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Chemical composition and antioxidant activity of *Polygonatum kingianum* processed by the traditional method of "Nine Cycles of Steaming and Sun-Drying"

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ABSTRACT

Polygonatum kingianum Coll. et (Hemsl) is a famous Chinese traditional food and medicine analogous plant. The rhizome of *P. kingianum* showed a decrease in levels of alkaloids, amino acids and derivatives, terpenoids, and an increase in organic acid and saccharides when it was processed by the traditional method of "Nine Cycles of Steaming and Sun-Drying". The relative content of 341 metabolites were increased (fold change, FC > 2; variable importance in projection, VIP > 1 and *P-value*, $P < 0.05$); while 456 metabolites were decreased (FC < 0.5, VIP > 1, and $P < 0.05$). The changes in chemical components result in a decrease in numb taste and an increase in sweetness. The increased antioxidant activity was observed in the processed samples. Together, this work has advanced the mechanism of reducing numb taste and enhancing antioxidant activity in the resource plants, such as *P. kingianum*, processed by the traditional method.

Introduction

Most traditional Chinese medicine (TCM) need to be processed through purifying, cutting and processing. The processing technology is a key link to ensure the quality of TCM slices, which is used for changing the properties of medicine, enhancing drug properties, or reducing toxicity (Qin et al., 2018). Therefore, an information about what happens during this processing is important not only for guaranteeing the

Abbreviations: P0, raw *P. kingianum*; TCM, Traditional Chinese medicine; RPK, Rhizome of *P. kingianum*; P1 - P9, 1-9 Steam drying cycle; Ala, alanine; Arg, L-arginine; Asp, aspartic acid; Cys, cysteine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine; Met, methionine; Phe, phenylalanine; Pro, proline; GABA, γ-aminobutyric acid; Fuc, Fucose; Ara, Arabinose; Gal, Galactose; Glc, Glucose; Xyl, Xylose; Man, Mannose; Fru, Fructose; Rib, Ribose; Gal-UA, Galacturonic Acid; Glc-UA, Glucuronic Acid; Man-UA, Mannuronic Acid; 5-HMF, 5-Hydroxymethylfurfural; DPPH·, 1,1-diphenyl-2-picrylhydrazyl; ABTS·+, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate); T-AOC, total antioxidant capacity; ·OH, Hydroxyl radical; VC, Vitamin C; SC, scavenging activities; LIT, Mass spectrum conditions mainly including Linear Ion Trap; QQQ, triple-quadrupole tandem mass spectrometry; Q TRAP, triple quadrupole compound linear ion trap mass spectrometer; HPLC, high performance liquid chromatography; UPLC-MS/MS, ultra-performance liquid chromatography-tandem mass spectrometry; UV, ultraviolet-visible spectroscopy; IC_{50}, half maximal inhibitory concentration; QC, quality control; VIP, variable importance in projection; *P*, *P-value*; PCA, Principal component analysis; ANOVA, Analysis of Variance; OPLS-DA, orthogonal partial least squares discrimination analysis; HCA, Hierarchical cluster analysis.

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quality of TCM but also for providing clues for modern pharmaceuticals.

Polygonatum kingianum Coll. et Hemsl is a perennial herb belonging to the Liliaceae family, Its dried rhizome has been used as a traditional medicine and food analogous plant in China for over 2000 years (Fan et al., 2020); it is also widely used as a food or vegetable in Asia and Southeast Asian countries, and is often made into dishes, tea, congee and wine (Shi et al., 2023). The rhizome of *P. kingianum* (RPK) is rich in active ingredients such as polysaccharides, alkaloids, steroidal saponins, amino acids and various trace elements (Shi et al., 2023). In TCM, RPK is frequently used to tone the body, extend life and promote satiety (Shi et al., 2023). Modern pharmacological studies have shown that RPK exhibits anticancer (Ma et al., 2019), anti-inflammatory, antibacterial

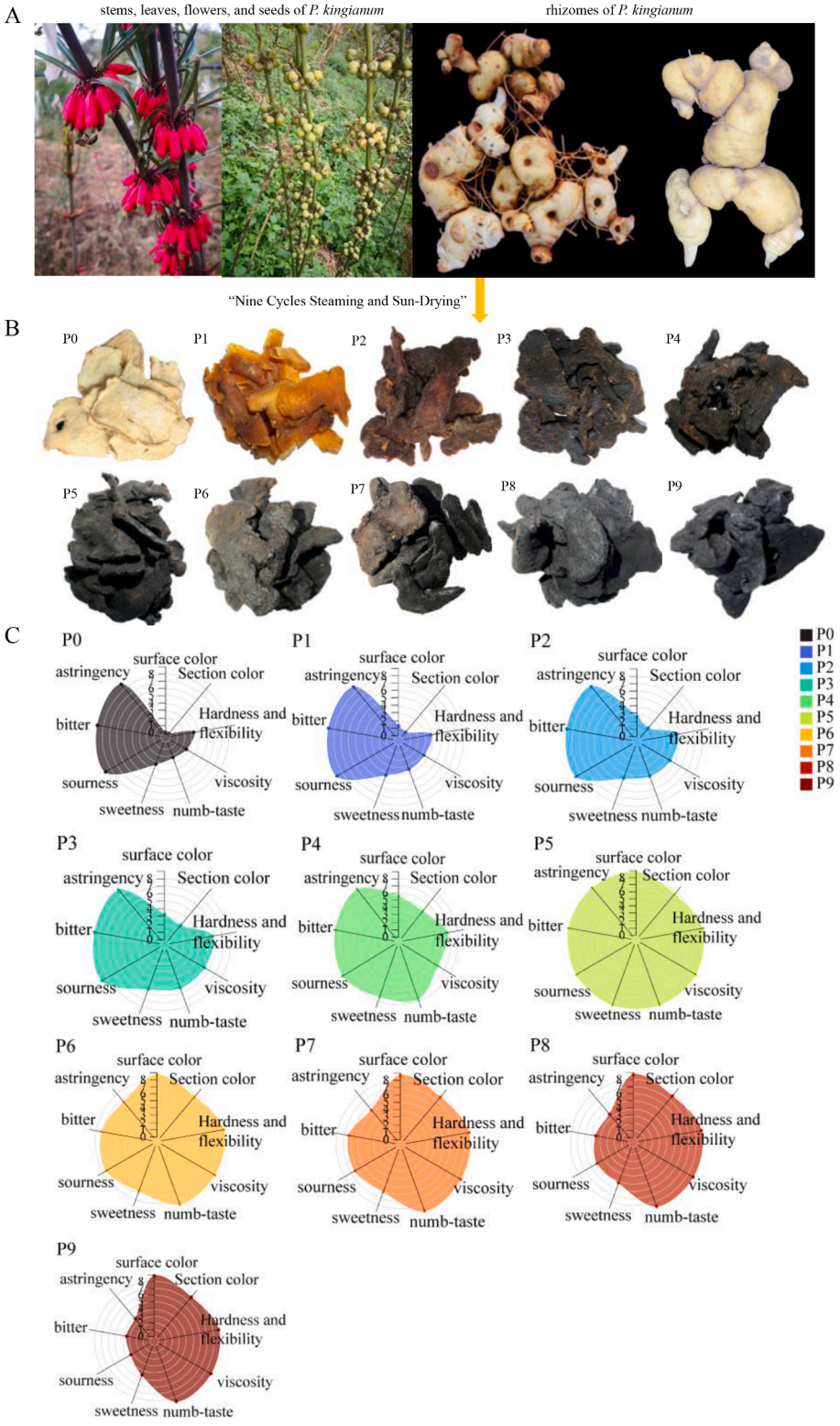

Fig. 1. The change in appearance and flavor of RPK during the processes. photograph of the plant and RPK (A), appearance of RPK during the process (B), sensory evaluation score of processed samples of RPK (C).

(Li et al., 2018), anti-fatigue (Shen et al., 2021) and antioxidant activities (Li et al., 2018). RPK can also be utilized to treat Alzheimer's disease (Wang et al., 2020), to prevent diabetes (Li et al., 2020) and osteoporosis (Cui et al., 2018).

The method of "Nine Cycles of Steaming and Sun-Drying" is a traditionally processed technology for TCM (Liao et al., 2022), which can not only improve the quality of medicinal materials, but also reduce toxic components to increase biological activity and achieve better medicinal effects (Cheng et al., 2021). Fresh RPK show a strongly numb taste, it must be traditionally processed through the method of "Nine Cycles of Steaming and Sun-Drying". During the process of RPK prepared by the method of "Nine Cycles of Steaming and Sun-Drying," the content of polysaccharide is significantly decreased during the period of three cycles of steaming processes, and then tend to be stabilized; the content of saponins is significantly increased from the first to fourth cycles of steaming processes, and then tend to be stabilized (Qing et al., 2020). However, it is an incomplete study that the effect of the method of "Nine Cycles of Steaming and Sun-Drying" on the quality of medicinal materials is only evaluated by the changes in the contents of RPK polysaccharides and other few components. Therefore, it is necessary to conduct an in-depth and systematic research to explore the changes in components during the process of "Nine Cycles Steaming and Sun-Drying". Additionally, the process-driven changes in antioxidant activity and flavor substances should be examined to establish a more comprehensive evaluation system.

In this work, the changes in the appearance and the content of polysaccharides, saponins, amino acids, phenolics and flavonoids were observed and analyzed through sensory evaluation and spectrophotometric methods during the traditional process of RPK. An extensively focused metabolomic technique based on ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) was also used to evaluate the shift in chemical metabolites. The change in antioxidant capacity was measured through scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH·), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS·+) and hydroxyl radical (·OH) and total antioxidant capacity (T-AOC). This work clarifies changes in chemical compositions and antioxidant activity during the preparation of RPK using the method of "Nine Cycles of Steaming and Sun-Drying", which would advance the knowledge about the traditional process of medicine and food analogous plants.

Materials and methods

The preparation of RPK

The fresh RPK was collected from the experimented sited located at Pu-er City, Yunnan, China (22.48° N and 100.58° E) (Fig. 1A). The sample was sliced before the steaming to reduce the steaming time and to ensure a more consistent quality of steamed sample (Yu et al., 2023). Sixteen kg of fresh RPK was sliced into 3 mm sections; then was prepared using "Nine Cycles of Steaming and Sun-Drying." Every cycle was steaming for 4 h, holding for 10 h and drying at 40 °C. At final preparation, the rhizomes were dried to a moisture of less than 15 %. The fresh samples were labeled P0, and the samples collected in one to nine cycles of preparation were labeled P1 to P9, respectively.

The sensory evaluation

Sensory evaluation of the processed samples was developed according to methods of the nature and flavor standard (Zheng et al., 2022). Nine skilled sensory testers assessed the processed samples' color, texture, taste, and odor. The study employed a quantitative descriptive analysis to assess several flavor attributes, such as astringency, sweetness, sourness, bitterness, and numbness on the tongue. The scoring criteria are shown in Table 1, ranging from 0 to 9.

Table 1
Processed samples Sensory sheet.

Classify	Index	Grade	Score
Colour	Surface color	Shiny black	9
		Black	6
		Dark brown	3
	Section color	Shiny black	9
		Black	6
		Dark brown	3
Texture	Hardness and flexibility	Soft and Moisturizing	9
		Soft texture	6
		Slightly hard and tough	3
	Viscosity	Strong viscosity	9
		Viscous	6
		Weak viscosity	3
Flavor	Numb-taste	No numb tongue sensation	9
		Having a tingling sensation on the tongue	6
		Strong numb tongue sensation	3
	Sweetness	Strong sweetness	9
		Sweet taste	6
		Weak sweetness	3
	Sourness	No sour taste	9
		Having a sour taste	6
		Sour	3
	Bitter	No bitterness	9
		Bitter taste	6
		Strong bitterness	3
	Astringency	No astringency	9
		Astringent	6
		Strong astringency	3

The determination of RPK basic components

The content of polysaccharides was determined using the Sulfate–Anthrone method, according to the report of Jin et al (Jin et al., 2018). The content of saponin in RPK was determined at a wavelength of 560 nm with the Vanillin–Perchloric acid–Glacial Acetic acid method (Navarro et al., 2018). The content of total amino acid was measured using the Ninhydrin Colorimetric Method at a wavelength of 570 nm (Jabeen et al., 2019). The content of polyphenolics was measured using the Folin–Ciocalteu methods at 765 nm wavelength (Rojas-Ocampo et al., 2021). The contents of flavonoids were measured using the $AlCl_3$–ACONa colorimetry method at 510 nm using rutin as a standard (Orsavova et al., 2023).

The determination of free amino acids

Reference standards (>98 % purity) for 16 amino acids, alanine (Ala, compound CID: 5950), L-arginine (Arg, compound CID: 6322), aspartic acid (Asp, compound CID: 5960), cysteine (Cys, compound CID: 5862), glutamic acid (Glu, compound CID: 33032), glycine (Gly, compound CID: 750), histidine (His, compound CID: 6274), isoleucine (Ile, compound CID: 6306), leucine (Leu, compound CID: 6106), serine (Ser, compound CID: 5951), threonine (Thr, compound CID: 6288), tyrosine (Tyr, compound CID: 6057), valine (Val, compound CID: 6287), methionine (Met, compound CID: 6137), phenylalanine (Phe, compound CID: 6140) and proline (Pro, compound CID: 145742), were purchased from Agilent Technologies (Beijing, China). Reference standards (>98 % purity) of γ-aminobutyric acid (GABA, compound CID: 119) were purchased from the National Institutes for Food and Drug Control (Beijing, China). The content of the 16 free amino acids in the prepared sample were determined using an Agilent 1200 series HPLC system having a Hypersil AA-ODS (2.1 × 200 mm, 5 μm) fitted with a C18 guard column (Agilent Technologies, Santa Clara, CA, USA) (Zhao et al., 2013).

The determination of monosaccharides

A standard solution of 11 monosaccharides, namely, fucose (Fuc, compound CID: 17106), arabinose (Ara, compound CID: 439195), galactose (Gal, compound CID: 6036), glucose (Glc, compound CID: 5793), xylose (Xyl, compound CID: 135191), mannose (Man, compound CID: 18950), fructose (Fru, compound CID: 2723872), ribose (Rib, compound CID: 10975657), galacturonic acid (Gal-UA, compound CID: 439215), glucuronic acid (Glc-UA, compound CID: 94715) and mannuronic acid (Man-UA, compound CID: 439630) were from SANSHUBIO. Samples (50 mg) were extracted with 700 μL of 80 % ethanol at 50 °C for 2 h, then added with H_2O (700 μL) and centrifuged at 10000 rpm for 3 min. The supernatant was analyzed on a Thermo ICS5000 + ion chromatography system (ICS5000+, Thermo Fisher Scientific, USA), using a Dionex™ column (CarboPac™ PA10, 250 mm × 4.0 mm, 10 μm). Mobile phase A was H_2O and mobile phase B was 100 mM NaOH in water; the sample injection was 20 μL; flow rate, 0.5 mL/min; the elution program was: volume ratio of solution A, B was 97.5:2.5 at 0 min, 80:20 at 30 min, 60:40 at 30.1 min, 60:40 at 45 min, 97.5:2.5 at 45.1 min, 97.5:2.5 at 60 min; the column temperature was set at 30 °C.

Antioxidant activities in vitro

T-AOC and scavenging capacities of DPPH·, ABTS·+ and ·OH were determined using commercial kits (Solarbio Science & Technology Co. Ltd, Beijing, China), according to the manufacturer's instructions. Ten samples from P0-P9 were extracted using the extraction solution in kits; the test concentration gradient is 1.5625, 3.125, 6.250, 12.500, 25.000, 50.000, 100.00 mg/mL; vitamin C (VC) solution was used as the positive control. Half maximal inhibitory concentrations (IC_{50}) of scavenging activities (SC) were analyzed to evaluate the antioxidant activities.

UPLC–MS/MS based widely targeted metabolome analysis

Samples of P0, P5 and P9 were analyzed using the metabolomics method at Metware Biotechnology Co., Ltd using ultra-performance liquid chromatography (UPLC, SHIMADZU Nexera X2, https://www.shimadzu.com.cn) combined with tandem mass spectrometry (MS/MS, Applied Biosystems 4500 Q TRAP, https://www.appliedbiosystems.com.cn). Samples were ground (30 Hz, 1.5 min) to powder with a grinding instrument (MM 400, Retsch). The powder (100 mg) was extracted with 1.2 mL 70 % methanol, and vortexed every 30 min for 30 s for a total of six times, which was extracted at 4 °C overnight. The extract was centrifuged at 12000 rpm for 10 min. The supernatant was filtered with microporous membrane (0.22 μm pore size), and subjected to metabolomics analysis. The chromatographic column was an Agilent SB-C18 (1.8 μm, 2.1 mm × 100 mm); mobile phase A was ultrapure water (0.1 % formic acid added) and phase B was acetonitrile (0.1 % formic acid added). The elution gradient was decreased in a previous report (Xiao et al., 2022). Mass spectrum conditions mainly including linear ion trap and triple-quadrupole tandem mass spectrometry (QQQ) scanning, performed on a triple quadrupole compound linear ion trap mass spectrometer (Q TRAP) and AB4500–Q TRAP–UPLC–MS/MS system, the detailed parameter settings of which are in a previous report (Xiao et al., 2022).

Statistical analysis

Statistical analysis was performed through the one-way analysis of variance (ANOVA) procedure of SPSS 26.0 (ANCOVA; SPSS 26.0, IBM Corp., Armonk, NY, USA). Significant differences among treatments were obtained based on *P-value* determined using the Tukey–Kramer test ($P < 0.05$). Comparison groups were subjected to principal component analyses (PCA) and (orthogonal) partial least-squares–discriminant analyses (OPLS–DAs) using Metabo Analyst 5.0 (https://www.metaboanalyst.ca/home.xhtml) (Li et al., 2022). Variable importance in the projection (VIP) was used to rank the overall contribution of each variable in the OPLS–DA model, and variables with a VIP > 1.0, $P < 0.05$, and fold change (FC) > 2 or < 0.5 were considered differentially changed metabolites (DCMs) (Ma et al., 2021). Fresh RPK was respectively sampled from three independent experimented plots, and these samples were parallelly processed and evaluated by the triple repetition.

Results and discussion

Changes in appearance during processing

Sensory evaluations of the processed samples are in Fig. 1B. The color of P0 was white and yellow; with the development of the process, the color became brown-black. After the fourth steaming, the color became black (Fig. 1B). The taste of fresh RPK has a numb-taste sensation, which disappears after the fourth steaming and becomes fragrant and sweet. After the sixth steaming, it changed to sour, bitter and astringent, and the sweetness gradually decreased (Fig. 1C). The change in flavor was consistent with the report of Zhang et al., who found that after five processing cycles, both bitterness and sweetness values decrease, and bitterness values begin to appear (Zhang et al., 2022). The color gradually became too black during the processing, which may be due to the Maillard reaction (Kaewtathip et al., 2022). Similar changes were observed in previous studies of the steaming and drying processes of *Polygonum multiflorum* Thunb. (Liu et al., 2009) and rhizomes of *Polygonatum cyrtonema* Hua (Jin et al., 2018).

Changes of chemical composition during the processing

Polysaccharides are the main active ingredients of RPK. After processing, the content of polysaccharides gradually decreased from 162.18 ± 0.84 mg/g in fresh samples (P0) to 97.72 ± 3.72 mg/g in the final processed samples (P9) (Fig. 2A). This decrease in polysaccharides was consistent with a previous report (Wu et al., 2022). Major monosaccharides in fresh samples (P0) were Fru and Glc with contents of 29.52 ± 11.84 and 15.38 ± 1.80 mg/g, respectively. These increased to 194.65 ± 6.85 and 41.66 ± 0.45 mg/g in P5 and then slightly decreased to 187.81 ± 4.58 and 40.90 ± 2.52 mg/g in P9, respectively. The content of Man increased from 0.25 ± 0.09 mg/g in P0 to 7.69 ± 0.24 mg/g in P5 and 10.12 ± 1.61 mg/g in P9 (Fig. 2B). After preparation, the contents of Fru, Glc and Man were increased 6.36-fold, 2.65-fold and 40.48-fold, respectively. The calculated sweetness index was Glc (1.12) and Fru (0.94) (Mao et al., 2019). Therefore, the increase in contents of Fru, Glc and Man is responsible for the increase in sweetness during processing (Jin et al., 2018). In addition, we found the content of Ara increased more than ten-fold, which was consistent with the report of Wu et al., who found the content of Ara in steamed rhizome was higher than that in the fresh rhizome (Wu et al., 2022). The decrease in polysaccharides and increase in monosaccharides indicated that during the processing of RPK, polysaccharides decompose into monosaccharides.

The contents of saponins, flavonoids and polyphenols in P0 were 27.06 ± 2.44, 9.67 ± 0.28 and 3.37 ± 0.10 mg/g, respectively. The saponin content steadily increased to 46.50 ± 4.68 mg/g in P9 (Fig. 2C). During the process of "Nine Cycles Steaming and Sun-Drying", the long-time high temperature might cause the transformation of steroidal saponins (Wang et al., 2017). For example, diosgenin is converted into aglycones and secondary glycosides, consequently leading to the increase in saponin content. However, saponins are one of the important active ingredients of RPK, and the increased saponin would obviously enhance the bioactivity. During the process of Nine cycles, the content of total flavonoid was increased from 9.67 ± 0.28 mg/g at the P0 to 17.67 ± 1.07 mg/g at the P2, and then was slightly decreased to 16.78 ± 0.08 mg/g at P3, and finally the maximum value of 22.00 ± 1.30 mg/g was observed at P9 (Fig. 2D). During the third steaming, the polyphenol content significantly increased to 8.35 ± 0.50 mg/g, then decreased to 5.76 ± 0.13 mg/g, and stabilized (Fig. 2E).

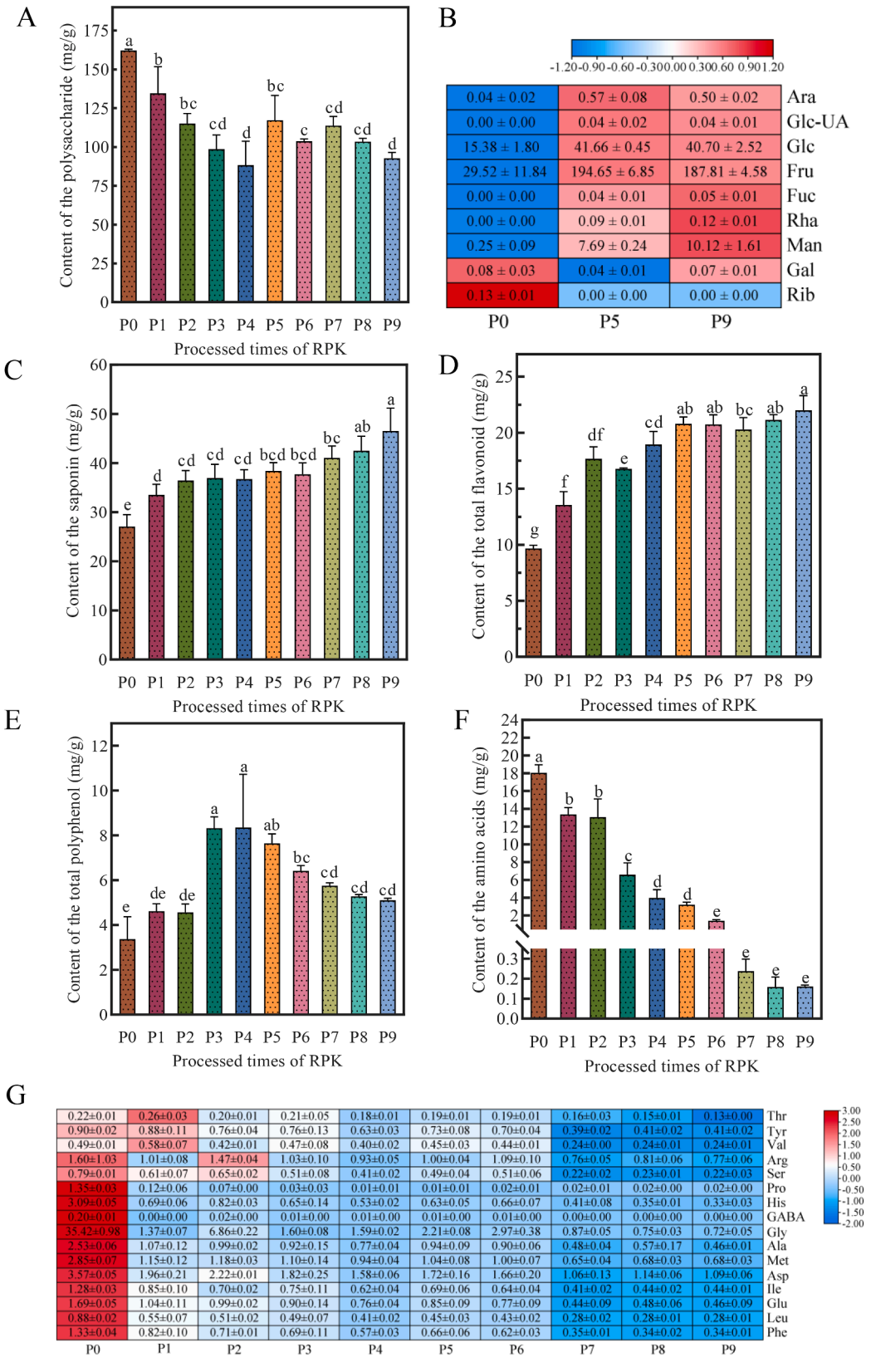

Fig. 2. Change in contents of polysaccharides (A), monosaccharide(B), saponins (C), flavonoids (D), polyphenols (E), total free amino acids (F), and free amino acids (G). Different letters in error bar represented significant differences ($P < 0.05$).

The content of free amino acids decreased from 18.05 ± 0.91 mg/g in P0 to 0.16 mg/g in the final processed sample (P9) (Fig. 2F). This decrease was verified through HPLC measurement of 16 amino acids (Fig. 2G), whose levels significantly decreased after the first, second or third steaming. This decrease in free amino acids was thought to be caused by the Maillard reaction (Wang et al., 2019), which resulted in the color gradually becoming black. Together, the contents of polysaccharides and free amino acids were decreased, while the contents of saponins and flavonoids were increased; in addition, the content of polyphenols were initially increased and then were slowly decreased during the process of nine cycles.

Widely targeted metabolome analysis

To investigate further the changes in chemical composition during processing, metabolites were extracted from P0, P5 and P9 samples and analyzed using a wide target UPLC–MS/MS-based metabolomics approach. By searching through both public and internal databases and comparing fragmentation patterns, retention periods, and *m/z* values with standards, a total of 1297 metabolites were identified (Fig. 3A). They were classified into 13 classes: lipids (183 metabolites), amino acids and derivatives (158 metabolites), flavonoids (151 metabolites), phenolic acids (138 metabolites), alkaloids (136 metabolites), organic acids (115 metabolites), terpenoids (58 metabolites), steroids (51 metabolites), nucleotides and derivatives (49 metabolites), lignans and coumarins (29 metabolites), quinones (15 metabolites), tannin (1 metabolite) and others (213 metabolites). They were further grouped into 54 sub-classes, such as free fatty acids (86 metabolites), saccharides (81 metabolites), steroidal saponins (49 metabolites) and flavones (37 metabolites). Our results were similar to the report by Wang et al., who identified a total of 1126 metabolites in four *Polygonati Rhizomes* with the most abundant being lipids (Wang et al., 2023). In addition, Sharma, et al. identified 314 compounds in *Polygonum verticillatum* rhizomes through UPLC–PDA–ESI/MS (Sharma et al., 2021). Wang et al. identified 335 metabolites in four types of *Polygonatum* using UPLC–ESI–MS/MS (Wang et al., 2022). Here, both identifications found a smaller number of metabolites than this work, and suggest our method has high efficiency.

In the PCA score plot, the QC samples are clustered together, and the total of PC1 and PC2 was 84.25 %, indicating that the instrumental chemistry system has good stability and repeatability (Fig. 3B). In this PCA, groups P5 and P9 are significantly separated. This result indicates

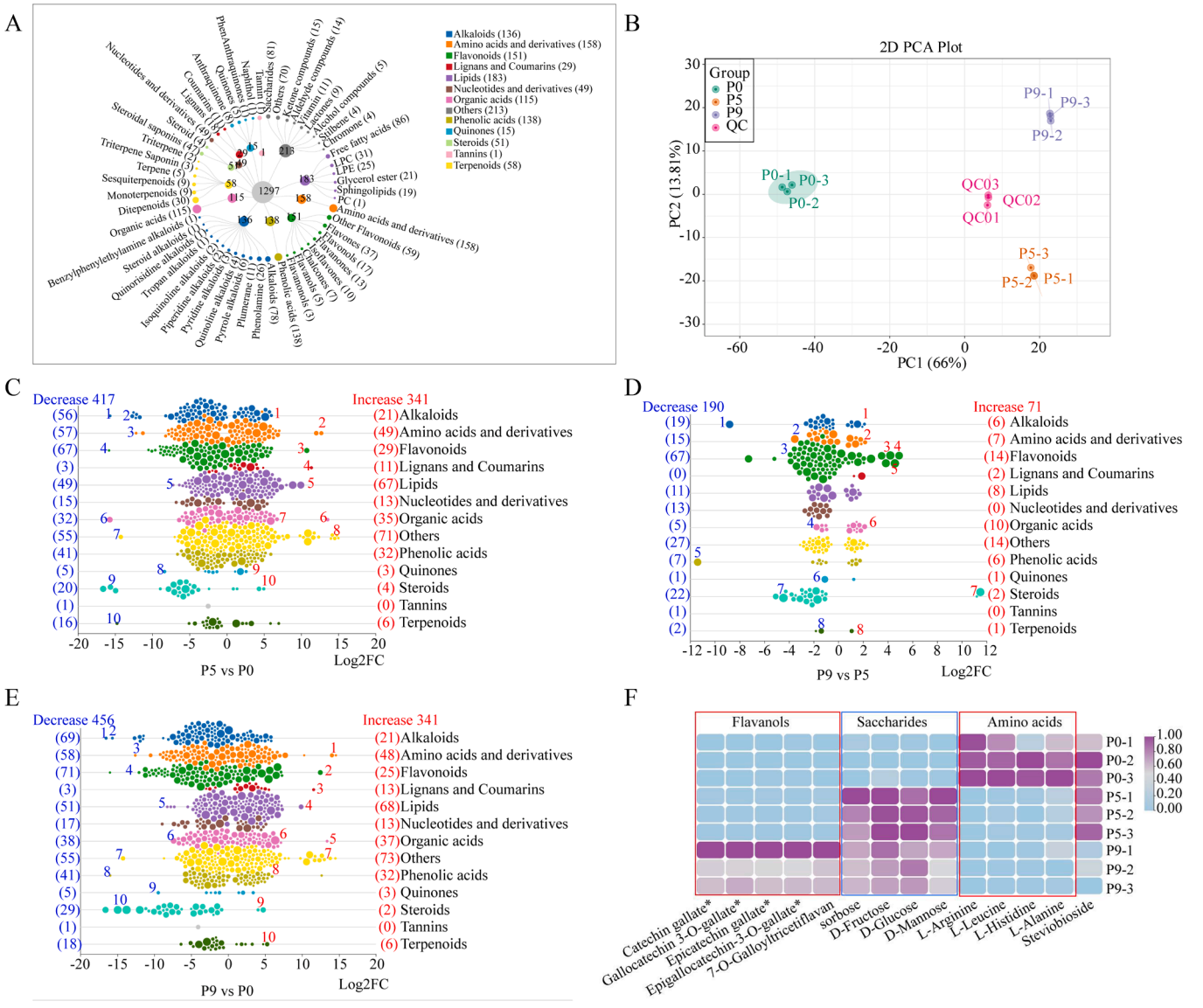

Fig. 3. Overview of metabolomics research on the processing of RPK. Classifications of identified metabolites (A), PCA analysis of P0, P5, and P9 samples (B), Beeswarm plot showing the differential changed metabolites (C-E), Heatmap analysis of core differential metabolites in RPK with different processing times (F).

that there is a significant change in metabolites among groups P0, P5 and P9. The Q^2 in OPLS–DA analysis of all comparison groups is higher than 0.95, indicating that the constructed model is suitable.

When groups P5 and P0 were compared, the relative levels of 341 metabolites increased (FC > 2, VIP > 1 and $P < 0.05$); these included lipids (67 metabolites), organic acids (35 metabolites), lignans and coumarins (11 metabolites), and flavonoids (29 metabolites). In contrast, the relative levels of 417 metabolites decreased (FC < 0.5, VIP > 1 and $P < 0.05$) and included alkaloids (56 metabolites), amino acids and derivatives (57 metabolites), flavonoids (67 metabolites), and polymers (49 metabolites) (Fig. 3C). Among them, relative levels of 57 amino acids and their derivatives were decreased, which was verified through HPLC detection. It was found that the contents of Leu, Ile, His, Ala and Arg decreased by 48.86 %, 46.09 %, 79.61 %, 62.85 % and 37.5 %, respectively (Fig. 2G).

When comparing groups P9 and P5, it was found that the relative levels of 71 metabolites (FC > 2, VIP > 1 and $P < 0.05$) were higher for alkaloids (6 metabolites), amino acids and derivatives (7 metabolites), flavonoids (14 metabolites), lignans and coumarins (2 metabolites), and lipids (8 metabolites). On the other hand, the relative levels of 190 metabolites (FC < 0.5, VIP > 1 and $P < 0.05$) were lower for alkaloids (19 metabolites), amino acids and derivatives (15 metabolites), flavonoids (67 metabolites), and lipids (11 metabolites) in the comparison between groups P9 and P5 (Fig. 3D) Among them, 15 compounds were organic acids, in which the relative contents of 10 organic acids increased. Organic acids can improve food flavor, and the hydrogen ions they produce can cause sourness (Shi et al., 2022), indicating that the sourness in the later stages of processing may come from organic acids. In addition, five flavanols were identified, namely, Catechin gallate*, Gallocatechin 3-*O*-gallate*, Epicatechin gallate*, Epigallocatechin-3-*O*-gallate* and 7-*O*-Galloyltricetiflavan; whose levels in P9 were significantly higher than in P0 and P5. In a previous report, Li Y et al. found that flavanols play an important role in the formation of astringent taste in the hickory seed coat (Li et al., 2022), therefore, we suggested that astringent taste in the later processed sample may come from flavanols (Fig. 3F).

In the comparison between P9 and P0, the relative levels of 341 metabolites were increased (FC > 2, VIP > 1 and $P < 0.05$), including alkaloids (21 metabolites), amino acids and derivatives (48 metabolites), flavonoids (25 metabolites), lignans and coumarins (13 metabolites) and lipids (68 metabolites); whereas the relative levels of 456 were decreased (FC < 0.5, VIP > 1 and $P < 0.05$ (Fig. 3E), such as alkaloids (69 metabolites), amino acids and derivatives (58 metabolites), flavonoids (71 metabolites) and lipids (51 metabolites). A total of 59 carbohydrate compounds were identified in the P0, P5 and P9, among which the relative content changes of Glc, Fru and Man were consistent with the HPLC detection results. The contents of Glc, Glc-UA, Fru, Man, Rha, Ara and Fuc in P5 and P9 were significantly higher than that of P0, that is, after "Nine Cycles of Steaming and Sun-Drying," macromolecular polysaccharides decompose into small-molecule monosaccharides. Moreover, the relative content of sorbose, Glc, Fru and Man was significantly increased after processing, with the highest in P5 followed by P9. Sorbose has the same sweetness as sucrose (Li et al., 2022), and sensory evaluation found that P5 has the highest sweetness. Therefore, sorbose, Glc, Fru and Man may contribute to the sweetness of RPK. Steviobioside, a terpenoid compound, is a sweetener (Philippaert et al., 2017) whose relative content is highest in P5 and lowest in P9, which once again confirms that the sweetness was more prominent during the fifth processing step (Fig. 3F).

As the processing progressed, 20 new compounds were generated from P0 to P5, which include Inoscavin B, Gingerglycolipid C, Tuberonic acid glucoside, Cormusglucoside H and Militarine. During the process from P0 to P9, 16 compounds were decomposed including Oxiglutatione, Salidroside, Isoquinoline and Indioside E. Six compounds were decomposed during the P0 to P5 processing and produced during the P5 to P9 processing, including Prunetin, pseudoginsenoside Rt3 and 5-HTP (Table S1). These newly generated or degradation differential metabolites include some functional substances, such as Timosaponin C which exhibits weak NO inhibition in cells (Wang et al., 2018). Prunetin is an *O*-methylated Isoflavone with anti-inflammatory activity. Prunetin is an effective inhibitor of human aldehyde dehydrogenase (Hu & Li, 2018). The formation of bioactive compounds supported the efficacy enhancement of "Nine Cycles of Steaming and Sun-Drying."

Changes in antioxidant activity

We compared the antioxidant activities of 10 samples from P0-P9 *in vitro*. The capacity of total antioxidant was gradually increased, accompanying with P9 samples exhibiting the highest value of total antioxidant capacity (Fig. 4A). The scavenging ability of ABTS·+ was the highest in the P5 samples, which showed an IC_{50} of 9.846 mg/mL (Fig. 4B). The DPPH· scavenging ability was gradually increased from the P0 to P9 samples, and the maximum value reached an IC_{50} of 2.10 mg/mL (Fig. 4C). Additionally, the P5 samples exhibited the highest ability of scavenging ·OH with an IC_{50} of 40.90 mg/mL (Fig. 4D). In the P9 samples, the IC_{50} for the activity scavenging ABTS·+, DPPH·, and ·OH were 17.40, 2.10, and 81.48 mg/mL, respectively. In a word, the antioxidant capacity of RPK was gradually increased with the increase of processing times. Previous researchers have found that with the increase of processing cycle of "Nine Cycles of Steaming and Sun-Drying", the antioxidant activity of *Polygonatum* showed an upward trend *in vitro* (Su et al., 2023; Yao et al., 2022), and the results of this study show a similar pattern.

Previous studies on the chemical composition changes in the traditional preparation of RPK have mainly focused on polysaccharides, saponins and 5-HMF, without paying attention to the changes in other compounds. In this work, we measured the changes in the content of polysaccharides, monosaccharide composition, saponins, amino acids, flavonoids and polyphenols, providing new evidence for the chemical composition changes during the traditional preparation of RPK. In addition, we employed extensively targeted metabolomics based on UPLC-MS/MS to comprehend the molecular alterations that occurred during RPK production. The overall differences in the traditional process of RPK metabolic profiles have not been thoroughly studied until now. In addition, processing can alter the flavor of RPK, but there is currently a lack of research on the identification of flavor substances. We found that the content of Glc, Fru, Man and sorbose significantly increased in the later stages of processing, with steviobioside being the highest in P5, which can explain why P5 has a sweeter taste. The increase in the content of flavanols and organic acids in the later stage may make the bitterness and astringency of RPK more intense. Second, other flavonoids, alkaloids, phenolic acids and other substances may also participate in the formation of flavor, but their accumulation is significant and needs further research to confirm. We also found there is a similar trend between the changes in antioxidant capacity and the changes in the composition of flavonoids and polyphenols. Our results indicated that processing could enhance the antioxidant activity of RPK, mainly derived from polyphenols and flavonoids.

Conclusion

During the traditional process of RPK using the "Nine Cycles of Steaming and Sun-Drying" method, the content of total polysaccharides and amino acids was obviously decreased, whereas the content of saponins, polyphenols and flavonoids was gradually increased. Metabonomic analysis has identified 1297 metabolites that might be classified into 13 categories. The relative content of amino acids and derivatives was decreased, while the relative content of saccharides was increased. The sourness and astringency were accompanied with the increase in organic acids and flavanols in the samples, while the sweetness of P5 samples was accompanied with the elevated levels of Fru, Glc, Man, sorbose and steviobioside. After the samples of RPK were processed, the

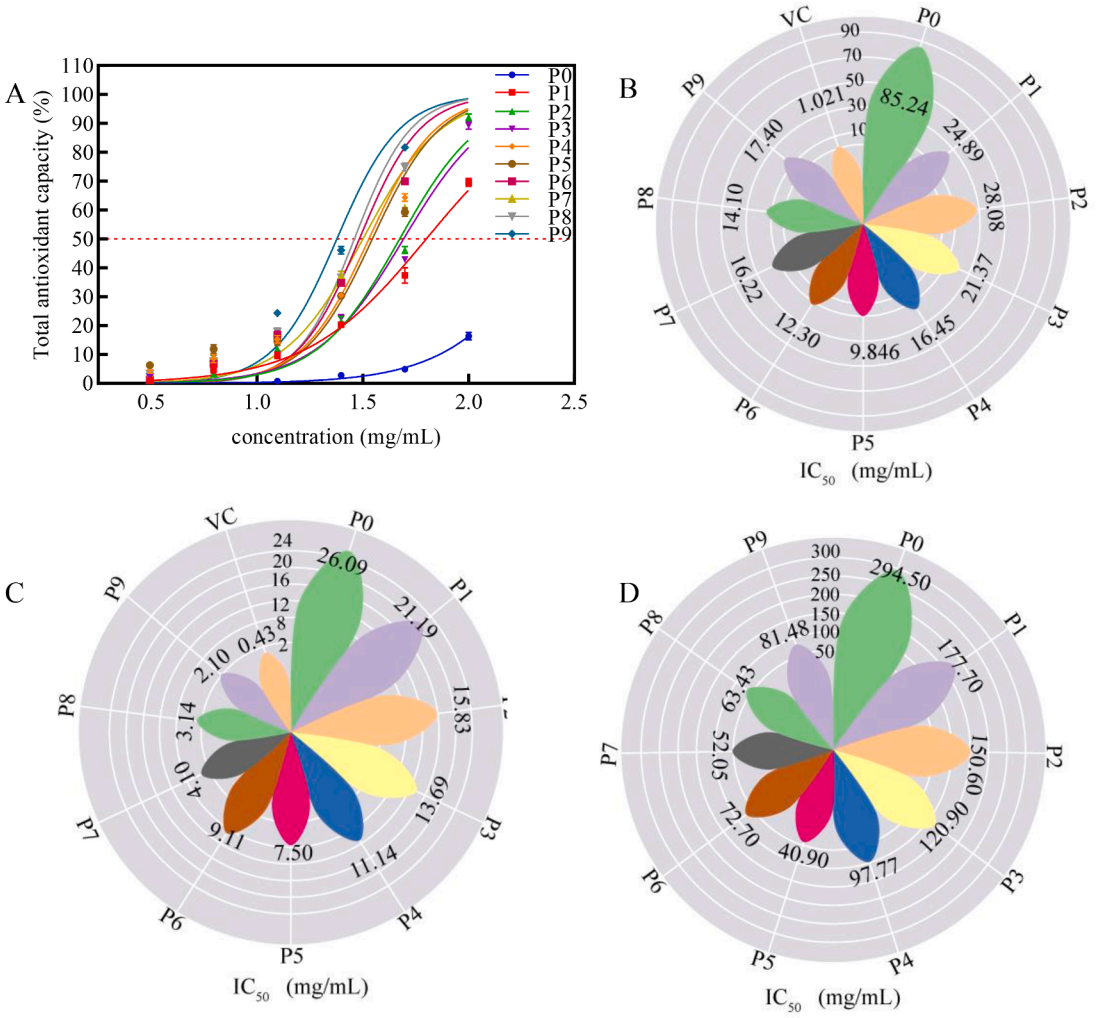

Fig. 4. Antioxidant activities of processed samples of RPK. Total antioxidant capacity (A), scavenging ability for ABTS·+ free radical (B), scavenging ability for DPPH· (C) and scavenging ability for ·OH (D).

antioxidant activities were increased *in vitro*. In conclusion, we have found that the levels of alkaloids, amino acids and derivatives and terpenoids is decreased, and organic acids and saccharides is increased in the samples of RPK during the process of preparation through the method of "Nine Cycles of Steaming and Sun-Drying". The process considerably decreases numb taste, increase sweet taste and enhance the antioxidant activities of RPK.

CRediT authorship contribution statement

Yanhui Guan: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Zhengwei Liang:** Investigation. **Ruoyu Li:** Investigation. **Yunjiao Guo:** Investigation. **Lingjing Dang:** Investigation. **Fuming Gong:** Investigation. **Susu Xu:** Investigation. **Teng Wang:** Investigation. **Nianguo Bo:** Investigation. **Shengchao Yang:** Supervision. **Weiwei Jiang:** Investigation. **Guanghui Zhang:** Supervision. **Ming Zhao:** Supervision. **Junwen Chen:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Ethical statement and sensory consent

All members volunteered to participate in the sensory evaluation and agreed to its publication. In addition, appropriate protocols were used to protect the rights and privacy of all participants during the execution of the research.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101292.

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