

Impact of drying methods on physicochemical properties of *Fritillaria hupehensis* (Hubeibeimu) flours

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Abstract

Fritillaria hupehensis (Hubeibeimu) widely grows in the mid-western zones of China. In the present work, we investigated the physicochemical compositions, antioxidant abilities, and thermal properties of the bulbs of *F. hupehensis* dehydrated by heat-pump drying (HD), vacuum drying (VD), natural drying (ND), freeze drying (FD), and microwave drying (MD). Total contents of nucleosides and nucleobases in *F. hupehensis* flours ranged from 727.64 to 1,654.25 µg/g, and total free amino acids ranged from 88.03 to 128.21 mg/g. FD flour had high contents of total nucleosides and nucleobases, and free amino acids. MD flour had low contents of total starch, amylose, protein, nucleosides and nucleobases, and VB₁ and VB₂, and high levels of total phenolic content (TPC) and antioxidant abilities. Furthermore, MD flour facilitated gelatinisation, while FD flour displayed opposite trend. HD flour had high total starch content, while ND flour weakened the bitter taste due to the percentage of sweet and bitter taste of amino acids.

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Introduction

The bulbs of *Fritillaria hupehensis*, officially named as “Hubeibeimu” in Chinese Pharmacopoeia, are widely distributed in the mid-western zones of China. The bulbs of *Fritillaria* are rich in bioactive components such as alkaloids, nucleosides, amino acids, sterols, and lignans, and they have been found to possess antioxidant, anticancer, anti-inflammatory, and antimicrobial properties (Peng *et al.*, 2013; Nile *et al.*, 2021). *Fritillaria* flour is not only used in herbal medicine, but also approved for addition to functional foods (Chen *et al.*, 2016).

For further preservation and consumption, fresh *F. hupehensis* should be dried and then ground into powder. The drying process is the basic technique for reducing the moisture content of plants to minimise the enzymatic degradation and microbial growth. In addition, the heat procedure is also beneficial for the release of anti-inflammatory bioactive compounds and essential oils from plants (Cheenkachorn *et al.*, 2022). However, inappropriate drying method could have adverse effects on plants by changing their colour, and destroying their bioactive chemicals.

The physicochemical properties and nutritional values of *Fritillaria* flour are determined by different drying methods. In practice, heat-pump drying (HD), vacuum drying (VD), natural drying (ND), freeze drying (FD), and microwave drying (MD) are common dehydration methods for agricultural products. These methods have different drying advantages. When compared with hot air drying, HD has lower energy consumption, higher drying efficiency, and better chemical retention (Xiong *et al.*, 2021). VD provides lower atmospheric pressure, thus resulting in higher drying rate (Xu *et al.*, 2021). Over decades, ND is commonly employed in the herb industry due to its simplicity and low cost. FD is costly, but it can maximally protect the appearance and chemicals in products. Due to the excellent drying efficiency, MD has been widely applied in the food industry (Behera and Balasubramanian, 2021).

In recent years, the effects of drying methods on *Fritillaria* were focused on *F. thunbergia* and *F. cirrhosa* varieties, while related information on *F. hupehensis* is unavailable. *F. hupehensis* is widely distributed in Hubei, Sichuan, and Hunan provinces of China, and it displays excellent functional effects. Therefore, it is necessary to investigate the effect of

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dehydration on the physicochemical properties of *F. hupehensis* flour. The present work thus aimed to compare the effects of different drying methods on the chemical components of *F. hupehensis* flour. Additionally, the antioxidant abilities and thermal properties of the resulting *F. hupehensis* flours were also investigated. The present work would provide an effective drying method for the production and application of *F. hupehensis* flour.

Materials and methods

Sample preparation

Fresh bulbs of *F. hupehensis* were collected from Lichuan City, Enshi Prefecture, Hubei Province, China. Fresh bulbs were cleaned and sliced, then dehydrated by the following methods: (i) HD was conducted using an electric heat-pump dryer (IKE, Weierxin, Foshan, China), with parameters set as: air flow, 1.5 m/s; temperature, 50°C; humidity, 10%; and time, 48 h. (ii) VD was conducted using a vacuum drying oven (DZF-6050; Suopu, Shanghai, China) at 55°C for 36 h. The pressure in the VD chamber was kept at 0 Pa. (iii) ND was conducted in the ventilation for 8 d with average room temperature of about 30°C. (iv) FD was conducted using a laboratory-scale freeze dryer (Scientz-18ND, Martin Christ, Germany). Sliced *F. hupehensis* was firstly frozen at -40°C, followed by freeze drying for 36 h, with absolute pressure of 20 - 40 Pa, and condenser temperature of -54°C. (v) MD was conducted at 300 W for 30 min on a domestic microwave oven (Galanz-P70F23P-G5, Guangdong, China). After dehydration, the moisture of samples was determined by a fast digital moisture analyser (MJ33, METTLER-TOLEDO, Zurich, Switzerland) at 105°C, and they were < 15%. The dried *F. hupehensis* slices were ground to powder, and passed through a 200-mesh sieve. The *F. hupehensis* flour samples were packed in plastic bags, and stored at 4°C in a refrigerator for further analysis.

Chemicals and reagents

Standards of nucleosides and nucleobases (uracil, cytidine, uridine, inosine, adenine, deoxyinosine, deoxythymidine, adenosine, and deoxyadenosine) were purchased from Yuanye Bio-Technology Co. Ltd. (Shanghai, China). ABTS kit was purchased from the Beyotime Institute of Biotechnology (Shanghai, China). Total starch and apparent amylose kits were purchased from Nanjing

Jiancheng Chemical Industrial (Nanjing, China). HPLC-grade methanol and formic acid was purchased from Thermo Fisher Scientific (Leicestershire, UK). All other analytical grade chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Physico-chemical properties

Proximate composition

Moisture, protein, ash, and crude fibre in *F. hupehensis* flours were determined by AOAC (2006). Total starch (TS) and apparent amylose (AAM) were calculated by Nanjing Jiancheng kits. The obtained results are shown in Table 1. Vitamins (thiamine, B₁; riboflavin, B₂) in *F. hupehensis* flours were tested by HPLC (LC-20AT, Shimadzu, Japan) method following a previous study (Al-Farga *et al.*, 2016).

Colour

The colour properties of different *F. hupehensis* powders were determined using a colorimeter (CM-5, MINOLTA, Japan). Parameters of L, a, and b represented lightness, redness to greenness, and yellowness to blueness, respectively. The total colour difference of different samples (ΔE) was calculated using Eq. 1:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (\text{Eq. 1})$$

Water binding capacity

About 3 g of *F. hupehensis* flour was added to 45 mL of distilled water, stirred at 700 rpm for 1 h at 25°C, and centrifuged at 4,000 rpm for 10 min to obtain the wet flour phase. The WBC was calculated using Eq. 2:

$$\text{WBC (\%)} = \frac{M_1 - M_2}{M_2} \times 100 \quad (\text{Eq. 2})$$

where, M₁ = weight of wet flour (g) and M₂ = weight of dry flour (g).

Swelling power and solubility

About 2% (w/v) aqueous flour suspensions were vibrated under water bath at temperatures of 55, 65, 75, 85, and 95°C. After cooling to the room temperature, the suspension was subsequently centrifuged at 4,000 rpm for 10 min to obtain the supernatant and swollen flour sediment. The supernatant was evaporated to dryness at 110°C. SP and SOL were calculated using Eqs. 4 and 5:

Table 1. Colour, moisture, ash, protein, vitamins (VB₂, VB₁), total starch (TS), apparent amylose (AAM), and water binding capacity (WBC) of *F. hupehensis* flours prepared by different processing methods.

Parameter	HD	VD	ND	FD	MD
Colour					
L	90.29 ± 0.06 ^a	86.50 ± 0.51 ^c	87.33 ± 0.23 ^b	90.38 ± 0.59 ^a	83.64 ± 0.28 ^d
a	-0.34 ± 0.01 ^d	0.33 ± 0.04 ^a	0.27 ± 0.03 ^{ab}	0.08 ± 0.06 ^c	0.23 ± 0.01 ^b
b	10.57 ± 0.05 ^d	11.45 ± 0.17 ^c	12.23 ± 0.06 ^b	9.00 ± 0.16 ^e	14.25 ± 0.11 ^a
ΔE	54.83 ± 0.06 ^a	51.08 ± 0.48 ^c	51.99 ± 0.24 ^b	54.77 ± 0.58 ^a	48.64 ± 0.26 ^d
Moisture (%)	8.34 ± 0.13 ^b	9.88 ± 0.35 ^a	8.84 ± 0.12 ^b	6.42 ± 0.05 ^d	7.50 ± 0.51 ^c
Ash (%)	3.68 ± 0.08 ^a	4.17 ± 0.56 ^a	4.25 ± 0.22 ^a	4.15 ± 0.35 ^a	4.59 ± 0.29 ^a
Protein (%)	0.55 ± 0.05 ^c	0.43 ± 0.02 ^d	0.78 ± 0.04 ^b	1.08 ± 0.11 ^a	0.26 ± 0.06 ^e
Vitamin (mg/kg)					
VB₁	0.26 ± 0.01 ^c	0.36 ± 0.01 ^b	0.41 ± 0.02 ^a	0.41 ± 0.01 ^a	0.12 ± 0.01 ^d
VB₂	0.09 ± 0.00 ^b	0.16 ± 0.02 ^a	0.18 ± 0.01 ^a	0.13 ± 0.02 ^{ab}	0.03 ± 0.00 ^c
TS (%)	65.58 ± 0.81 ^a	58.29 ± 0.53 ^c	62.35 ± 1.01 ^{bc}	64.65 ± 0.26 ^{bc}	56.03 ± 0.24 ^d
AAM (%)	8.58 ± 0.46 ^a	8.97 ± 0.32 ^a	8.38 ± 0.05 ^a	7.84 ± 0.14 ^b	5.40 ± 0.47 ^c
WBC (%)	116.74 ± 0.86 ^d	133.05 ± 4.01 ^c	129.41 ± 1.76 ^c	141.53 ± 5.71 ^b	213.07 ± 1.41 ^a

Values are mean ± SD of triplicate ($n = 3$). Means followed by different lowercase letters in the same row are significantly different ($p < 0.05$).

$$SP (\%) = \frac{P \times 100}{W \times (100 - SOL)} \quad (\text{Eq. 3})$$

$$SOL (\text{g/g}) = \frac{A}{W} \times 100 \quad (\text{Eq. 4})$$

where, A = dried supernatant weight, W = dry flour weight, and P = wet flour sediment weight.

Total phenolic and flavonoid contents

F. hupehensis flour (1.0 g) was mixed with 10 mL of 80% ethanol, and extracted for 30 min in the ultrasonic bath at 60°C. The extraction was repeated twice, and centrifuged to collect the supernatant to a final volume of 20 mL. The total phenolic content (TPC) and total flavonoid content (TFC) of extracts were determined using the Folin-Ciocalteu's and NaNO₂-AlCl₃-NaOH method, respectively (Wang *et al.*, 2018). TPC value was expressed as gallic acid equivalent (mg GAE/g), and TFC value was represented as rutin equivalent (mg RTE/g).

Antioxidant activities

Antioxidant activities of *F. hupehensis* flours were evaluated by DPPH, FRAP, and ABTS assays following previous procedures (Lu *et al.*, 2018; Wang *et al.*, 2018). DPPH and FRAP values were calculated as equivalent units of ascorbic acid per gram of *F.*

hupehensis flour (μmol AAE/g DW), while ABTS value was expressed as micromole Trolox equivalent per gram of *F. hupehensis* flour (mmol TE/g DW).

Nucleosides and nucleobases

Nucleosides and nucleobases in *F. hupehensis* flours were analysed following a previous study with minor modifications (Peng *et al.*, 2013). About 2.0 g of *F. hupehensis* powder was dissolved in 20 mL of purified water, and extracted by ultrasonic (300 W, KQ-300DE, Kunshan ultrasonic instruments, Jiangsu) for 1 h at room temperature. The extract was centrifuged and filtered, and then injected into a LC-20AT HPLC system (Shimadzu, Japan) equipped with an AQ-C₁₈ analytical column (5 μm, 4.6 × 250 mm; Shimadzu, Japan). The mobile phases were: A = distilled water/formic acid (0.1%, v/v); and B = 100% methanol. The linear gradient program was set as: 0 min, 95% A; 15 min, 95% A; 25 min, 80% A; 35 min, 55% A; 40 min, 55% A; and 45 min, 95% A. The flow rate was controlled at 0.8 mL/min, and the detection wavelength was set at 260 nm. The column was maintained at 30°C with 10 μL sample injected for HPLC analysis, and the obtained HPLC chromatograms of nucleosides and nucleobases are shown in Figure 1.

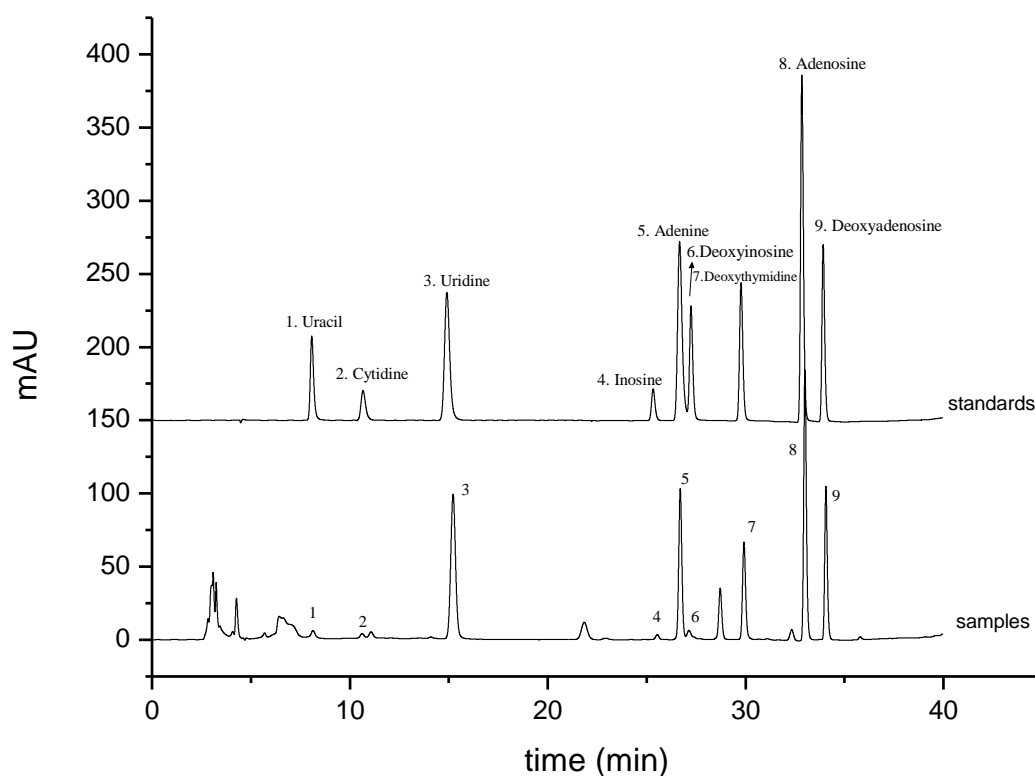


Figure 1. HPLC chromatograms of nucleosides and nucleobases. 1: uracil; 2: cytidine; 3: uridine; 4: inosine; 5: adenine; 6: deoxyinosine; 7: deoxythymidine; 8: adenosine; and 9: deoxyadenosine.

Free amino acids

Free amino acids (FAAs) in the *F. hupehensis* flours were analysed by an amino acid analyser (L-8900, Hitachi, Katsuda, Japan). About 0.2 g of *F. hupehensis* powder was mixed with 20 mL of HCl (0.1 mol/L), and kept at room temperature for 2 h, and then, the mixture was centrifuged to obtain the supernatant. Then, the same volume of trichloroacetic acid (10%) was added in the supernatant, and kept at room temperature for another 1 h. After adjusting the pH value to 2.2, the supernatant was filtered through a 0.22 μm membrane, and then injected into an amino acid analyser.

Thermal properties

A differential scanning calorimetry (DSC200, NETZSCH, Selb, Germany) was used to test the thermal characteristics of *F. hupehensis* flours following a method reported previously (Jan *et al.*, 2017; Mukwevho and Emmambux, 2022). Flour samples (5 mg) mixed with distilled water (10 μL) was hermetically sealed in aluminium pans, and then equilibrated for 12 h at room temperature. Scans were run at the heating rate of 10°C/min from 10 to 120°C. An empty aluminium pan was used as a reference.

Statistical analysis

All experiments were repeated three times, and the data were expressed as mean \pm relative standard deviation (RSD). One-way analysis of variance (ANOVA) was used to compare the significant differences in mean values, and $p < 0.05$ (Duncan's *post hoc* test) was considered as significant. All statistical analyses were performed on IBM SPSS Statistics version 20.0.

Results and discussion

Colour, WBC, and proximate composition

Colour, WBC, and proximate composition provide basic nutrient information of *F. hupehensis* flours. Table 1 shows the colour, moisture, protein, ash, vitamins (VB₂, VB₆), total starch, apparent amylose, and water binding capacity of *F. hupehensis* flours prepared by different drying methods. ΔE values for FD flour (54.77) and HD (54.83) were significantly higher than that of ND flour (51.99), VD (51.08), and MD flour (48.64), thus indicating that FD and HD flours showed relatively high colour difference. ΔE value is used as a colour quality indicator of dried sample, and a value above 6.0 is

considered as a visible colour for consumers (Lenaerts *et al.*, 2018). In the present work, the colour of all dried *F. hupehensis* flours was clearly visible. In fact, FD and HD flours displayed lighter colour due to their higher L value, and lower a and b values. For other drying method, the high temperature was reached during MD, and non-enzymatic browning reactions could be favoured. Slow drying of ND comprised long dehydration time with low temperature which might have promoted enzymatic browning reactions. Higher moisture occurred during the initial VD phase which could also have prompted the enzymatic browning reactions (Sun *et al.*, 2021).

The moisture contents of the five *F. hupehensis* flours were lower than 10%, thus suitable for long-term preservation. Ash content is related to the total mineral content in foods. The ash contents in *F. hupehensis* flours had no significant difference among them. Ash contents ranged from 3.68 to 4.59%, which were slightly higher than that of commercial wheat varieties (Jalgaonkar and Jha, 2016; Memon *et al.*, 2020). Protein contents in different samples were significantly different. Heating denatured the protein structures, thus resulting in the loss of protein. It has been reported that soluble proteins decreased with increasing temperatures (Murphy and Marks, 2000), in accordance with our results. The soluble protein content of FD flour was 1.08%, while protein contents in ND, VD, HD, and MD flours were much lower as compared to FD flour. As a whole, the protein content of *F. hupehensis* was lower than that of *F. thunbergii* flour reported previously (Chen *et al.*, 2016).

VB₁ and VB₂ serve as crucial co-enzymes in various metabolic processes in life, and they are always supplemented as micronutrients of wheat flour (Al-Farga *et al.*, 2016). *F. hupehensis* flours presented slightly lower VB₁ and VB₂ values as compared to wheat flours (Al-Farga *et al.*, 2016; Xie *et al.*, 2018). Vitamins B are water soluble and heat-labile, thus tend to be damaged by high temperature penetrating (Arruda *et al.*, 2013). Degradation of vitamins B in food follows the first-order reaction kinetics, and is influenced by temperature, oxygen concentration, pH, and oxidation reduction parameters (Sarkar *et al.*, 2020). In the present work, VB₁ concentrations in *F. hupehensis* flours were much higher than those of VB₂. ND was a mild dehydration progress without light irradiation, and FD enabled rapid drying at low temperatures.

Consequently, ND and FD flours had the highest VB₁ content without significant difference. VB₂ in ND flour was slightly higher than FD flour, which could have been due to the pH changes caused by rapid dehydration of FD.

The total starch contents of *F. hupehensis* flours ranged from 56.03 to 65.58%. The lowest starch content was found in MD flour, while the highest content in HD flour. Starch hydrolysis enzymes, including α -amylase, β -amylase, and glucoamylase, were reported to be active between 55 and 60°C (Correia and Beirão-da-Costa, 2012). The apparent amylose (AAM) levels in FD and MD flours were significantly lower than the others. The WBC of *F. hupehensis* flours ranged from 116.74% (HD flour) to 213.07% (MD flour), thus indicating that microwave might alter the starch structure of *F. hupehensis* flour to hold the maximum water volume.

Swelling power and solubility

The SP values of *F. hupehensis* flours ranged from 3.09 to 9.81 g/g, while SOL values were in the range of 24.51 - 39.50% (Table 2), which increased with increasing temperature. It was reported that amylose could strongly inhibit the SP of flour (Chen *et al.*, 2016). Similarly, FD flour contained a lower amylose content (7.84%) with a higher SP value at each temperature. However, MD flour had low amylose content and SP. The possible reason was that the microwave procedure destroyed the amylose chains, and lessened the hydrogen bonding forces. It was proved that SP could be affected by comprehensive factors such as the interactions of amylose and amylopectin, and the arrangement of starch crystals and non-starch components (Wyller *et al.*, 2017). The mechanism for changes in the SP of different *F. hupehensis* flours should be further investigated.

As compared to the SOL value of FD flour, ND flour increased by 7.48% in 90°C, but MD, VD, and HD flours decreased by 5.54, 8.38, and 10.03%, respectively. Higher SOL value indicates that more amylose is leached from the starch granule (Yu *et al.*, 2021). The SOL value is also affected by the inner structure and rearranging of starch granules (Yu *et al.*, 2021). In the present work, thermal treatment (MD, VD, and HD) significantly decreased the SOL of *F. hupehensis* flours, which could have been attributed to the enhanced intramolecular bonding, and prevented the leaching of amylose from the starch granule.

Table 2. Swelling power (SP) and solubility (SOL) of *F. hupehensis* flours prepared by different processing methods.

Parameter		HD	VD	ND	FD	MD
SP (g/g)	55°C	3.25 ± 0.15 ^a	3.35 ± 0.12 ^a	3.34 ± 0.22 ^a	3.60 ± 0.21 ^a	3.09 ± 0.20 ^a
	65°C	4.61 ± 0.11 ^{ab}	5.05 ± 0.26 ^{ab}	4.78 ± 0.18 ^{ab}	5.25 ± 0.18 ^a	4.36 ± 0.24 ^b
	75°C	5.81 ± 0.12 ^b	6.19 ± 0.17 ^b	5.87 ± 0.18 ^b	6.86 ± 0.21 ^a	5.70 ± 0.15 ^b
	85°C	6.54 ± 0.17 ^{bc}	7.19 ± 0.12 ^b	6.81 ± 0.25 ^{bc}	8.26 ± 0.31 ^a	6.21 ± 0.24 ^c
	95°C	7.72 ± 0.33 ^{bc}	8.21 ± 0.22 ^b	7.86 ± 0.32 ^{bc}	9.81 ± 0.46 ^a	6.76 ± 0.21 ^c
SOL (%)	55°C	24.51 ± 0.78 ^b	25.63 ± 0.47 ^b	29.24 ± 0.22 ^a	27.03 ± 1.22 ^{ab}	25.95 ± 0.35 ^b
	65°C	25.22 ± 0.32 ^c	26.58 ± 0.28 ^{bc}	30.65 ± 0.23 ^a	28.11 ± 0.58 ^b	27.35 ± 0.87 ^b
	75°C	26.16 ± 1.11 ^c	28.18 ± 0.98 ^{bc}	32.52 ± 0.63 ^a	29.63 ± 0.47 ^{ab}	29.05 ± 0.75 ^{bc}
	85°C	28.64 ± 0.75 ^c	29.54 ± 0.87 ^{bc}	34.08 ± 0.89 ^a	32.54 ± 1.23 ^{ab}	30.91 ± 0.09 ^{abc}
	95°C	30.05 ± 0.49 ^c	30.65 ± 0.98 ^c	35.90 ± 0.92 ^a	33.40 ± 0.28 ^b	31.55 ± 0.48 ^{bc}

Values are mean ± SD of triplicate ($n = 3$). Means followed by different lowercase letters in the same row are significantly different ($p < 0.05$).

TPC, TFC, and antioxidant ability

Results of TPC, TFC, and antioxidant abilities (DPPH, FRAP, and ABTS) of *F. hupehensis* flours are presented in Table 3. TPC ranged from 0.38 to 0.75 mg/g. MD flour had the highest TPC value, while HD flour had the lowest. High drying temperature positively affected the structural properties of plant fibre, and prompted the release of TPCs (Khudyakov *et al.*, 2022). Therefore, MD flour had higher TPC than FD flour. TFC ranged from 0.49

to 1.86 mg/g DW. FD flour had the highest TFC value, followed by MD, VD, ND, and HD. Flavonoids are rich in OH compounds, and their destruction would cause the degradation of flavonoid compounds during drying (Sun *et al.*, 2022). The changes in TFC and TPC values in *F. hupehensis* flours processed by various methods were not similar, which was consistent with the results in apple and ginger slices (Khudyakov *et al.*, 2022; Sun *et al.*, 2022).

Table 3. Total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant abilities (DPPH, FRAP, ABTS) of *F. hupehensis* flours prepared by different processing methods.

Sample	TPC	TFC	DPPH	FRAP	ABTS
	mg GAE/g	mg RTE/g	µmol AAE/g	µmol AAE/g	mmol TE/g
HD	0.38 ± 0.02 ^d	0.49 ± 0.04 ^c	1.85 ± 0.05 ^c	0.95 ± 0.02 ^c	1.23 ± 0.06 ^c
VD	0.52 ± 0.02 ^c	0.58 ± 0.07 ^c	2.01 ± 0.04 ^{bc}	1.43 ± 0.05 ^c	3.17 ± 0.11 ^b
ND	0.54 ± 0.03 ^c	0.57 ± 0.08 ^c	2.13 ± 0.08 ^b	1.86 ± 0.11 ^d	3.38 ± 0.35 ^b
FD	0.65 ± 0.03 ^b	1.86 ± 0.22 ^a	3.01 ± 0.04 ^a	2.71 ± 0.07 ^b	3.79 ± 0.09 ^b
MD	0.75 ± 0.03 ^a	1.40 ± 0.22 ^b	3.13 ± 0.06 ^a	3.13 ± 0.25 ^a	5.25 ± 0.38 ^a

Values are mean ± SD of triplicate ($n = 3$). Means followed by different lowercase letters in the same row are significantly different ($p < 0.05$).

Antioxidant analyses revealed significant differences in the antioxidant abilities of *F. hupehensis* flours. DPPH values ranged from 1.85 to 3.13 µmol AAE/g DW. MD flour had the highest DPPH value, and HD flour had the lowest. As for FRAP and ABTS values, they had the similar trends as DPPH in *F. hupehensis* flours. In addition, good

linear relationships was found between antioxidant properties and TPC contents (DPPH, $R^2 = 0.87$; FRAP, $R^2 = 0.96$; ABTS, $R^2 = 0.95$), thus showing that phenolics could be the main antioxidants in *F. hupehensis* flours. Similar phenomenon was also observed in previous studies (Lu *et al.*, 2018; Krishnan *et al.*, 2020).

Thermal processing decreases the nutritional value of food stuffs due to the loss of thermolabile compounds by oxidation. In the present work, MD flour had the highest TPC value, and the best antioxidant ability, followed by FD, ND, VD, and HD flours, which might have been due to their different polyphenol oxidase (PPO) activities. Due to the energy-enzyme interaction, microwave could inactivate PPO more effectively. HD could have higher PPO enzymatic activity depending on the appropriate drying temperature (Valadez-Carmona *et al.*, 2017).

Nucleosides and nucleobases

Nucleosides and nucleobases were reported as the important bioactive ingredients in traditional Chinese medicines (Shashidhar *et al.*, 2013; Chen *et al.*, 2019). Most of nucleosides and nucleobases have been found to show a variety of beneficial functions such as relieving physical fatigue, improving memory, inhibiting tumour growth, and immunomodulation (Shashidhar *et al.*, 2013; Chen *et al.*, 2019). Nucleosides and nucleobases are important components in *F. hupehensis* bulbs. Nine kinds of them (uracil, cytidine, uridine, inosine, adenine, deoxyinosine, deoxythymidine, adenosine, and deoxyadenosine) were detected in the *Fritillaria* flours. The HPLC chromatograms of nucleosides and nucleobases are shown in Figure 1, and their corresponding contents are presented in Table 4.

The nucleosides and nucleobases in *F. hupehensis* flours ranged from 727.64 to 1,654.25 µg/g. These results were lower than those of *F.*

taipaiensis, but significantly higher than those reported in *F. hupehensis* (Cao *et al.*, 2010; Peng *et al.*, 2013). Among these nine nucleosides and nucleobases, both uridine and adenosine were the dominant components in *F. hupehensis*, and their contents accounted for more than 50% of the total nucleosides and nucleobases. This finding was in accordance to a previous study (Cao *et al.*, 2010). However, uracil, cytidine, deoxyinosine, deoxythymidine, and deoxyadenosine were not reported in *F. hupehensis* previously (Cao *et al.*, 2010). The contents of nucleosides and nucleobases in dried *F. hupehensis* were directly influenced by drying methods. The elevated temperature could have activated the relevant degrading enzymes to decrease their contents (Artymowicz *et al.*, 2021). Therefore, FD flour displayed the highest level of total nucleosides and nucleobases (1654.25 µg/g). Moreover, the contents of nucleosides and nucleobases decreased in the following order: MD < HD < ND < VD < FD. Deoxythymidine and deoxyadenosine decreased intensively during MD, and this showed that deoxynucleotides were unstable during microwave treatment.

Free amino acid

The FFA contents of dried *F. hupehensis* flours are presented in Table 5. Seventeen amino acids were found, with their total contents ranging from 88.03 to 128.21 mg/g. Functional ingredients such as phenolics and alkaloids in traditional Chinese plants were generally investigated by researchers, while

Table 4. Contents of nine nucleoside and nucleobase components (µg/g) in *F. hupehensis* flours prepared by different processing methods.

Nucleoside and nucleobase	HD	VD	ND	FD	MD
Uracil	9.18 ± 0.81 ^d	25.12 ± 1.26 ^{bc}	44.55 ± 2.05 ^a	27.71 ± 1.23 ^b	21.52 ± 1.22 ^c
Cytidine	24.71 ± 1.79 ^d	43.14 ± 1.94 ^c	26.31 ± 1.83 ^d	60.26 ± 3.05 ^b	81.57 ± 4.21 ^a
Uridine	333.05 ± 7.93 ^c	374.06 ± 2.82 ^b	358.93 ± 10.01 ^{bc}	510.19 ± 7.71 ^a	185.56 ± 8.64 ^d
Inosine	57.27 ± 2.57 ^c	86.05 ± 3.25 ^b	102.67 ± 5.53 ^a	82.65 ± 6.32 ^b	66.90 ± 1.28 ^c
Adenine	98.45 ± 5.35 ^b	112.96 ± 0.62 ^b	105.44 ± 8.31 ^b	154.02 ± 2.68 ^a	74.28 ± 1.56 ^c
Deoxyinosine	20.81 ± 2.33 ^c	55.69 ± 2.01 ^a	58.65 ± 2.81 ^a	39.65 ± 2.05 ^b	15.65 ± 0.48 ^c
Deoxythymidine	124.39 ± 4.11 ^c	142.14 ± 0.37 ^b	117.31 ± 5.34 ^c	171.66 ± 3.07 ^a	16.52 ± 0.85 ^d
Adenosine	286.24 ± 12.68 ^{bc}	301.20 ± 2.32 ^b	261.76 ± 6.59 ^c	394.30 ± 2.03 ^a	220.96 ± 4.43 ^d
Deoxyadenosine	123.61 ± 6.67 ^c	164.83 ± 0.79 ^b	123.77 ± 2.69 ^c	213.82 ± 0.56 ^a	44.66 ± 1.04 ^d
total	1077.72 ± 40.22 ^d	1305.18 ± 6.66 ^b	1199.39 ± 31.22 ^c	1654.25 ± 23.09 ^a	727.64 ± 10.34 ^e

Values are mean ± SD of triplicate ($n = 3$). Means followed by different lowercase letters in the same row are significantly different ($p < 0.05$).

Table 5. Contents of free amino acids (mg/g) in *F. hupehensis* flours prepared by different processing methods.

Amino acid	HD	VD	ND	FD	MD
Asp	8.50 ± 0.14 ^b	7.88 ± 0.11 ^b	8.59 ± 0.40 ^b	10.92 ± 0.44 ^a	11.42 ± 0.25 ^a
Glu	7.43 ± 0.61 ^b	8.86 ± 0.32 ^a	9.78 ± 0.21 ^a	10.09 ± 0.34 ^a	9.87 ± 0.21 ^a
Ala	7.41 ± 0.51 ^{ab}	3.68 ± 0.14 ^c	6.25 ± 0.14 ^b	7.77 ± 0.61 ^a	2.49 ± 0.21 ^c
Gly	4.70 ± 0.24 ^{ab}	4.33 ± 0.25 ^b	5.71 ± 0.13 ^a	5.05 ± 0.55 ^{ab}	5.43 ± 0.13 ^{ab}
Pro	3.30 ± 0.13 ^b	3.36 ± 0.31 ^b	4.35 ± 0.3203 ^{ab}	5.41 ± 0.42 ^a	4.91 ± 0.43 ^a
Ser	3.96 ± 0.18 ^c	3.96 ± 0.21 ^c	4.46 ± 0.28 ^{bc}	5.27 ± 0.29 ^{ab}	5.36 ± 0.16 ^a
Thr	2.75 ± 0.08 ^c	3.00 ± 0.22 ^{bc}	3.58 ± 0.30 ^{ab}	3.82 ± 0.13 ^a	4.14 ± 0.09 ^a
Arg	23.53 ± 1.51 ^b	22.92 ± 0.37 ^b	21.05 ± 0.75 ^b	23.24 ± 0.88 ^b	33.07 ± 0.17 ^a
His	2.25 ± 0.03 ^b	1.75 ± 0.06 ^c	1.90 ± 0.10 ^{bc}	3.13 ± 0.11 ^a	2.24 ± 0.14 ^b
Leu	9.27 ± 0.85 ^{ab}	7.75 ± 0.52 ^b	8.08 ± 0.61 ^b	11.35 ± 0.31 ^a	7.28 ± 0.42 ^b
Ile	1.72 ± 0.18 ^c	0.95 ± 0.08 ^c	1.25 ± 0.13 ^c	2.94 ± 0.24 ^b	3.85 ± 0.37 ^a
Met	nd	nd	nd	nd	0.64 ± 0.02 ^a
Phe	7.84 ± 0.33 ^b	6.27 ± 0.57 ^c	6.26 ± 0.08 ^c	10.91 ± 0.08 ^a	7.24 ± 0.35 ^{bc}
Val	4.95 ± 0.32 ^b	3.74 ± 0.25 ^c	5.01 ± 0.37 ^b	6.07 ± 0.25 ^a	4.72 ± 0.08 ^{bc}
Tyr	7.36 ± 0.35 ^b	3.45 ± 0.41 ^c	6.14 ± 0.33 ^b	10.79 ± 0.85 ^a	3.15 ± 0.07 ^c
Lys	5.32 ± 0.62 ^b	4.13 ± 0.23 ^b	4.39 ± 0.18 ^b	8.53 ± 0.72 ^a	5.71 ± 0.30 ^b
Cys	2.52 ± 0.16 ^a	2.00 ± 0.31 ^a	2.58 ± 0.07 ^a	2.92 ± 0.57 ^a	0.60 ± 0.06 ^b
MSG-like	15.93 ± 1.06 ^c	16.74 ± 0.61 ^{bc}	18.37 ± 0.86 ^b	21.01 ± 1.10 ^a	21.29 ± 0.65 ^a
Sweet	22.12 ± 1.61 ^{bc}	18.33 ± 1.60 ^c	24.35 ± 1.65 ^{ab}	27.32 ± 2.83 ^a	22.33 ± 1.44 ^{abc}
Bitter	49.56 ± 4.55 ^b	43.38 ± 2.62 ^b	43.55 ± 2.88 ^b	57.64 ± 2.64 ^a	59.04 ± 2.19 ^a
Tasteless	15.20 ± 1.60 ^b	9.58 ± 1.34 ^c	13.11 ± 0.82 ^{bc}	22.24 ± 3.06 ^a	9.46 ± 0.61 ^c
Total	102.81 ± 8.82 ^{bc}	88.03 ± 6.16 ^c	99.38 ± 6.22 ^{bc}	128.21 ± 9.60 ^a	112.12 ± 4.89 ^{ab}

nd: not detected; MSG-like: Asp + Glu; sweet: Ala + Gly + Ser + Thr + pro; bitter: Arg + His + Ile + Leu + Met + Phe + Val; and tasteless: Lys + Tyr + Cys. Values are mean ± SD of triplicate ($n = 3$). Means followed by different lowercase letters in the same row are significantly different ($p < 0.05$).

FFA were still ignored (Qureshi *et al.*, 2013; Li *et al.*, 2019). The total amino acid levels in *F. hupehensis* flours were significantly higher than those of *Coptis* herbs (Li *et al.*, 2019).

Consistent with earlier reports, drying methods affected the FFA contents of *F. hupehensis* (Ghribi *et al.*, 2015; Hu *et al.*, 2020). The content of total FFA in different dried *F. hupehensis* flours decreased in the order of FD > MD > HD > ND > VD. Thermal treatment has been proven to show double impacts on FFA. It does not only reduce the content of free amino acids through the Strecker degradation and Maillard reaction, but also generates new free amino acids by proteolysis (Li *et al.*, 2011). The greater loss of FFA in VD flour might have been due to the greater intake of amino acids by Maillard reaction but low proteolysis release.

The content of bitter-taste amino acids, taking up to 43.82 - 52.67% of the total amino acids, was the predominant ingredient, followed by sweet (19.91 - 24.50%), MSG-like (15.49 - 19.02%), and tasteless (8.44 - 17.35%) amino acids. It has been reported that the taste of *Fritillaria* bulbs is typically bitter (Cunningham *et al.*, 2018), and the large accumulation of bitter-taste amino acids could contribute to this. Arginine (21.05 - 33.07 mg/g) was the major amino acid among others in *F. hupehensis* flour. Arginine has been associated with the vasodilator effects of nitric oxide, and is also beneficial for muscle development and repair (Gong *et al.*, 2021). MD flour had the highest percentage of bitter-taste amino acids, and lowest sweet-taste amino acids. ND flour had the highest percentage of sweet-taste amino acids, and lowest percentage of bitter-

taste amino acids. Consequently, MD might favour the release of bitter-taste amino acids, and ND might weaken the bitter taste of dried *F. hupehensis* flour. The content of FFA in *F. hupehensis* was analysed for the first time.

Differential scanning calorimetry analysis

Differential scanning calorimetry (DSC) was applied to analyse the gelatinisation process and characterisation of the *F. hupehensis* flours (Table 6). The onset of the gelatinisation temperature (T_o) was observed between 65.23 to 73.27°C, the peak gelatinisation temperature (T_p) and the end set gelatinisation temperature (T_c) were in the range of 74.53 - 77.80 and 80.43 - 77.80°C, respectively. It has been reported that saccharide, amylose, lipid, and protein contents in the starch granules could affect the thermal parameters of flours (Lapčíková *et al.*, 2021; Li *et al.*, 2022). For MD flour, T_o ($65.23 \pm 0.23^\circ\text{C}$) and T_p ($74.53 \pm 0.06^\circ\text{C}$) values shifted to relatively low temperatures, and exhibited wide range of ΔT ($15.20 \pm 0.23^\circ\text{C}$), while ND flour had the high T_o ($73.27 \pm 0.01^\circ\text{C}$) and T_p ($77.80 \pm 0.39^\circ\text{C}$) value, and narrow range of ΔT ($10.17 \pm 0.11^\circ\text{C}$). These differences could be explained by their different granulated architecture, and the different composition

of amylose-amylose and amylose-lipid complexes (Li *et al.*, 2022). Certain amount of starch within MD flour might have pre-gelatinised, thus resulting in lower gelatinisation temperature (Zhu *et al.*, 2020; Li *et al.*, 2022). ND flour, without special treatment, could comprise a more ordered and compact crystalline structure; therefore, higher temperature was required to melt the starch crystals.

The ΔH of *F. hupehensis* flours ranged as 3.01 - 3.85 J/g, and their sequences were: MD < HD < ND < VD < FD. Previous study indicated that microwave treatment could partially gelatinise these flours, and decrease their corresponding ΔH value (Villanueva *et al.*, 2018). The gelatinisation enthalpy ΔH was determined by the molecular structure and water binding ability of the flour. Therefore, the ΔH value might be related to the swelling power of the flour sample (Lapčíková *et al.*, 2021). Accordingly, ΔH values obtained in the present work were in agreement with their swelling capacities. The higher degree of crystalline structure in the flour will need more energy to accomplish gelatinisation (Shen *et al.*, 2020). Microwave treatment could disrupt the crystal structure of *F. hupehensis* flour, and led to a decreased ΔH value.

Table 6. Thermal characteristics of *F. hupehensis* flours prepared by different processing methods.

Thermal parameter	HD	VD	ND	FD	MD
T_o	72.40 ± 0.15^c	71.23 ± 0.15^d	73.27 ± 0.01^a	72.80 ± 0.06^b	65.23 ± 0.23^e
T_p	77.67 ± 0.67^a	76.23 ± 0.34^b	77.80 ± 0.39^a	77.67 ± 0.22^a	74.53 ± 0.06^c
T_c	83.83 ± 0.20^a	82.53 ± 0.06^b	83.43 ± 0.11^a	83.60 ± 0.10^a	80.43 ± 0.45^c
ΔT ($^\circ\text{C}$)	11.43 ± 0.21^b	11.30 ± 0.26^b	10.17 ± 0.11^d	10.80 ± 0.36^c	15.20 ± 0.23^a
ΔH (J/g)	3.18 ± 0.32^b	3.41 ± 0.35^{ab}	3.27 ± 0.43^b	3.85 ± 0.26^a	3.01 ± 0.03^b

Values are mean \pm SD of triplicate ($n = 3$). Means followed by different lowercase letters in the same row are significantly different ($p < 0.05$).

Conclusion

In the present work, the physicochemical composition, antioxidant ability, and thermal properties of *F. hupehensis* flour, processed by HD, VD, ND, FD, and MD were investigated. Noticeable differences were found in these *F. hupehensis* flours, and MD flour was significantly different from others. MD flour had lower concentrations of total starch, amylose, protein, nucleosides and nucleobases, and VB₁ and VB₂, but a higher TPC, WBC value, antioxidant capacity. The highest content of protein,

VB₁, free amino acids, nucleosides and nucleobases, and TFC value were found in FD flour, thus making it ideal for the preservation of chemical ingredients in *F. hupehensis* flour. MD flour was easier to be gelatinised, whereas FD flour was the opposite. HD flour had the highest total starch while ND flour imparted bitter taste of the dried *F. hupehensis*. As a conclusion, different drying methods would serve different processing needs of *F. hupehensis* flour. The present work provided valuable information for further production and application of *F. hupehensis* flour in the functional food industry.

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