Effects of different sea buckthorn leaf tea processing technologies on nutrient level and fecal microflora in vitro

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Summary

Three sea buckthorn leaf tea products, namely, tea dried at room temperature (SDRIED), conventionally processed green tea (SGREEN) and black tea (SBLACK), were compared for their effects on human fecal microflora and the levels of related nutrients. Content analysis confirmed higher total polysaccharides for SGREEN (165.8 ± 0.1 g·kg^{-1}) than for SBLACK (100.0 ± 0.1 g·kg^{-1}) and SDRIED (115.8 ± 0.1 g·kg^{-1}), higher total quercetin for SGREEN (6.0 ± 0.2 g·kg^{-1}) and SDRIED (7.7 ± 0.2 g·kg^{-1}) than for SBLACK (2.4 ± 0.1 g·kg^{-1}), and higher total gallic acid for SBLACK (0.60 ± 0.01 g·kg^{-1}) than for SGREEN (0.46 ± 0.01g·kg^{-1}) and SDRIED (0.07 ± 0.01 g·kg^{-1}). An in vitro digestion assay demonstrated that the order of nutrient contents remained unchanged after digestion. Furthermore, an in vitro fermentation assay indicated that only SGREEN treatment could increase the content of the probiotic *Bifidobacterium* and decrease the content of the pathogenic *Clostridium perfringens* simultaneously. These findings show that green tea processing technology is more suitable for making sea buckthorn leaf tea. For the first time, improved fecal microflora rather than antioxidant activity was selected to evaluate different leaf tea products.

Keywords

sea buckthorn leaf tea; processing technology; nutrient level; fecal microflora function; in vitro digestion; in vitro fermentation

Sea buckthorn (*Hippophae rhamnoides* L.) is a plant with the ability to prevent soil erosion [1]. It has been planted extensively all over the world. Its male leaves are low in caffeine, high in polyphenols [2], and exhibit various functions. Their extract is also safe for drinking and has been made into tea. Three approaches have been used to process sea buckthorn leaves into tea: drying at room temperature, conventional processing as green tea, and conventional processing as black tea. However, it has remained unclear which is the best alternative.

In contrast to other types of leaf tea, the main functional effect of sea buckthorn leaf tea is defecation [3], rather than to improve antioxidant activity [4]. This is due to its flavonoids, which mainly exist in the conjugated form and are linked to a saccharide moiety via a C-3 bond [4], as well as the predominant phenolic acid gallic acid [5], which is absorbed via the paracellular route [6]. Neither of these is markedly absorbed in the blood, resulting in increased phenolic uptake by the colon. Ke et al. [7] found that the growth of harmful bacteria could be inhibited by quercetin extracted from guava leaves, and Tzounis et al. [8] further concluded that flavanol monomers could change the composition of human fecal microflora, both of which indicate that polyphenols could influence the colon microflora.

Another related group of nutrients whose effects cannot be ruled out is the polysaccharides, which have been regarded as a prebiotic; their

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effects have been verified in many experiments. Lui et al. [9] found that hot-water-soluble polysaccharides extracted from sea buckthorn leaves were from the cell wall. Barry et al. [10] also proved that pectin, a cell-wall polysaccharide, was useful for promoting the intestinal health of cats.

Based on these findings, we speculated that not only polysaccharides but also polyphenols influenced the colon microflora. Accordingly, in this study, both polysaccharides and polyphenols were used to evaluate the merits of different approaches to process sea buckthorn leaf tea, a comparison that has not been reported previously.

In addition, when consumed, sea buckthorn leaf tea passes through the highly acidic stomach (pH 1–3) and the alkaline intestinal tract (small intestine juice, pH 5.8; large intestine juice, pH 6.5) and reacts with a variety of hydrolytic enzymes, so its nutrients could be lost or liberated. Therefore, the influence of the human digestive system should not be overlooked. An in vitro digestion model was thus adopted to investigate digestive impacts on nutrients.

Furthermore, an in vitro fermentation model was used to examine the impact of treatment with different sea buckthorn leaf tea digestates on colon flora. Three taxa in the flora, *Bifidobacterium*, *Escherichia coli* and *Clostridium perfringens*, were selected for this study as representative components of the flora, as they collectively represent the range of characteristics of human colon bacteria, namely Gram positive or negative; being strictly anaerobic, facultatively anaerobic, or aerobic; and being probiotic, neutral, or harmful.

Summarizing all of the related factors, the objectives of this study were as follows:

- to analyse the effects of different types of processing on the contents of polysaccharides, free and bound gallic acid, quercetin and quercetin-3-glucoside in three kinds of sea buckthorn leaf tea;
- to observe the differences in related nutrients after digestion compared with before;
- to compare the changes in the numbers of *Bifidobacterium* spp., *E. coli* and *C. perfringens* after fermenting digestate powder of the three kinds of sea buckthorn leaf tea with human fecal inoculum.

MATERIALS AND METHODS

Preparation of three kinds of sea buckthorn leaf tea

The fresh male leaves of sea buckthorn were collected in wild sea buckthorn forests in Shanxi Guangling, China, between July and August 2014.

The sea buckthorn leaves were dried at room temperature (samples marked as SDRIED). Sea buckthorn green tea and black tea were produced using the same sea buckthorn leaves.

The process for producing sea buckthorn green tea included picking fresh sea buckthorn leaves, spreading them out for 24 h at room temperature, and then removing the green leaves, rolling them, and drying them at 90 °C until moisture was less than 8 % (samples marked as SGREEN).

The process for producing sea buckthorn black tea included spreading out fresh sea buckthorn leaves, rolling them, and then fermentation at 28 °C for 6 h and drying them at 90 °C until moisture was less than 8 % (samples marked as SBLACK). No additional bacteria were added during fermentation.

The three samples were ground into a fine powder using a 250-μm screen with a high-speed homogenizer (FSH-2; Jintan Tianjing Lab Instruments Manufacturing, Jintan, China), then kept in sealed dark plastic bags in a desiccator at room temperature and used within one week. All chemicals used in this study were purchased from Beijing Kebio Bio-Technology (Beijing, China).

In vitro digestion pre-treatment

The in vitro digestion procedure was done following Liu et al. [11], with a modification of the digestion time, in accordance with a review of human digestion models for food applications [12].

Five grams of powder from each type of tea infusion was mixed with 250 ml of boiling water for 15 min and then centrifuged. The supernatant was collected and mixed with 250 ml of saline (140 mmol·l^{-1} NaCl, 5 mmol·l^{-1} KCl). Then, pH was decreased to 2.0 by adding 1 mol·l^{-1} HCl to the homogenate, and pepsin was added to a final concentration of 1.3 mg·ml^{-1}. The digestate was incubated in a shaking water bath (TS-100B Thermostatic Shaker; Shanghai Tensuc Lab Instruments Manufacturing, Shanghai, China) at 37 °C for 1 h. The pH value of the digestate was increased to 5.8 by adding 1 mol·l^{-1} $NaHCO_3$. Porcine bile extract and pancreatin were then added to final concentrations of 1.1 mg·ml^{-1} and 0.175 mg·ml^{-1}, respectively. The pH value was increased to 6.5 and the mixture was incubated at 37 °C for 2 h. Finally, the digestates were freeze-dried to a powder and stored at –4 °C for further study.

Monosaccharide composition of polysaccharide analysis

The monosaccharide assays were conducted in accordance with the work of Zhang et al. [13]. In brief, the samples were prepared after being

washed with 70% ethanol and a mixture of chloroform and methanol (1:1, v/v). Two milligrams of prepared sample were hydrolysed in 2 mol·l^{-1} trifluoroacetic acid at 121 °C for 90 min. The released glycosides were reduced with sodium borohydride (10 mg·ml^{-1} in 1 mol·l^{-1} ammonium hydroxide). The alditol acetates were generated and analysed using an Agilent 7890 series gas chromatograph equipped with a 5975C MS detector (Agilent Technologies, Santa Clara, California, USA). The derivatives were separated on an SP-2380 column (Agilent Technologies) with a constant flow of 1 ml·min^{-1} helium. Quantification was based on the internal standard myo-inositol.

Quercetin and quercetin-3-glucoside analysis

Quercetin and quercetin-3-glucoside were analysed following the method of YANG et al. [14] with some modifications. In brief, 0.1 g of sample was mixed with 20 ml of ethanol and extracted using ultrasound for 25 min. Then, the sample was centrifuged at 1 792 ×*g* for 20 min. The supernatant was removed, and the remaining pellet was again extracted with ethanol. The supernatants were pooled and evaporated at 45 °C to dryness. The solution was then reconstituted in methanol to 10 ml, passed through a microfilter (pore size 0.45 μm), and stored at –20 °C until analysis.

Samples were analysed using a high-performance liquid chromatography (HPLC) procedure (Agilent 1100, Agilent Technologies) with an XDB-C18 column (250 × 4.6 mm, 5 μm particle size; Agilent Technologies) and an Agilent G1315B detector set at 360 nm. The mobile phase was a 0.05 mol·l^{-1} potassium dihydrogen phosphate buffer solution:methanol (40:60, v/v) at a flow rate of 0.8 ml·min^{-1}. Injections of 10 μl were made. The contents in the samples were calculated based on a calibration curve.

Gallic acid analysis

Gallic acid analysis was performed in accordance with the work of ARIMBOOR et al. [5]. Five grams of sample were mixed with 25 ml of 60% methanol and aerated into nitrogen gas. The mixture was then centrifuged, first at 4 ×*g* for 30 min and then at 448 ×*g* for 15 min. The supernatant was pooled and evaporated at 50 °C to dryness, and a crude extract of the polyphenols was obtained.

Crude phenolic extracts were dissolved in 25 ml of deionized water, acidified with 6 mol·l^{-1} HCl to pH 2, and filtered to remove the precipitated phospholipids. They were then extracted with 25 ml of diethyl ether at room temperature. This procedure was repeated five times. The diethyl ether extracts were pooled and evaporated under vacuum at ≤40 °C. The free gallic acid fraction was obtained.

The water phase was neutralized with 2 mol·l^{-1} NaOH and evaporated under vacuum at 40 °C almost to dryness. The residue was then treated with 20 ml of 4 mol·l^{-1} NaOH in a nitrogen atmosphere for 4 h at room temperature. The reaction mixture was subsequently acidified to pH 2 with 6 mol·l^{-1} HCl, extracted with diethyl ether as before, and analysed as gallic acid esters.

The water phase was then neutralized and dried as before. The residue was hydrolysed with 50 ml of 2 mol·l^{-1} HCl for 30 min at 95 °C. Next, the mixture was cooled, adjusted to pH 2, and extracted with diethyl ether as before. This extract was referred to as gallic acid glycosides. All of the extracts were dissolved in 8 ml of 80% methanol and kept at –20 °C until HPLC analysis.

The HPLC (Agilent 1100; Agilent Technologies) analysis was performed in accordance with the work of DENG et al. [15] with some modifications. The mobile phase consisted of methanol and 1.5% acetic acid (3:97, v/v) at a flow rate of 1.0 ml·min^{-1} with an XDB-C18 column. Absorption was detected at 272 nm using an Agilent G1315B detector. Injections of 10 μl were made. The contents in the samples were calculated based on a calibration curve.

Loss of polysaccharide, quercetin and gallic acid substances after digestion

The loss of the tested nutrients was determined by comparison of the contents of nutrients in three different sea buckthorn tea digestion powders before and after digestion.

The value of loss (L) in percent was calculated as:

$$L = \frac{(W_a - W_b)}{W_a} \times 100 \quad (1)$$

where W_a, W_b is the amount of the tested nutrients before and after digestion, respectively.

In vitro fermentation

In vitro fermentation was assayed in accordance with the work of NOACK et al. [16]. Fecal samples from three healthy adult volunteers (two males and one female) free from gastrointestinal disease (aged 21–22) were pooled. The donors had not received antibiotics for three months prior to the study.

Fecal samples of 12.5 g were mixed with 250 ml of phosphate-buffered saline (pH 7.3). The supernatants were pooled after being left to stand. The fecal supernatant was introduced to brain heart

infusion (BHI) medium (Beijing Land Bridge Technology, Beijing, China) at a rate of 5 % (v/v), and then cultured at 37 °C for 24 h to make the fecal inoculum. Subsequently, 0.05 g of the digestate powder of three samples of sea buckthorn leaf tea water (freeze-dried using the tea infusion after passing through the in vitro digestion model) was added to 20 ml of BHI broth and fermented in triplicate anaerobically at 37 °C for 24 h.

All cultures were diluted to 10^{-7} with sterile water. They were then plated onto *Bifidobacterium* agar (Beijing Land Bridge Technology) and cultured at 37 °C for 24 h under fastidious anaerobic conditions to detect the *Bifidobacterium* group, plated onto *E. coli* chromogenic medium (Beijing Land Bridge Technology) and cultured at 37 °C for 18 h with shaking to detect the *E. coli* group, and plated onto sulfite polymyxin sulfadiazine agar base (Beijing Land Bridge Technology) and cultured at 37 °C for 24 h under anaerobic conditions to detect the *C. perfringens* group. The numbers of colony-forming units were determined by counting the colonies on the plates.

RESULTS AND DISCUSSION

Effect of processing on the nutrient level

Polysaccharides

The monosaccharide composition of polysaccharides in the freeze-dried powder of the three types of sea buckthorn leaf tea water is presented in Fig. 1A. It is indicated that the polysaccharides were largely derived from the cell wall, coinciding with the findings of Liu et al. [17]. Polysaccharides in sea buckthorn leaf tea mainly included glucose, arabinose, galactose and xylose. Among these monosaccharides, glucose and xylose are the main components of hemicelluloses, whereas arabinose, galactose and rhamnose come from pectin.

As for the monosaccharide content, differences were observed in the three major components: glucose, galactose and arabinose. The glucose content in sea buckthorn leaves processed using the conventional approach for green tea (SGREEN) nearly doubled, and the galactose level in SGREEN was also higher ($p < 0.05$) than those of sea buckthorn leaves dried at room temperature (SDRIED) or sea buckthorn leaves processed using the conventional approach for black tea (SBLACK). Moreover, the arabinose contents in SGREEN and SDRIED were both higher ($p < 0.05$) than that in SBLACK, although there were no differences between SGREEN and SDRIED.

The contents of total polysaccharides, pectin, and hemicellulose in the three types of sea buckthorn leaf tea are summarized in Fig. 1B. The findings suggest that processing has a significant effect on polysaccharides. The pectin content of SBLACK was clearly lower than that of SDRIED. In contrast, the hemicellulose content of SBLACK was higher ($p < 0.05$) than that of SDRIED. The greatest dissolution of pectin and hemicellulose was noted for SGREEN. The order of total polysaccharide contents from highest to lowest was as follows: SGREEN, SDRIED and SBLACK.

Comparison of these three types of sea buckthorn leaf tea indicates that breakage of the cell wall in SGREEN stimulated greater release of polysaccharides and no fermentation that would destroy them, resulting in a higher level of preserved polysaccharides. In contrast, SBLACK processing enhanced the activity of pectinase and cellulose during fermentation, leading to the greatest loss of pectin and other polysaccharides. Finally, SDRIED, with poor cell wall breakage and no enzymatic lysis, was associated with intermediate polysaccharide content.

Quercetin and quercetin-3-glucoside

A previous study showed that three kinds of flavonoids, namely, quercetin, kaempferol and myricetin, could be analysed in sea buckthorn leaf tea. However, only kaempferol and myricetin aglycones, but not their glycosides, were available for purchase, so only quercetin and quercetin-3-glucoside could be obtained commercially. Hence, quercetin and quercetin-3-glucoside were chosen to represent flavonoids in this study.

The contents of quercetin and quercetin-3-glucoside are presented in Fig. 1C. As this figure shows, flavonoids in sea buckthorn leaf tea are mainly present in the form of glycosides. In terms of quercetin content, there was little difference among the three types of sea buckthorn leaf tea. However, the content of quercetin-3-glucoside in SGREEN was higher ($p < 0.05$) than that in SDRIED. They were both markedly higher than that of SBLACK, mostly due to quercetin-3-glucoside being hydrolysed by β-glycosidase during fermentation. Accordingly, when comparing the total quercetin contents, values for SGREEN and SDRIED were more than double that for SBLACK.

In particular, there were no significant differences in total quercetin contents between SDRIED and SGREEN. The most likely explanation for this is that not only quercetin substances but also oxidative and hydrolytic enzymes in SGREEN were released as the cell walls were broken, leading to enzymatic lysis of quercetin to

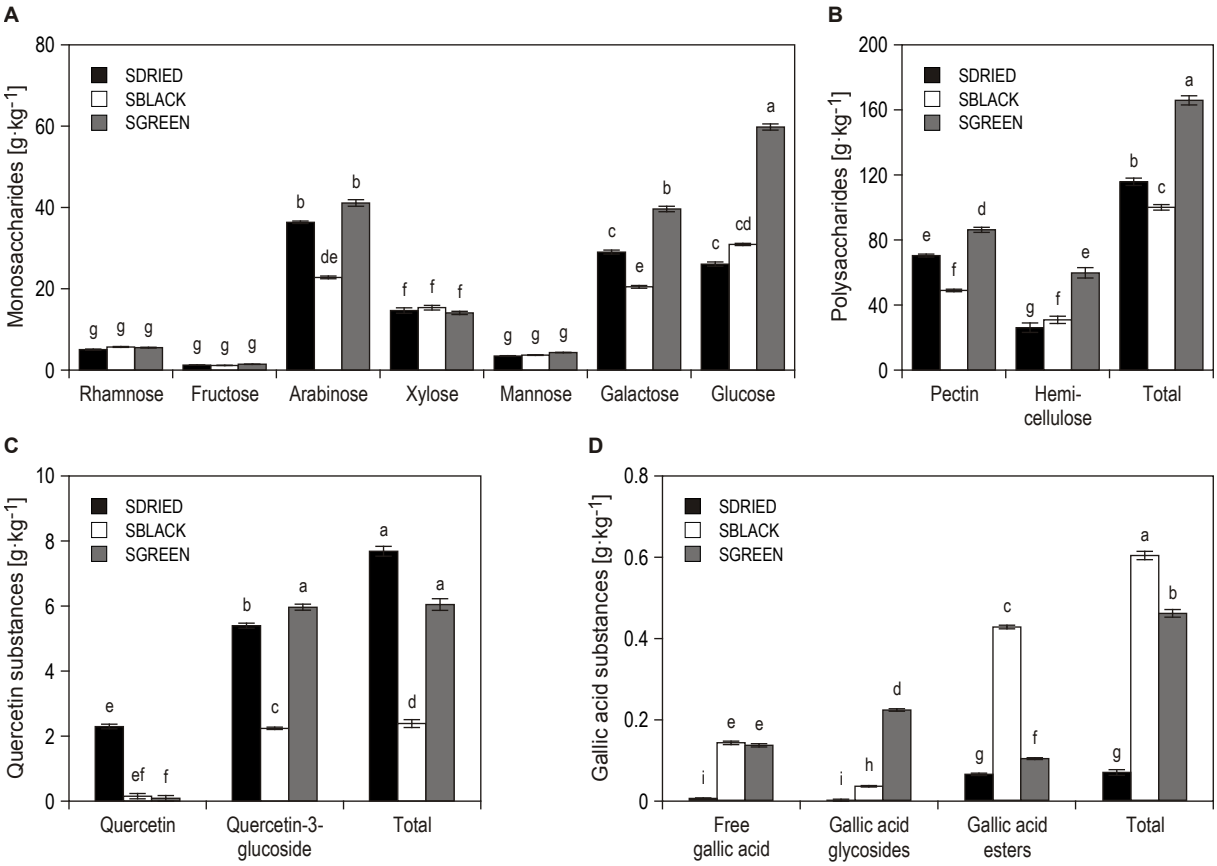

Fig. 1. Content of nutrients in three types of sea buckthorn leaf tea infusion.

A – monosaccharides, B – polysaccharides, C – quercetin substances, D – gallic acid substances.
Mean values of three replicates ± standard deviation are presented. Values with different letters are significantly different ($p < 0.05$). The contents of tested nutrients are expressed per kilogram of dry weight.
SDRIED – sea buckthorn leaves dried at room temperature, SGREEN – sea buckthorn leaves produced using conventional green tea technology, SBLACK – sea buckthorn leaves produced using conventional black tea technology.

offset the greater release of quercetin. Alternatively, compared with SDRIED, the process for SGREEN was complex and took a long time, resulting in the contained quercetin being oxidized much more than that in SDRIED.

Gallic acid

The effect of processing on gallic acid content is presented in Fig. 1D. In contrast to the finding that SBLACK had the lowest contents of total quercetin and polysaccharides, the largest content of total gallic acid was observed for SBLACK. Statistical analysis showed that the total gallic acid content in SBLACK was higher than that in SGREEN. Although no significant difference in free gallic acid contents was detected between SBLACK and SGREEN, substantial variation was still observed in the levels of both gallic acid glycosides and esters. In SGREEN, gallic acid bound as glycoside was dominant, with a level far higher ($p < 0.05$) than that of gallic acid ester. Instead, in SBLACK, gallic acid was mainly bound as esters. Its content was also significantly higher ($p < 0.05$) than that of gallic acid glycoside. Finally, only a low content of free and bound gallic acid could be found in SDRIED.

These findings suggest that black tea processing is beneficial for the liberation of gallic acid, especially for gallic acid esters. It is likely that the fermentation in black tea processing way leads to sufficient breakage of cell walls, which stimulates the release of gallic acid esters, and there is no specific enzyme that cleaves ester bonds to destroy them. Although many other nutrients were oxidized or intensively hydrolysed by enzymes, gallic acid, especially gallic acid esters, were liberated and relatively well preserved.

Effect of digestion on nutrient level

Polysaccharides

The effect of digestion on polysaccharides is presented in Tab. 1. It is demonstrated that hemi-

cellulose increased and pectin decreased obviously after digestion.

Results indicated that the content of hemicellulose clearly increased and of pectin clearly decreased after digestion. The results further indicated that pectin was largely destroyed due to being an acidic polysaccharide that can be easily neutralized by the alkalinity of the small intestine. The greatest loss of pectin occurred for SGREEN, followed by SDRIED and SBLACK. These data suggest that the loss of pectin largely involved its liberation from the cell wall and its loss by in vitro digestion.

In contrast, a dramatic increase was noted in hemicellulose after digestion. The level of glucose, the main component of hemicellulose, sharply increased in all samples. The hemicellulose content increased for SDRIED, SBLACK and SGREEN.

After digestion, total polysaccharides increased by 10.7 % ± 5.5 % in SBLACK, decreased by 2.2% ± 2.2% in SDRIED, and lost most in SGREEN, by 15.9% ± 8.9% after digestion, but total polysaccharide contents remains unchanged. The largest content of polysaccharide could still be seen in SGREEN, while the lower contents ($p < 0.05$) were in SDRIED and SBLACK.

Quercetin and quercetin-3-glucoside

Tab. 1 also shows the effect of digestion on quercetin substances, which reveals a tendency for large losses of both quercetin glycones and glycosides after digestion, with greater destruction of the latter (62–85 %) than of the former (33–72 %). This is mostly due to the lower stability of quercetin glycosides than of quercetin glycones in the alkaline environment of the intestines. Boyer et al. [18] also found that the loss of quercetin 3-O-glucoside was higher than that of quercetin in digestive juice with pH 6.5.

The proportions of quercetin aglycone loss were 85.1 % ± 8.4 % for SDRIED, 33.3 % ± 6.1 % for SBLACK, and –17.7 % ± 1.0 % for SGREEN. Thus, the content of quercetin aglycone in SGREEN increased rather than decreased. This was probably due to glycosidic bonds being cleaved by the acidic conditions in the stomach, and quercetin aglycone being liberated. In contrast, the proportions of loss of quercetin-3-glucoside from higher to lower were in the following order: SBLACK, SDRIED and SGREEN. This order did not differ between before and after digestion. The total quercetin content in SGREEN was significantly higher ($p < 0.05$) than that in SDRIED,

Tab. 1. The impact of in vitro digestion on the level of polysaccharides, quercetin substances and gallic acid substances.

	Sea buckthorn leaf tea		
	SDRIED	SBLACK	SGREEN
Polysaccharides			
Polysaccharides [g·kg^{-1}]	112.54 ± 4.07^{b}	110.66 ± 4.76^{b}	139.42 ± 3.48^{a}
Pectin loss [%]	45.0 ± 1.4	39.8 ± 2.6	63.7 ± 1.8
Hemicellulose loss [%]	–70.9 ± 7.5	–66.1 ± 7.8	–38.5 ± 6.1
Total loss [%]	2.2 ± 2.2	–10.7 ± 5.5	15.9 ± 8.9
Quercetin substances			
Total quercetin [mg·kg^{-1}]	54.29 ± 0.72^{d}	23.35 ± 0.35^{c}	60.60 ± 0.88^{e}
Quercetin loss [%]	85.1 ± 8.5	33.3 ± 6.1	–17.7 ± 7.0
Quercetin-3-glucoside loss [%]	72.1 ± 0.3	79.5 ± 0.3	62.0 ± 0.6
Total loss [%]	3.4 ± 0.1	2.1 ± 0.4	–0.2 ± 0.1
Gallic acid substances			
Total gallic acid [mg·kg^{-1}]	16.37 ± 0.13^{h}	79.26 ± 0.90^{f}	70.69 ± 0.67^{g}
Free gallic acid loss [%]	52.7 ± 2.1	0.9 ± 2.0	–0.5 ± 2.1
Gallic acid glycoside loss [%]	–60.4 ± 27.1	–32.4 ± 10.3	12.3 ± 2.6
Gallic acid ester loss [%]	–56.0 ± 7.7	27.6 ± 3.6	–7.2 ± 9.3

Mean values of three replicates ± standard deviation are presented. Values within the same row followed by different letters are significantly different ($p < 0.05$). Polysaccharides, total quercetin and total gallic acid are the contents of digestate powder in three types of sea buckthorn leaf tea water after digestion and expressed per kilogram of dry weight. The percentage of nutrients loss was calculated according to Eq. 1.

SDRIED – sea buckthorn leaves dried at room temperature, SGREEN – sea buckthorn leaves produced using conventional green tea technology, SBLACK – sea buckthorn leaves produced using conventional black tea technology.

which was also significantly higher ($p < 0.05$) than that in SBLACK. This indicated that not only quercetin glycone but also quercetin glucoside can be protected by water-soluble polysaccharides.

Gallic acid

The impact of in vitro digestion on gallic acid is presented in Tab. 1, which again shows that the loss of gallic acid mainly involved its liberation and loss by in vitro digestion. SBLACK, with the highest gallic acid ester content before digestion, lost the most. In contrast, the level of gallic acid glycosides in SBLACK was increased, despite its lower gallic acid glycoside content before digestion. Similarly, the levels of both gallic acid glycosides and esters in SDRIED increased.

Moreover, similar to quercetin, the conversion of gallic acid glycosides in SGREEN into free gallic acid slightly increased the free gallic acid content because gallic acid glycosidic bonds were cleaved by the acidic environment in the stomach.

After digestion, the total content of gallic acid was still the highest in SBLACK, followed by SGREEN and SDRIED.

Effect of processing on human fecal microflora

Results on the impact of the process for producing sea buckthorn leaf tea on fecal microflora are presented in Fig. 2. Because the digestate powder added to the fecal inoculum was the freeze-dried material from tea infusion after it was passed through the in vitro digestion model described above, the amount added was too small to have a marked effect on the population of fecal microflora.

Statistical analysis and in vitro research showed that the growth of the probiotic *Bifidobacterium* group bacteria was increased only by SGREEN treatment, and that the growth of the pathogenetically neutral *E. coli* group was inhibited only by adding SBLACK. At the same time, the growth of pathogenic *C. perfringens* was inhibited by both SBLACK and SGREEN treatments. The antimicrobial effect of SBLACK was significantly stronger ($p < 0.05$) than that of SGREEN. The lack of an effect of SDRIED treatment, in terms of increasing or decreasing the colon microflora compared with the controls, failed to support the suggestion that SDRIED infusion has a laxative effect, probably due to the addition of too little digestate powder in the present study.

In this study, 0.05 g of digestate powder of the three types of sea buckthorn tea infusion was added to fecal inoculum, and the corresponding amounts of three nutrients in these powders could be calculated using the data on the total

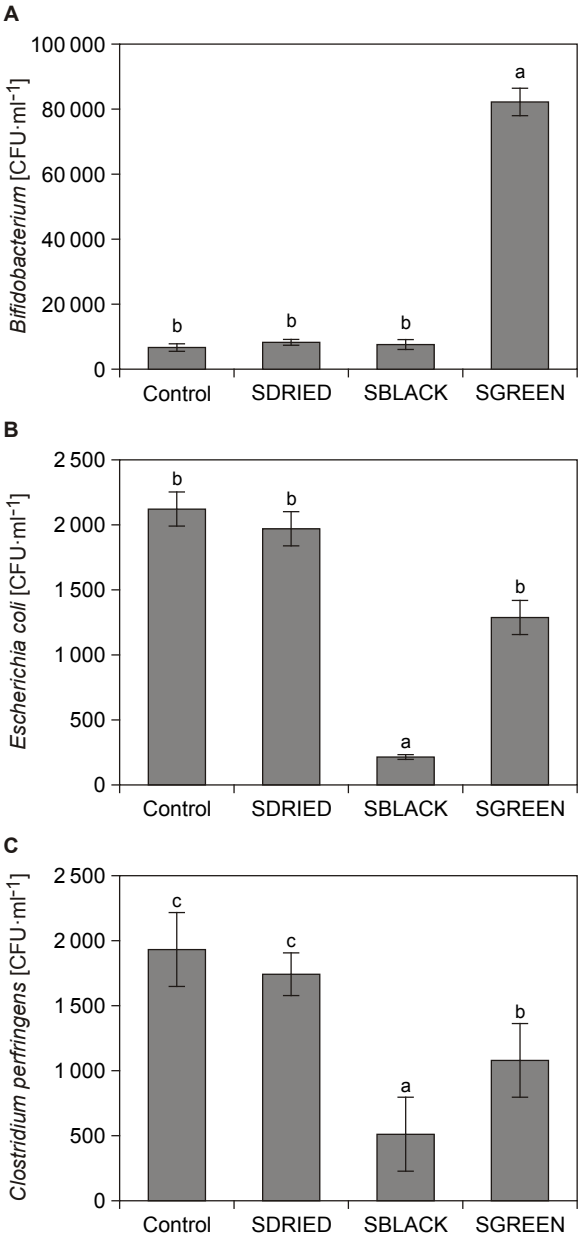

Fig. 2. Effects of three sea buckthorn leaf tea products on the representative flora in human colon.

Mean values of three replicates ± standard deviation are presented. Values with different letters are significantly different ($p < 0.05$).
SDRIED – sea buckthorn leaves were dried at room temperature, SGREEN – sea buckthorn leaves were produced using conventional green tea technology, SBLACK – sea buckthorn leaves were produced using conventional black tea technology.

content of three nutrients listed in Tab. 1. For example, polysaccharide content in SDRIED was 112.54 g·kg^{-1}, as shown in Tab. 1. The amount of polysaccharides in 0.05 g of digestate powder was 0.05 g × 112.54 g·kg^{-1} = 5.63 mg. Accordingly, the order of the levels of total polysaccharides

was as follows: SGREEN (6.97 mg) > SDRIED (5.63 mg) > SDRIED (5.53 mg); that of total quercetin was SGREEN (3.03 mg) > SDRIED (2.71 mg) > SBLACK (1.17 mg); and that of total gallic acid was SBLACK (3.96 mg) > SGREEN (3.53 mg) > SDRIED (0.82 mg). This implies that the increase in the *Bifidobacterium* group was mainly attributable to polysaccharides, and the decrease in the *C. perfringens* may have been related to gallic acid. As for *E. coli*, the cause of the inhibitory effect remains unclear. The experiment described here demonstrated that not only polysaccharides but also polyphenols may affect the microflora in the human colon. However, complex characteristics were revealed, which means that further research on this issue is warranted.

Based on these in vitro results, we can conclude that green tea processing technology is more suitable for sea buckthorn leaf tea. Although the inhibitory effects of SBLACK on both *E. coli* and *C. perfringens* were stronger than SGREEN treatment, the probiotic *Bifidobacterium* could not be stimulated by adding SBLACK alone. Superior effects of an increase in *Bifidobacterium* and inhibition of *C. perfringens* could be seen only upon SGREEN treatment. Nonetheless, given the complexity of human intestinal flora, further in vivo studies should be conducted to verify these results.

CONCLUSION

SGREEN processing was beneficial for the release of pectin and hemicellulose as well as the preservation of quercetin glucoside; in contrast, SBLACK processing increased the total gallic acid content. After in vitro digestion, all nutrients were largely destroyed, except for hemicellulose; however, the order of nutrient levels remained unchanged after digestion. SGREEN treatment supported *Bifidobacterium* growth and suppressed *C. perfringens* growth; SBLACK treatment inhibited *C. perfringens* growth stronger than SGREEN, but did not support the growth of *Bifidobacterium*. The presented data reveal that green tea processing technology is more suitable for making sea buckthorn leaf tea. To the best of our knowledge, this is the first attempt to compare different types of leaf tea according to their effects on improving the human colon microflora rather than improving antioxidant activity.

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