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The Common Features and Mechanisms of Ginger Juice Processing Technology Based on the Composition and Gastrointestinal Effects of Chinese Herbs

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Abstract

Context: All Chinese herbal medicines should be processed before they are used in clinics offering Traditional Chinese Medicine (TCM). Ginger juice is one of the process assistants and a widely-applied agent in processing many herbal medicines.

Objective: In this study, the ginger juice was investigated with its processing mechanism according to its common processing function on herbal medicines, which included *Rhizoma pinelliae* (RP, the tuber of Pinellia ternata (Thunb.) Breit.), officinal magnolia bark (OMB, the root bark of Coptis chinensis Franch.), and *Rhizoma coptidis* (RC, the rhizoma of Magnolia officinalis Rehd. et Wils).

Materials and Methods: The composition, especially the essential oil, of the raw ingredients and ginger juice-processed products and their gastrointestinal effect on model rats with functional dyspepsia (FD) were compared, the relationship between the changes of composition and pharmaceutical effects was analysed.

Results: The gastric residue rate of the rats in the drug-treated group was significantly lower than in the control group ($P<0.05$), while the intestinal propulsive rates were markedly higher ($P<0.05$). The motilin and gastrin level in serum of the drug-treated groups had significantly increased ($P<0.05$) compared with the control group. Compared with the raw product group, there was an apparent reduction in gastric residual rate for ginger juice processed Rhizoma Pinelliae (GJRP)and ginger juice processed Rhizoma Coptidis (GJRC) ($P<0.05$). The GJ processed products groups showed a common increase in the intestinal propulsive rate ($P<0.05$), the motilin level of GJRP and ginger juice processed officinal magnolia bark (GJOMB) groups was significantly increased ($P<0.05$) and gastrin levels of all ginger-processed groups were significantly increased ($P<0.05$). Meanwhile, eight types of common components were found in ginger juice, ginger juice-processed RP, OMB, and RC, in which farnesene, nerolidol, dragosantol, and a-elemene, each with pharmacological activity showed a positive relationship with gastrointestinal function of processed drugs through Pearson's correlation analysis.

Discussion and conclusion: This work provided a better understanding of ginger juice processing mechanisms and a guide to research into the common processing features of processing assistants in Chinese herbal medicine.

Keywords: Ginger juice-processing; *Rhizoma pinelliae*; Officinal magnolia bark; *Rhizoma coptidis*; Essential oil; Gastrointestinal effects

Introduction

Ginger is the fresh rhizome of *Zingiberaceae* (*Zingiber officinale Rosc*) with pungent and warm properties in TCM theory [1]. The chemical composition of ginger mainly includes essential oil, gingerol, and diphenyl heptane [2]. Ginger, normally used as herbal medicine, can accelerate blood circulation, stimulate the secretion of gastric juice and excite intestinal motility [3]. It is also one of the assistants applied in the processing of traditional Chinese herbal medicines [4]. After being processed by ginger juice, the cold properties of some Chinese drugs could be moderated, the effects of the drugs on the gastrointestinal tract could be strengthened and some side-effects, such as irritation, could be relieved [5]. In 2010 Chinese Pharmacopoeia, there are eight Chinese herbal medicines record as requiring processing by ginger juice. Here, three typical ginger juice-processed drugs were used as examples: *Rhizoma Pinelliae* (RP), the dry rhizoma of *Pinellia ternata* (Thunb) Breit, used in treating cold phlegm coughs [6], officinal magnolia bark (OMB), the bark of *Magnolia officinalis* Rehd et Wils or *Magnolia officinalis* Rehd. Et wils. var. *biloba* Rehd et Wils, used in regulating the function of the stomach [7], and *Rhizoma Coptidis* (RC), the rhizome of *Coptis chinensis* Franch, *Coptis deltoidea* C.Y. Cheng et Hsiao or *Coptis teeta* Wall, used in treating pyretic toxicity [8], are the main drugs processed with ginger juice according to TCM theory. It is believed that if practitioners do not include processing information, there can be no complete and correct use of these drugs in TCM. The essential oil, recognised as having antimicrobial, qi-regulating, diaphoretic, and analgesic properties, can be found both in ginger and in these drugs.

Gas chromatography-mass spectrometry (GC-MS) is used to analyse the essential oils in drugs. It is a conventional and useful method for analysing complex mixtures, as well as the preferred option to analyse essential oils in Chinese medicine. For example, the chemical composition of the essential oil of *Curcuma zedoaria* was analysed by GC-MS and was shown to have a high content of epicurzerenone (46.6%) and curdione (13.7%) [9]. The essential oil

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in the root of *Kadsura longepedunculata* Finet et Gagnepand and the fruit of *Schisandra sphenanthera* Rehd. et Wills. were also investigated by GC-MS: the cadinene type compounds and their derivatives were found to have been rich in essential oils (54.2% and 39.7% respectively) and δ-cadinene was the major component present in such oils [10].

As reported, many Chinese herbal medicines can promote gastroenteric effects. These may be mainly measured by determining the rate of gastric emptying and intestinal propulsion, and the level of motilin (MTL) and gastrin (GAS) respectively [11-15]. For example, the Chinese formula 'Xiang sha liu jun Granules' (a formulation made with ten different Chinese herbal medicine) can strengthen gastroenteric effects by promoting spleen deficiency syndrome, changing the rate of gastric emptying, slowing peristalsis in the small intestine resulting in a lower gastric residual rate and intestinal propulsion rate, while increasing MTL and GAS levels compared to the model control group [16]. *Linderae Radix* extract was also reported as promoting gastroenteric effects by adjusting the levels of MTL and GAS [17].

In this study, the essential oil in six different samples of raw and ginger juice-processed RP, OMB, and RC were analysed by GC-MS. Rats were administered with the raw and ginger juice-processed products, the rate of gastric residue and intestinal propulsion, and the levels of motilin (MTL) and gastrin (GAS) were determined to measure the effect on gastroenteric motivity of these samples. The relationship between their chemical components and pharmaceutical effects was analysed to find the possible common features in ginger juice processing technology.

Materials and Methods

Materials

A gas chromatograph coupled with a mass selective detector (Agilent-6890N/59731) was used for the analysis of essential oils. A high-speed refrigerated centrifuge and microplate reader were also used. ELISA (enzyme linked immunosorbent assay) kits for MTL and GT were purchased from Shanghai changjin company and was prepared to measure the concentrations of rat motilin and gastrin. All chemicals were of analytical grade, including anhydrous ethanol, petroleum ether, and chloroform, which were obtained from Nanchang tianbang company. Nutritional semi-pastes were also supplied from Nanchang tianbang company. RB, OMB, and RC were purchased from the Tian Qi Hall medicine plant company (in Zhangshu city, China). Ginger-processed-RB, -OMB, and -RC were made in our own laboratory according to the 2010 Chinese Pharmacopoeia (the herbral medicine were added ginger juice to mix well, then fry them in a pan for several minutes when the ginger guice were absorbed thoroughly). The ginger was obtained from Shenzhen agricultural products company. The concentration of indicative components of 6-gingerol in the ginger was determined as 0.08% and this conformed 2010 Chinese Pharmacopoeia (the concentration of 6-gingerol in ginger must be at least 0.05%). Male Sprague-Dawley rat, Specific Pathogen-Free (SPF) grade, mass 100-120 g was supplied by Academy of Hunan Slack King Animal, under license number HNASLKJ20120967. The procedures involving animals and their care conform to the Guiding Principles for the Care and Use of Laboratory Animals of China.

Essential oil extraction

Three herbs and their ginger-processing products were powdered and passed through a 40 mesh sieve: 500 g medical powder and 500 g ginger pieces were provided and the mixture subjected to six-fold dilution and overnight soaking. Subsequently, they were subjected to hydro-distillation for 10 h until the volatile oil content became stable. The volatile oils were collected and dissolved in moderate ethyl acetate separately before drying over anhydrous Na_2SO_4 for preparation.

Chromatographic condition

The essential oil content in the samples was determined by GC-MS, which was equipped with an HP-5MS 5% phenyl methyl siloxane capillary (30 mm × 250 μm × 0.25 μm). The ion source (150 eV, at a temperature of 230°C) was operated in electron ionisation mode over a scan range of 33 to 550 amu. The column oven temperature was between 60 and 300°C and was programmed to vary as follows: from 60°C (held for 2 min) to 280°C (held for 10 min) at a rate of 8°C/min. The total separation time was 56 minutes. Injector and mass transfer line temperatures were set to 250°C and 280°C, respectively. The injection volume was 1 μL at a ratio of 1:50. Helium was used as the carrier gas at a constant flow rate of 1 ml/min.

The preparation of herbal decoctions

Three herbs and their ginger-processed products were powdered and passed through a 50 mesh sieve: 150 g of each were added to 10 times the amount of water, then the samples were soaked for 30 minutes. After boiling for 1 h and filtration, the residue was diluted six-fold in water to boil for another 30 minutes. The filtrate was merged and concentrated to 1 $g.ml^{-1}$ and 150 ml decoctions of each herb were produced and stored at 4°C.

Grouping and administration

After adaptation to the laboratory environment for 3 days, the rats were randomly assigned to 9 groups: control group (GC), model group (MG), domperidone group (DG), RP group, GJRP group, RC group, GJRC group, OMB group, and the GJOMB group. In every group, there were at least 6 rats. The FD model rats were processed by the tail-provoked method [18]. The rats were then dosed by intragastric administration with the herbal decoctions (1 gml^{-1}) in infusion form at a dose of 3 ml (containing approximately 3 g crude drugs) each time while CP and MP were given as 3 ml saline, the dose of DG was in accordance with the ten times dosage mass conversion from human to rat. Each group was treated once a day under continuous administration for 14 days. On the 14^{th} day, the rats were given drugs 30 minutes before surgery. During these days, rats were in a conventional free-feeding and water supply environment.

Specimen collection

The rats were otherwise fasted apart from free access to water for 12 h and a ration of 4 ml nutritional semi-paste. After 20 minutes, the rats were anaesthetised by single intraperitoneal injection of 10% chloral hydrate at 0.3 mL/100 g. After laparotomy, the pylorus and oesophageal openings of their stomachs were clamped by arterial forceps before removal of the stomach. The full stomach and small intestine of the rats were collected. After being dried on absorbent paper, the stomach was weighed and opened along the greater curvature: the contents were cleaned with distilled water. The stomach was weighed again after water in the outer wall of the stomach had been drained. The difference between the full mass and net mass of the stomach was considered as the residual mass of the inner stomach. The length from the front end of the toner to the pyloric sphincter (L_1) and the length from the pyloric sphincter to the end of the small intestine (L_2) were measured. The intestinal propulsion rate (IPR) was identified as ratio L_1:L_2. After collection from the abdominal aortic artery of the rats, the blood was centrifuged at 3,500 rpm, without anticoagulant, for 10 minutes and stored at 4°C.

The measurement of motilin and gastrin concentrations in rats

The motilin and gastrin concentrations in the rats were measured according to the instructions on the ELISA kits. The OD value of the standard products was selected as the abscissa (*X*-axis), and the concentrations of standard products as the ordinate (*Y*-axis) to draw the MTL, GT test kit standard curves.

Statistical analysis

All experiments were performed at least six times: the results were presented as mean value ± standard error (SE). The statistical analysis was done using SPSS 19's independent-samples T test. A value of $P<0.05$ was considered significant. Pearson correlation analysis was applied to the relative analysis of common ingredients in all ginger juice processed samples and their pharmacodynamic indices by using SPSS 19.

Discussion

The main component of ginger was volatile oil which can dilate blood vessels, improve microcirculation, stimulate the gastric mucosa, increase gastric mucosal blood flow, strengthen mucosal tissue metabolism and promote gastric secretion [19]. The gastrointestinal effect is one of its major functions [20]. Therefore, the volatile oil in GJ and GJ-processed herbs was analysed by GC-MS: the gastrointestinal effect of these herbs and their processed products was determined.

The volatile components changed in GJ-processed herbs whatever their composition: the gastrointestinal effects of GJ-processed herbs were strengthened compared with raw herbs. Eight common compositions were found both in GJ and GJ-processed products, in which there were four compositions having positive correlation with gastrointestinal effect through Pearson correlation analysis. Among the four compositions, farnesene and dragosantol were not present in the raw herbs, but found to be the common compositions in all the three GJ-processed herbs: nerolidol and -elemene could be found in the raw OMB but their concentrations increased in GJOMB.

According to the literature, farnesene could be found as the main essential oil composition in *Dalbergia odorifera* with the effect of anti-inflammatory, analgesia and sedation [21,22]. Dragosantol was reported

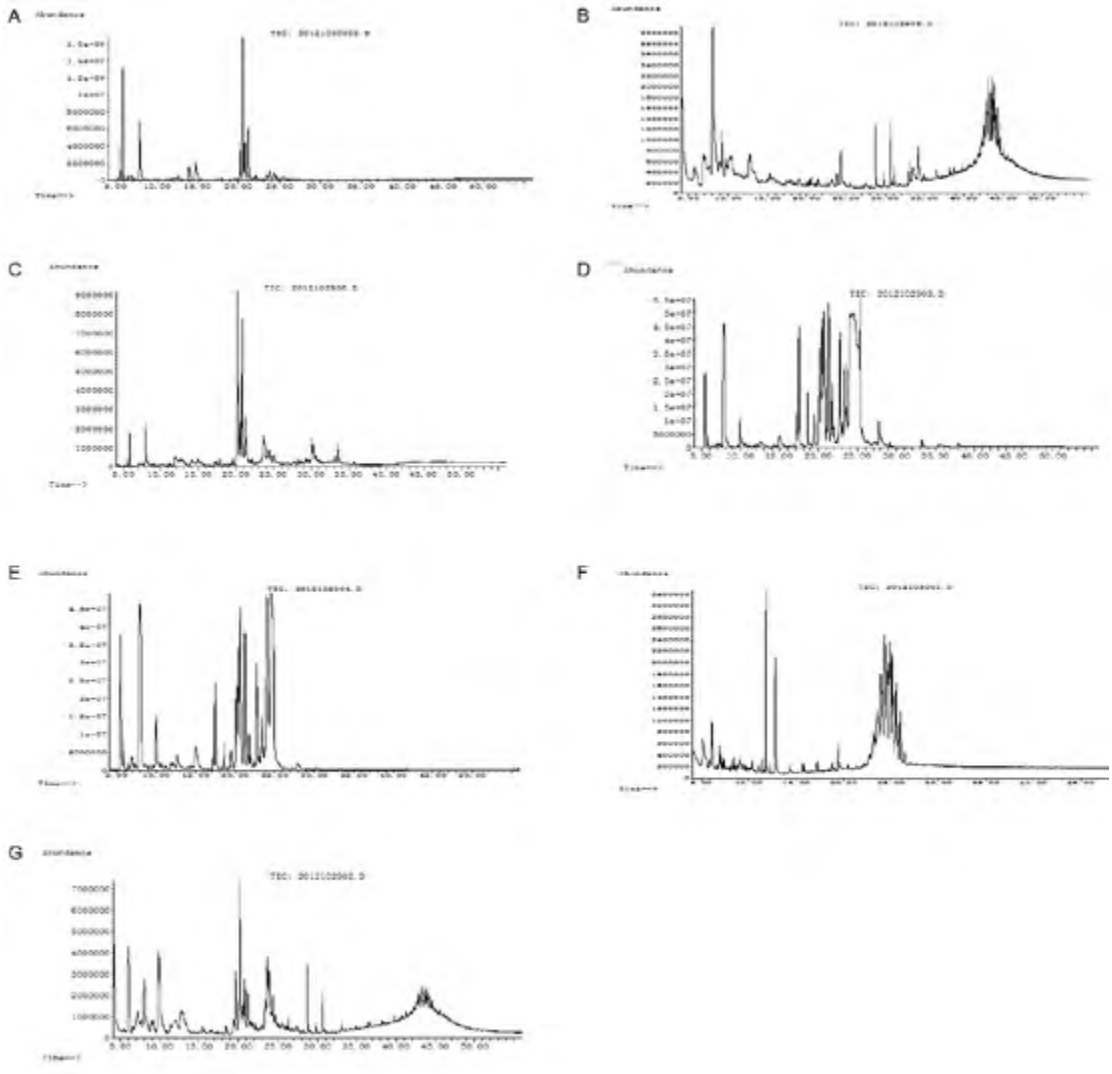

Figure 1: GC-MS total ion chromatogram. A-ginger, B- RC, C- GJRC, D- OMB, E- GJOMB, F- RP, G- GJRP.

to be stable and skin-compatible, having both anti-inflammatory and antibacterial effects. Nerolidol has the effect of an antioxidant [23] and -elemene is considered as an anticarcinogenic which can promote the circulation of qi and relieve pain to cure indigestion and distending pain [24]. The results indicated that the changes of the volatile oil in the GJ processed herbs were directly related to the increase of their gastrointestinal effects.

Results

A typical total ion chromatogram (TIC) of the essential oil fraction extracted from ginger, RC, GJRC, OMB, GJOMB, RP, and GJRP is shown in Figure 1. Although the TICs of these samples were complicated, most of the chromatographic peaks were well separated. Mass spectral data for the compositional peaks were analysed according to the NIST 05 mass spectral library. The identification of compounds was performed on the basis of their similarity. The common chemical compositions of GJRP, GJRC, and GJOMB were initially identified (Table 1). The composition and relative content of volatile compounds changed significantly after ginger juice processing: 26 major compounds were identified, among which eight common components were found, namely: camphene, borneol, α-terpineol, farnesene, selinene, nerolidol, bisabolol, and α-elemene. The gastric residue rate of the group under administration was significantly lower than in the control group (P<0.05), while the intestinal propulsive rates were markedly higher than the control group (P<0.05). Compared with the raw product group, there was an apparent reduction in gastric residue rate for GJRP and GJRC (P<0.05). The products processed by ginger juice showed a common increase in intestinal propulsive rate (P<0.05) (Figures 2 and 3).

Compared with the control group, the motilin and gastrin levels in the serum of the drug-treated groups had significantly increased (P<0.05). Compared with the raw product group, the motilin level in the GJRP and GJOMB groups was significantly increased (P<0.05), the gastrin levels of all the ginger-processed groups were significantly increased (P<0.05) (Figures 4 and 5). The group (GJRC) with the lowest gastric residual rate was indexed as 100 points, the group (GJRC) with the highest intestinal propulsive rate was also indexed as 100 points. The group (GJOMB) with the highest concentration of motilin in serum was taken as 100 points, the group (GJRC) with the highest concentration of gastrin in serum was taken as 100 points. The average value of the other groups were sequentially converted into the appropriate score. Each of the four scores accounted for 25% to obtain the total scores to present the value of gastrointestinal function on the Y-axis. The common ingredients: camphene, borneol, α-terpineol,

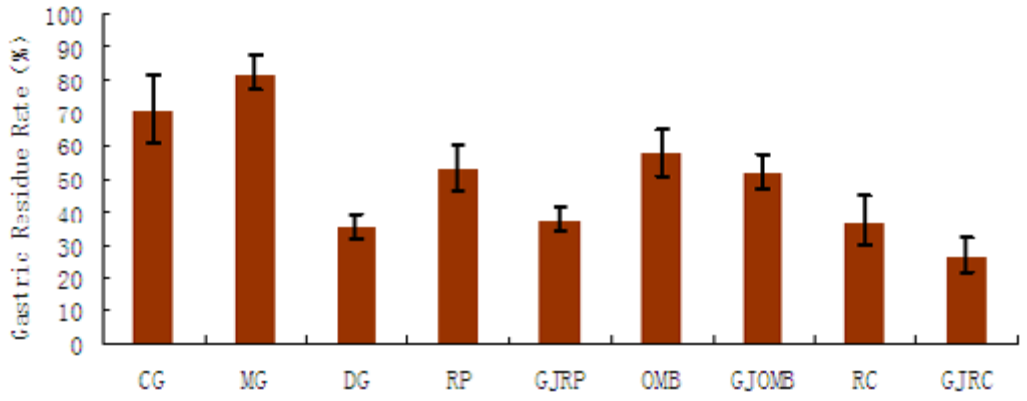

Figure 2: Gastric residue rate of samples. *Denotes significant differences between CG and the all drugs treated groups, MG and all drugs treated groups, RP and GJRP, RC and GJRC, OMB and GJOMB, P<0.05. The columns represent the mean values ± S.E. from six independent determinations.

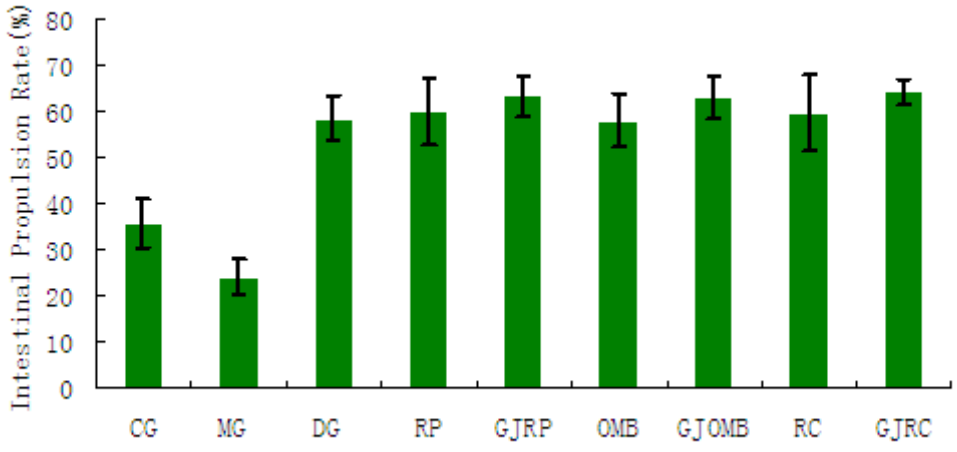

Figure 3: Intestinal propulsion rate of samples. *Denotes significant differences between CG and the all drugs treated groups, MG and all drugs treated groups, RP and GJRP, RC and GJRC, OMB and GJOMB, P<0.05. The columns represent the mean values ± S.E.from six independent determinations.

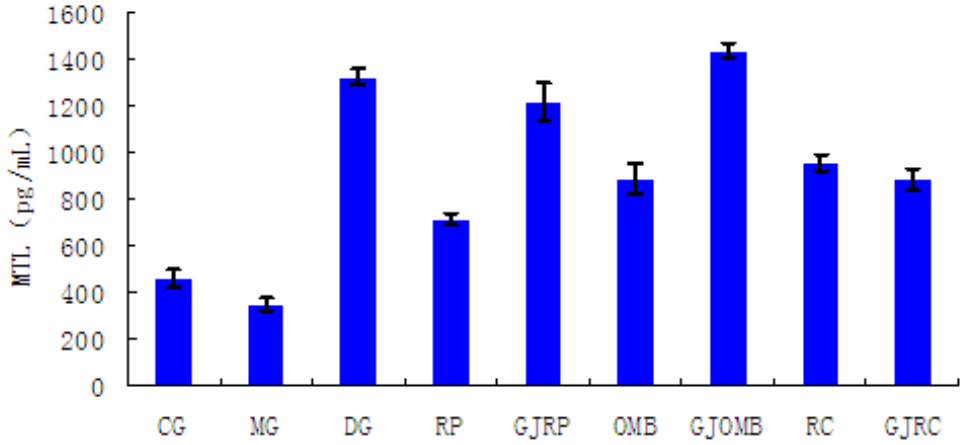

Figure 4: Motilin level of serum of samples. *Denotes significant differences between CG and the all drugs treated groups, MG and all drugs treated groups, RP and GJRP, OMB and GJOMB, *P*<0.05.The columns represent the mean values ± S.E. from six independent determinations.

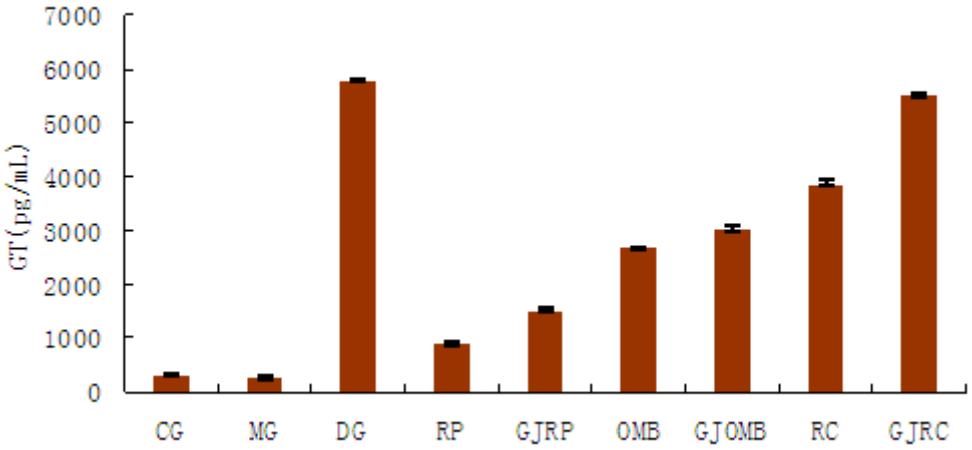

Figure 5: Gastrin level of serum of samples. *Denotes significant differences between CG and the all drugs treated groups, MG and all drugs treated groups, RP and GJRP, RC and GJRC, OMB and GJOMB, *P*<0.05. The columns represent the mean values ± S.E. from six independent determinations.

No.	t/ min	Name	Formula	Area/ %						
				Ginger	RC	OMB	RP	GJRC	GJOMB	GJRP
1	5.44	a-Pinene	$C_{10}H_{16}$	3.73		1.51		0.48	5.50	
2	5.79	Camphene	$C_{10}H_{16}$	13.33		0.26		2.61	0.78	7.43
3	6.47	β-Pinene	$C_{10}H_{16}$	0.56		0.08			0.19	
4	6.86	β-Myrcene	$C_{10}H_{16}$	1.23					0.70	
5	7.21	α-Phellandrene	$C_{10}H_{16}$	0.44		0.24			0.21	
6	7.87	Eucalyptol	$C_{10}H_{18}O$	11.18				5.66		
7	11.91	Borneol	$C_{10}H_{18}O$	0.41		0.05		3.83	0.41	3.47
8	12.57	α-Terpineol	$C_{10}H_{18}O$	0.83		0.63		1.79	1.41	2.79
9	14.74	α-Citral	$C_{10}H_{16}O$	4.97				1.31	0.13	
10	17.43	Copaene	$C_{15}H_{24}$	0.20		3.22		0.85	2.40	
11	17.84	β-Elemene	$C_{15}H_{24}$	0.49		0.10		0.69		0.17
12	19.47	farnesene	$C_{15}H_{24}$	0.41				0.47	0.15	0.31
13	19.60	alloaromadendrene	$C_{15}H_{24}$	0.24		0.06		0.28	0.08	
14	20.17	α-curcumene	$C_{15}H_{22}$	6.52				19.18		8.76
15	20.78	β-Bisabolene	$C_{15}H_{24}$	7.05				14.22		2.97
16	21.99	selinene	$C_{15}H_{24}$	1.15		5.27		4.90	4.59	1.92

17	22.15	nerolidol	$C_{15}H_{26}O$	0.86		0.17		0.86	0.15	0.31
18	22.87	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	$C_{15}H_{26}O$	0.37					0.09	
19	23.38	Dragosantol	$C_{15}H_{26}O$	1.11				1.24	0.02	0.31
20	23.78	Azulene,1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl) -.	$C_{15}H_{24}$	3.23		0.92		0.60		0.49
21	24.03	α-elemene	$C_{15}H_{24}$	0.57		0.17		7.68	0.10	4.00
22	24.36	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha. .alpha.,4a,8-tetramethyl-,	$C_{15}H_{26}O$	1.98	2.86	19.97		2.85	2.78	
23	24.64	1H-Benzocyclohep tene,2,4a,5,6,7,8 ,9,9a-octahydro-3,5,5-trimethyl-9-methylene-,	$C_{15}H_{24}$	0.75				1.24		1.14
24	25.46	cis-Z-.alpha.-Bisabolene epoxide	$C_{15}H_{24}$	0.74				0.62	0.46	
25	25.58	1-Formyl-2,2-dimethyl -3-trans-(3-methyl-but-2-enyl)-6-methylidene -cyclohexane	$C_{15}H_{24}O$	0.59				1.44		0.68
26	26.44	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-,(Z,E)-	$C_{15}H_{24}$	0.16				0.60		

Table 1: Possible common chemical composition of the samples by GC-MS.

Sample	Common ingredients (area %)								Value
	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	*Y*
RP					0	0	0	0	51.35
GJRP	7.34	3.47	2.79	0.31	1.92	0.31	0.31	4.00	68.52
RC	0	0	0	0	0	0	0	0	70.59
GJRC	2.61	3.83	1.79	0.47	4.90	0.86	1.24	7.68	90.32
OMB	0.26	0.05	0.63	0	5.27	0.17	0	0.17	58.48
GJOMB	0.78	0.41	1.41	0.15	4.59	0.15	0.02	0.10	72.48

Table 2: Common ingredients (relative contents) x_1 to x_8 and gastrointestinal comprehensive value *Y*.

		x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	*Y*
	Pearson correlation	0.290	0.684	0.511	0.814*	0.429	0.815*	0.816*	0.772*	1
Y	**Significance**	0.289	0.067	0.150	0.024	0.198	0.024	0.024	0.036	
	N	6	6	6	6	6	6	6	6	6

Note: *Correlation is significant at the 0.05 level (1-tailed)

Table 3: Pearson correlation analysis.

farnesene, selinene, nerolidol, dragosantol, and -elemene were named x_1 to x_8 (Table 2), and farnesene, nerolidol, dragosantol, and -elemene showed significant positive correlation with gastrointestinal function (P<0.05) (Table 3).

Conclusions

The results provided a clue with which an understanding of the common functions and features of GJ processing technology could be attained. Further study should be aimed at exploring the correlation between the changed component of herbs processed by ginger juice and other pharmacodynamic indices to lay a foundation for revealing the effects of ginger-processed herbs.

Acknowledgments

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