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ORIGINAL PAPER

Effects of ball milling micronization on amino acids profile and antioxidant activities of *Polygonatumcyrtonema* Hua tuber powder

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Abstract
Recently, edible and medicinal plants have attracted intensive attention as functional foods because of their biological activities. However, there has been little research on the influence of ball milling micronization on the properties of the *Polygonatum* powder. In this study, ball milling was used to micronize *Polygonatumcyrtonema* Hua tuber coarse powder to observe the effects on amino acids profile and antioxidant activities. Different ball milling times produced significant differences in the total polyphenols, amino acids, and soluble sugar contents ($P < 0.05$). The multiple linearities between total amino acids content, soluble sugar content, and ABTS radical scavenging activity revealed the relationship between antioxidant activities and ingredients. Moreover, the different cluster tendency by principal components analysis (PCA) and cluster analysis indicated the effects of different ball milling times. It was also highlighted that asparagine, α-aminobutyric acid, and valine can be used as the biomarkers to describe the effects of ball milling time by orthogonal partial least squares discriminant analysis (OPLS-DA). In summary, amino acids profile and antioxidant activities of *Polygonatumcyrtonema* Hua tuber powder were significantly influenced by different ball milling time. The present study provided a foundation for research on the potential application of *Polygonatum* tuber micronization and the development of dietary supplements and functional foods.

Keywords *Polygonatum* · Amino acid · Orthogonal partial least squares discriminant analysis · Biomarker · Antioxidant activity

Introduction

Food products with active ingredients obtained from edible and medicinal plants provide valuable resources for the development of dietary supplements for health protection [1]. Generally, the physicochemical properties, such as solubility, flowability, water holding capacity, and extractability of some valuable ingredients can be affected by the particle size of medicinal plants extracts [2, 3]. The nutritional efficacy of active ingredients is the main factor that must be considered when designing functional foods [4]. Micronization, as alternative powder strategy, benefits the improvement and retention of food functionalities [5, 6].

Many particle size reduction techniques have been proposed for micronization, such as antisolvent micronization [7], ball milling [8], high-pressure homogenization [9], supercritical solution micronization [10], and airflow pulverization [11]. Among the techniques, ball milling methods have been widely used to treat natural food materials or food additives to explore their properties [12].

Ball milling micronization has been developed to reduce particle sizes ($P < 0.05$) to alter the functional properties of food samples for new applications in food and life sciences [13, 14]. It was reported that the particle size of wheat flour had a significant effect on its functional properties [15]. Wu et al.[16] also investigated the improvement of the ball milling process on the digestible properties of scallop

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(*Chlamysfarreri*) proteins, which provided valuable information on the application of scallop proteins as an ingredient in food products.

Moreover, ball milling processing produced changes in antioxidant capacity and bioavailability [17]. Bioactive compounds in edible and medicinal plant products are increasingly being used for their functional properties. Research on the application of functional foods has attracted increased attention because of their health improving properties [18]. *Polygonatum* species, as multipurpose edible and medicinal plants, can be applied in pharmaceutical materials for their anti-aging, antioxidation, antifatigue, antimicrobials, anticancer and anti-vascular activities [19].

However, to date, there has been little research on the influence of ball milling processing on the properties of *Polygonatum* powder. Studying ball milling micronization treatments could provide insights into their application in the food industry. Therefore, the present study aimed to observe the particle size distribution and antioxidant activities of micronized *Polygonatum* tuber coarse powder after different ball milling times. In addition, we used principal components analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) to effectively describe the change of amino acids profile and related metabolites in different treatments.

Materials and methods

Sample preparation

Plant materials

Fresh Polygonatum cyrtonema Hua tuber samples were collected and sent to the laboratory after cleaning to remove soil and other impurities. All samples were oven-dried at 50–60 °C for 96 h (Average moisture content < 8%). The dried samples were stored at − 20 °C for further research.

Preparation of micronized samples

A laboratory grinding mill was used to grind the dried *Polygonatum cyrtonema* Hua tuber samples for 1 min. A pause of 10 s was performed for each 10 s treatment to dissipate the heat generated. The coarse powder was subjected to pulverization by ball milling (Ball mill mixer XQW–2A, Changsha Tencan Powder, Changsha, China) for 20, 60, and 120 min. For ball milling, powder and agate balls (1 mm), in a volume ratio of 4:1, were placed into an agate grinding bowl (50 mL). The samples were then ground by the agate balls at 450 rpm and the micronized samples were collected at 20, 60, and 120 min. Samples without ball milling (0 min) were used as the control. After the collection, the micronized samples were stored in a freezer at − 20 °C in double Ziploc bags for further use. The samples were mixed with 80% ethanol–water solution at a final concentration of 50 mg mL^{-1}. The mixture was vortex mixed for 2 h at room temperature and used for antioxidant activity tests after filtration through Whatman filter paper.

Morphological characterization

Different ball milling treatments can produce different surface morphologies, which can be characterized using scanning electron microscopy. Different treatment samples (0, 20, 60, 120 min) were prepared for the characterization using a scanning electron microscope (FEI 430 NanoSEM, Hillsboro, OR, USA).

Particle size distribution

After characterization by scanning electron microscopy, three images at the same magnification for one sample were used to count and measure the particles. The diameter size distributions of 90 particles were analyzed using the Nano Measurer software (https://nano-measurer.software.informer.com/). Thirty continuous particles in every imaging center were gathered and ninety particles for three images of the same sample were counted and analyzed.

Total polyphenols, total amino acids, and soluble sugar contents

Folin-Ciocalteu's reagent solution was used to treat the samples according to previous research [20] for the determination of the total polyphenols content at 765 nm. Total amino acids content was performed according to the ELISA Kits instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Soluble sugar content was determined using the anthrone colorimetric method [21].

Free amino acids and related metabolites

Free amino acids and related metabolites were determined using an amino acid analyzer according to previous research [22]. In brief, the samples were mixed and milled with HCl solution (0.02 mol L^{-1}), transferred into 10 mL capped glass tubes, and 4 mL of ultra-purified water was added. The solution was then vortex mixed at 4 °C for 10 min and centrifuged at 4000×*g* for 15 min. One milliliter of the supernatant was mixed with 1 mL of N-hexane, the mixture was centrifuged at 11,000×*g* for 10 min, and then the supernatant was discarded. The pellet was mixed with 1 mL of 8% sulfosalicylic acid and vortex mixed for 10 min. After that, the solution was centrifuged at 16,000×*g* for 10 min at 4 °C. The supernatant

was dried (TVE–1100, Eyela, Tokyo, Japan), dissolved in citrate buffer (pH 2.2), filtered through a 0.22 μm membrane, and then analyzed in an automatic amino acid analyzer (S-433D, Sykam, German) at 570 and 440 nm. A mixed amino acids solution (Sykam, German) was used as the standard.

Fourier transform infrared spectroscopy (FTIR) analysis

The samples with the same weight collected from different milling times were used for the FTIR verification. Spectra between 4000 and 500 cm^{-1} were recorded using an FTIR spectrometer (Nicolet iS5, Thermo Fisher Scientific Inc., MA, USA). The spectral resolution was 4 cm^{-1}. Measurements were performed at room temperature using 1 mg samples, and potassium bromide powder was used as the control. Experiments were collected and evaluated in triplicate.

Antioxidant activities

According to previous researches [23, 24], the DPPH radicals (2,2-diphenyl-1-picrylhydrazyl) with the concentration of 0.04 mg mL^{-1} and samples solution (50 mg mL^{-1}) were used to determine the DPPH scavenging activity at 517 nm. The ABTS radical (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity was also proposed according to the references [23, 24] and the value was determined at 734 nm. Total antioxidant capacity was also verified using a T-AOC ELISA Kit (Shanghai MLBio, Shanghai, China) according to the instructions.

Statistical analysis

PCA was used to identify and determine the amino acids and related metabolites at ball milling times, to better understand the amino acid changes induced by the treatments [25]. In addition, OPLS-DA was employed to effectively screen the biomarkers of the amino acids and related metabolites in different treatments by SIMCA-P 14.1 software (Umetrics, Sweden). In addition, variable importance in projection (VIP) > 1.5 influenced mostly the extracted OPLS-DA models according to previous research [26, 27].

Moreover, stepwise regression analysis using the Matlab software was used to establish a linear relationship among the total polyphenols, total amino acids, and soluble sugar contents and antioxidant activities. The mean values and standard deviations of each analysis are reported. All data were analyzed using analysis of variance (ANOVA). A difference was considered statistically significant at $P < 0.05$.

Results and discussion

Morphological characterization and particle size distribution

Particle size distribution of the powder became narrower because of the increase in shearing force with the extension of the milling time and there were increased numbers of smaller particles as milling time increased (Fig. 1a–d). As shown in Fig. 1e, 60% of the particles in the coarse powder were more than 100 μm in size. The samples collected at 60 min contained particle with more than 88% distribution below 50 μm and others were 50–100 μm. Thus, milling achieved the requirement of micronization, and the percentage of particles above 50 μm in size was dramatically decreased in the 60 min samples compared with that in 20 min samples. The particle size distribution was further narrowed in 120 min, in which particle sizes below 50 μm increased to 99%, while there were only 4% below 50 μm in the 0 min samples. The different ball milling times induced different particle size distributions between the treated samples and the coarse powder ($P < 0.05$).

Total polyphenols content

Differences were observed for the total polyphenols content when the samples were treated with different ball milling times. The total polyphenols content increased with the extension of the milling time (Fig. 2a). The total polyphenols content increased dramatically when the collection time increased from 20 to 60 min, probably because of the smaller particle size. The more homogeneous particle size distribution produced a marked increase in the total polyphenols content. The total polyphenols content collected at 120 min (29.29 ± 0.07 mg g^{-1}) was also significantly increased compared with that of the time 0 min ($P < 0.05$). It was likely that cell breakage occurred because of the smaller particle size, inducing the release of polyphenols. The results were consistent with those of previous research that showed that micronization increased the total polyphenols content [28].

However, the increase in the total polyphenols content between the 120 and 60 min samples was not so dramatic as that between the 60 and 20 min samples. One possible explanation was that increased milling time destroyed some of the polyphenol compounds. This result was in line with previous research. Ball milling of achenes produced more bioactive compounds, including ellagic acid (19.72%) and total phenols (52.37%) [29]. In *Inonotus obliquus*, finely ground powders produced by ball milling

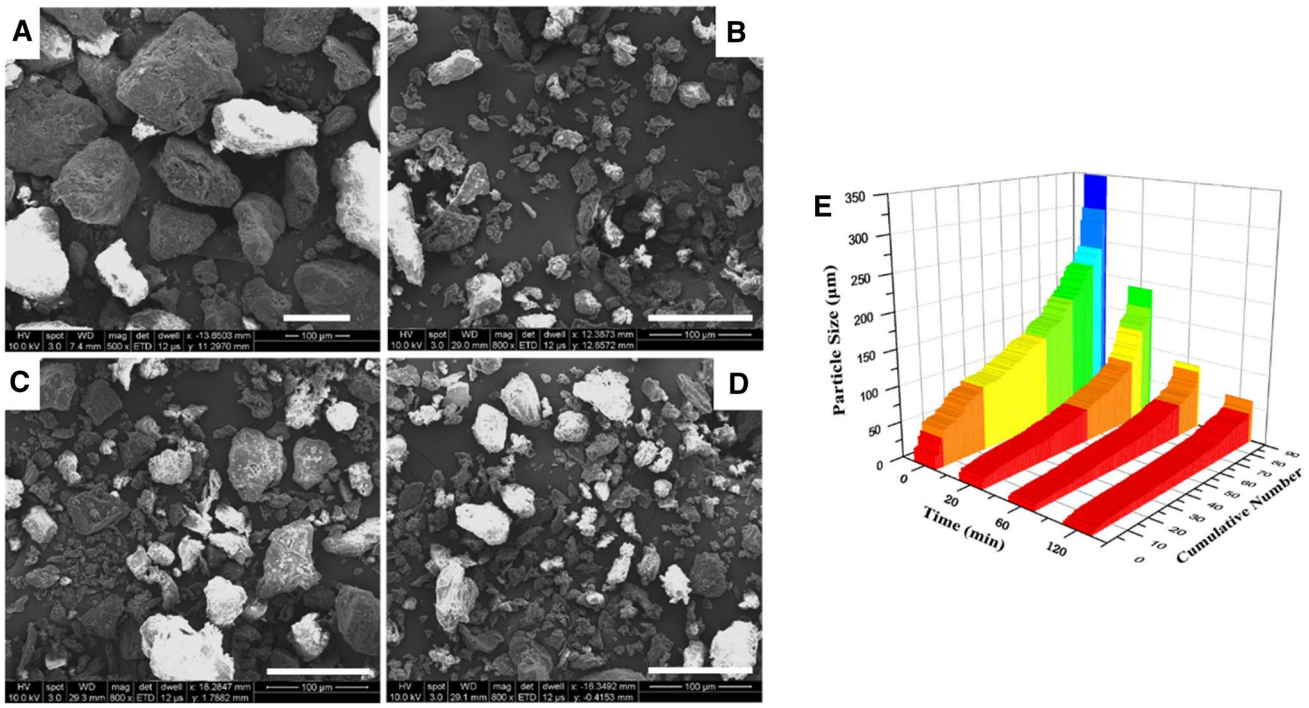

Fig. 1 Morphology characterization by scanning electron microscope for the *Polygonatum* tuber powder with different ball milling times. **a** coarse powder **b** milling for 20 min **c** 60 min, and **d** 120 min. **e** The particle size distribution for different ball milling times. Scale bar (bottom right) is 100 μm

coarse powders resulted in a four-fold increase in the yield of polyphenols [30]. Thus, using an appropriate ball milling time could be a powerful strategy to increase the levels of extracted bioactive compounds, especially total polyphenols.

Total amino acids content

Total amino acids content was an important indicator of food nutritional content. The determination of total amino acids has been proposed for nutritional verification [31]. Ball milling changed the total amino acids content (Fig. 2b) and a significant increase in total amino acids content was observed in the samples collected at 20 min (1027.48 ± 11.48 μmol g^{-1}) compared with that of control samples. Nevertheless, the total amino acids content was decreased at 60 min samples ($P < 0.05$) and stayed at the same level in the 120 min samples. Thus, prolonged milling time more than 60 min produced a negative effect on the total amino acids content.

Soluble sugar content

As reported in previous researches, there were many polysaccharides and oligosaccharides in *Polygonatum* tubers [32, 33], which contributed to the antioxidant activities and other bioactivities. Moreover, soluble sugar content can also be used to verify the quality of functional plant products. The results of Fig. 2c indicated that the soluble sugar contents were significantly different between the treatments. The soluble sugar content of the samples collected at 60 min reached 472.58 ± 2.78 mg g^{-1}. No difference in total sugar content was observed between the coarse powder and the samples collected after 20 min of milling ($P > 0.05$). However, the sugar content of the samples increased significantly in the 60 min samples but then decreased in the 120 min samples ($P < 0.05$). Thus, extended the milling time beyond 60 min probably resulted in sugar compounds degradation.

Amino acids and related metabolites

Results of amino acids and related metabolites of the samples were shown in Fig. 3 according to the standard curves. Twenty-five amino acids and related metabolites were determined from all the samples. The four most abundant free amino acids in all samples were asparagine, glutamic acid, arginine, and histidine. Further analysis was performed to describe the change produced from the treatments.

PCA and cluster analysis

PCA was employed to identify and describe the amino acid and related metabolites of different ball milling treatments.

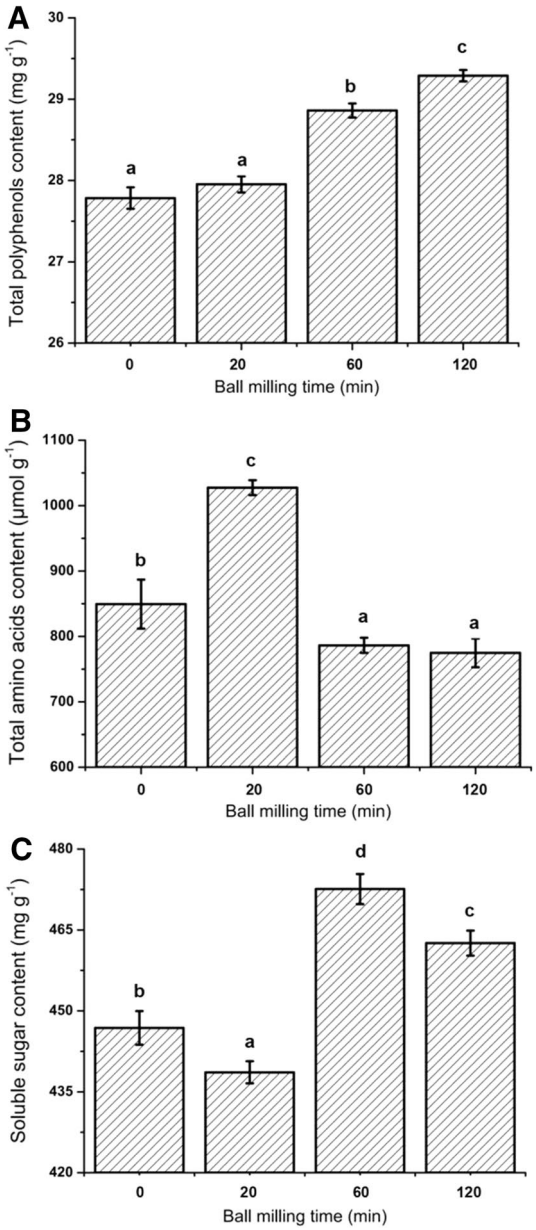

Fig. 2 Total polyphenols content (**A**), total amino acids content (**B**), and soluble sugar content (**C**) of the treated *Polygonatum* tuber powder samples. Different lower-case letters (a–c) in the same figure represent significant differences ($P < 0.05$)

The results showed that the first three principal components explained 97.97% of the variability, as assessed with the aid of log transformation and data centering. As shown in Fig. 4a, the first principal component (PC1) explained 63.66% of the variability, with positive coefficients for all compounds. The second principal component (PC2) and the third principal component (PC3) explained 32.70% and 1.61% of the variability, respectively. These findings supported the view that the first three principal components can be employed to describe amino acids and related metabolites in the ball milling treatments. The four treatments were distributed in different regions, indicating the feasibility of PCA. The results also showed that there was a tendency to cluster between the 60 min group and 120 min group because of their adjacent distribution (Fig. 4a). Thus, we concluded that PCA could effectively discriminate amino acids and related metabolites of the different treatments.

The determination of amino acids and related metabolites indicated that, compared with the control samples, the proline content of the samples had increased by 82% at 20 min, 110% at 60 min, and 66.5% at 120 min ($P < 0.05$). The initial increase in proline content was followed by a decrease at 120 min, which was the same trend as that observed for the change of the soluble sugar content and total antioxidant capacity.

As shown in Fig. 4b, a heatmap produced by software HemI [34] was used to describe the changes in the levels of 25 amino acids and related metabolites under the different treatments. The average linkage in hierarchical cluster analysis (Pearson distance) was determined, which indicated that the control samples and 20 min samples tended to cluster, while the 60 and 120 min samples tended to cluster. The clustering results were also in line with the PCA results.

OPLS-DA results

OPLS-DA was capable of discriminating amino acids and related metabolites as potential biomarkers of ball milling treatments. OPLS-DA results were shown in Fig. 5 and explained the differences between groups. The coefficient plot showed the increase or decrease for each of the amino acids and related metabolites identified. As shown in the OPLS-DA analysis, the VIP scores allowed a ranking of amino acids and related metabolites according to how much they influenced different samples. The VIP scores of amino acids and related metabolites > 1.5 were described in Fig. 5 BDF and the results indicated the different effects of ball milling time at the biochemical level.

As shown in Fig. 6, the levels of asparagine, α-aminobutyric acid, and valine were significantly different ($P < 0.05$) between 20 and 60 min groups, highlighting the significant changes of amino acids and related metabolites by different ball milling times. These metabolites can be used as biomarkers to describe the effects for amino acids profile produced by ball milling.

The change in amino acid levels was in line with amino acid synthesis being very sensitive to processing [26]. MetaboAnalyst software 3.0 showed the potential pathways including valine, leucine and isoleucine biosynthesis, alanine, aspartate, and glutamate metabolism, using the biomarkers screened by OPLS-DA. Here, we have proposed the potential pathway associated with ball milling time by KEGG (Kyoto Encyclopedia of Genes and Genomes)

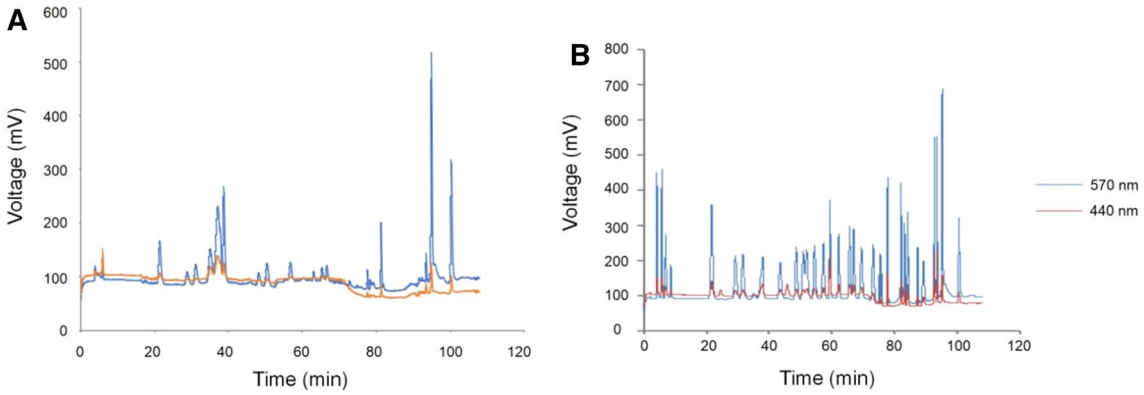

Fig. 3 Amino acids and related metabolites of the treated samples (**a**) and the standard solutions (**b**) using the amino acid analyzer

database [35]. An overall profile of the metabolic caused by ball milling was shown in Fig. 7 and the results demonstrated that amino acid synthesis and degradation were associated with ball milling processing.

FTIR analysis

As shown in the FTIR spectrum (Fig. 8), the same weight of the samples collected from different milling times produced different curves, which could be used to verify the related fingerprint spectrum of the chemical bonds [36]. In the spectrum, the strong absorption band near 3425 cm^{-1} could be attributed to the hydrogen bonds of polyphenols compounds or amide compounds. The adsorption bands near 1452 cm^{-1} and 1641 cm^{-1} mainly represented aromatic compounds for verification of C=C stretching of aromatic rings. The absorption band near 2930 cm^{-1} was the O–H bond of carboxylic acid or the C=N bond of the amide compounds, and the absorption band near 1033 cm^{-1} was caused by carbonyl compounds, aromatic compounds, or vinyl-containing compounds. Therefore, it could be inferred that the samples probably consisted of polyphenol compounds, amide compounds, carboxylic acid, carbonyl compounds, aromatic compounds, and vinyl-containing compounds.

The absorption of the chemical bonds changed according to the length of ball milling. The absorbances at 1033 cm^{-1}, 1452 cm^{-1}, 1641 cm^{-1}, and 2930 cm^{-1} of the samples collected at 20 and 60 min were increased compared with those of the coarse powder. However, the absorbances were decreased for the samples collected at 120 min and were even smaller than those of the coarse powder, indicating that 120 min milling time induced significant changes compared to that of the samples collected at 20 or 60 min and the control samples ($P < 0.05$). The change in the absorption also reflected the influence of different ball milling times among the samples.

Antioxidant activities

Free radical scavenging activity was a general mechanism used to measure the activities of natural antioxidants. Antioxidant activity can also be used as an important factor in characterizing the nutritional efficacy of nutrients [20]. DPPH and ABTS radical scavenging activities and total antioxidant capacity have been used to assess the changes in antioxidant activity for functional ingredients [37, 38].

Figure 9a showed the DPPH radical scavenging activity of *Polygonatum* tuber powder between different treatments. Significant differences were observed between the control samples and treated samples ($P < 0.05$). However, no difference was observed for the samples collected at 60 and 120 min ($P > 0.05$). Moreover, those samples with small diameter particles showed promising DPPH radical scavenging activity compared with the other samples ($P < 0.05$). Our results were consistent with previous reports, which showed that increasing the ball milling time can improve the DPPH radical scavenging activities of persimmon seed, peel, and calyx powders [39]. It was also reported that ball milled achenes could be added into fruit wine to increase the DPPH radical scavenging activities [29].

Total antioxidant capacity was also employed to represent the nutritional efficacy of the finely ground powders of *Polygonatum* tuber powder. The total antioxidant capacity of the samples collected at 20 min were significantly higher compared with those of the samples collected at 0 min (Fig. 9b). Moreover, the samples collected at 60 min presented the highest total antioxidant capacity. However, as milling time increased to 120 min, the total antioxidant capacity was decreased compared with that of the samples collected at 60 min ($P < 0.05$). These findings describing that milling increased the total antioxidant capacity were consistent with previous research [40].

Samples collected at 60 min contained the highest ABTS radical scavenging activity among the treated samples

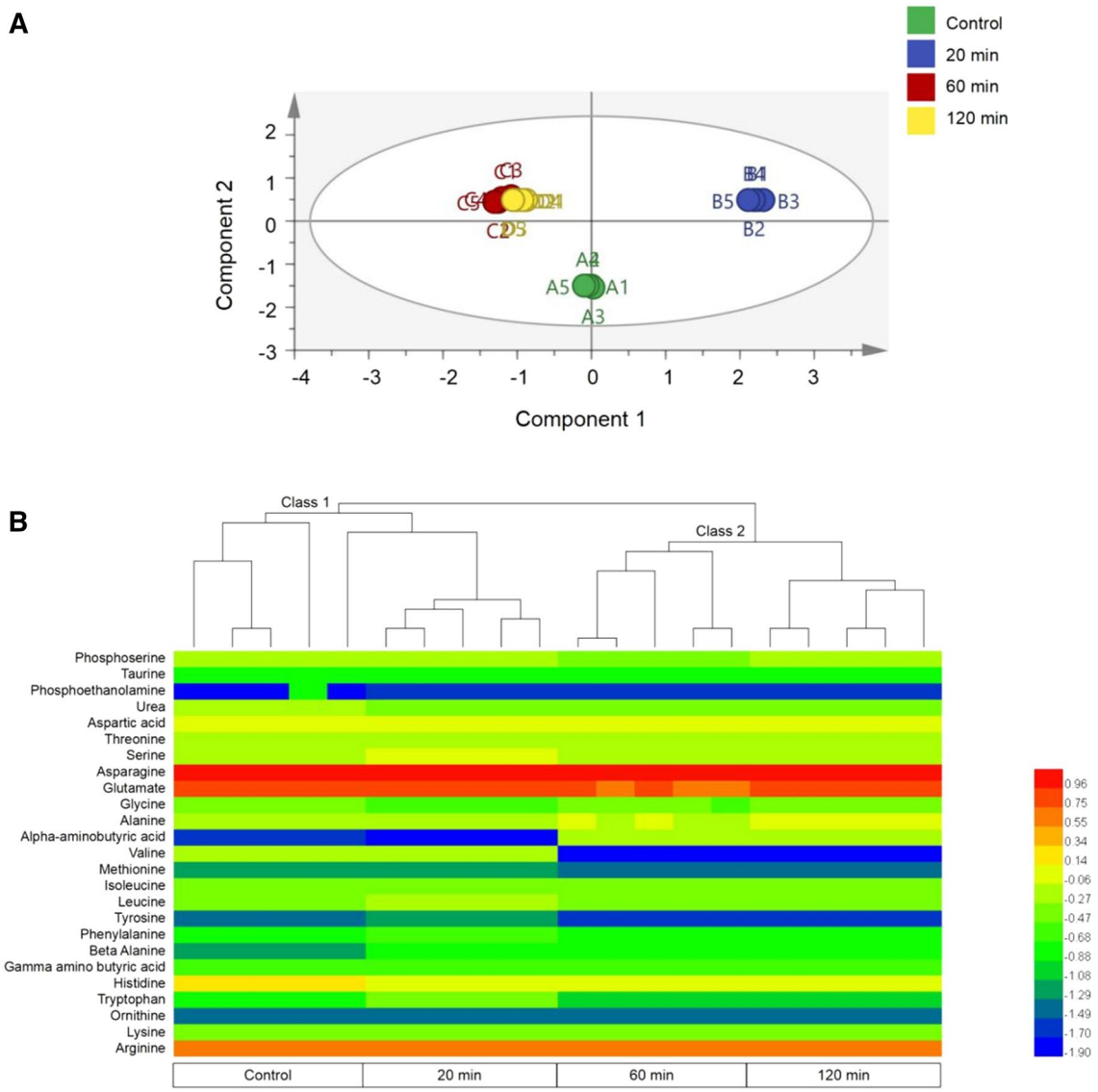

Fig. 4 **a** Principal components analysis score plot of the top two principal components for amino acids and related metabolites for all treatments. **b** A heatmap of amino acids and related metabolites for different ball milling times, generated using the software Heatmap illustrator (HemI 1.0). Class 1 and 2 represent the different tendency to cluster, as assessed using hierarchical cluster analysis

(Fig. 9c). Significant differences were observed between the samples collected at 60 min and the other treatments ($P < 0.05$), indicating that extension of the milling time induced a negative effect on the ABTS radical scavenging activity. The current study of *Polygonatum* tuber powder presented excellent ABTS radical scavenging activity compared with that of many previously studied fruits, such as blueberry [24] and *Carissa opaca* fruit [41].

Stepwise regression analysis

Generally, the increase in the total polyphenols, soluble sugar, and total amino acids contents resulted in an increase in the radical scavenging activity. The results of DPPH and ABTS radical scavenging activities and total antioxidant capacity highlighted the promising antioxidant activities of *Polygonatum* tuber powder. The samples collected at 60 min provided the most promising antioxidant activities and negative effects were produced by the extension of the ball milling time. Using correlation analysis and coefficient simulation, linear relationships were established to clarify the influence of the antioxidant activities produced by modulating the total polyphenols, soluble sugar, and total amino acids contents.

Pearson analysis was employed to study the linear correlation coefficient and *P* value between the total polyphenols,

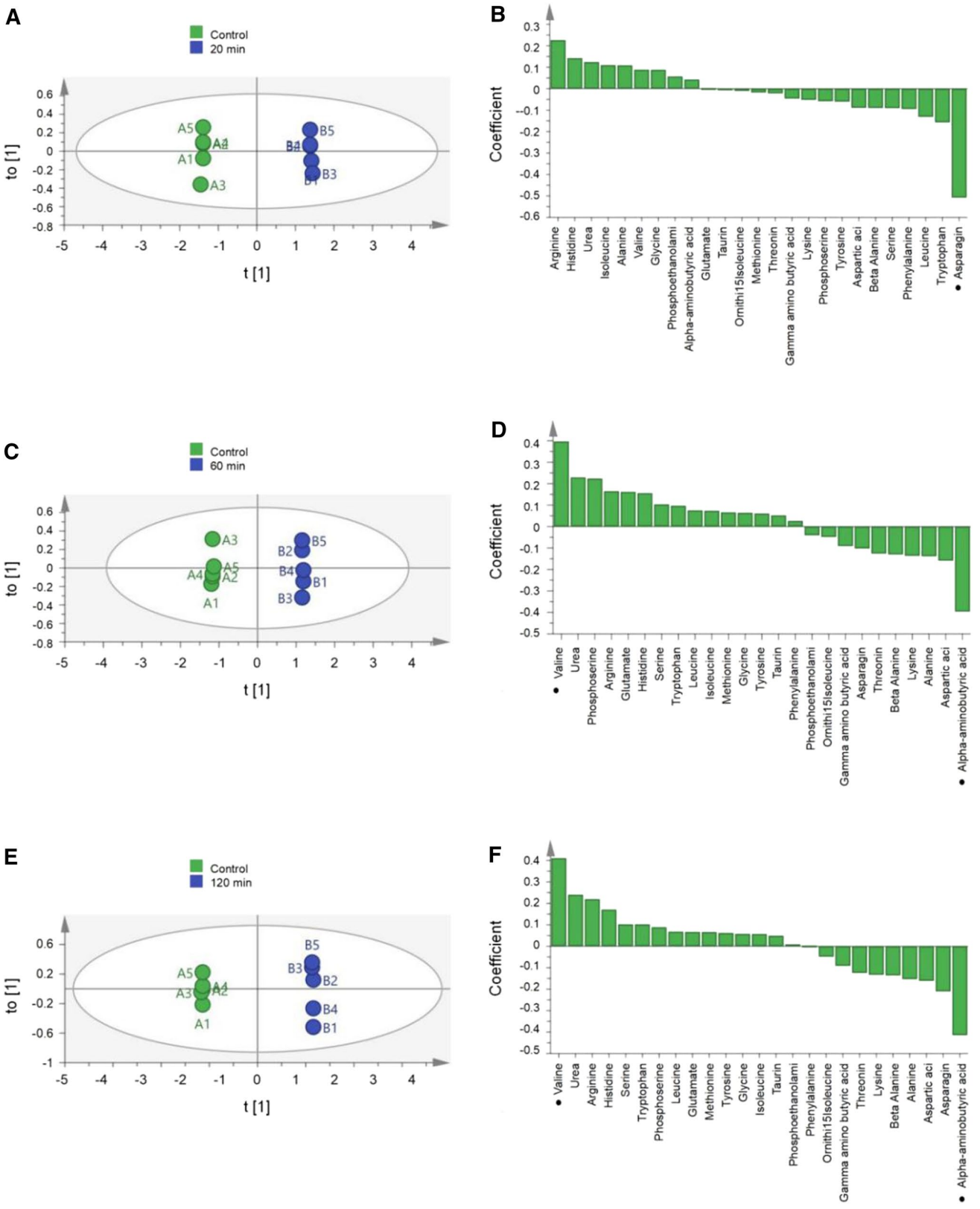

Fig. 5 OPLS-DA scores (**a**) and coefficient plot (**b**) of the control group and 20 min group. OPLS-DA scores (**c**) and coefficient plot (**d**) of the control group and 60 min group. OPLS-DA scores (**e**) and coefficient plot (**f**) of the control group and 120 min group. The black dots indicate which metabolites contribute significantly to the OPLS-DA models (VIP > 1.5). R^2 X = 0.985, Q^2 Y = 0.911. OPLS-DA = orthogonal partial least squares discriminant analysis

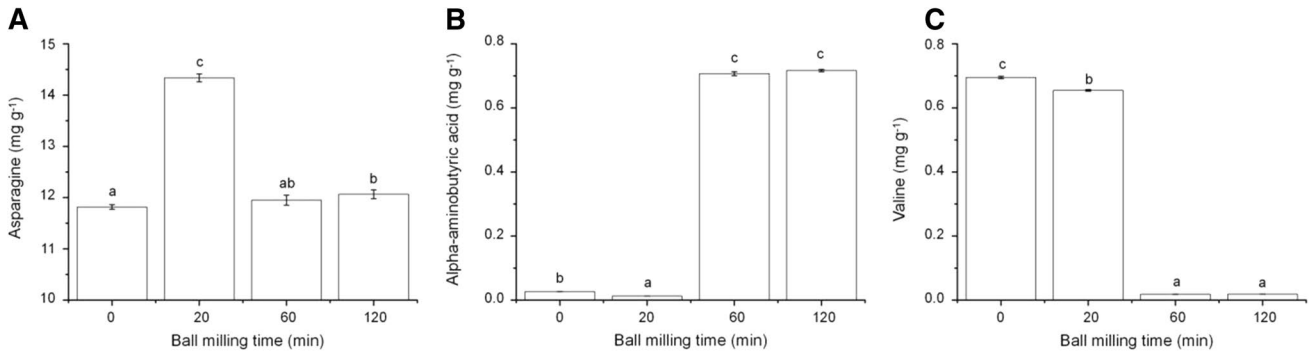

Fig. 6 Different concentration of the biomarkers from the different ball milling time **A** asparagine, **B** α-aminobutyric acid, and **C** valine. Letters a–d represent significant differences ($P<0.05$)

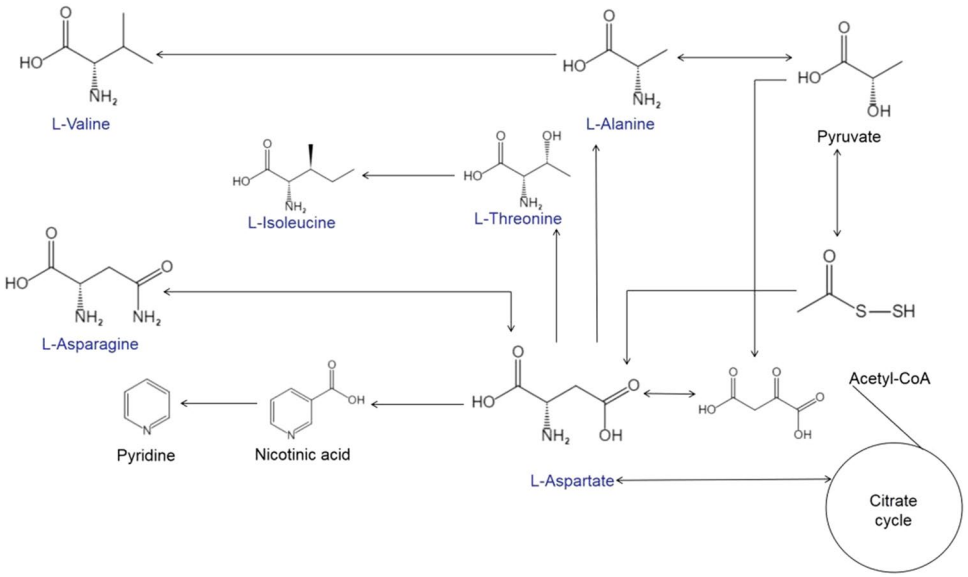

Fig. 7 Potential metabolic pathways of the biomarkers influenced by ball milling treatments produced from KEGG (Kyoto Encyclopedia of Genes and Genomes) database

soluble sugar, total amino acids contents, and antioxidant activities [42]. The results indicated that the linear relationship between the soluble sugar content and ABTS radical scavenging activity was significant ($P<0.05$) (Fig. 10a). The linear equation was as follows.

$$y = 0.2x - 7.6 \quad R^2 = 0.851$$

where y is the ABTS radical scavenging activity and x is the soluble sugar content.

The linear relationship between the total polyphenols content and DPPH radical scavenging activity was also significant (Fig. 10b). The linear equation was as follows.

$$y = 2.9x + 11 \quad R^2 = 0.889$$

where y is the DPPH radical scavenging activity and x is the total polyphenols content.

Stepwise regression analysis was used to establish the linear relationship between the functional ingredients and antioxidant activities. The stepwise regression analysis equation [43] representing the linear correlative relationship was as follows:

$$y = \beta_0 + \beta_1 x_1 + \beta_k x_k$$

where β_0 is regression constant, β_k is independent variables, x_k is the partial regression coefficient, and k is the number of the independent variables.

The multiple linear regression equation only containing the significant regression variables (Fig. 10c) was as follows.

$$y = 0.0208x_1 + 0.3341x_2 - 87.9051 \quad R^2 = 0.996$$

where y is the ABTS radical scavenging activity, x_1 is the total amino acids content, and x_2 is the soluble sugar content.

The results indicated that the linear correlative relationship was significant between the total amino acids content and the soluble sugar content and ABTS radical scavenging activity.

The changes in antioxidant activities were approximately consistent with the changes in the total polyphenol, soluble

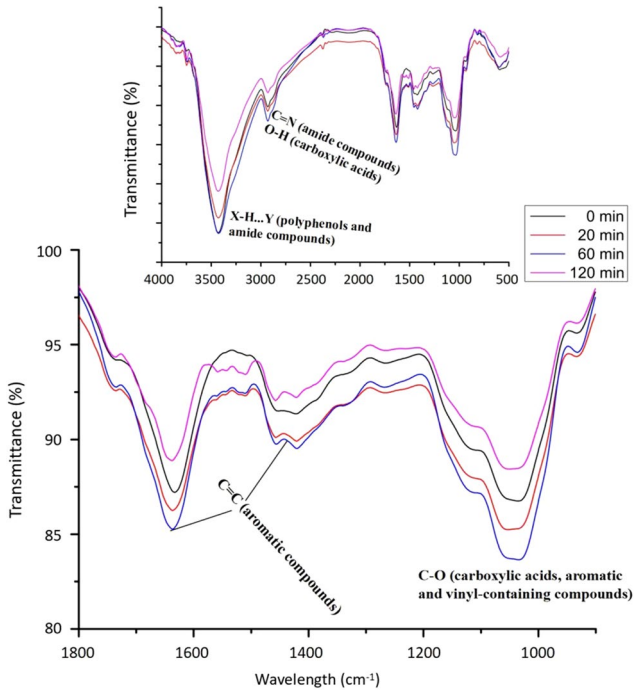

Fig. 8 Fourier transform infrared (FTIR) spectrum for the treated *Polygonatum* tuber powder samples with different ball milling processing. The bonds and the probable compounds in brackets at different wavelengths are shown

sugar, and total amino acid contents. Multiple linearities between the total amino acids content, soluble sugar content, and the ABTS radical scavenging activity was established. Moreover, the results indicated that ABTS and DPPH radical scavenging activities were significantly associated with soluble sugar content. Moreover, *Polygonatumcyrtonema* Hua polysaccharides presented good antioxidant activities, which agreed with the changes observed in previous studies [44, 45]. As shown in Fig. 11, a scheme was proposed to demonstrate that amino acids profile and antioxidant activities of micronized *Polygonatumcyrtonema* Hua tuber powder were significantly influenced by different ball milling time.

Conclusion

The present study showed the effects of ball milling micronization on amino acids profile and antioxidant activities of *Polygonatumcyrtonema* Hua tuber powder with different particle size distribution. The multiple linearities between total amino acids content, soluble sugar content, and ABTS radical scavenging activity revealed the relationship between antioxidant activities and ingredients. Moreover, the different cluster tendency by PCA and cluster analysis indicated the effects of different ball milling times. It was also stressed that asparagine, α-aminobutyric acid, and valine can be used

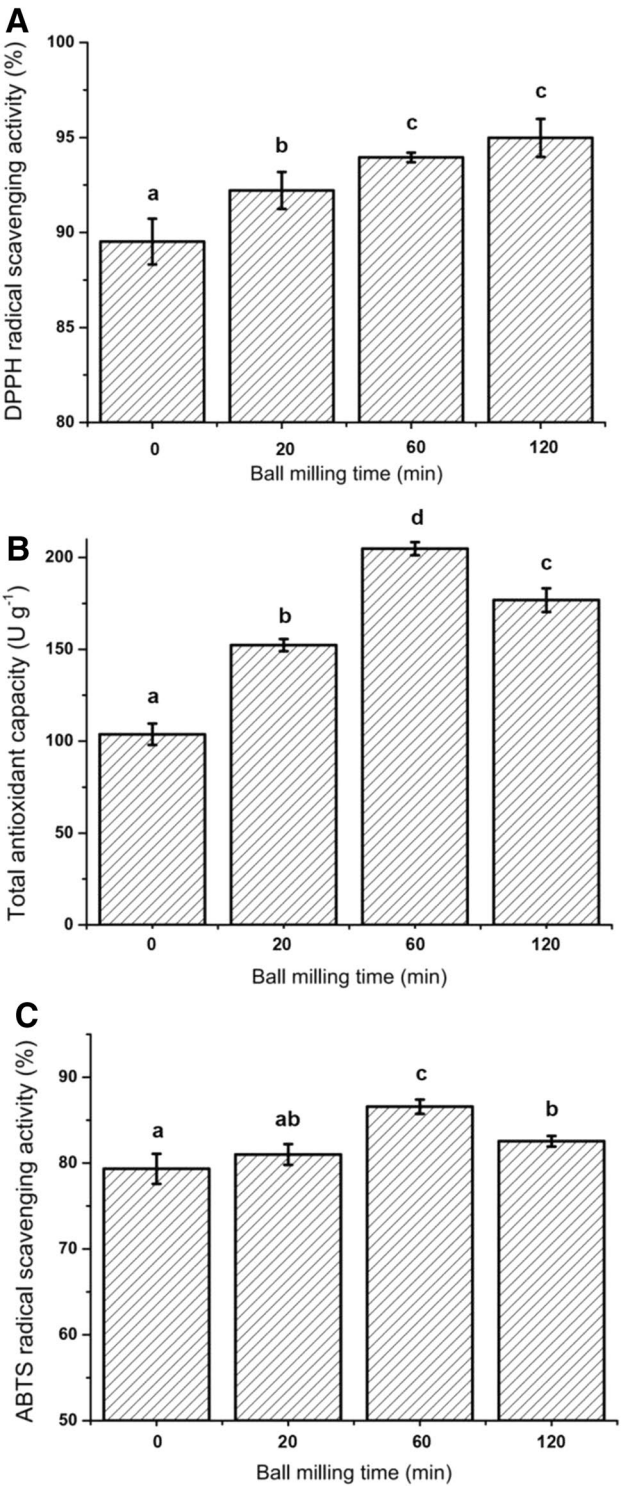

Fig. 9 Antioxidant activities of the treated *Polygonatum* tuber powder samples. **A** DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity **B** total antioxidant capacity and **C** ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity. Different lower-case letters (a–d) in the same figure mean significant differences ($P < 0.05$)

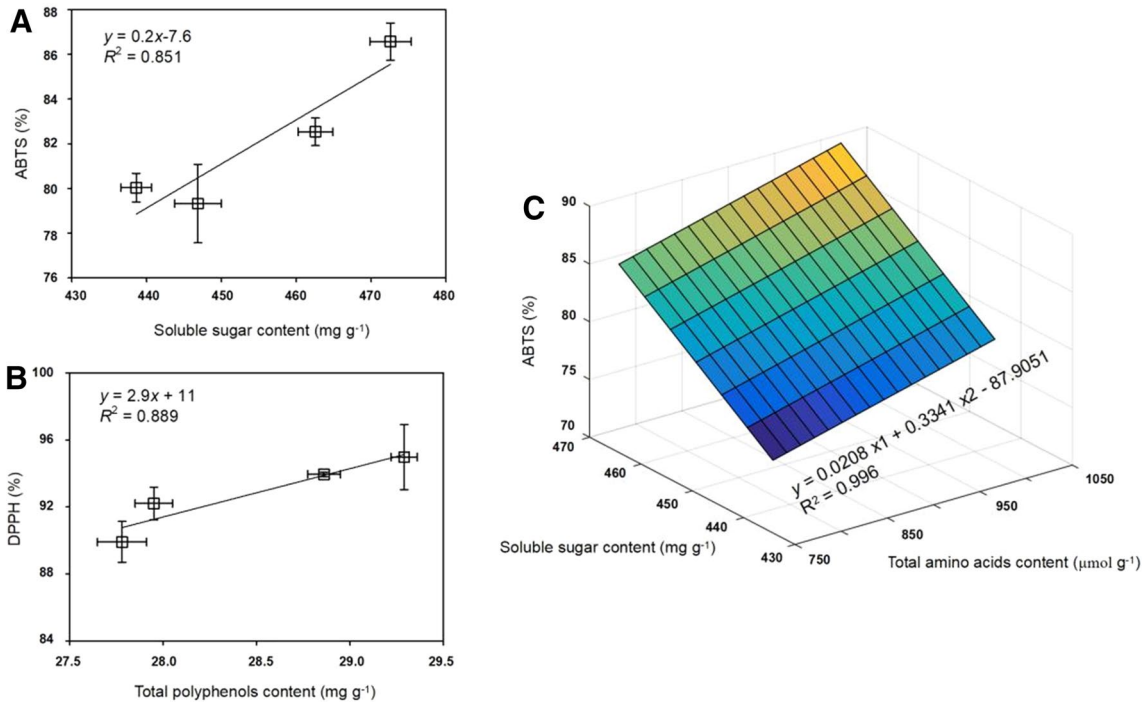

Fig. 10 The linear relationship between the soluble sugar content and ABTS radical scavenging activity (**a**). The linear relationship between total amino acids content and DPPH radical scavenging activity (**b**). Multiple linear correlative relationships between the total polyphenols content and the soluble sugar content and ABTS radical scavenging activity (**c**). DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

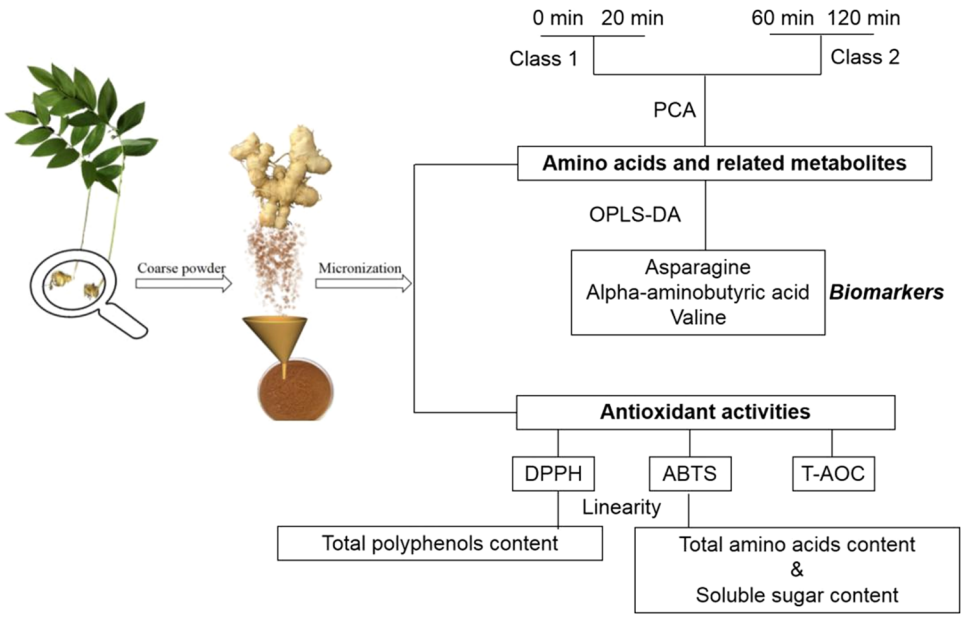

Fig. 11 Illustration of the effects of the amino acids and related metabolites profile and the linearity between antioxidant activities and the total polyphenols, total amino acids, and soluble sugar contents. *T-AOC* = Total antioxidant capacity, *DPPH* = 2,2-diphenyl-1-picrylhydrazyl, *ABTS* = 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid, *OPLS-DA* = orthogonal partial least squares discriminant analysis, *PCA* = Principal components analysis

as the biomarkers to describe the effects of ball milling time by OPLS-DA. Altogether, amino acids profile and antioxidant activities of *Polygonatumcyrtonema* Hua tuber powder were significantly influenced by different ball milling times. These findings provided the foundation for future research on the potential application of *Polygonatum* tuber powder and the development of dietary supplements and functional foods.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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