid production during the colonic fermentation of RS and other dietary fibers, and to clarify their possible interactions unknown in the present time.

Conclusion

Probiotics and tea polyphenols, added into a simulated colonic fermentation system of maize RS, had significant impacts on the production of acetic, butyric, and lactic acid. The probiotics enhanced the production of acetic acid and especially of butyric acid, while only some probiotics increased the production of lactic acid. Tea polyphenols enhanced lactic acid production but inhibited acetic and butyric acid production. The probiotics weakened the inhibitory impacts of tea polyphenols and therefore improved acetic and butyric acid production. The influences of other probiotics and phenolic compounds on the colonic fermentation of RS and other dietary fibers should be investigated in future studies.

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References

- Abuajah CI, Ogbonna AC, Osuji CM (2014) Functional components and medicinal properties of food: A review. J Food Sci Technol. doi:10.1007/s13197-014-1396-5
- Ahmed R, Segal I, Hassan H (2000) Fermentation of dietary starch in humans. Am J Gastroenterol 95:1017–1020
- Ajila CM, Prasada Rao UJS (2013) Mango peel dietary fibre: Composition and associated bound phenolics. J Funct Food 5: 444–450
- Araya M, Morelli L, Reid G, Sanders M, Stanton C, Pineiro M, Ben Embarek P (2002) Guidelines for the evaluation of probiotics in

- food. Report of a Joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food, London, Ontario, Canada
- Ashraf R, Shah NP (2011) Selective and differential enumerations of *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium* spp. in yoghurt—a review. *Int J Food Microbiol* 149:194–208
- Atlas RM (2004) Handbook of Microbiological Media vol 1. CRC Press
- Barker SB, Summerson WH (1941) The colorimetric determination of lactic acid in biological material. *J Biol Chem* 138:535–554
- Bauer JL, Harbaum-Piayda B, Schwarz K (2012) Phenolic compounds from hydrolyzed and extracted fiber-rich by-products. *LWT-Food Sci Technol* 47:246–254
- Bird AR, Brown IL, Topping DL (2006) Low and high amylose maize starches acetylated by a commercial or a laboratory process both deliver acetate to the large bowel of rats. *Food Hydrocoll* 20:1135–1140
- Bourquin LD, Titgemeyer EC, Fahey GC Jr (1996) Fermentation of various dietary fiber sources by human fecal bacteria. *Nutr Res* 16: 1119–1131
- Bourriaud C, Robins RJ, Martin L, Kozłowski F, Tenailleau E, Cherbut C, Michel C (2005) Lactate is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is evident. *J Appl Microbiol* 99:201–212
- Brites CM, Trigo MJ, Carrapico B, Alvina M, Bessa RJ (2011) Maize and resistant starch enriched breads reduce postprandial glycemic responses in rats. *Nutr Res* 31:302–308
- Butel MJ (2014) Probiotics, gut microbiota and health. *Med Mal Infect* 44:1–8
- Crittenden R, Karppinen S, Ojanen S, Tenkanen M, Fagerström R, Mättö J, Saarela M, Mattila-Sandholm T, Poutanen K (2002) *In vitro* fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. *J Sci Food Agric* 82:781–789
- Devi PB, Vijayabharathi R, Sathyabama S, Malleshi NG, Priyadarisini VB (2014) Health benefits of finger millet (*Eleusine coracana* L.) polyphenols and dietary fiber: A review. *J Food Sci Technol* 51: 1021–1040
- Dona AC, Pages G, Gilbert RG, Kuchel PW (2010) Digestion of starch: *In vivo* and *in vitro* kinetic models used to characterise oligosaccharide or glucose release. *Carbohydr Polym* 80: 599–617
- Duncan SH, Louis P, Flint HJ (2004) Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol* 70:5810–5817
- Fenster K, Rankin S, Steele J (2003) Accumulation of short-chain ethyl esters by esterases of lactic acid bacteria under conditions simulating ripening parmesan cheese. *J Dairy Sci* 86:2818–2825
- Fuentes-Zaragoza E, Riquelme-Navarrete MJ, Sánchez-Zapata E, Pérez-Álvarez JA (2010) Resistant starch as functional ingredient: A review. *Food Res Int* 43:931–942
- Grun CH, van Dorsten FA, Jacobs DM, Le Belleguic M, van Velzen EJJ, Bingham MO, Janssen HG, van Duynhoven JPM (2008) GC-MS methods for metabolic profiling of microbial fermentation products of dietary polyphenols in human and *in vitro* intervention studies. *J Chromatogr B* 871:212–219
- Jaquet M, Rochat I, Moulin J, Cavin C, Bibiloni R (2009) Impact of coffee consumption on the gut microbiota: A human volunteer study. *Int J Food Microbiol* 130:117–121
- Jonathan MC, van den Borne JJGC, van Wiechen P, Souza da Silva C, Schols HA, Gruppen H (2012) *In vitro* fermentation of 12 dietary fibres by faecal inoculum from pigs and humans. *Food Chem* 133: 889–897
- Kanwar J, Mujtaba Taskeen IM, Huo CD, Chan TH, Dou QP (2012) Recent advances on tea polyphenols. *Front Biosci* 4:111–131
- Kaplan H, Hutkins RW (2000) Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. *Appl Environ Microbiol* 66: 2682–2684
- Lebet V, Arrigoni E, Amadò R (1998) Measurement of fermentation products and substrate disappearance during incubation of dietary fibre sources with human faecal flora. *LWT-Food Sci Technol* 31: 473–479
- Lee HC, Jenner AM, Low CS, Lee YK (2006) Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Res Microbiol* 157:876–884
- Liu RP, Xu GF (2008) Effects of resistant starch on colonic preneoplastic aberrant crypt foci in rats. *Food Chem Toxicol* 46:2672–2679
- Lopez HW, Levrat-Verny MA, Coudray C, Besson C, Krespine V, Messenger A, Demigné C, Rémésy C (2001) Class 2 resistant starches lower plasma and liver lipids and improve mineral retention in rats. *J Nutr* 131:1283–1289
- Morrison DJ, Mackay WG, Edwards CA, Preston T, Dodson B, Weaver LT (2006) Butyrate production from oligofructose fermentation by the human faecal flora: What is the contribution of extracellular acetate and lactate. *Brit J Nutr* 96:570–577
- Parkar SG, Stevenson DE, Skinner MA (2008) The potential influence of fruit polyphenols on colonic microflora and human gut health. *Int J Food Microbiol* 124:295–298
- Purwani EY, Purwadaria T, Suhartono MT (2012) Fermentation RS3 derived from sago and rice starch with *Clostridium butyricum* BCC B2571 or *Eubacterium rectale* DSM 17629. *Anaerobe* 18: 55–61
- Rodríguez-Cabezas ME, Camuesco D, Arribas B, Garrido-Mesa N, Comalada M, Bailón E, Cueto-Sola M, Utrilla P, Guerra-Hernández E, Pérez-Roca C, Gálvez J, Zarzuelo A (2010) The combination of fructooligosaccharides and resistant starch shows prebiotic additive effects in rats. *Clin Nutr* 29:832–839
- Scharlau D, Borowicki A, Habermann N, Hofmann T, Klenow S, Miene C, Munjal U, Stein K, Gleis M (2009) Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutat Res* 682:39–53
- Siew-Wai L, Zi-Ni T, Karim AA, Hani NM, Rosma A (2010) Fermentation of metroxylon sago resistant starch type III by *Lactobacillus* sp. and *Bifidobacterium bifidum*. *J Agric Food Chem* 58:2274–2278
- Souza da Silva C, Bolhuis JE, Gerrits WJ, Kemp B, van den Borne JJ (2013) Effects of dietary fibers with different fermentation characteristics on feeding motivation in adult female pigs. *Physiol Behav* 110:148–157
- Sreenivas KM, Lele SS (2013) Prebiotic activity of gourd family vegetable fibres using *in vitro* fermentation. *Food Biosci* 1:26–30
- Stewart ML, Timm DA, Slavin JL (2008) Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an *in vitro* fermentation system. *Nutr Res* 28:329–334
- Vijayalaxmi S, Jayalakshmi S, Sreeramulu K (2014) Polyphenols from different agricultural residues: Extraction, identification and their antioxidant properties. *J Food Sci Technol*. doi:10.1007/s13197-014-1295-9
- Wang ZJ, Ohnaka K, Morita M, Toyomura K, Kono S, Ueki T, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Maekawa T, Yasunami Y, Takenaka K, Ichimiya H, Terasaka R (2013) Dietary polyphenols and colorectal cancer risk: The Fukuoka colorectal cancer study. *World J Gastroenterol* 19: 2683–2690
- Williams BA, Mikkelsen D, le Pailh L, Gidley MJ (2011) *In vitro* fermentation kinetics and end-products of cereal arabinoxylans and (1,3;1,4)- β -glucans by porcine faeces. *J Cereal Sci* 53:53–58
- Yang JY, Martinez I, Walter J, Keshavarzian A, Rose DJ (2013) *In vitro* characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe* 23:74–81

- Zhao XH, Lin Y (2009) Resistant starch prepared from high-amylose maize starch with citric acid hydrolysis and its simulated fermentation *in vitro*. Eur Food Res Technol 228:1015–1021
- Zhao XH, Zhu CL (2013) Synergic effect of exogenous microorganism combination on propionic and butyric acid formation during *in vitro* colonic fermentation of maize resistant starch. Starch-Starke 65: 660–665
- Zhou ZK, Cao XH, Zhou JY (2013) Effect of resistant starch structure on short-chain fatty acids production by human gut microbiota fermentation *in vitro*. Starch-Starke 65:509–516
- Zhu CL, Zhao XH (2013) *In vitro* fermentation of a retrograded maize starch by healthy adult fecal extract and impacts of exogenous microorganisms on three acids production. Starch-Starke 65: 330–337