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RESEARCH

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Comprehensive analysis of nutritional profile, in vitro antioxidant activity of litchi and its effects on postprandial glycemic response and plasma proteome in humans

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Abstract

Litchi (*Litchi chinensis* Sonn.) is a fruit grown in subtropical to tropical areas. Its effects on postprandial glycemic response and the potential benefits for human remain unclear. This study quantified the nutritional components of litchi pulp by high-performance liquid chromatograph (HPLC) or HPLC-MS/MS methods, and conducted a trail among healthy volunteers to observe the dynamic blood glucose and plasma proteome alteration after eating litchi. Litchi was rich in glucose, fructose, sucrose, various amino acids and vitamins. Among 3 litchi cultivars, "Feizixiao" possessed the highest vitamin content and the strongest antioxidant activity. After eating litchi, blood glucose rose rapidly to peak within 30 min, and then dropped to the initial fasting blood glucose level around 120 min, and continued to drop, with a trough phenomenon during 120–180 min. The glycemic index of litchi was 66 and glycemic load was 11 (first reported). In the plasma proteome, brain acid soluble protein 1 (BASP1), Tsukushi (TSKU), protein MTSS 1 (MTSS1), vinculin (VCL), peptidyl-prolyl *cis-trans* isomerase A (PPIA) and tubulin alpha-1B chain (TUBA1B) were identified as important proteins related to the function of litchi. In conclusion, litchi is rich in nutrients and may have various health benefits for humans.

Keywords Litchi, Nutritional components, Antioxidant activity, Glycemic response, Proteome

Introduction

Litchi (*Litchi chinensis* Sonn.) is a fruit grown in subtropical to tropical areas and its pulp (arils) can be eaten by human, with a sweet and juicy taste [1]. In recent years, litchi has gradually developed into an important economic crop, grown in more than 20 countries and regions, of which China is the largest growing region [1, 2]. Litchi is rich in amino acids, vitamin, minerals and phytochemicals, along with a high nutritional value [3, 4]. Increasing evidence exhibits that litchi possesses the functions of lowering blood glucose, lowering blood lipids, preventing obesity, enhancing immune function, protecting liver and improving cognitive impairment [1, 5–9]. Therefore, litchi has a bright prospect of being

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developed as a functional food, and now has been popular in many countries and regions [1, 2].

However, with the export of litchi to the world, its safety has been increasingly concerned. Several studies showed that litchi pulp potentially caused "litchi disease", which was characterized by hypoglycemic symptoms such as dizziness, heart palpitations and sweating after eating litchi. It was frequently reported during the litchi ripening season in several regions including China, India, Bangladesh, and Vietnam [10–12]. Evidence pointed out that "litchi disease" was attributed to eating too much litchi on an empty stomach [12]. Deng et al. [13] early speculated that the high concentration of fructose in litchi pulp stimulated islet beta cells to rapidly release a large amount of insulin, thus reducing blood glucose levels. Shrivastava et al. [14] further found that hypoglycin A (HGA) and methylene cyclopropyl glycine (MCPG) in litchi were potentially related to the occurrence of hypoglycemia. When the body is in a state of prolonged fasting, the glucose levels in the blood will decrease. The decreased glucose levels trigger the process of glycogenolysis. However, in some individuals with less glycogen reserves, gluconeogenesis takes over glycogenolysis to replenish the glucose levels. HGA and MCPG from litchi are involved in fatty acid oxidation and gluconeogenesis pathways, by blocking several steps in the former process. Depleted glycogen stores and suppression of gluconeogenesis synergistically cause hypoglycemia [15]. Previous research focused on the possible causes of litchi induced hypoglycemia, but studies regarding the blood glucose fluctuations after litchi consumption were notably absent. Notably, blood glucose fluctuations directly reflect the glycemic response of the human body and play a decisive role in determining the occurrence of hypoglycemia. Monitoring the dynamic blood glucose fluctuations after litchi consumption may help identify the critical periods when litchi-related hypoglycemia occurs.

Additionally, previous studies reported that litchi was rich in sugars [1]. Sugar intake is closely related to glycemic response, which makes us wonder whether ingesting a certain amount of litchi will induce a short-term spike in blood glucose levels in the human body. In medicine, controlling blood glucose fluctuations is recommended for some people, especially individuals with diabetes or/and cardiovascular disease, and the elderly (because of the decreasing of insulin sensitivity as aging) [16–18]. Glycemic index (GI) is a relative value that indicates the speed and extent of blood glucose rise after food intake, and glycemic load (GL) further indicates the total effect of food on glycemic response [19]. The 2 indicators are important tools to help people control blood glucose fluctuations and guide a healthy diet. However, the GI and GL of litchi fruit remain unknown.

Litchi has a rich nutritional value and various potential health benefits. This may be attributed to the abundant vitamin and bioactive compounds in litchi pulp. A large amount of evidence has shown that litchi is rich in vitamin C (VC), which is a hydrophilic vitamin and a powerful antioxidant that can trap hydroxyl and superoxide radicals in the body [20, 21]. Notably, some other vitamin (such as niacin) also possess antioxidant activity and offer numerous health benefits [22], but research on other vitamin in litchi is relatively lacking. Therefore, a comprehensive quantitative analysis of various vitamin, as well as the in vitro antioxidant activity, will provide a reference basis for assessment of the nutritional value of litchi.

The in vivo research, especially population-based controlled trials, can offer higher-level evidence for clarifying the nutritional value and health benefits of functional foods. However, few clinical trials have been reported on litchi and other tropical fruits [4, 23]. Evaluation of the health benefits and molecular mechanisms of action in human studies could provide commercial opportunities to the growers to allow them to expand their business opportunities by promoting fresh healthy fruits in the market and then to functional foods [23]. In medicine, genes, proteins and metabolites are all involved in a variety of physiological activities in humans, and are closely related to the health of the body. Among these, proteins are the final effectors of physiological pathways and the direct manifestations of life phenomena [24]. Proteins in the blood can be well used as biomarkers for diagnosis, prognosis, and monitoring of treatment response. Recently, omics technology provides a new perspective for elucidating human health and disease mechanisms [25]. Identification of various changes in plasma proteins in human body may provide clues for understanding the potential health benefits of litchi, and provide opportunities for litchi to be used as a functional food in the future.

In this study, we selected 3 representative litchi cultivars from southern China (an area of litchi origin), and comprehensively quantified the various nutritional compositions (such as sugar compositions, total proteins, fatty acids, various amino acids and vitamin) for evaluating the nutritional value of litchi. We quantified the in vitro antioxidant activity of 3 litchi cultivars, and observed that litchi 'Feizixiao' had the highest vitamin content and strongest antioxidant activity, which implied that the higher the vitamin, the stronger the antioxidant activity. Furthermore, a clinical control trail was conducted to observe the effect of litchi on postprandial glycemic response within 240 min, so as to observe the trough phenomenon of glycemic curve, which was ignored in previous food-related studies because of only 120 min of monitoring. The GI and GL values were calculated to guide the rational consumption of litchi fruit.

To our knowledge, these values are the first reported. Additionally, an in-depth characterization of plasma proteome alteration after litchi consumption was further performed to comprehensive assessment of the health benefits of litchi and this exploratory work furnished some implications for functional foods development in the future.

Materials and methods

Litchi, glucose and chemical materials

Litchi 'Feizixiao' (FZX), 'Guiwei' (GW) and 'Nuomici' (NMC) were picked from a fruit farm in Huidong County, Guangdong, China, during the litchi ripening season in June, 1 day before each test, and transported to the test site by cold chain. The outer shell and core of litchi were peeled off by the staff on site, leaving the pulp, part of which was immediately used for on-site test, and the other part was frozen at −80 ℃ for subsequent composition detection. Edible grade anhydrous glucose was purchased from Haiwangfuyao Pharmaceutical Co., Ltd. (China).

Mass spectrum grade formic acid, methanol, acetonitrile, and isopropyl alcohol were purchased from Merck (Germany). Zinc acetate, potassium ferrocyanide, copper sulfate, potassium sulfate, sulfuric acid, boric acid, methyl red indicator, bromocresol green indicator, and sodium hydroxide were purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (China).

Measurements of sugar compositions

The contents of fructose, glucose, sucrose, maltose and lactose in litchi pulp were determined according to national standard GB 5009.8−2016 [26]. High-performance liquid chromatograph with evaporative light-scattering detection (HPLC-ELSD, Agilent, USA) and amino column (250 mm × 4.6 mm, 5 μm, Waters, USA) were used for the analysis of sugar composition [27]. Each litchi cultivar was tested 3 times and the average value was calculated. Details of the experimental procedures were as follows: weigh 10.0 g of litchi pulp, crush it, transfer the mixture to a 100 mL volumetric flask, slowly add 5.0 mL each of zinc acetate solution and potassium ferrocyanide solution, and then add ultra-pure water to the mark. After 30 min of ultrasound, 3 centrifugations and filtration (0.45 μm microporous filter membrane) were performed for subsequent HPLC analysis. HPLC parameters were as follows. Mobile phase: 70% acetonitrile and 30% ultrapure water; flow rate: 1.0 mL/min; injection volume: 20 μL; column temperature: 40 ℃; differential refraction detector temperature: 40 ℃; drift tube temperature range: 80–90 ℃; nitrogen pressure: 350 kPa; impactor: off.

Measurements of total soluble solids

The content of total soluble solids (TSS) was determined by digital refractometer (ATAGO, Japan). A total of 15 litchi fruits were randomly selected from each variety, and the TSS content of the pulp was measured using a refractometer and recorded.

Measurements of other nutritional compositions

The total protein content in litchi pulp was determined by automatic Kjeldahl nitrogen analyzer (FOSS Kjeltec8400, Denmark) based on Kjeldahl method for nitrogen determination, referring to the national standard GB 5009.5−2016 [28]. Weigh 2–5 g of thoroughly mixed sample, accurate to 0.001 g, into the digestion tube, then add 0.4 g of copper sulfate, 6 g of potassium sulfate and 20 mL of sulfuric acid in the digestion furnace for digestion. When the temperature of the digestion furnace reaches 420 ℃, digestion was continued for 1 h. At this time, the liquid in the digestion tube was green and transparent. After taking out and cooling, 50 mL water was added to realize the process of automatic liquid addition, distillation, titration and recording titration data on the automatic Kjeldahl nitrogen analyzer. Amino acid, fatty acid, and vitamin contents, including VA, VB_1, VB_2, VB_3-niacin, VB_3-nicotinamide, VB_6, VC, VD, and VE, were determined using HPLC-MS/MS method by Shenzhen Academy of Metrology & Quality Inspection (SMQ).

Preparation of litchi water extracts

The homogenate of litchi pulp was prepared according to the ratio of 1:10 (litchi pulp:ultrapure water) using a juicer, and then extracted for 30 min at a constant temperature of 60 ℃ in a water bath. After centrifugation (3 000 r/min, 5 min), the supernatant was retained, and finally filtered by 0.22 μm water filtration membrane.

Measurements of antioxidant activity

The antioxidant activity of the litchi water extracts was calculated from the Trolox calibration curve and was expressed as μg of the Trolox equivalent per 1 mL of extract solution. The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method was used to assess the antioxidant activity of water extracts from 3 litchi cultivars, as described in previous studies [29, 30]. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined, using the method proposed by Brand-Williams et al. [31]. The ferric-reducing antioxidant power (FRAP) was determined, using the method proposed by Benzie and Strain [32]. All measurements were performed according to the instruction manual of the corresponding kits and detailed procedures could be referred to the corresponding references. Specifically, the

details of the ABTS method were as follows: prepare the $ABTS^{+}\bullet$ stock solution by mixing ABTS with potassium persulfate (in a molar ratio of 1.0:0.5) in the dark for 12 h to form $ABTS^{+}\bullet$. Dilute the stock solution with distilled water 40 times to obtain the working solution. Mix 10 μL of the litchi water extract with 190 μL of $ABTS^{+}\bullet$ working solution and let it react for 6 min. Lastly, measure the decrease in absorbance at a wavelength of 414 nm and calculate the free radical scavenging rate. The details of the DPPH method were as follows: prepare the DPPH working solution according to the instructions of the kit. Mix 150 μL of the litchi water extract with 150 μL of DPPH working solution, and let it react at 25 ℃ in the dark for 30 min and then centrifuge. Lastly, measure the absorbance of the supernatant at a wavelength of 517 nm and calculate the free radical scavenging rate. The details of the FRAP method were as follows: prepare the FRAP working solution by mixing acetic acid buffer (pH 3.6), 2,4,6-tripyridyltriazine (TPTZ) solution and $FeCl_3$ solution in a volume ratio of 10:1:1. Mix 5 μL of the litchi water extract, 25 μL of distilled water with 170 μL of FRAP working solution and let it react at 25 °C for 10 min. Lastly, measure the absorbance at a wavelength of 590 nm and calculate the total antioxidant activity. The ABTS (product number: G0128W), DPPH (product number: G0142W), and FRAP (product number: G0115W) kits were all purchased from Suzhou Grace Biotechnology Co., Ltd. (China).

Experimental objects and procedure of the self-control trial

Healthy young volunteers aged 18–25 years were recruited for physical examination and biochemical tests (including blood glucose, insulin levels, thyroid function, kidney function, and liver function), and 20 objects were finally selected, half male and half female. Inclusion criteria were as follows: 1) body mass index (BMI): 18.5–24 kg/m^2; 2) no litchi allergy; 3) no diabetes (or impaired glucose tolerance), other metabolic diseases, digestive system diseases, endocrine system diseases and mental diseases; 4) no family history of diabetes; 5) no nutrient supplements, oral contraceptives, acetylsalicylic acid, steroids, protease inhibitors and antipsychotics that affect glucose tolerance have been taken in the past 3 months. The trail was conducted in accordance with the Decla-ration of Helsinki, and approved by the Ethics Committee of the National Institute of Nutrition and Health of the Chinese Center for Disease Control and Prevention (protocol code 2023–006). The participants were provided with a privacy notice informing them of data protection and the purpose of the study as well as a consent form. All participants have signed informed consent forms.

The day before each test, participants were asked to start fasting after 10:00 p.m. and arrive at the test site at 8:00 a.m. the next day. After 15 min of sitting and quieting down, each participant was asked to eat 300 g of fresh litchi (containing about 50 g of carbohydrates) [33] or 50 g of glucose within 5 min. According to the standard for determination of food glycemic index (China Health Industry Standard WS/T 652—2019), GI is defined as the ratio of the incremental area under the curve (iAUC) of blood glucose within a certain period of time (≥ 2 h) after consuming a food containing 50 g of available carbohydrates to the corresponding increase area after consuming a reference food (50 g of glucose). The GI is expressed as a percentage, and the GI value of glucose is 100 (typically as a reference value)[34].

A total of 5 separate tests (3 litchi tests and 2 glucose tests) were conducted on all participants on an empty stomach in the morning, with an interval of more than 72 h among each test. In each test, dynamic blood glucose levels at multiple time points (at the 0^{th}, 15^{th}, 30^{th}, 45^{th}, 60^{th}, 90^{th}, 120^{th}, 180^{th}, and 240^{th} min) were measured using a glucose meter (Abbott, UK).

Plasma proteome measurement

Non-targeted proteomics were performed on plasma samples collected before (at the 0^{th} min) and after (at the 240^{th} min) litchi/glucose consumption. Eight objects (4 males and 4 females) were randomly selected from the participants who completed all 5 tests, and a total of 64 plasma samples from the eight objects collected in all litchi tests and the first oral glucose test were tested. The plasma samples were enriched with low abundance protein using the EasyPept Deep low abundance protein enrichment and pre-treatment preparation kit (Shanghai Omicsolution Co., Ltd., China). The sample pre-treatment included denaturation, reduction, alkylation, enzyme digestion and desalination [25]. Fragments with mass to charge ratios between 350 and 1 500 were scanned using mass spectrum (Orbitrap Eclipse Tribrid, Thermo Fisher, USA), based on data-independent acquisition (DIA) mode. Each sample was remeasured twice and the mean value was used for analysis.

Statistical analysis

Descriptive statistical analyses were performed. Normal test and the variance homogeneity test were conducted on all data, including sugar components of 3 litchi cultivars and blood glucose at each time point of 5 tests. When the data met normal distribution, the mean ± standard deviation was shown, otherwise the median and quartiles were shown. *t*-Test was selected to compare the difference when normal distribution and

homogeneity of variance were both met, otherwise non-parametric test was performed. Proteomic data processing was as follows: a) Retention of proteins expressed in 66.7% and above samples; b) $\log_2$ conversion; c) filling in missing values; d) normalization; e) check the distribution of data matrix. Principal co-ordinates analysis (PCoA) and difference analyses (using package "limma") were performed to observe the changes of plasma proteins after litchi and glucose consumption. The *P*-values of multiple tests were adjusted by Benjamini-Hochberg method and false discovery rate (FDR) was used as the key indicator for screening differentially expressed proteins. Venn diagram was utilized to display different and shared regulated proteins. R 4.2.3, R-Studio and GraphPad Prism 8.0.1 were used for statistical analysis and graphing. A bilateral test with a two-sided *P* (or FDR) of <0.05 indicated a significant threshold.

Results and discussion

Nutritional profile of litchi pulp

As shown in Table 1, glucose, fructose and sucrose are the main sugar compositions in litchi, and maltose and lactose are not detected. Total sugar contents of 3 litchi cultivars were (19.40 ± 0.51) g/100 g (FZX), (19.90 ± 0.37) g/100 g (GW), and (15.50 ± 1.61) g/100 g (NMC), respectively. TSS can directly reflect soluble sugar contents, which is an important index to evaluate the sweetness and quality of fruit[35]. The TSS contents of 3 litchi cultivars were (19.58 ± 1.27)% (FZX), (19.79 ± 0.77)% (GW), and (14.95 ± 1.76)% (NMC), closely to the results of total sugar contents. Among 3 litchi cultivars, the content of glucose and fructose in litchi 'FZX' was the highest, followed by litchi 'GW', and litchi 'NMC' was the lowest, whereas the content of sucrose in litchi 'FZX' was far lower than that of litchi 'GW' ($P<0.001$) and 'NMC' ($P<0.01$) (Fig. 1A). No significant difference in total

Table 1 Sugar composition and total soluble solid content in fresh litchi pulp

Ingredient	FZX	GW	NMC	Average value
Glucose (g/100 g)	8.03 ± 0.29 (41.4%)^{abc}	6.10 ± 0.14 (30.7%)^{abc}	4.27 ± 0.12 (27.5%)^{abc}	6.13 ± 1.64 (37.7%)
Fructose (g/100 g)	8.20 ± 0.28 (42.3%)^{abc}	6.10 ± 0.22 (30.7%)^{abc}	4.20 ± 0.08 (27.1%)^{abc}	6.17 ± 1.75 (37.4%)
Sucrose (g/100 g)	0.76 ± 0.13 (3.9%)^{ab}	5.87 ± 0.29 (29.5%)^{a}	5.60 ± 0.69 (36.1%)^{b}	5.5 (0.9, 5.9) (24.9%)
Maltose (g/100 g)	–	–	–	–
Lactose (g/100 g)	–	–	–	–
Sum of the above sugars	16.97 ± 0.51 (87.5%)	18.07 ± 0.15 (90.8%)	14.10 ± 1.10 (91.0%)	16.38 ± 1.88 (89.7%)
Total sugar (g/100 g)	19.40 ± 0.51	19.90 ± 0.37	15.50 ± 1.61	19.2 (17.3, 20.0)
TSS (%)	19.58 ± 1.27^{a}	19.79 ± 0.77^{b}	14.95 ± 1.76^{ab}	18.9 (16.5, 19.9)

Note: The normal test was performed. If the data met the normal distribution, it was presented as mean values ± standard deviations, otherwise it was presented as median (quartile 1, quartile 3). Percentage (%) indicates its proportion of total sugar content. "–" indicates that it is not detected. Values with superscript letter a, b, and c are significantly different across columns ($P<0.05$)

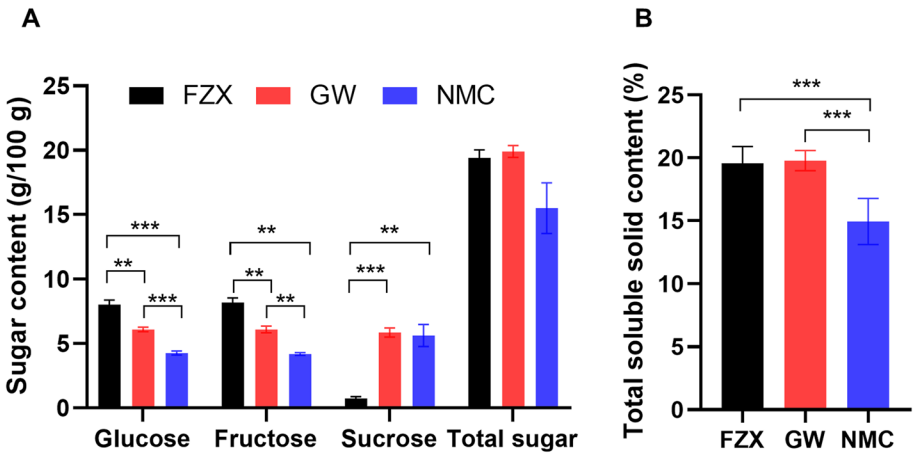


Fig. 1 Sugar composition and total soluble solid content in litchi pulp. **A** Differences in sugar composition among 3 litchi cultivars. A total of 3 measurements were performed for each litchi cultivar. **B** Differences in total soluble solid content among 3 litchi cultivars. A total of 15 measurements were performed for each litchi cultivar. If the data met normal distribution and variance homogeneity, *t*-test was used to observe the differences; otherwise, non-parametric test was used. $^{**}P<0.01$, $^{***}P<0.001$

sugar content among 3 litchi cultivars was observed ($P > 0.05$) (Fig. 1B), and the median value of all litchi was 19.2 g/100 g (quartile ranges, 17.3–20.0 g/100 g).

As shown in Table 2, the content of total proteins in 3 litchi cultivars is close, with an average value of about 816 mg/100 g. Fatty acids, including palmitic acid ($C_{16:0}$), linoleic acid ($C_{18:2}$) and α-linolenic acid (α-$C_{18:3}$), were detected in litchi 'GW', while only linoleic acid was detected in litchi 'FZX', and only α-linolenic acid was detected in litchi 'NMC'. A total of 15 kinds of amino acids were detected, including aspartate, threonine, serine, glutamic acid, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, arginine, and proline, while methionine was not detected in litchi pulp. The average content of alanine, glutamic acid, aspartate and proline were all above 40 mg/100 g, and they 4 contribute about 50% of the total content of all amino acids quantified (Fig. 2A).

Of the 9 kinds of vitamin, VB_2, VB_3-niacin, VB_3-nicotinamide, VB_6 and VC were detected in all litchi cultivars, while VA, VD and VE were not detected, and VB_1 was detected with a value closely to the minimum detection limit. VB_3-niacin was the most abundant vitamin, accounting for 52%–78% of the total vitamin content, followed by VC and VB_6. Additionally, a relatively elevated VB_2 content was observed in litchi 'NMC' (Fig. 2B). Among 3 litchi cultivars, the content of vitamin in litchi 'FZX' was the highest, followed by litchi 'GW', and the content of vitamin in litchi 'NMC' was the lowest (Table 2).

In this part, the nutritional compositions of 3 litchi cultivars were quantified comprehensively. The TSS contents of 3 litchi cultivars were 14.95%–19.79%, and litchi was rich in glucose, fructose and sucrose. These determined a good taste of litchi. Additionally, litchi was rich in proteins and various amino acids. Among the amino acids detected, valine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine are all essential amino acids for humans. And the abundant vitamin contents also contributed to a high nutritional value of litchi pulp. Furthermore, the nutritional maps visualized various amino acids and vitamin compositions in litchi, filling the knowledge gaps in previous studies.

Table 2 Proteins, amino acids, fatty acids and vitamin content (mg/100 g) of fresh litchi pulp

Nutritional composition	FZX	GW	NMC	Average value	Minimum detection limit
Total proteins	820	820	810	816	–-
Fatty acids					
$C_{16:0}$	–	8.30	–	2.77	6.6
$C_{18:2}$	3.98	4.47	–	2.82	3.3
α-$C_{18:3}$	–	7.46	7.02	4.83	3.3
Amino acids					
Aspartate	54	61	59	58	0.36
Threonine	18	21	21	20	0.48
Serine	28	32	24	28	0.60
Glutamic acid	78	64	67	70	0.70
Glycine	24	22	24	23	0.84
Alanine	72	83	78	78	9.70
Valine	29	26	27	27	0.32
Methionine	–	–	–	–	7.50
Isoleucine	21	19	21	20	1.30
Leucine	31	32	35	33	3.60
Tyrosine	11	12	13	12	9.50
Phenylalanine	18	16	21	18	8.30
Lysine	30	34	39	34	0.44
Histidine	9	8	10	9	2.00
Arginine	18	16	20	18	6.50
Proline	51	48	25	41	8.70
Vitamin					
VA	–-	–-	–-		0.030
VB_1	1.04	-	1.11	0.72	1.00
VB_2	3.14	2.50	8.30	4.65	1.00
VB_3-niacin	125	71.4	40.4	78.9	5.00
VB_3-nicotinamide	3.24	3.35	4.41	3.67	2.00
VB_6	10.6	8.39	12.5	10.5	2.00
VC	17.1	7.0	10.1	11.4	2.0
VD	–-	–-	–-	–-	0.002
VE	–-	–-	–-	–-	0.120

Note: "–-" indicates that it is not detected

Antioxidant activity of water extracts from three litchi cultivars

ABTS, DDPH and FRAP have been extensively used to evaluate the antioxidant activity of fruit and vegetable juices and their extracts[36, 37]. Table 3 shows the total antioxidant activity of water extracts from 3 litchi cultivars. The radicals-scavenging rates of litchi 'FZX' were 50.76% (ABTS), 61.31% (DDPH) and 56.20% (FRAP), respectively, being the highest as compared to that of the other 2 litchi cultivars. The total antioxidant activity of litchi 'GW' and 'NMC' was relatively close, about 30.6%–51.5%.

The vitamin contents may partly contribute to the antioxidant activity of litchi. VB_3-niacin and VC are both water-soluble vitamin and VC is a powerful antioxidant, contributing to the antioxidant system of fruit[37]. Our study showed that litchi pulp was rich in VB_3-niacin and VC, especially litchi 'FZX', and litchi water extracts had a powerful antioxidant activity of ABTS, DDPH and FRAP radicals. The vitamin content was largely consistent with the antioxidant activity of 3 litchi cultivars, which the higher the vitamin content, the stronger the antioxidant activity.

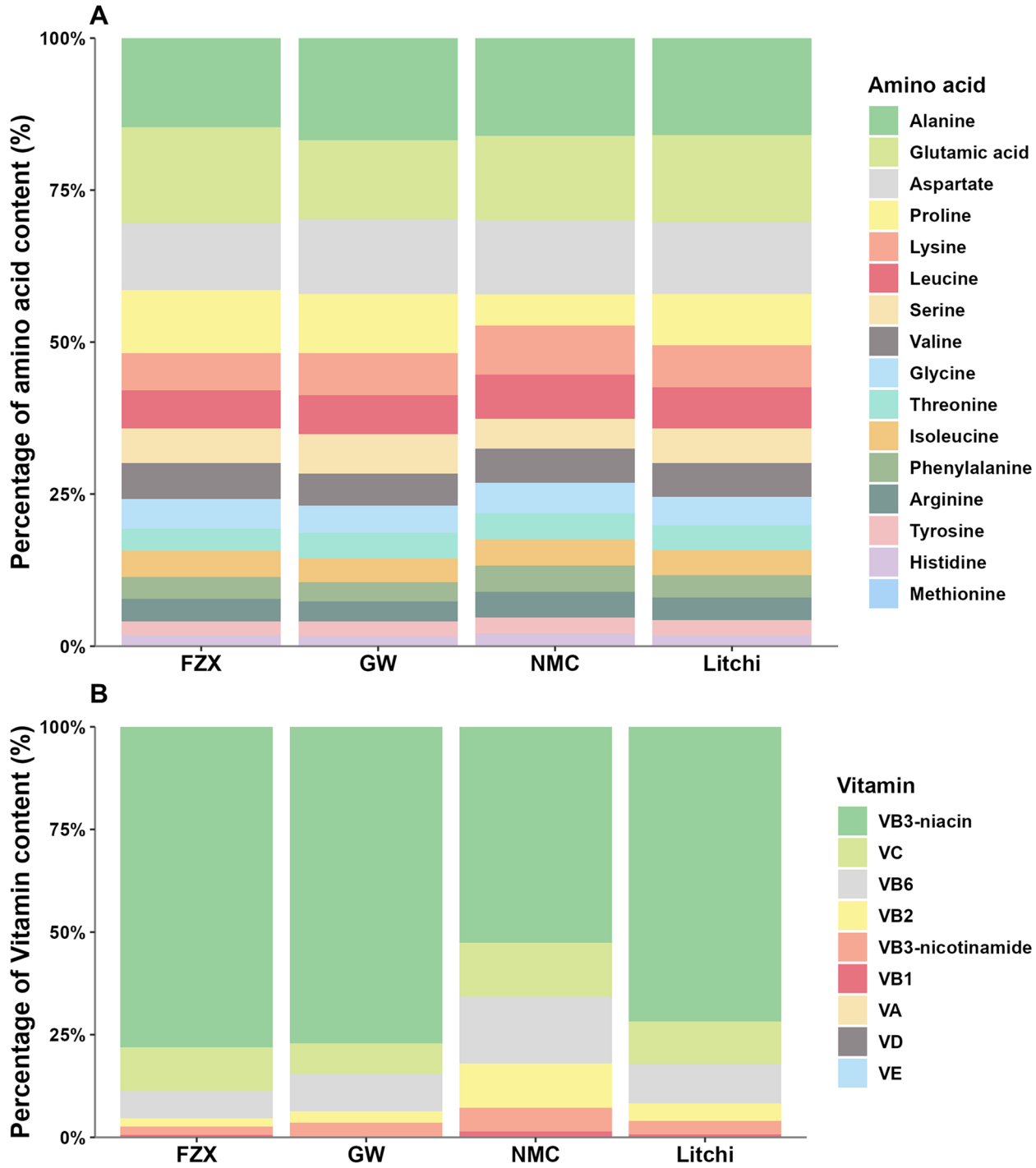


Fig. 2 Amino acid and vitamin profile of three litchi cultivars. **A** Amino acid composition percentage map of 3 litchi cultivars. **B** Vitamin composition percentage map of 3 litchi cultivars

Effects of litchi pulp on glycemic response in humans

In all 5 tests, no hypoglycemic symptoms, allergic reactions, or other discomfort were reported by the volunteers or observed. The glycemic increase curves of the 2 separate oral glucose tests almost overlapped (Fig. 3A), indicating that the quality of trail was well controlled.

In litchi tests, after consuming 300 g of litchi pulp, the glycemic curves were observed to rise rapidly within

Table 3 Total antioxidant activity of water extracts from 3 litchi cultivars

Index	FZX	GW	NMC	Average value
ABTS				
Radical-scavenging ability (μg Trolox/mL)	40.48	26.14	21.29	29.30 ± 9.52
Radical-scavenging rate (%)	50.76	37.90	33.55	40.73 ± 8.54
DPPH				
Radical-scavenging ability (μg Trolox/mL)	15.31	12.72	12.03	13.35 ± 2.24
Radical-scavenging rate (%)	61.31	51.46	48.81	53.86 ± 8.50
FRAP				
Restore Fe^{3+} to Fe^{2+} ability (μg Trolox/mL)	7.39	2.27	2.64	4.10 ± 2.67
Rate (%)	56.20	30.61	33.50	40.10 ± 12.79

Note: The total antioxidant activity is shown as the equivalent dose of Trolox, an antioxidant used as a standard. Multiple samples from the same litchi cultivar were mixed to detect its antioxidant capacity. Each litchi cultivar was tested twice, and the average value and standard deviation were calculated

30 min, with a trend closely to that of the oral glucose tests, and then drop to the initial fasting blood glucose level around 120th min. During the 120–180 min, a trough value was observed, and at the 240th min the blood glucose level was observed to returned to the initial fasting blood glucose level again. Notably, as compared to the oral glucose tests, the peak value observed in litchi tests was at the 30th min, earlier than that of glucose tests (at the 45th min), and the peak value of litchi tests was lower than that of glucose tests (Figs. 3B-C). The blood glucose increase curves of the 3 litchi cultivars were close, but the glycemic increment at the 60th min in litchi 'FZX' test was higher than that in litchi 'NMC' test ($P < 0.05$) (Figs. 3D-E).

The glycemic response is closely related to the composition and content of sugars in the food consumed. The glycemic increments of litchi tests in the first 30 min were close to that observed in glucose tests, which may be attributed to the large amount of glucose in litchi pulp. 300 g litchi pulp contains about 12.8–24.1 g of glucose, accounting for 27.5%–41.4% of the total sugars. The glucose could be absorbed into circulatory system through the small intestine, rapidly increasing blood glucose levels. Of the 3 litchi cultivars, litchi 'FZX' contained the most glucose, which explained the higher level of its glycemic increment at the 60th min than that observed in litchi 'GW' and 'NMC' tests. Fructose was also absorbed by body in the small intestine, but its GI was only 23, much lower than that of glucose (100). Jang et al. [38] observed that when consumed in low doses, fructose first began to be metabolized in the small intestine, with about 42% of dietary fructose being converted to glucose, 30% to organic acids, and 14% being transported to the liver. For a healthy adult weighing about 60 kg, the limit of fructose that could be metabolized in the small intestine on an empty stomach was about 30 g. According to the quantitative results of sugar in our study, 300 g of litchi contained about 12.6–24.6 g of fructose, lower than the limit value. Therefore, while these fructose in litchi might be partially absorbed and converted into glucose, they contributed slightly to the glycemic increment on human. This explained why the glycemic curves were observed to begin to drop earlier in the litchi tests than in the glucose tests.

At the 120th min, blood glucose increase curve in all litchi and glucose tests were observed to return to the initial level and then continue to drop and reach a trough value. This may be related to the regulation of insulin secretion. After glucose entered the circulation system, the body began to secrete insulin to lower the blood glucose levels by increasing the consumption. Then signals about the drop in blood glucose levels would be further fed back to the islet cells to inhibit insulin secretion [39]. A delay existed in this signaling process, so contributing to the appearance of the trough value in the glycemic curve. This suggests that the risk period of the onset of "litchi disease" may be 2 h after eating a large amount of litchi on an empty stomach.

GI and GL of the three litchi cultivars

The incremental area under the curves (iAUCs) were calculated and the iAUCs in litchi tests were all lower than that in glucose tests ($P < 0.001$), but no significant difference was observed among the 3 litchi cultivars ($P > 0.05$) (Fig. 3F). The GI of litchi 'FZX', 'GW' and 'NMC' were 76, 64 and 58, respectively, and no significant difference was observed among them ($P > 0.05$). The average GI of litchi was 66, classified as a medium GI food (GI, 55–70), and the average GL was 11, just reaching the lower limit of medium GL foods (GL, 10–20) (Table 4).

The GI of litchi was relatively high compared to some common fruits, such as apples with a GI of 36, pears with 36, grapes with 43, oranges with 43, bananas with 52, pineapples with 66, and watermelons with 72. However,

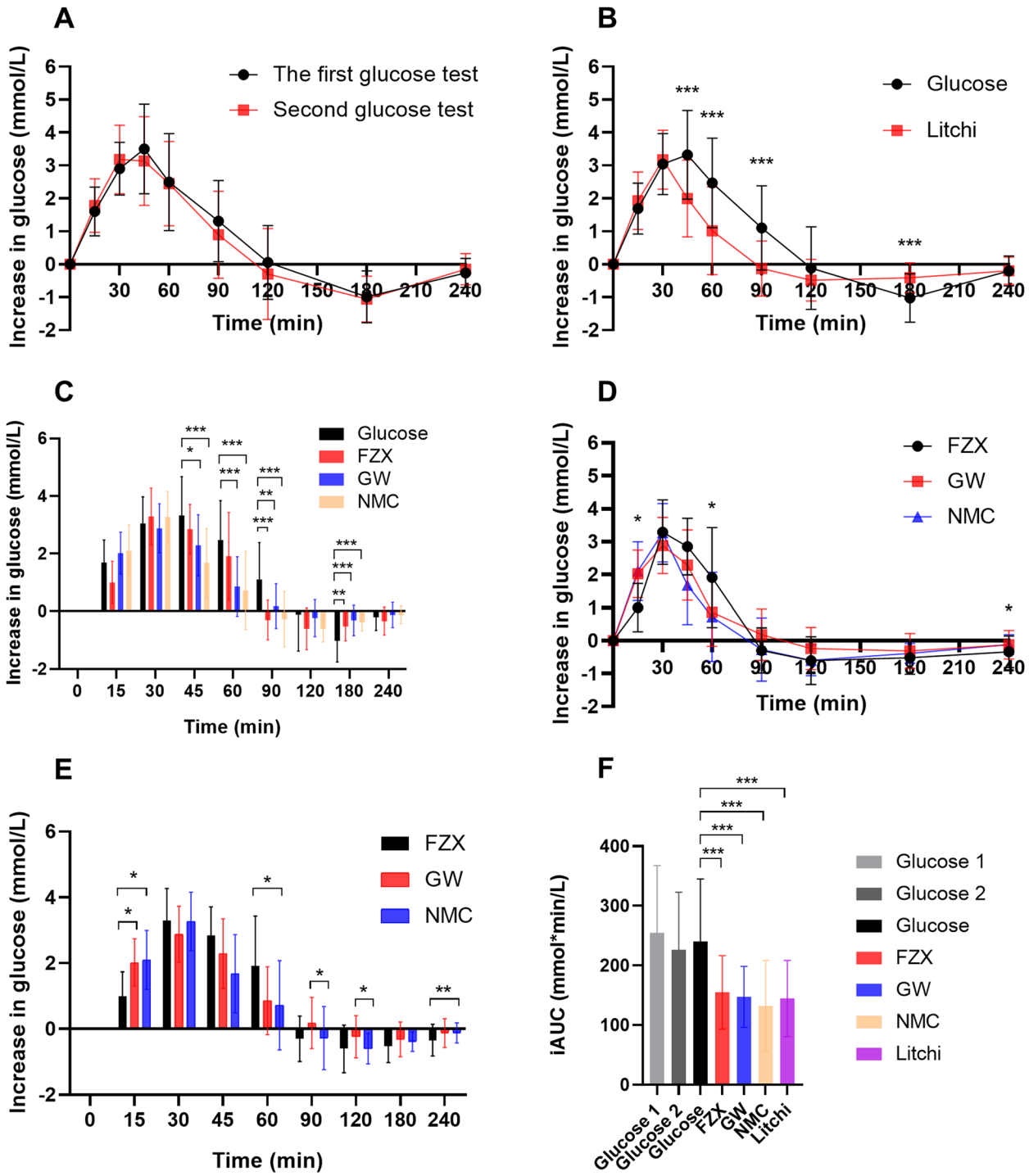


Fig. 3 Glycemic increment curves in glucose and litchi tests. **A** Glycemic increment curves in 2 separate glucose tests. **B** Differences between glucose and litchi tests. "Glucose" indicated the combined results of 2 separate glucose tests and "Litchi" indicated the combined result of 3 separate litchi tests. **C** Differences in 3 separate litchi tests, as compared to glucose tests. **D**-**E** Differences among 3 separate litchi tests. **F** iAUC (240 min). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 4 GI and GL of 3 litchi cultivars

Index	FZX	GW	NMC	Average value	*P* value
GI	76.44	63.93	57.85	66.08	0.274
GL	12.74	10.66	9.64	11.01	–

Note: The *P* value shows the results of non-parametric test of GI among the 3 litchi varieties

the GL of litchi was relatively low, probably due to its elevated water content. Considering the sweet taste and rich nutrition, moderate consumption of litchi is recommended for the general or diabetic populations.

To our knowledge, the GI and GL values were the first reported to guide the rational consumption of litchi fruit. Notably, since the subjects of the trail were all healthy young volunteers from the litchi origin, considering that individual differences are unavoidable, the generalization of the results to other regions and populations should be cautious.

Subgroup analysis

Considering the differences in some physical and biochemical indicators between male and female groups (Table S1), the subgroup analysis was further performed, and no significant difference was observed in the glycemic response of litchi between gender groups (Fig. 4).

Effects of litchi pulp on human plasma proteome alteration

A total of 1 752 proteins of 64 plasma samples in glucose and litchi tests were identified, and 718 proteins expressed in 66.7% and above samples were included for follow-up analysis. PCoA analysis showed that plasma proteome was changed in both glucose and litchi tests ($P=0.001$) (Figs. 5A-B). Compared with that before litchi consumption (at 0 min), 87 (34 up-regulated and 53 down-regulated) proteins were significantly regulated after litchi consumption (at the 240th min) (Figs. 5C-D). Considering rhythm and other factors, 61 proteins were retained after excluding the same significantly regulated proteins in glucose test (Fig. 5E). Among them, brain acid soluble protein 1 (BASP1), Tsukushi (TSKU), protein MTSS 1 (MTSS1), vinculin (VCL), peptidyl-prolyl *cis-trans* isomerase A (PPIA), and tubulin alpha-1B chain (TUBA1B) were potentially important proteins related to litchi function ($|\log_2$ fold change (FC)$| > 1$ and FDR < 0.001) (Table S2). The protein-protein interaction (PPI) network shows that VCL, transgelin-2 (TAGLN2), TUBA1B, tubulin beta chain (TUBB), tubulin alpha-4A chain (TUBA4A), 14-3-3 protein zeta/delta (YWHAZ), integrin beta-3 (ITGB3), and integrin alpha-IIb (ITGA2B) are potentially important proteins related to litchi function (Fig. 5F).The results of Gene Ontology (GO) function enrichment of the 61 proteins were mainly blood coagulation, hemostasis, platelet aggregation, regulation of body fluid levels, homotypic cell-cell adhesion, secretory granule lumen, focal adhesion, vesicle lumen, cell-substrate junction, cadherin binding, and structural constituent of cytoskeleton (Fig. 5G). The results of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were mainly complement and coagulation cascades, regulation of actin cytoskeleton, phagosome, gap junction, motor proteins, pathogenic Escherichia coli infection, and rap1 signaling pathway (Fig. 5H).

The proteome alteration of 3 litchi tests was displayed in Fig. 6. No significantly regulated proteins were observed in litchi 'FZX' test, while 81 proteins (the largest number of significantly regulated proteins) were observed in litchi 'GW' test, and details were shown in Table S3. The PPI network shows that VCL, ITGB3, ITGA2B, and YWHAZ are potentially important proteins both related to litchi 'GW' and 'NMC', similar to the combined results of 3 litchi tests. The shared GO functions of litchi 'GW' and 'NMC' were mainly blood coagulation, coagulation, platelet aggregation, homotypic cell-cell adhesion, focal adhesion, cell-substrate junction, and platelet alpha granule (Figs. 7A-B). The shared KEGG pathway were mainly regulation of actin cytoskeleton, salmonella infection, P13K-Akt signaling pathway, focal adhesion, and fluid shear stress and atherosclerosis (Figs. 7C-D).

The identified proteins reflected the potential functions of litchi in the human body. Table S4 shows the litchi-related important proteins and their functions. Among them, VCL, TAGLN2, YWHAZ, and MTSS1 were involved in the anti-tumor process reported by previous studies [40–43]. Litchi contained abundant polyphenols and flavonoid compounds, including quercetin, rutin, (−)-epicatechin, procyanidin A1, proanthocyanidin B2, kaempferol, etc., which were closely related to the health benefits for humans [4]. Świeca et al. [40] observed that plant extracts rich in phenolic compounds considerably reduced the activity and proliferation ability of human stomach cancer cells. At the same time, the level of VCL protein changed, which might be involved in this process. Wang et al. [41] found that quercetin antagonized the growth and invasion ability of esophagus cancer cells and induced cell apoptosis by inhibiting the expression of TAGLN2. Hu et al. [42] revealed that quercetin inhibited glycolysis and cell proliferation of oral squamous cell carcinoma by inhibiting the G3BP1/YWHAZ axis. Pérez-Durán et al. [43] found that (−)-epicatechin inhibited metastatic-associated proliferation, migration, and invasion of murine breast cancer cells in vitro with the change in the relative expression level of the gene MTSS1. Increasing studies indicated that litchi and its phytochemicals possessed

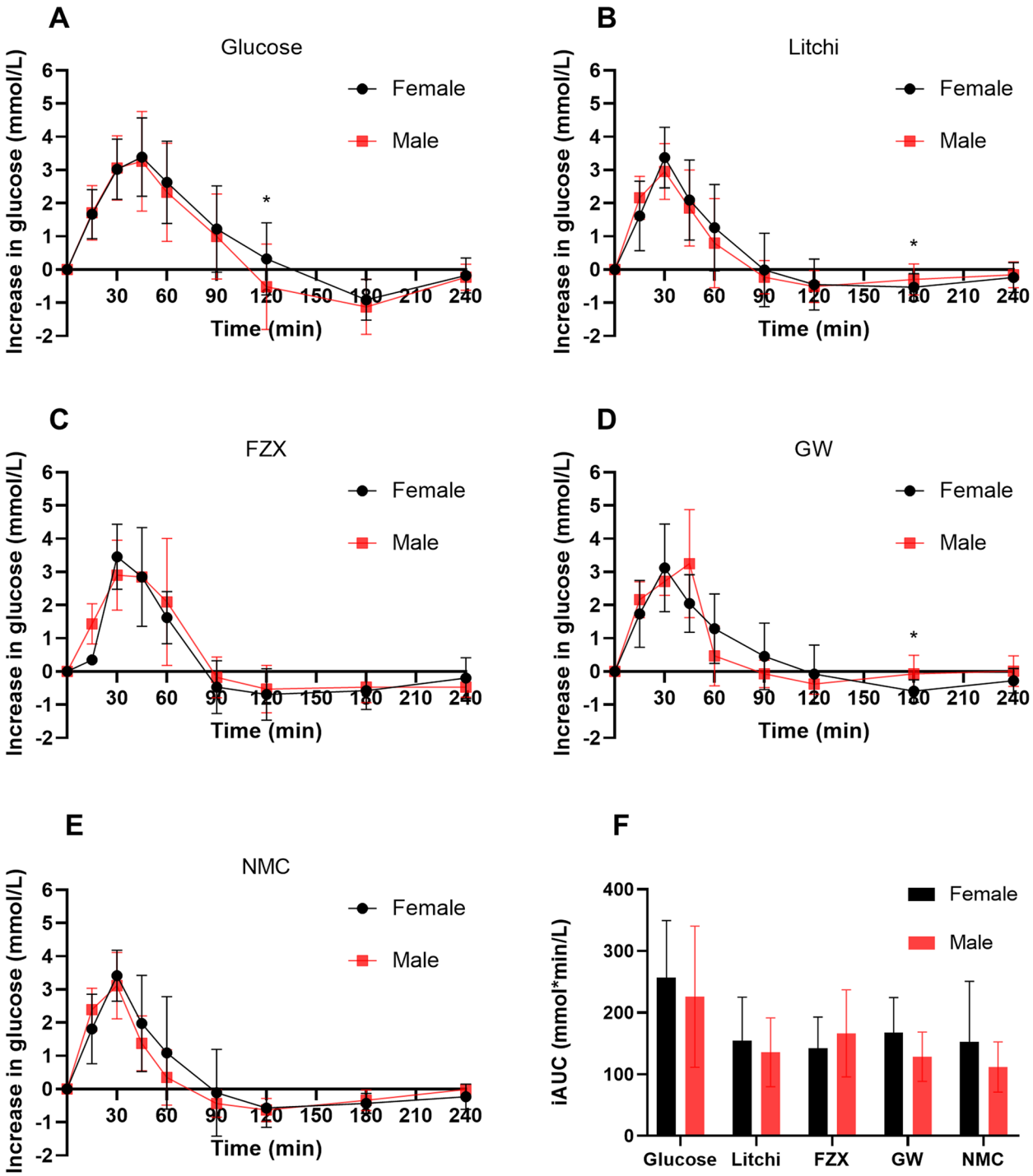


Fig. 4 Differences in glycemic increment curves between male and female groups. Glycemic increment curves of **A** the combined results of 2 glucose tests, **B** the combined result of 3 litchi tests, **C** litchi 'FZX' test, **D** litchi 'GW' test, **E** litchi 'NMC' test, grouped by gender. **F** iAUC of blood glucose response grouped by gender (240 min). *$P < 0.05$

the potential to be used in anti-tumor treatment [8, 44]. Our study revealed that several cancer-related proteins were changed after consuming litchi, which provided population-level evidence for further elucidating the potential molecular mechanisms underlying the anti-tumor activity of litchi. In addition,

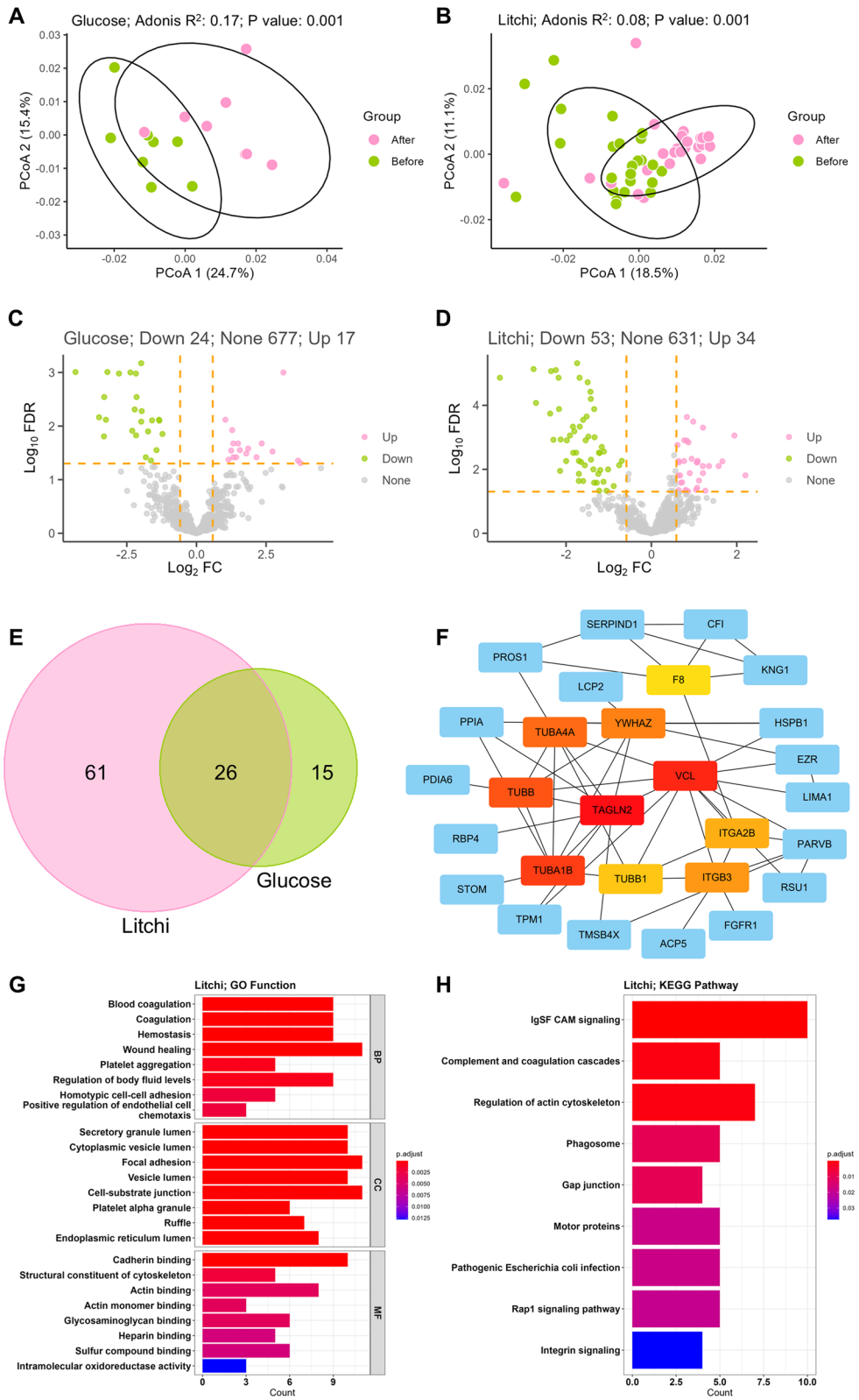


Fig. 5 Proteome alterations in human plasma in the glucose and litchi tests. **A**-**B** PCoA of plasma proteome before (at 0 min) and after (at the 240th min) glucose and litchi tests. **C**-**D** Proteome alteration at the 240th min in glucose and litchi tests, as compared to the 0th min. **E** Overlap of proteome alteration in glucose and litchi tests. **F** PPI network, **G** GO enrichment analysis, and **H** KEGG pathway analysis of the differentially expressed proteins related to the function of litchi

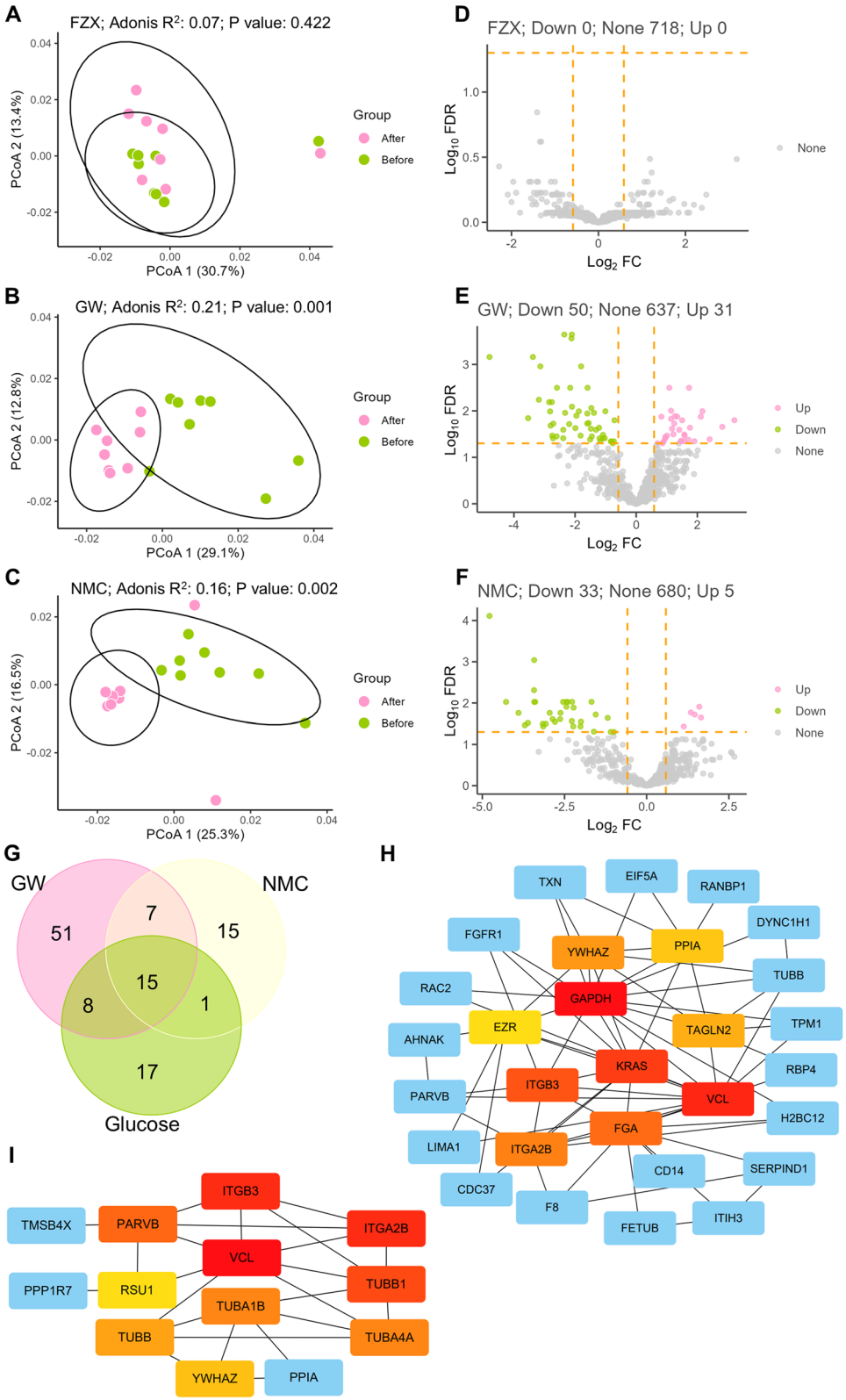


Fig. 6 Differential and shared proteome alteration among three litchi tests. **A-C** PCoA of plasma proteome before (at 0 min) and after (at the 240th min) 3 litchi tests. **D-F** Proteome alteration at the 240th min in 3 litchi tests, as compared to 0 min. **G** Overlap of proteome alteration in litchi 'GW' and 'NMC' tests. **H-I** PPI network of the differentially expressed proteins related to the function of litchi 'GW' and 'NMC'

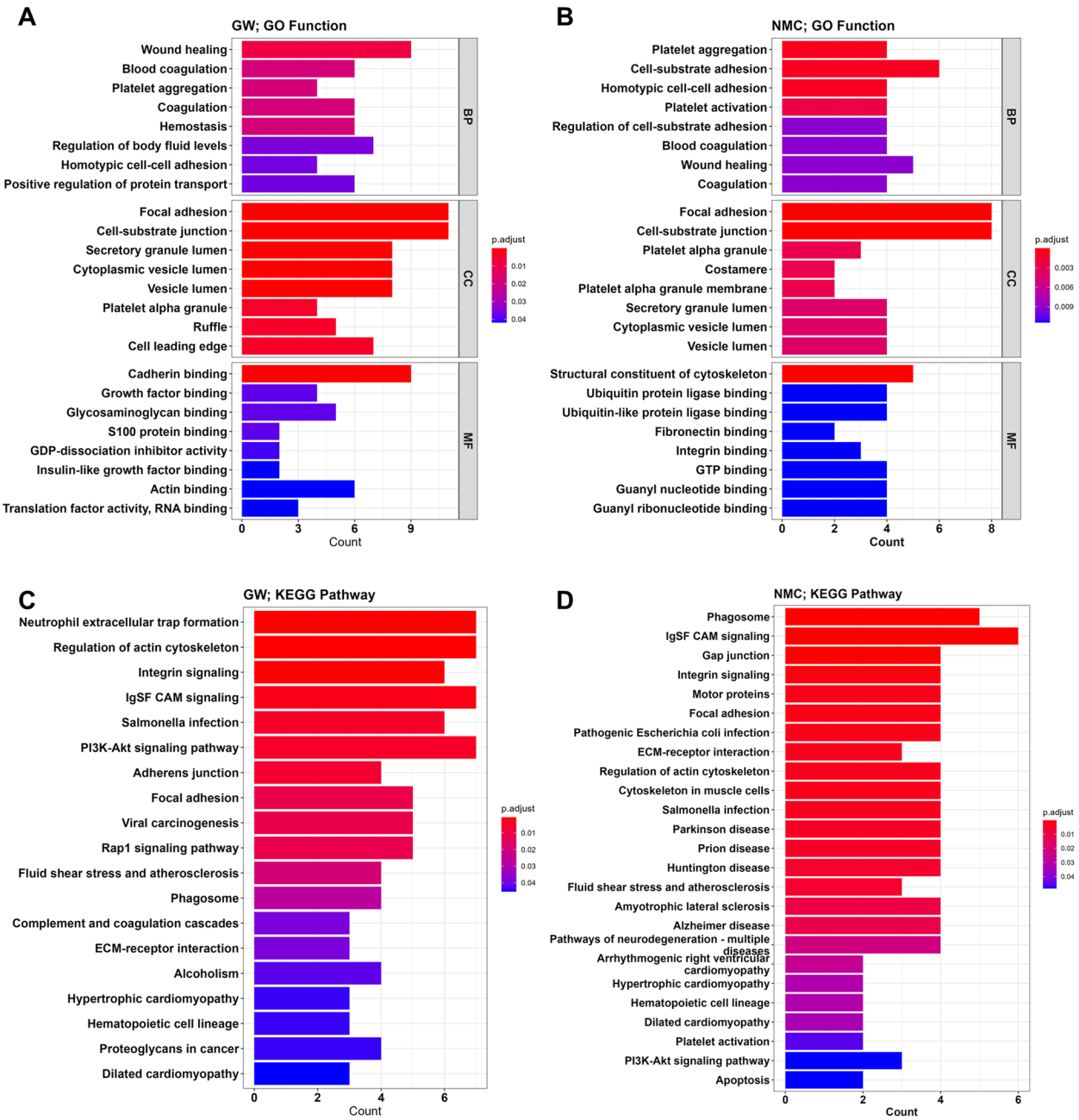


Fig. 7 GO function enrichment and KEGG pathway analysis. **A-B** GO enrichment analysis, and **C-D** KEGG pathway analysis of the differentially expressed proteins related to the function of litchi 'GW' and 'NMC'

some other proteins identified by our study, including BASP1, insulin-like growth factor-binding protein 7 (IGFBP7), heat shock protein beta-1 (HSPB1), and TUBA1B were also involved in the anti-aging and anti-tumor process as reported by previous studies [45–47]. Further research is needed in the future to explore the potential of the bioactive substances in litchi as adjuvant drugs for clinical anti-tumor immunotherapy.

Additionally, litchi also has various other potential health benefits. Xiong et al. [48] and Chen et al. [49] found that the polyphenols and flavonoids in the extract of litchi could improve cognitive function and inhibit the excessive phosphorylation of Tau protein, which was associated with the development of Alzheimer's disease (AD). Many studies demonstrated that plant-derived polyphenols, flavonoids and other

phytochemicals could regulate HSPB1 and brain-derived neurotrophic factor (BDNF) to improve neurological function, including improvements in spatial learning and memory [50–52]. Our study identified the alteration of HSPB1 and BDNF by proteome, furnishing clues and evidence for further research on the potential mechanism by which litchi improve cognition in the future. The GO function enrichment and KEGG pathway analysis methods can cluster multiple proteins based on their functions and further associate them with specific biological phenotypes, thereby presenting a clear functional orientation. Our study showed that the functions of the identified proteins mainly included blood coagulation, coagulation, hemostasis, platelet aggregation, and platelet alpha granule, which were closely related to the incident and development of atherosclerosis [53]. Our previous research found that proanthocyanidins extracted from litchi could ameliorate atherosclerosis in apolipoprotein-E knockout mice [54, 55]. Sung et al. [56] revealed that the extract from litchi inhibited platelet aggregation and significantly prolonged coagulation times in rat platelet-poor plasma, which indicated the antithrombotic effect of the litchi extract and suggest that litchi may be a new natural source for the development of antiplatelet, anticoagulant and thrombolytic therapeutics for thrombotic and cardiovascular diseases.

Other GO function identified in this study, such as cell-substrate junction, cadherin binding, and structural constituent of cytoskeleton interrelated and jointly contributed to the normal physiological structure and function of the organism. Protein VCL is an important constituent of both cell-cell and cell-matrix junctions, where it plays crucial roles in the regulation of cell adhesion and migration. When activated, it mediates the linkage between cadherins (cell-cell) or integrins (cell-matrix) and the actin cytoskeleton through interactions with various proteins [57]. These functions are closely related to vascular damage and cardiovascular health [58].

Conclusion

This study quantified the sugar compositions, total proteins, fatty acids, various amino acids and vitamin of 3 litchi cultivar, and mapped a nutritional profile to visualize various amino acids and vitamin compositions. The results showed that litchi pulp was rich in glucose, fructose, sucrose, vitamin, and amino acids. VB_3-niacin was the most abundant vitamin in litchi, accounting for 52%–78% of the total vitamin content, followed by VC and VB_6. Additionally, litchi contained a variety of essential amino acids for the human body. These indicated that litchi possessed rich nutritional value.

We further assessed the radical scavenging ability of litchi water extracts and found that the vitamin contents were largely consistent with the results of antioxidant activity, which litchi 'FZX' had the highest vitamin content and the strongest antioxidant activity among 3 litchi cultivars.

A clinical controlled trial was conducted to monitor the dynamic blood glucose fluctuations after litchi consumption, and a trough phenomenon was observed during the 120–180 min, which suggests that the risk period of the onset of "litchi disease" may be 2 h after eating a large amount of litchi on an empty stomach. The GI and GL values were calculated to guide the rational consumption of litchi fruit. The average GI of litchi was 66, classified as a medium GI food, and the average GL was 11, just reaching the lower limit of medium GL foods. To our knowledge, these values are the first reported.

Additionally, an in-depth characterization of plasma proteome alteration after litchi consumption was further performed to comprehensive assess of the health benefits of litchi, and some proteins were identified, including BASP1, TSKU, MTSS1, VCL, PPIA, TUBA1B, TAGLN2, TUBB, TUBA4A, YWHAZ, ITGB3, ITGA2B, TUBB1, and F8. These proteins indicated that litchi possessed various health benefits such as anti-tumor, anti-aging, improvement of cognitive function, improvement of atherosclerosis and protection of cardiovascular health. This exploratory work furnished some implications for functional foods development in the future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44462-026-00068-2.

Supplementary Material 1.

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Authors' contributions

L.W. and S.L. wrote the main manuscript text. L.W., W.Y., S.L., W.Y. and W.X. completed the methodology, and data curation. L.W., W.Y., and S.L. completed the formal analysis and visualization. J.W., L.L., and Z.W. completed the conceptualization, and supervision. J.W. and F.Q. provided funding acquisition. All authors reviewed the manuscript.

Data availability

Data will be made available on request.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Decla-ration of Helsinki, and approved by the Ethics Committee of the National Institute of Nutrition and

Health of the Chinese Center for Disease Control and Prevention (protocol code 2023–006). The participants were provided with a privacy notice informing them of data protection and the purpose of the study as well as a consent form. All participants have signed informed consent forms.

Competing interest

The authors declare no competing interests.

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