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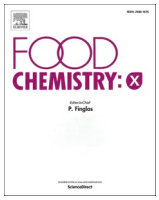

Comparison of phenotypic and phytochemical profiles of 20 *Lycium barbarum* L. goji berry varieties during hot air-drying

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ABSTRACT

The variety and drying stage of goji berries (GBs) may affect their final physical and chemical properties. This study investigated the differences in physical phenotypic parameters and major chemical composition parameters of 20 GBs varieties during four stages of hot air-drying (HD). The results indicated that the color difference values L*, a*, and b* decreased during the HD process. The contents of all amino acids decreased, with significant reductions in amino acids involved in the Maillard reaction. Correspondingly, the level of 5-hydroxymethylfurfural, a Maillard chemical reaction intermediates, increased. Furthermore, the decreased L* values were closely linked to the decomposition of carotenoids. Notably, the differences in constituents among different varieties of dried GBs were smaller than those in fresh GBs. These findings provide a theoretical basis for optimization of the GBs drying process, contributing to the expansion of GBs breeding programs and their use in global functional food and pharmaceutical industries.

1. Introduction

The Goji berry (*Lycium barbarum* L.) is a dicotyledonous plant belonging to the Solanaceae family (Hu et al., 2022). Goji berrys (GBs) plants are widely distributed in arid to semi-arid regions, including parts of North and South America, Africa, and Eurasia (Duan et al., 2023), although they originated in Asia, mainly in the Ningxia region of western China (Fatchurrahman et al., 2022). Moreover, China has the largest area of Goji berry(GB) cultivation and production in the world, and GBs are a key commercial crop in Ningxia Province. The Goji Berries Market size was valued at USD 1.51 Billion in 2024 and the total Goji Berries revenue is expected to grow at a CAGR of 3.9 % from 2025 to 2032, reaching nearly USD 2.05 Billion(Maximize Market Research MMR Report: Goji Berries Market - Global Industry Analysis and Forecast, 2025-2032).

Since GBs contain flavonoids, polysaccharides, unsaturated fatty acids, vitamin C, carotenoids, amino acids, and trace minerals (Gong et al., 2022; C. Yang et al., 2022), its extracts have antioxidant and free radical scavenging properties (Zhang et al., 2022). Therefore, GBs have the effects of protecting the liver and nervous system, promoting mitochondrial biogenesis, and improving the energy balance (Zhou et al., 2022), as well as significant effects in promoting growth, inhibiting apoptosis, anti-inflammatory, promoting gastrointestinal tract regulation (Huang et al., 2022; C. Yang et al., 2022). In addition, GBs have beneficial effects in protecting against many diseases, such as alcoholic liver injury, type 2 diabetes mellitus, Alzheimer's disease, and Parkinson's disease (Rajkowska et al., 2022).

Due to chemical changes and microbial decay, the raw GBs spoil rapidly after harvest, and thus drying is used to extend the shelf life of GBs (Rajkowska et al., 2022). Meanwhile, dried GBs can be used in traditional Chinese medicine. Currently, about 80 % of GBs in China need to be dried after harvest (Tang et al., 2023). At present, the drying methods used in the GBs industry are hot air-drying (HD), vacuum pulsation drying, and freeze-drying methods. Considering the cost-effectiveness, HD is still the main drying method used for GBs (Zhao et al., 2019). The HD of GBs usually involves drying in stages at different temperatures (Xu et al., 2022). The first stage usually involves a temperature of 40–50 °C for a duration of 5–10 h (Tang et al., 2023). During this stage, the surface water of the goji berry fruit evaporates and the fruit becomes soft, while the skin remains smooth (Tang et al., 2023).

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The temperature of the second stage is between 45 and 55 °C and the duration is typically 10–15 h (Zhao et al., 2019). At this stage, the water in the fruit is transferred to the epidermis and continues to evaporate, resulting in shrinkage of the epidermis (Tang et al., 2023). The third stage involves a temperature of 50–60 °C and lasts for 15–25 h. During this stage, the fruit cells rupture and the fruit skin shrinks completely, leading to a final moisture content of the dried fruit of between 10 % and 15 % (Zhao et al., 2019). Variable-temperature drying is more effective in reducing discoloration and saving more time compared with constant high-temperature drying.

Previous studies have shown that the quality of GBs tends to change during HD, and one of the most important changes is the alteration in color during HD. This is mainly due to the alteration of pigments in the fruit, resulting from carotenoid degradation, enzymatic browning, non-enzymatic browning, and other causes of fruit color darkening (Hu et al., 2022). At the same time, the sugar, protein, and carotenoid contents of the fruit may also be reduced due to degradation (Tang et al., 2023). After HD, the GBs contain higher levels of polysaccharides, especially low molecular weight polysaccharides, characterized by high antioxidant activity and high levels of Maillard reaction products (MRPs) (Tang et al., 2023). Moreover, different varieties of GBs vary in their contents of phytochemicals and nutrients, leading to variations in their physicochemical qualities after drying (Yang et al., 2022). However, current research is based on comparisons between fresh fruit and fruit after completion of drying (Tang et al., 2023; Zhao et al., 2019), and there is a lack of research on the phenotypic and phytochemical profiles of goji berries during the drying process, as well as on differences between goji berry varieties.

A diversification of genetic resources represents the backbone of plant trait improvement, and its importance in the formation of different fruit varieties cannot be denied (Martinidou et al., 2024). Since GBs represent a medicinal germplasm resource, changes in the quality of different varieties during the drying process are particularly important. In addition to the beneficial properties for human health, it is important to understand changes in quality arising from the processing of GBs, specifically in terms of optimization of drying processes. Thus, the present study utilized state-of-the-art chromatographic techniques to analyze GBs from 20 varieties at four stages of HD, with an evaluation of phytochemical characteristics, including their sugar, carotenoid, amino acid, and 5-hydroxymethylfurfural (5-HMF) components. The aim is to reveal the changes in phenotype parameters and key chemical components of 20 goji berry varieties during the four stages of HD, as well as their correlations, and to screen excellent germplasm resources that maintain good quality during the hot air drying process. This study will provide a theoretical basis for optimization of the GB drying process, contributing to the expansion of GB breeding programs and their use in the global functional food and pharmaceutical industries.

2. Materials and methods

2.1. Materials

The 20 GB varieties analyzed in this study (listed in Table 1) were harvested in 2022 from the Chinese National Goji Berry Germplasm Repository (latitude 38°38′50″N, longitude 106°9′13″E), representing key genetic resources under standardized cultivation conditions. To preserve bioactive compounds, GB samples were transported under controlled conditions (4 °C) and entered the drying process within two hours of harvesting. Glucose, fructose, sucrose, quinic acid, and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO, USA). Citric acid, oxalic acid, malic acid, tartaric acid, and fumaric acid were purchased from Sangon Biotech (Shanghai, China). 5-HMF was purchased from Yuanye (Shanghai, China). Tetrahydrofuran was purchased from Shuangshuang (Shandong, China). Potassium carbonate, sodium ascorbate, buffer solution, tetrapotassium hexacyanoferrate trihydrate, zinc acetate, phenol, anthrone, and dichloromethane were obtained from Damao (Tianjin, China). Phenolphthalein complexone, sodium hydroxide, potassium sodium tartrate, acetic acid, phenolphthalein, hydrochloric acid, and Vitamin C were from Guangfu (Tianjin, China). Absolute ethyl alcohol, phosphoric acid, ammonium hydrogen phosphate, and other reagents were purchased from Liberace (Yinchuan, China). All other chemicals were of analytical grade.

Table 1
Shrinkage rate and Planting location of 20 GBs varieties Shrinkage rate and Planting location of 20 GBs varieties.

GBs sample name	Shrinkage rate of HD	Species	Planting location
N02	45.07 % ± 0.15^{abcd}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N03	40.27 % ± 0.14^{bcde}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N104	38.63 % ± 0.17^{bcde}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N17	32.62 % ± 0.13^{cdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
X9	62.94 % ± 0.11^{a}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
NNQ6	34.34 % ± 0.15^{cdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
NNQ7	52.01 % ± 0.12^{abc}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
BH	33.89 % ± 0.20^{cdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N816	42.24 % ± 0.21^{abcd}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N56	45.58 % ± 0.22^{abcd}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
NNQ15	35.82 % ± 0.16^{cdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N810	28.57 % ± 0.15^{def}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
Z168	17.89 % ± 0.26^{f}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
Z44	37.03 % ± 0.21^{bcdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
N35	24.10 % ± 0.24^{ef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
DMY	31.95 % ± 0.31^{cdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
JQ4	39.72 % ± 0.27^{bcde}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
NQ1	56.20 % ± 0.11^{ab}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
NQ5	44.04 % ± 0.14^{bcde}	*Lycium barbarum* L.	Yinchuan, Ningxia, China
SN1	32.61 % ± 0.20^{cdef}	*Lycium barbarum* L.	Yinchuan, Ningxia, China

According to Tukey's HSD test, there were significant differences ($P = 0.05$) in the shrinkage rate test for each GBs variety for different letters (Gong et al., 2022).

2.2. Hot air-drying process

The HD process was performed as previously described (Tang et al., 2023) with slight modifications. For the HD pretreatment, the pretreatment solution was prepared with 0.2 % Na_2CO_3 supplemented by 0.3 % K_2CO_3, in which fresh GBs were immersed for 30 s to achieve dewaxing. The HD process involved drying at 45 °C for 5 h, followed by 50 °C for 10 h, and finally at 55 °C for 15 h (1807H/SSYG Saishangyangguang Company, Yinchuan, China). Samples were collected at different time points and were labeled FG for fresh GBs, GS1 for GBs collected after 10 h of drying, GS2 for GBs collected after 25 h of drying, and DG for dried GBs. The collected samples were stored at 4 °C for further analysis.

2.3. Assessments of fruit appearance

Hardness was determined following the method of Zhang et al. (2024) with minor modifications. The revised experimental parameters are specified as follows. The hardness of the GBs was measured using a texture analyzer (Texture Technologies, New York City, NY, USA). The speeds before and after measurement were 20 mm/min and 40 mm/min, respectively, while the trigger force was set to 0.5 N and the detection distance to 5 mm. Ten measurements were taken on each sample and the average value was calculated.

The fruit area (FA) and length-to-width ratio (LWR) were scanned using an automatic seed testing analyzer (Khan et al., 2024). Ten measurements were conducted on each sample and the average value was calculated.

The color of the GBs was determined with a CM-5 spectrophotometer (Konica Minolta, Osaka, Japan), measuring L* (lightness), a* (red-green), and b* (yellow-blue) (Tang et al., 2023). Ten measurements were made on each sample and the average value was calculated.

The shrinkage rate (SR) was determined as described (Yang et al., 2020). The SR was measured by a displacement method and was calculated according to the formula:

$$SR = \frac{FA_1 - FA_2}{FA_1} \times 100\% \quad (1)$$

where FA_1 and FA_2 are the fruit areas (mm^2) of the fresh and dried samples, respectively.

Moisture content was measured using a moisture analyzer (HX204, Mettler Toledo, Switzerland) with the standard drying program, at a drying temperature of 150 °C and a shutdown criterion of 1 mg/50 s (Zhu et al., 2018). All comparisons of fruit components were made based on dry matter.

2.4. Detection of amino acids and their derivatives

The amino acid content was analyzed following the method described by Zhao et al. (2024) with minor modifications. Fifty-gram samples of GBs from the different varieties and 4 stages of drying were placed in 50 mL sterile centrifuge tubes and were sent at low temperature (0–4 °C) to Metabo-Profile Biotechnology (Shanghai) Co., Ltd. for Quantitative Detection of Amino Acids in Biological Samples Using Targeted Metabolism Technology Platform (Metabo Profile, Shanghai, P. R. China). The columns used were an ACQUITY UPLC HSS T3 C18 1.7 μM VanGuard pre-column (2.1 × 5 mm) and an ACQUITY UPLC HSS T3 C18 1.7 μM analytical column (2.1 × 100 mm). The mobile phase consisted of solvent A containing pure water (FairLawn, NJ, USA) with 0.1 % formic acid (FairLawn) and solvent B consisting of acetonitrile (FairLawn) with 0.1 % formic acid. The elution conditions were 95 % A and 5 % B for 5 min, followed by 35 % A and 65 % B, and finally, at 6.01 min, 95 % A and 5 % B, with these conditions maintained until the completion of 8 min of operation. The column temperature was maintained at 40 °C, with a flow rate of 0.60 mL/min and an injection volume of 5 μL. Amino acid detection was performed using ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) (ACQUITY UPLC Xavo TQ S, Waters Corp., Milford, MA, USA).

2.5. Detection of sugars

2.5.1. Detection of total sugar

The total sugar content of GBs was determined as described using the 3,5-dinitrosalicylic acid (DNS) method with glucose as the standard (Tang et al., 2023). Five measurements were made for each sample and the average value was calculated.

2.5.2. Detection of sucrose (Suc) and fructose (Fru)

Sugar extraction was performed as described by Zhao et al. (2015). Three grams (accurate to 0.0001 g) were weighed and rapidly ground to powder with liquid nitrogen in a mortar. The samples were then mixed with 20 mL of 80 % (*v*/v) ethanol solution and transferred to a heart-shaped bottle, followed by the addition of a further 55 mL of 80 % (v/v) ethanol solution. Following reflux extraction for 60 min, the mixture was filtered at 80 °C; this extraction procedure was repeated three more times. The filtered extracts were combined and cooled to room temperature, after which the volume was made up to 100 mL. Four milliliters of the filtrate were then placed in a glass tube, and dried with a suction pump, and 1 mL of pyridine was added and shaken until the filtrate dissolved. This was followed by the addition of 0.4 mL of hexamethyldisilazylamine and 0.2 mL of trimethylsilyl chloride in an ice water bath. The mixture was allowed to stand at 20 °C for 30 min and was then centrifuged followed by collection of the supernatant for the next step of gas chromatography (GC) analysis. The chromatographic column used was a 30 mm × 0.25 mm × 25 μm Rtx-5 quartz capillary column (Restek, USA). The conditions involved an initial temperature of 180 °C maintained for 20 min, and increased at 20 °C/min to 280 °C over 10 min and the FID detector temperature was 300 °C, the inlet temperature was 280 °C. The flow rate of N_2 was 30 mL/min, the flow rate of H_2 was 30 mL/min, the airflow velocity was 300 mL/min, the split ratio was 20:1, and the injection volume was 1 μL. The final sugar concentration was calculated according to the standard curve and expressed as mg/g FW. All sugar standards were purchased from Sigma Aldrich (USA).

2.5.3. Detection of Lycium barbarum polysaccharides (LBP)

The extraction and determination of LBP were conducted as described by Zhao et al. (2015), with slight modifications. Following the collection of the GB samples, approximately 3.0 g of the sample was ground and placed in a conical flask. Thereafter, 75 mL of 80 % (*v*/v) ethanol solution was added, and the mixture was subjected to reflux extraction for 1 h. After filtration at 80 °C, the residues were washed with a heated 80 % ethanol solution (about 25 mL) and placed in a flask, followed by the addition of 75 mL of distilled water. After extraction in a boiling water bath for 1 h, the residue was repeatedly washed with distilled water, and the washing solution was mixed with the filtrate to yield a total volume of 50 mL. A volume of 0.3 mL of the sample solution was then added to 2 mL of distilled water and 1 mL of phenol solution, followed immediately by the addition of 5 mL of concentrated sulfuric acid with continuous mixing, after which the mixture was allowed to stand at room temperature for 5 min. The mixture was then placed in a boiling water bath for 15 min, after which it was removed and cooled to room temperature, and the absorbance at 490 nm was measured. The glucose content was calculated according to the standard curve of glucose ($y = 0.1533x - 0.0269$, $R^2 = 0.9972$, where y is the glucose content and x is the absorbance). Finally, the LBP content was calculated according to the following formula.

$$W(mg/g) = \frac{\rho \times 250 \times f}{m \times V \times 10^3} \quad (2)$$

where W is the LBP content (mg/g), ρ is the glucose content (g), f is the conversion coefficient of polysaccharide glucose conversion rate (3.19), m is the sample mass (g), and V is the measured volume (0.3 mL).

2.6. Detection of 5-hydroxymethylfurfural analysis

The extraction and derivatization of 5-HMF was analyzed as previously described with slight modifications (Geirola et al., 2024). GB samples (5 g) were weighed and ground, after which 30 mL of distilled water was added and the mixture was shaken well. Then, 2 mL of 10 % potassium ferrocyanide solution and 2 mL of zinc acetate solution was added with thorough mixing. The samples were then centrifuged at 1000 rpm for 3 min, after which the clear supernatants were transferred to 200 mL volumetric flasks and made up to volume with distilled water.

After appropriate dilution, the absorbance of the solution was measured at 284 nm.

$$5-\mathrm{HMF(ug/g)}=\frac{\mathrm{C}\times\mathrm{V}\times\mathrm{N}}{\mathrm{m}} \quad (3)$$

where C represents the absorbance values used in the calibration curve to obtain the concentration of the standard solution, V indicates the volume of sample extract in mL, N indicates the sample dilution factor, and m represents the dry sample mass.

2.7. Total acid analysis

The total acid content was determined as described (Shi et al., 2023), using titration with 0.1 M NaOH to pH 8.2. The pH value was measured using a pH meter (HI5221, HANNA Instruments, USA).

2.8. Carotenoid analysis

The carotenoid contents were analyzed as previously described (Zhao et al., 2016) with slight modifications. GBs samples (5 g) were weighed and ground, after which tetrahydrofuran solution was added in a 1:5 material:liquid ratio for ultrasonic extraction for 10 min. This was repeated multiple times until the filter residue was colorless. The solution was then placed in a rotary evaporator at 45 °C until almost dry. The material was then dissolved in a 25 mL volumetric flask with dichloromethane solution, diluted appropriately, and absorbances were

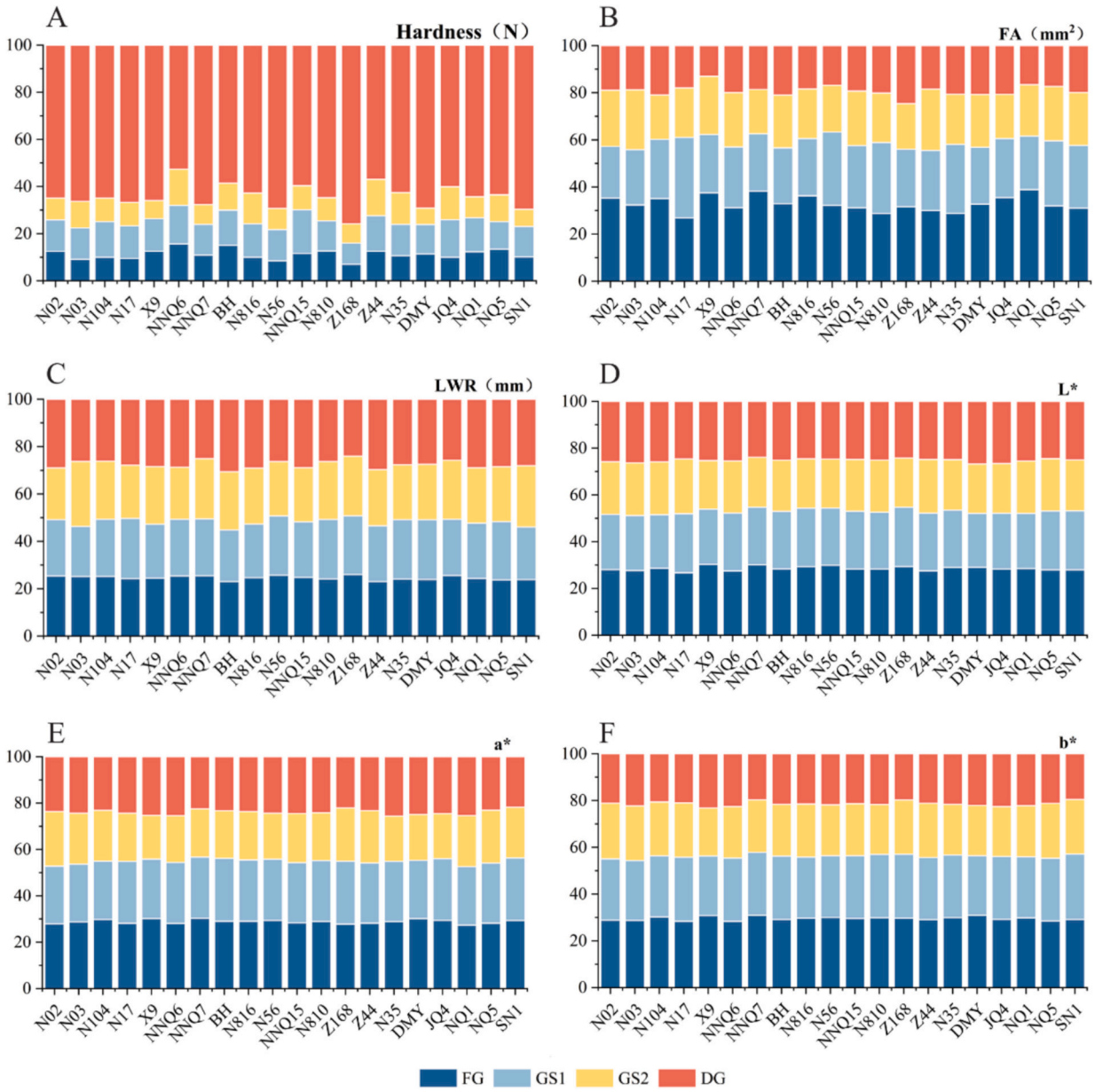

Fig. 1. Changes in the phenotypic profiles of goji berries during hot air-drying process. A: Hardness of goji berries from different varieties at different stages of drying; B: Fruit Area (FA) of goji berries from different varieties at different stages of drying; C: Length-to-width ratio (LWR) of goji berries from different varieties at different stages of drying; D: Color difference L* value of goji berries from different varieties at different stages of drying; E: Color difference a* value of goji berries from different varieties at different stages of drying; F: Color difference b* value of goji berries from different varieties at different stages of drying; G:Appearance of goji berries from different varieties at different stages of drying. FG represents fresh GBs, GS1 represents GBs collected after drying for 10 h, GS2 represents GBs collected after drying for 25 h, and DG represents dried GBs. Data from different stages are expressed as a percentage of the total data. This figure was drawn using the Origin 2024.

measured at 460 nm.

$$\text{The total carotenoid}(ug/g) = \frac{C \times V \times N}{m \times 10^6} \quad (4)$$

where C is the absorbance value used in the calibration curve to obtain the standard carotenoid solution concentration, V represents the total volume of the sample extract in mL, N is the sample dilution, and m represents the dry sample mass.

2.9. Statistical analysis

Ten statistical replicates were used for the determination of phenotypic parameters while three replicates were used for examination of the phytochemical profiles. The results are presented as mean ± standard deviation. Data were analyzed with SPSS version 21.0 (IBM Corp., Armonk, NY, USA) using one-way analysis of variance (ANOVA) and Duncan's test to evaluate the differences between mean values, with differences considered significant at $P < 0.05$. Figures were generated using Origin software (version 8.6) and R software.

3. Results and discussion

3.1. Changes in the appearance of goji berries

The appearance of goji berries is closely related to their commercial value. Fig. 1 shows the changes in appearance of the different GB varieties. The hardness of the fruit affects its taste and is also an indirect reflection of its moisture content (Fig. 1A). Differences in fruit hardness were not significant during the first three stages, although the hardness was significantly greater when the fruit was fully dried, relative to the earlier three stages. This indicated that when the water loss reaches a certain level, the hardness of the fruit increases significantly ($P < 0.05$). Within a specific range of moisture content (10–15 %), the less the hardness of the dry GBs, the better the taste of the fruit, while greater hardness is associated with better taste and quality after storage in fresh GBs (Fatchurrahman et al., 2022). The highest degree of hardness in the FG stage was observed in the 'X9' and 'BH' varieties, while 'NNQ6' showed the lowest hardness in the dried fruit. The FA was found to decrease significantly in all varieties during HD (Fig. 1B). This is possibly due to HD-induced breakdown and shrinkage of the cellular structure, resulting in wrinkling and the collapse of the fruit epidermis, markedly reducing the fruit area (Tang et al., 2023). There were no significant differences in the LWR values among the different GB varieties during the HD process (Fig. 1C), indicating that the shape of the GB fruit did not change significantly ($P < 0.05$). The GB varieties 'X9' and 'N1' showed higher SR than other varieties during the HD process, with SR values of 65.21 % and 57.26 %, respectively, while markedly reduced shrinkage was seen in the 'Z168' and 'N35' varieties, with SR values of 21.91 % and 28.36 %, respectively. The lower the SR, the smaller the change in FA, which is more conducive to better commodity value. Significant increases in SR typically occur during the middle and late stages of HD when drying fruit and vegetables (Wen et al., 2022).

Higher values of L*, a*, and b* at the DG stage are indicative of higher commodity value. Due to the generation of MR and pigment degradation, the L*, a*, and b* values of fruit tend to decrease after HD (Tang et al., 2023). A decrease in the color difference value a* indicates a reduction in the red color of the fruit (Fig. 1E). This change may be due to the degradation of red substances and pigments within the fruit. In the FG stage, the highest a* value was observed in the 'DMY' variety, while in the DG stage, the highest a* value was found in 'N35'. The one with the smallest change in a* value in HD is 'NQ1'. The color difference b* value was observed to decrease gradually from the fresh to dry fruit (Fig. 1F), indicating a gradual reduction in the yellow color of the fruit, likely caused by the gradual degradation of carotenoids and other components (Ouyang et al., 2020). During the FG stage, the highest b* values were found in 'N35' and 'N56', while at the DG stage, the highest b* value was observed in 'X9'.

The color difference L* value (Fig. 1D), representing the brightness of the fruit, gradually decreased, indicating that the brightness of the fruit reduced slowly during the drying process. The 'NNQ6' variety exhibited the least variation in the b* value during HD. In the FG stage, the highest L* values were recorded in 'N56' and 'X9', while in the DG stage, the highest L* values were seen in 'N35'. The variety showing the least change in L* value during the HD process was designated 'N03'. L* and a* represent the main indicators of color degradation. This is consistent with previous studies, where the values of L* and a* decrease with increasing drying time (Bi et al., 2022).

3.2. Changes in amino acid components of goji berries

Fig. 2 presents the dynamic changes in amino acid and sugar composition across different drying stages (FG, GS1, GS2, DG) for various GBs cultivars. The left panels (Fig. 2A, C, E, G) demonstrate the progressive alterations in amino acid profiles during dehydration. Meanwhile, the right panels (Fig. 2B, D, F, H) display the corresponding variations in sugar content. In this study, a total of 33 amino acids and their derivatives were detected in fresh GBs, including 8 essential amino acids and 13 amino acid derivatives (Fig. 2). The amino acids with higher contents in GBs were proline, alanine, serine, glutamine, and threonine, accounting for between 67.54 and 77.32 % of the amino acid contents. A significant variation in the content of the same amino acid was observed among the different GB varieties, although the predominant types of amino acids remained constant. In the FG stage, marked differences in the levels of specific amino acids were observed among different varieties ($P < 0.05$) (Fig. 2A), while in the DG stage, the differences in amino acid contents between the different varieties decreased (Fig. 2G). Overall, during the HD process, the overall amino acid contents decrease gradually.

The levels of sarcosine, aminoadipic acid, and β-alanine were found to vary significantly between the fresh and the dried fruit in most varieties. The aminoadipic acid level decreased during the drying process, reaching its lowest value in the dried fruit. Aminoadipic acid is a metabolic intermediate in the lysine synthesis pathway (Zhang et al., 2023), and α-amino adipic acid is also a substrate of the Maillard reaction and is, therefore, fully consumed and converted during the HD process. Sarcosine and β-alanine both formed relatively small proportions of the overall amino acid content of GBs, and the large coefficient of variation was mainly due to the relatively high contents in some varieties, such as fresh 'JQ4' and 'NNQ15', while there was little difference between the varieties in the dried fruits.

During the HD process, protein hydrolysis may release free amino acids, while degradation and the Maillard reaction may reduce amino acids (Yang et al., 2020). Therefore, the observed decrease in amino acid content could be attributed to the consumption of amino acids as substrates in the Maillard reaction. At the same time, intensification of the Maillard reaction reduces the pH of the reaction system, resulting in the acidic hydrolysis of oligopeptides and an increase in the content of free amino acids in the system (Ouyang, 2021). A previous study has shown that lemons lose five amino acids when subjected to HD for 6 h, and glycine, threonine, and isoleucine represent new amino acid components introduced into the sample during the drying process (Xie et al., 2022). In this study, however, the overall amino acid content in GBs decreased significantly during HD, although there were no significant changes in the specific amino acid types.

3.3. Changes in sugars

3.3.1. Changes in sucrose and fructose

Sucrose participates in the Maillard reaction after being hydrolyzed and converted into reducing sugars (fructose) during HD (Cao et al., 2020). In a system containing moderate water contents, sucrose can

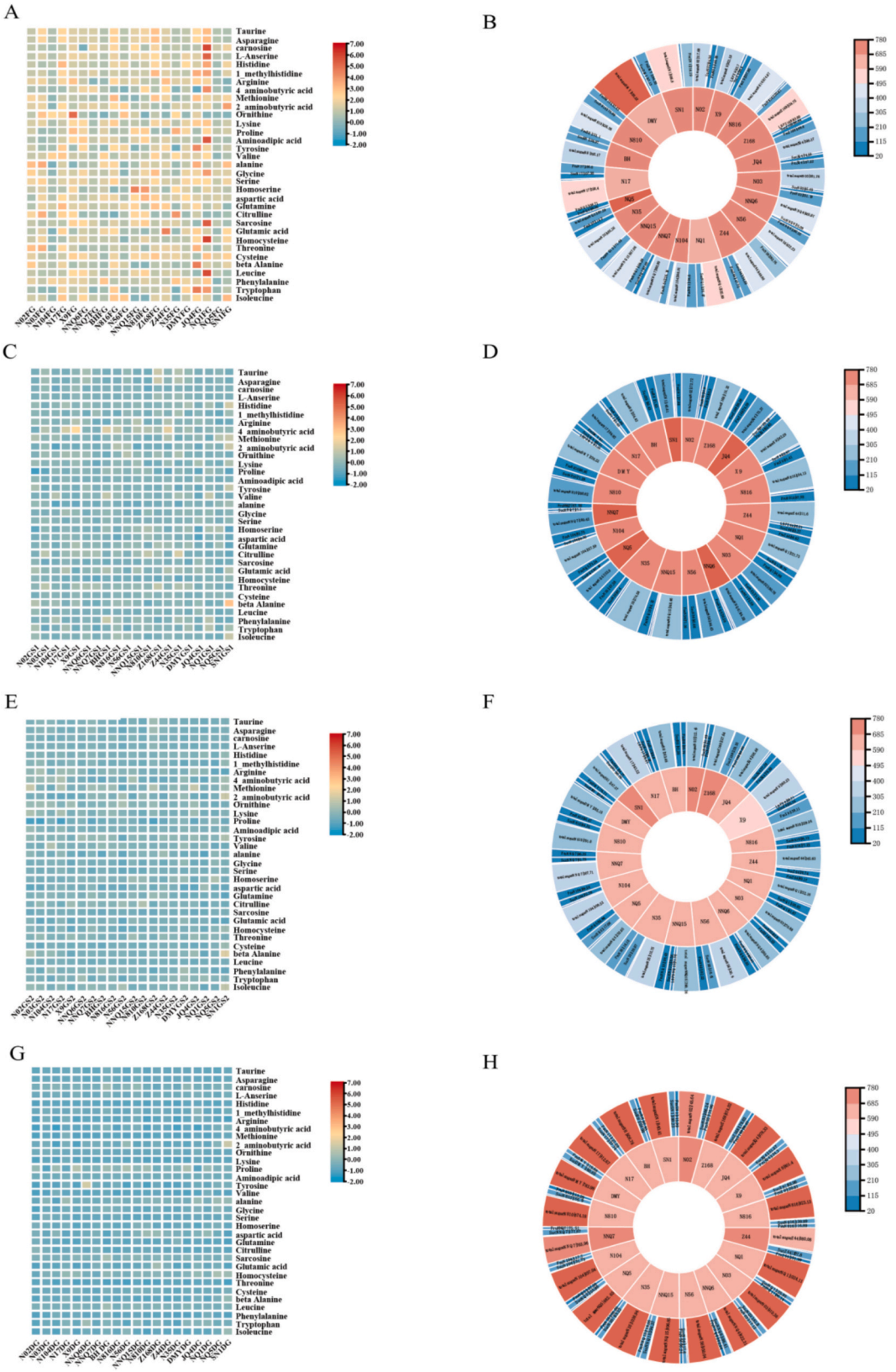

Fig. 2. Changes in amino acid contents and sugar during hot air-drying of different goji berry varieties. A: Amino acid of goji berries from different varieties at FG stages; B: Total sugar, *Lycium barbarum* polysaccharides (LBP), Sucrose (Suc) and fructose (Fru) of goji berries from different varieties at FG stages; C: Amino acid of goji berries from different varieties at GS1 stages; D: Total sugar, *Lycium barbarum* polysaccharides (LBP), Sucrose (Suc) and fructose (Fru) of goji berries from different varieties at GS1 stages; E: Amino acid of goji berries from different varieties at GS2 stages; F: Total sugar, *Lycium barbarum* polysaccharides (LBP), Sucrose (Suc) and fructose (Fru) of goji berries from different varieties at GS2 stages; G: Amino acid of goji berries from different varieties at DG stages; H: Total sugar, *Lycium barbarum* polysaccharides (LBP), Sucrose (Suc) and fructose (Fru) of goji berries from different varieties at DG stages. The amino acid heatmaps of the four stages use the same color scale range, and the sugar content of the four stages also uses the same color scale range. This figure was drawn using the Origin 2024.

produce more HMF than fructose and glucose in acidic media, while in systems with low water contents, the presence of amino acids leads to a decrease in the amount of HMF formed by sucrose and fructose (Ouyang et al., 2020). In this study, sucrose levels exhibited an overall increasing trend throughout the drying process, particularly during the HD stages (Fig. 2B, D, F, H). In other words, in the later HD stages, the rate of sucrose degradation decreases, and the sucrose content increases (Fig. 2H). Furthermore, significant differences were observed among the different varieties during the FG stage ($P < 0.05$) (Fig. 2B). The highest sucrose levels were found in 'N810', differing significantly from those in other varieties ($P < 0.05$). In the subsequent GS1 stage, the highest sucrose content was recorded in 'N810', where it differed significantly ($P < 0.05$) relative to the contents observed in the other varieties (Fig. 2D). In the GS2 stage, the highest sucrose levels were observed in 'N35', which were significantly higher ($P < 0.05$) than those in the other varieties (Fig. 2F). In the DG stage, the sucrose levels were higher in 'NNQ15' (Fig. 2H). Sucrose has been identified as a contributing factor to the development of diabetes and other diseases (Gomez et al., 2024), and the sucrose contents of the final dried GBs are thus an important consideration. The differences in sucrose content among the varieties were more pronounced during the FG stage than in the DG stage. In FG, the varieties with lower sucrose content were 'N104' and 'NNQ15', which showed significant differences compared to other varieties ($P < 0.05$). In DG, the variety with the lowest sucrose content was 'N56', differing significantly from the levels in other varieties ($P < 0.05$).

During HD, sucrose hydrolysis produces fructose, while the Maillard reaction consumes fructose. The fructose levels generally showed a continuous downward trend, indicating that the consumption of fructose in HD was always greater than its production (Fig. 2B, D, F, H). The highest fructose content in FG was found in 'NQ5', significantly different from that in the other varieties ($P < 0.05$). In GS1, the highest fructose content in GS1 was recorded in 'N35' ($P < 0.05$), while the highest levels in GS2 were seen in 'X9' with a significant difference ($P < 0.05$) compared to other varieties. The highest fructose content in DG was observed in 'N17', differing significantly from that in the other varieties ($P < 0.05$).

3.3.2. Changes of Lycium barbarum polysaccharides

LBP are one of the important active ingredients in GBs. Previous studies have shown that after drying, GBs contain higher concentrations of low molecular-weight polysaccharides, which are associated with higher nutritional value following HD. In FD (Fig. 2B), the variety that showed markedly higher LBP levels was 'Z168' ($P < 0.05$), while the highest contents in GS1 were observed in 'Z44' ($P < 0.05$), in GS2 in 'NNQ15' ($P < 0.05$), and in DG (Fig. 2H), in 'N810' ($P < 0.05$), where the level was 46.41 mg/g, which was 208.82 % higher than that in 'BH' with the lowest LBP content (15.02 mg/g) and 33.70 % higher than that in the traditional variety 'NQ1' (34.71 mg/g). During the HD process, 'NQ5', 'DMY', and 'N810' showed consistently high levels of LBP, suggesting their potential as specialized varieties for LBP production. The MR in GBs during HD can induce polysaccharide aggregation or binding with other components. Therefore, GBs after HD may contain abundant polysaccharides.

3.4. Changes in 5-hydroxymethyl furfural

5-HMF is a typical product of the Maillard reaction, and is formed during the initial stage of the MR when food is heated (Bi et al., 2022). During fruit dehydration, 5-HMF results mainly from the formation of (Z) -3,4-deoxyketone during the MR from 3-deoxyglucosone (Bi et al., 2022). Overall, the 5-HMF contents of GBs increased continuously during the HD process ($P < 0.05$), consistent with the continuous increases in 5-HMF observed during the drying of sour jujube (Li, Chen, et al., 2022). Previous studies have shown that changes in 5-HMF contents have significant effects on non-enzymatic browning (NEB) at a temperature of 50 °C (Li, Chen, et al., 2022), and at drying temperatures close to 100 °C, the contents of 5-HMF increase with the increase in temperature (Ouyang et al., 2020). The overall accumulation of HMF may thus related to the duration of heat application (Kayacan et al., 2020).

There were significant differences in the contents of 5-HMF in fresh GBs from different varieties ($P < 0.05$) (Fig. 3A). In FG, the highest levels of 5-HMF were recorded in the 'DMY', 'Z168', and 'JQ4' varieties, differing significantly from those in the other varieties ($P < 0.05$). In GS1, markedly higher levels of 5-HMF were found in 'DMY' and 'NQ1', relative to the other varieties ($P < 0.05$). In GS2, the varieties with higher levels of 5-HMF were 'JQ4', 'N810', and 'NQ1', with significant differences from those in the other varieties ($P < 0.05$). In DG, the highest content of 5-HMF was seen in 'NQ1' ($P < 0.05$). The variety showing the least change in 5-HMF content during HD was 'NNQ7'. Although the toxicological relevance of 5-HMF is not yet clear, it is considered to have adverse effects on human and animal health (Martins et al., 2022). 'NNQ7' and 'BH' were found to have relatively low 5-HMF contents in the FG and DG stages, while 'NNQ7' had the lowest content in DG at 1061.38 μg/g, differing significantly from that in the other varieties ($P < 0.05$). Moreover, NNQ7 produced the least amount of 5-HMF during the HD process.

3.5. Changes in total acid

During the entire drying process, the total acid content of the GBs gradually decreased. Research has demonstrated that during the HD process, amino acids are initially involved in the Maillard reaction, leading to a decline in pH as the heating time progresses. The extent of this decline is directly proportional to the consumption of amino acids (Ouyang et al., 2020). Secondly, the reducing sugars in the reaction system are easily degraded to produce compounds such as formic acid and acetic acid when heated at a higher pH (Li, Yang, et al., 2022). Other types of acids, such as propionic acid, citric acid, lactic acid, saccharin acid, formic acid, and levopropionic acid, may also be produced during the heating process (Li, Yang, et al., 2022). The occurrence of the Maillard reaction, a condensation reaction of carbonyl ammonia, has been identified as a factor contributing to the reduction in pH in the reaction system (Ouyang et al., 2020). At the same time, acidic substances with a buffering ability such as formic acid, acetone aldehyde, acetic acid, and glyoxal produced in the intermediate stage of the Maillard reaction inhibit the acidity of the reaction system (Ouyang et al., 2020), slowing the decline in pH during the later stages of drying. In this study, the total acid content of FG has a significant impact on the taste of the fruit (Fig. 3C). The total acid content of FG was found to vary significantly among the different varieties, with the varieties with higher total acid contents being 'N02' ($P < 0.05$). In GS1, the highest total acid content was found in 'NNQ15', differing significantly from that in other varieties ($P < 0.05$). In GS2, 'Z168' had the highest total acid content and showed significant differences compared to other varieties ($P < 0.05$), while in DG, the total acid content was higher in 'NNQ7' and 'N56'.

3.6. Changes in carotenoids

Carotenoids are red-colored substances and represent one of the main bioactive substances in GBs. During the HD process, the carotenoid content of GBs decreased overall, although there were significant differences among the different varieties (Fig. 3B). In FG, the 'N17' exhibits the highest carotenoid content, showing significant differences compared to other varieties ($P < 0.05$). This characteristic suggests its potential as a premium raw material for deep-processed goji berry products aimed at vision improvement. In GS1, 'DMY' showed significantly higher carotenoid contents while 'JQ4' had the significantly highest carotenoid contents in both GS2 and DG ($P < 0.05$). The least change in carotenoid levels seen during HD was in 'N03'. It was found that the degradation of carotenoids in some varieties increased overall

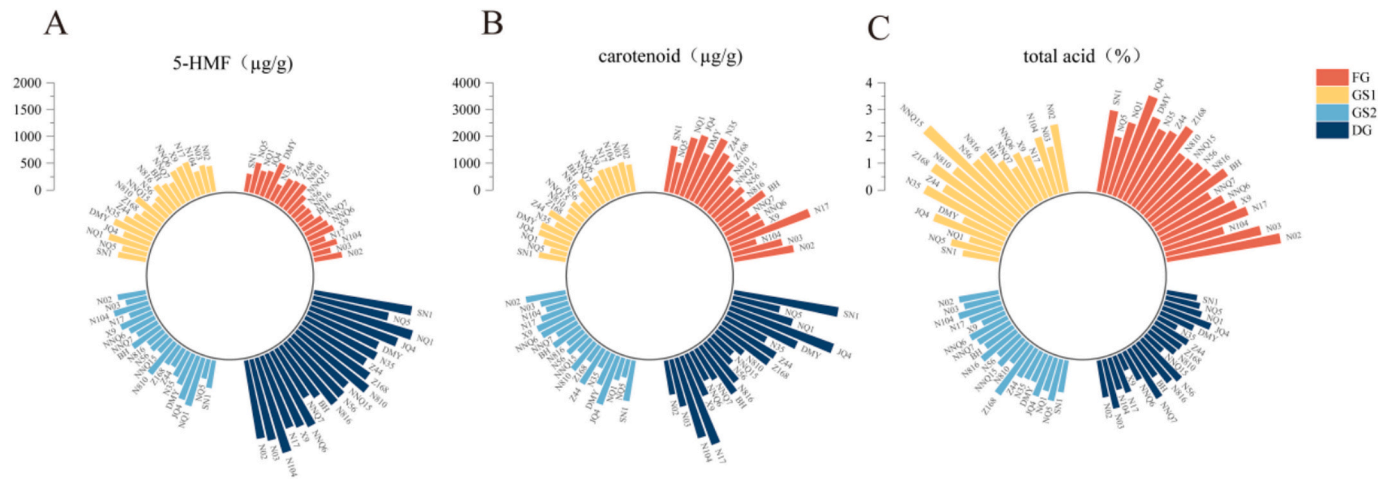

Fig. 3. Changes in total acid, carotenoid and 5-hydroxymethyl furfural during hot air-drying of different varieties of goji berries. A: Total acid of goji berries from different varieties at different stages of drying; B: Carotenoid of goji berries from different varieties at different stages of drying; C: 5-hydroxymethyl furfural (5-HMF) of goji berries from different varieties at different stages of drying. This figure was drawn using the Origin 2024.

during the drying process (Grasso-Kelley, Liu, Halik, & Douglas, 2021). There may be two reasons for this observation. Firstly, GBs are intact fruits, and during drying, carotenoids are released as cells rupture and collapse (Zhang et al., 2018). Secondly, the overall temperature during the HD process of GBs was between 40 and 60 °C, and the overall heating temperature is relatively mild, resulting in relatively small losses of carotenoids (Li, Chen, et al., 2022).

3.7. Variations in phenotypic and phytochemical profiles during hot air-drying of different goji berry varieties

Fig. 4 shows the variations in the phenotypic and phytochemical profiles of different GB varieties during the four HD stages. The coefficient of variation displays the magnitude of differences between different varieties in terms of specific indicators. In this context, it provides a quantitative metric for the extent of variation among different varieties in terms of specific indicators. It is evident that as the coefficient of variation increases, the discrepancy in the indicator among different varieties also increases. In the FG stage, the components with coefficients of variation greater than 50 % included sucrose (59.12 %), carnosine (58.00 %), ornithine (74.01 %), aminoadipic acid (60.04 %), citrulline (57.38 %), sarcosine (54.27 %), β-alanine (57.80 %), and tryptophan (56.39 %). In the GS1 stage, components with a coefficient of variation greater than 50 % included sucrose (57.29 %), aminoadipic acid (53.76 %), sarcosine (77.20 %), phenylalanine (69.84 %), and β-alanine (55.19 %). In the GS2 stage, the components with a coefficient of variation greater than 50 % included aminoadipic acid (53.84 %), sarcosine (67.66 %), and phenylalanine (58.87 %). In the DG stage, the components with a coefficient of variation greater than 50 % included carnosine (60.68 %), methionine (59.75 %), ornithine (54.04 %), aminoadipic acid (60.04 %), tyrosine (73.44 %), citrulline (54.71 %), sarcosine (56.49 %), phenylalanine (90.34 %), β-alanine (55.20 %), and isoleucine (62.04 %). Among them, amino adipic acid, creatine, and phenylalanine showed significant inter-variety variability at each stage, which may be useful for the identification of goji berry varieties.

During the FG to DG stage of each variety, the coefficients of variation of almost all amino acids were $>$ 50 %, while the coefficients of variation of carnosine, ornithine, amino-adipic acid, sarcosine, and β-alanine were $>$ 100 %. At the same time, the coefficients of variation for total sugar and sucrose exceeded 50 %. In general, changes in the material components of the GBs during the drying process were greater than those among the varieties at the same stage.

3.8. Partial least squares discriminant analysis (PLS-DA)

Using the phenotypic and phytochemical profiles of GBs as dependent variables and the different stages as independent variables, PLS-DA was found to effectively distinguish GB samples from the four stages (Fig. 5A). The independent variable fitting index (R2x) in this analysis was found to be 0.584, while the dependent variable fitting index (R2y) was 0.11, and the observation that the model prediction indices Q^2 and R^2 exceeded 0.5 indicated that the model fitting results were acceptable. After 200 permutation tests (Fig. 5B), the intersection point between the Q2 regression line and the vertical axis was less than 0, indicating that the model was not overfitted and that the model validation was effective. It is hypothesized that these findings could be utilized for the differential analysis of phenotypic and phytochemical profiles of GBs at different stages.

The PLS-DA showed that there were significant differences in the composition of fresh fruits during the HD process compared to the accelerated drying stage, the decelerated drying stage, and the dried fruits. Compared with the components in the accelerated drying stage (GS1), the decelerated drying stage (GS2), and the fresh fruit stage (FG), there were significant differences in the GB components in the dried fruit stage (DG), although no significant differences in the fruit components were seen between the GS1 and GS2 stages. This indicates that during HD of GBs, a series of reactions and substance transformations have occurred, and the components of the dried and fresh fruits have undergone significant changes. The GS1 and GS2 stages are important stages involving material transformation.

3.9. Correlation analysis

As illustrated in Fig. 6, a correlation analysis was conducted on 50 quality indicators during the four HD stages of 20 goji berry varieties. In production, after HD, GBs need to go through a color screening process to remove darker or abnormally colored GBs, allowing the remaining fruit to be packaged and sold, ultimately forming a commodity. Therefore, the color difference L*, a*, and b* values of the dried fruit are important indicators reflecting the value of GBs products. In this study, a significant positive correlation was observed between the color difference L* and b* values of the dried fruit and the fructose content of GS2 ($P < 0.01$). The higher the content of fructose in GS2, the greater the color difference L* and b* values of the dried fruit, with greater brightness and yellowing of the dried fruit, and the higher the overall quality of the fruit. The fructose content in the FG stage was significantly positively correlated with b * in the DG stage ($P < 0.05$). Fructose is an

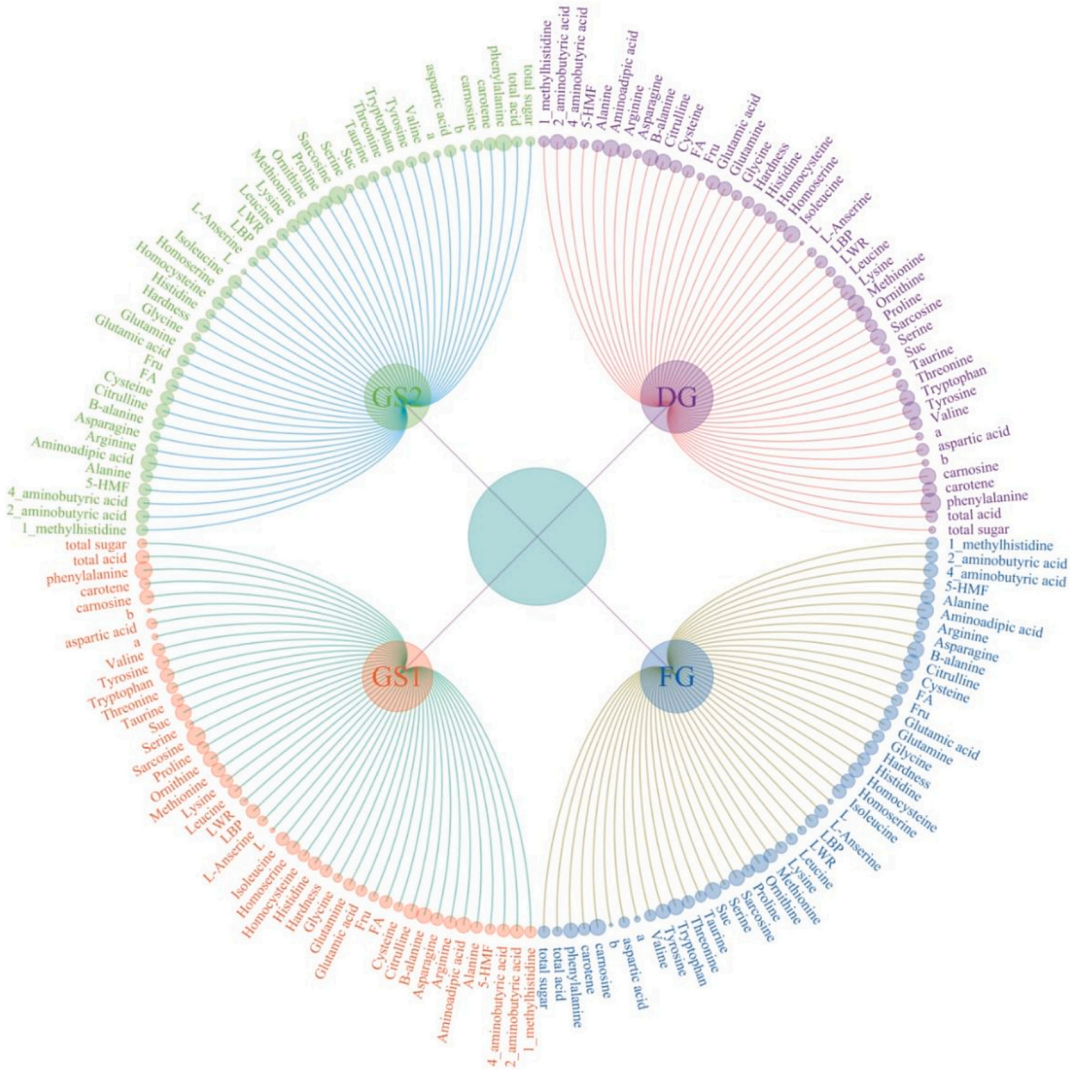

Fig. 4. Variations in Fruit Quality during HD among Different Varieties of GBs. FG: Comparison of coefficients of variation of phenotypic and phytochemical profile during the fresh GBs of different varieties; GS1: Comparison of coefficients of variation of phenotypic and phytochemical profile during the GBs of different varieties after 10 h of drying; GS2: Comparison of coefficients of variation of phenotypic and phytochemical profile during the GBs of different varieties after 25 h of drying; DG: Comparison of coefficients of variation of phenotypic and phytochemical profile during the dry GBs of different varieties. Different dot sizes indicate the size of the coefficient of variation. This figure was drawn using the R project (version 3.5.3).

important substrate in the Maillard reaction. When fructose is heated alone or in combination with aspartic acid at pH 5.5 and 7.0, chlorogenic acid increases the formation of HMF (Li, Yang, et al., 2022). Fructose, together with arginine, lysine, aspartic acid, and threonine, can generate melanin-like substances with different contents, molecular weights, and structures in a simulated system (Kim & Lee, 2009). This may affect the color of GBs in the final DG stage of HD.

The color difference L* value in the DG stage was found to be significantly negatively correlated with the carotenoid content during the FG stage ($P < 0.05$), indicating that the higher carotenoid contents during the FG stage are associated with lower L* values in the DG stage (Fig. 7). This may be due to degradation of carotenoids during the drying process, thus reducing the final L* values. In addition, there was a significant positive correlation ($P < 0.05$) between the ornithine content in FG and the L * value in DG. In addition, the content of glutamic acid in FG was significantly negatively correlated with L* in DG ($P < 0.05$). The contents of arginine and ornithine in FG were also found to be significantly positively correlated with the a* value in DG ($P < 0.05$).

The total sugar content during the FG stage was significantly positively correlated with the 5-HMF level in the GS1 stage ($P < 0.01$). Furthermore, serine, histidine, proline, aminoadipic acid, sarcosine, homocysteine, β-alanine, leucine, and tryptophan levels in the FG stage were significantly positively associated with 5-HMF contents in the GS1 stage ($P < 0.05$). These results suggest high levels of MR during the early stages of HD, and these amino acids actively participate in the MR in GBs during the HD process. The color difference a* value of DG was found to be significantly positively correlated with 5-HMF in GS1 ($P < 0.01$). Higher levels of 5-HMF in GS1 were associated with higher a* values in DG, indicating less red coloration of the dried fruit. This phenomenon may be attributed to the production of melanin by 5-HMF during GS1, leading to an increase in the a* value (Bork et al., 2022). However, the accumulation of HMF does not fully reflect the degree of fruit browning in HD (Kanzler & Haase, 2020).

The content of homocysteine in FG showed significant or extremely significant positive correlations with 5-HMF in GS1, 5-HMF in GS2, and 5-HMF in DG. Serine in GS1 was significantly positively correlated with 5-HMF in GS2 and 5-HMF in DG. The trend of changes in the serine content showed significant or extremely significant negative correlations with glutamine in GS1, 5-HMF in GS2, and 5-HMF in DG. The levels of these three amino acids showed a continuous downward trend during

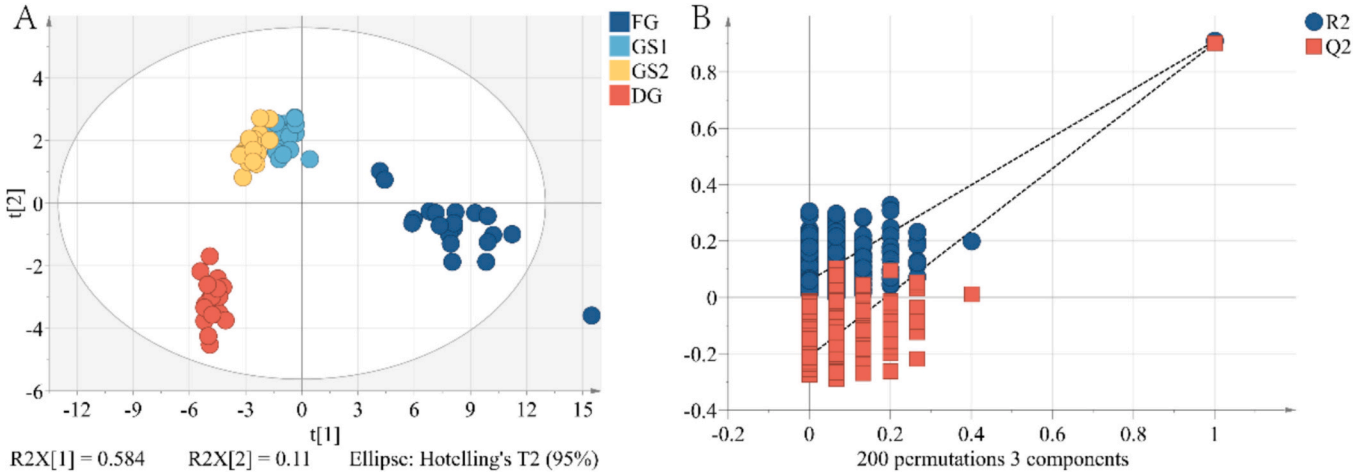

Fig. 5. Partial Least Squares Discriminant Analysis (PLS-DA) showing changes in components during hot air-drying of different varieties of goji berries. PLS-DA score plots (left) and internal validation tests with 200 permutations each (right) of PLS-DA models derived from elements. R2X[1] and R2Xo[1] indicate the values for components T[1] and To[1]. Ellipses represent the results of Hotelling T2 tests with 95 % confidence. The permutation plots (right) display the coefficients of correlation between the original and permuted Y variables on the X-axis versus cumulative R2 and Q2 on the Y-axis and show the regression lines. This figure was drawn using the SIMCA (version 14.1).

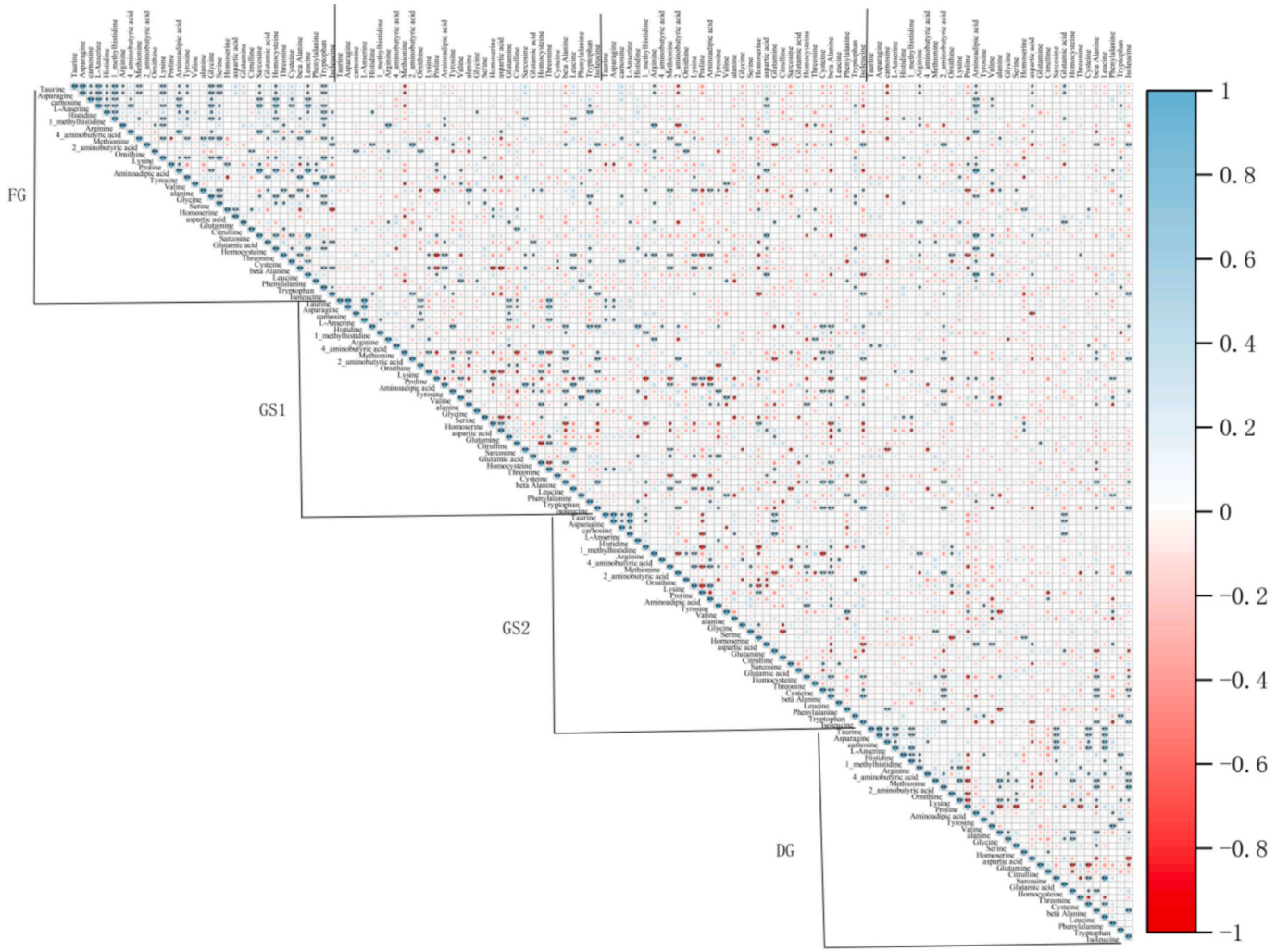

Fig. 6. Correlation analysis of quality changes during the hot air-drying process of goji berries. Correlation plot for all of the parameters studied with a significance level of 0.05. Data were analyzed by SPASS software (version 21.0) using Pearson's correlation coefficients. The color range from blue to red indicates low to high correlation coefficients. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

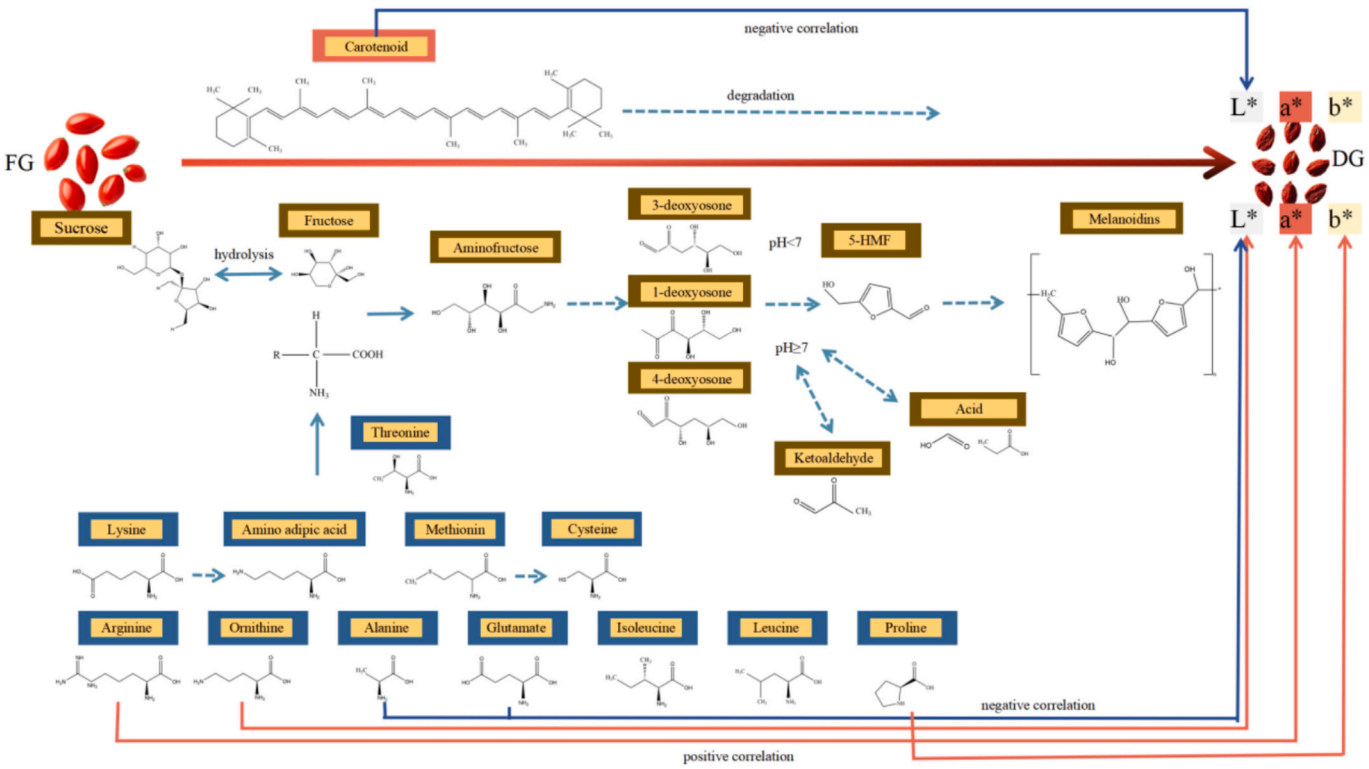

Fig. 7. Color changes during the hot air-drying process of goji berries Based on the detected relevant components, the Maillard reaction process during the hot air drying of goji berries was simplified. And the identification of components in fresh fruits that affect the color difference of dried fruits.

the HD process. Structural differences in amino compounds can affect the rate of the Maillard reaction and the reaction products. Amino groups at the end or ε- amino groups are more effective due to their smaller steric hindrance effects and α- amino acids are more prone to the Maillard reaction (Ouyang et al., 2020; Zhang et al., 2023). However, in reaction systems with lower moisture levels or during prolonged heating at high temperatures, the presence of amino acids may inhibit the production of 5-HMF (Wong et al., 2015).

In addition to participating in the Maillard reaction, amino acids also undergo mutual transformation, leading to changes in the final amino acid content. For example, in terms of the lysine and amino adipic acid contents in the fresh and dried fruit, Lysine in GS2 was significantly correlated with aminoadipic acid in GS1 and aminoadipic acid in GS2. Aminoadipic acid participates in both lysine synthesis and the Maillard reaction during HD (Zhang et al., 2023). Significant or extremely significant correlations were observed between methionine levels in the dried fruit and homocysteine in GS2 and homocysteine in dried fruits, methionine in GS2 and homocysteine, and methionine in GS1 and homocysteine in GS1. This is attributable to known pivotal role of homocysteine as an intermediate product in the metabolic processes of methionine and cysteine (Zhang et al., 2018).

4. Conclusion

This comprehensive evaluation of the physicochemical qualities of different varieties of GBs during the four stages of HD vividly describes the inherent physicochemical diversity of these varieties and paves the way for optimization of the HD process for different GB varieties. The findings of this study revealed that the contents of amino acids and their derivatives as MR substrates decreased significantly during the HD process, while the contents of the MR intermediate product 5-HMF increased significantly. Furthermore, the degradation of carotenoid color-developing compounds had a significant effect on the final color quality of the dried GBs This study identified excellent GB germplasm, which can be utilized in future breeding programs for the enhancement of fruit quality traits. The fresh ‘X9’ and ‘N35’ fruits have high LBP and carotenoid contents, and therefore have the potential to be promoted and sold as fresh fruit varieties, while the ‘N17’ and ‘NNQ6’ varieties showed only small changes in the color difference values during the drying process and also contained high levels of LBP and carotenoids, making them more suitable for use in traditional Chinese medicine. The ‘NQ5’, ‘DMY’, and ‘N810’ varieties contain high levels of LBP and could therefore be used as specialized varieties for LBP production. The findings of this study emphasize the remarkable physicochemical properties of GBs and their potential applications in the food and pharmaceutical industries, where they would contribute to the production of nutritionally rich natural products, as well as promote the protection of goji berry biodiversity. This study is based on the unified process of GB drying during production, and future research is needed to optimize the drying process for different GB varieties.

CRediT authorship contribution statement

Ting Huang: Writing – original draft, Data curation. **Ning Jia:** Visualization, Software, Methodology. **Lunxuan Zhu:** Visualization. **Wen Jiang:** Investigation, Conceptualization. **Aobai Tu:** Software. **Ken Qin:** Methodology, Funding acquisition. **Xiaojin Yuan:** Supervision. **Juxiu Li:** Writing – review & editing, Funding acquisition.

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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.

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Data availability

Data will be made available on request.

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