

ORIGINAL RESEARCH

Open Access



Superfine grinding modulates the volatile profile and enhances physical properties, chemical components and bio-activities of *Carthamus tinctorius* L. by microstructural destruction

Tianjian Guo¹, Yu Li¹, Jun Li¹, Xiao Chen², Ghulam Murtaza³, Han Cheng¹, Xincan Xiao¹, Leiling Shi⁴, Ruixi Gao¹ and Xianju Huang^{1,2*}

Abstract

In this study, safflower powders with varying particle sizes were prepared by conventional and superfine grinding methods. Results of particle size distribution, morphology, color, flow and hydration properties, Fourier transform infrared spectra, and volatile components showed that superfine grinding reduced the particle size to the micron scale and markedly increased the specific surface area, enhanced cell wall disruption and flowability, and improved brightness as well as red-yellow coloration. Hydration properties exhibited increases in solubility and swelling capacity but decrease in water-holding capacity. Fourier transform infrared spectroscopy spectra confirmed that no structural changes occurred, while gas chromatography–mass spectrometry analysis revealed distinct volatile profiles, with superfine powders showing stronger fruity and floral attributes. Furthermore, the availabilities of bioactive compounds such as hydroxysafflor yellow A, flavonoids, and polysaccharides were elevated, correlating with enhanced antioxidant and anticoagulant activities. These findings demonstrate that superfine grinding enhances the physico-chemical characteristics, volatile profiles, and bioactivity of safflower powder, thus offering valuable potential for its use in food, nutraceutical, and pharmaceutical applications.

Keywords *Carthamus tinctorius* L., Superfine grinding, Volatile profile, Microstructural destruction, Physical properties

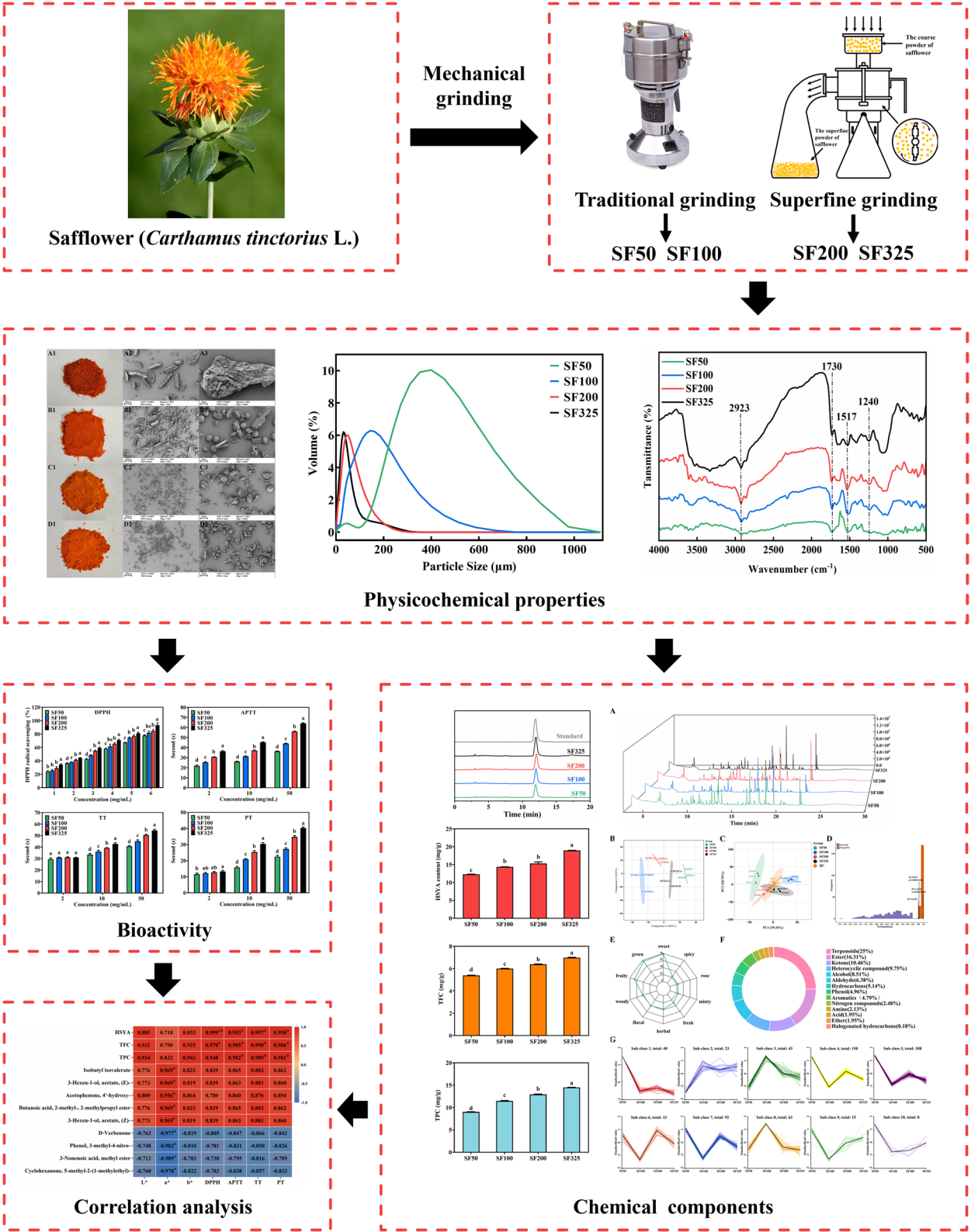
*Correspondence:

Xianju Huang

xianju@mail.scuec.edu.cn

Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Safflower (*Carthamus tinctorius* L.) is the dried tubular inflorescence of an annual or biennial species belonging to the family Asteraceae [1]. It is typically processed into dried flowers for various uses and has been distributed across Asia, North America, and South America. Safflower is rich in functional components, including flavonoids, quinone chalcones, alkaloids, steroids, organic acids, polyacetylenes, and bioactive polysaccharides, which have demonstrated significant medicinal value [2]. It exhibits notable pharmacological properties, including anticoagulant, cardiovascular-protective, anti-inflammatory, and antioxidant activities, and has shown therapeutic efficacy against hypertension, hyperlipidemia, coronary heart disease, and angina pectoris [3]. When processed into powder, safflower effectively retains its nutritional components and can be used to produce safflower wine, tea, and other products. Safflower also serves as a natural pigment and flavoring agent in food processing, further enhancing its versatility as a plant resource [4].

Superfine grinding is an emerging food processing technology primarily employed to produce micron, submicron, or nano-scale (100 μm to 1 nm) powder products. The working principle of superfine grinding involves utilizing high-speed rotating blades to impact particles, thereby reducing their size, followed by separation via cyclone airflow (Fig. 1A). The reduction in particle size achieved through superfine grinding increases the specific surface area of the particles and disrupts their cellular structures. This process facilitates the full exposure of bioactive constituents and may have an impact on the powder's bioactivity [5]. Additionally, superfine grinding can improve product edibility and taste, demonstrating substantial application potential in the production of nutritional and functional foods [6].

Currently, research on safflower powder remains limited, with few studies addressing its physicochemical properties, volatile constituents, and bioactivities across different particle sizes. This study aims to examine the impact of particle size reduction via superfine grinding on the physicochemical properties, volatile components, and bioactivity of safflower powder. Multiple indicators were analyzed, including particle size distribution, morphology, flow property, hydration property, volatile components, bioactive chemical components content, antioxidant activity, and anticoagulant activity. Findings of this study would provide a theoretical foundation for the application of safflower powder in the food, nutraceutical, and pharmaceutical industries.

Materials and methods

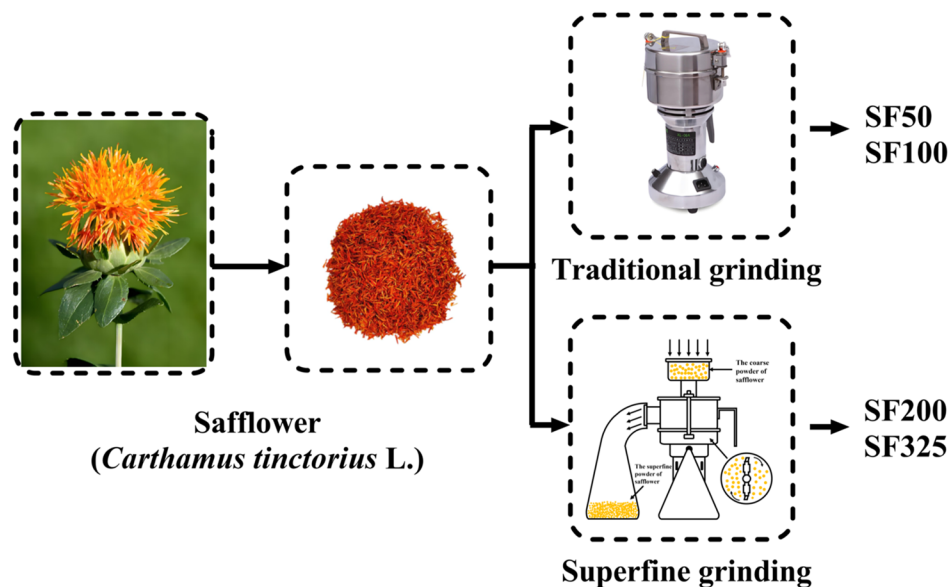
Materials

Safflower (*Carthamus tinctorius* L.) was sourced from Yili City, Xinjiang Province, and purchased from Hubei Yaosheng Traditional Chinese Medicine Technology Co., Ltd. (Zaoyang, China). Ethyl alcohol ($\geq 99.7\%$), n-butanol ($\geq 99.5\%$), chloroform ($\geq 99\%$), concentrated sulfuric acid ($\geq 95\%$), acetonitrile ($\geq 99.5\%$), phosphoric acid ($\geq 85\%$), phenol ($\geq 99\%$), anhydrous glucose ($\geq 99\%$), sodium nitrite (NaNO_2) ($\geq 98\%$), aluminum chloride (AlCl_3) ($\geq 99\%$), and sodium hydroxide (NaOH) ($\geq 96\%$) were supplied by Shanghai Hushi Laboratory Equipment Co., Ltd. (Shanghai, China). Potassium bromide (KBr, spectroscopic grade, $\geq 99\%$) was supplied by Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Hydroxysafflor yellow A (HSYA) ($\geq 98\%$, HPLC, Lot No. A091B212053) and Rutin ($\geq 97\%$, HPLC, Lot No. D221B235230) were obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) ($\geq 96\%$, Lot No. C17432088) was obtained from Shandong Keyuan Biochemical Co., Ltd. (Shandong, China). Freeze-dried rabbit plasma (sodium citrate anticoagulated, pooled, Lot No. 20250326) was supplied by Qingdao Hi-tech Industrial Park Hope Biotechnology Co., Ltd. (Qingdao, China) and stored at $2-8^\circ\text{C}$ for later use. Activated partial thromboplastin time (APTT), thrombin time (TT), and prothrombin time (PT) assay kits (Cat. Nos. GCTC0306, GCTC0335, GCTC0307) were supplied by Beijing Vokai Biotechnology Co., Ltd. (Beijing, China) and stored at 4°C for later use. The APTT kit contained sodium citrate anticoagulant, ellagic acid solution, and CaCl_2 solution; the TT kit contained sodium citrate anticoagulant and thrombin solution; and the PT kit contained sodium citrate anticoagulant and thromboplastin solution. All of the experiments were carried out using ultrapure deionized water (Milli-Q grade).

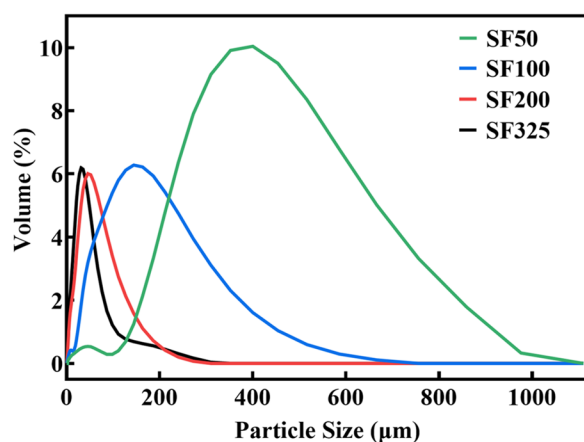
Preparation of safflower powder

Fresh safflower was air-dried at room temperature ($25 \pm 2^\circ\text{C}$, relative humidity 45–55%) until constant weight before grinding. The dried material was coarsely ground using a high-speed universal grinder (FW250, Zhengzhou Kefeng Instrument and Equipment Co., Ltd., Zhengzhou, China) for 2 min to obtain the coarse powder of safflower. The ground material was then sieved using an electric vibrating screen machine (Type 200, Xinxiang Zhongtai Machinery Co., Ltd., Xinxiang, China) shaker through 50-mesh (300 μm) and 100-mesh (150 μm) sieves. The SF 50 and SF100 corresponded to the samples between the 50-mesh and 100-mesh, and between the 100-mesh and 200-mesh, respectively. Subsequently, the SF100 was pulverized in a small ultra-micro grinder (WF-18, Wenzhou Dingli Medical Devices

A



B



C

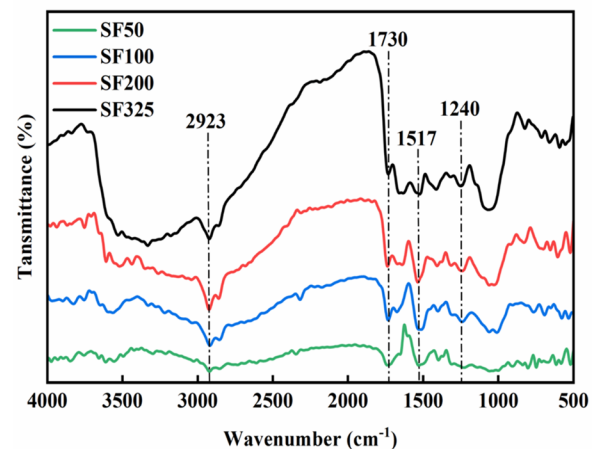


Fig. 1 Preparation and physical properties of safflower powders. **A** Preparation of safflower powder; **B** Particle size distribution of safflower powders; **C** FTIR of safflower powders. Values with different superscript letters in the same graph are significantly different ($P < 0.05$)

Co., Ltd., Wenzhou, China) for 1 min. This pulverized material was then sieved through 200-mesh (75 μm) and 325-mesh (45 μm) sieves to obtain superfine safflower powder. The SF200 and SF325 samples corresponded to powders that passed through a 200-mesh sieve but were retained on a 325-mesh sieve, and to powders finer than 325-mesh, respectively. Both the high-speed universal grinder and the small ultra-micro grinder operated at a speed of 25,000 rpm. To minimize heat accumulation and prevent the thermal degradation of phytochemicals, an intermittent grinding cycle was employed, consisting of 30 s of operation followed by a 10-min cooling period. Throughout this process, the maximum temperature was

strictly maintained below 40 °C. To ensure the reliability of the preparation process, three independent batches of superfine safflower powders were prepared for each particle size level.

Determination of particle size analysis

The particle size and surface area of the pulverized powders were analyzed using a laser diffraction particle size analyzer (Mastersizer 3000+Ultra, Malvern Panalytical, UK), with deionized water as the dispersing agent, to obtain the D_{10} , D_{50} , and D_{90} values and specific surface area (SSA) of each powder. Furthermore, the span, which represents the width of particle size distribution, and cell

wall breakage ratio (Φ), were determined using the following equations, respectively:

$$\text{Span} = (D_{90} - D_{10})/D_{50} \quad (1)$$

$$\phi = 1 - (1 - 10/D_{50})^3 \quad (2)$$

Determination of scanning electron microscopy

Powder samples were mounted on aluminum stubs with conductive carbon tape, vacuum-dried, and sputter-coated with a 10 nm gold layer (Q150R Plus, Quorum, UK). Surface morphology was observed using a scanning electron microscope (SEM, Sigma 360, ZEISS, Germany) at an accelerating voltage of 5 kV, with images captured at magnifications of $\times 50$, $\times 100$, and $\times 200$.

Determination of color difference

The color parameters (L^* , a^* , and b^*) of safflower powder were measured using a colorimeter (LS173, Shenzhen Linshang Technology Co., Ltd., Shenzhen, China), with three measurements per sample averaged. The measuring light source was an LED, with an 8 mm measuring aperture, and the results were displayed in terms of chromaticity values. A standard white plate ($L^* = 97.08$, $a^* = -0.45$ and $b^* = -0.12$) was used for calibration. The color difference values (ΔE^*) of powders were calculated according to equation:

$$\Delta E^* = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2} \quad (3)$$

where L_0 , a_0 , b_0 and L^* , a^* , b^* are the color parameters of SF50 sample and other ones, respectively.

Determination of flow property

Tap density

The tap density of safflower powder was determined following the procedure described by Huang [7]. A known mass of safflower powder (M_1) was transferred into a 10 mL measuring cylinder using a glass funnel. Then it was shaken on thick sponge until the volume no longer decreased, and the final compacted volume (V) was recorded. The tap density was calculated according to equation:

$$\text{Tap density (mg/mL)} = M_1/V \quad (4)$$

Angle of repose and angle of slide

The angles of repose and slide were determined according to the Zhang's method [8]. The funnel was fixed to a test tube stand and positioned above a sheet of grid paper. The 10 g of safflower powder was slowly and uniformly poured into the funnel until the apex of the powder pile

reached the funnel outlet. The height of the cone (H), as well as the radius of the cone's base (R), was measured. The angle of repose was calculated according to equation:

$$\text{Angle of repose (}^\circ\text{)} = \arctan H/R \quad (5)$$

A glass plate was placed horizontally on the desktop, and its length (L) was measured, and one end of the glass plate was secured. The 10 g of safflower powder was placed at the non-fixed end of the glass plate. The glass plate was then slowly lifted until the safflower powder started to slide. The height (H) of the non-fixed end was recorded. The angle of slide was then calculated using the following formula:

$$\text{Angle of slide (}^\circ\text{)} = \arcsin H/L \quad (6)$$

Determination of hydration property

Water solubility index

The water solubility index (WSI) of safflower was measured using the method of Yi et al. with some modifications [9]. A 0.5 g portion (M_0) of safflower powder was accurately weighed and thoroughly mixed with 50 mL of purified water. The mixture was heated in a water bath at 80 °C for 30 min, followed by centrifugation at 6,000 rpm for 10 min at 4 °C. The supernatant was filtered and transferred into a pre-weighed dry beaker (M_1). The beaker was dried in a 70 °C drying oven until a constant weight was reached. The final weight of the beaker (M_2) was recorded. The WSI was calculated using the following formula:

$$\text{WSI (\%)} = (M_2 - M_1)/M_0 \quad (7)$$

Water-holding capacity

The method of Zhang et al. was adopted and slightly modified to measure water-holding capacity (WHC) [8]. A 0.5 g of safflower powder (M_0) was weighed and mixed with 50 mL of purified water in pre-weighed centrifuge tubes (M_1). The mixture was subsequently placed on a shaker and agitated at 100 rpm for 30 min. The sample was then centrifuged at 6,000 rpm for 15 min at room temperature. The supernatant was discarded, and the precipitate was blotted to remove surface moisture. The centrifuge tubes were subsequently weighed to determine the mass (M_2). The WHC was calculated using the following formula:

$$\text{WHC (g/g)} = (M_2 - M_1 - M_0)/M_0 \quad (8)$$

Swelling capacity (SC)

The swelling capacity (SC) was determined according to the method described by Weng et al. [10]. A 0.5 g of the safflower powder (M) was weighed and uniformly loaded

into a graduated test tube using a glass funnel. The initial volume (V_1) was recorded. Subsequently, 5 mL of purified water was slowly added, and the mixture was thoroughly mixed. After being sealed and left to stand at room temperature ($25 \pm 2^\circ\text{C}$) for 24 h to ensure complete water absorption and sedimentation, the settled volume (V_2) was recorded. The SC was calculated using the following formula:

$$\text{SC (mL/g)} = (V_2 - V_1)/M \quad (9)$$

Fourier transform infrared spectroscopy analysis

Safflower powder and potassium bromide (KBr) were dried at 80°C for 4 h before analysis. 1 mg of safflower powder was thoroughly mixed with KBr at a ratio of 1:100, and the mixture was then compressed into a semi-transparent pellet for Fourier transform infrared spectroscopy (FTIR) analysis. FT-IR spectra were recorded at room temperature using a Nicolet 6700 FTIR spectrophotometer (Thermo Fisher Scientific, USA) in the range of $4000\text{--}400\text{ cm}^{-1}$, with 32 scans at a resolution of 4 cm^{-1} . The crystallinity index of cellulose was calculated from the FTIR spectra according to the method of Zhao et al. [11], which is based on the absorption band ratios A_{1374}/A_{2900} and A_{1374}/A_{668} . The FTIR spectral data were preprocessed by baseline correction, Savitzky–Golay smoothing (5-point window), and normalization.

Characterization of volatile components

The volatile components of safflower powders were analyzed using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS/MS, Agilent 8890–7000D, Agilent Technologies, USA). The detailed sample preparation, extraction, and instrumental conditions are provided in the Supplementary Materials (Section S1).

Determination of bioactive ingredient content

Hydroxysafflor yellow A content

The HPLC method was conducted in accordance with the guidelines of the Chinese Pharmacopoeia (2020 edition) [12]. To measure the hydroxysafflor yellow A (HSYA) content, 0.4 g of safflower powder was mixed with 50 mL of 25% methanol, then the solution was weighed. The solution was sonicated for 40 min, cooled, and weighed again. The lost weight was replenished with 25% methanol, and the solution was then filtered through a $0.22\text{ }\mu\text{m}$ microporous membrane filter to obtain the sample solution.

The HPLC method was developed using an Agilent 1200 series HPLC system (Agilent Technologies, Santa

Clara, CA, USA) equipped with a WondaSil C18-WR column ($4.6 \times 250\text{ mm}$, $5.0\text{ }\mu\text{m}$). The mobile phase was composed of methanol, acetonitrile, and 0.7% phosphoric acid solution (26:2:72), with a flow rate of 1.0 mL/min and an injection volume of $10\text{ }\mu\text{L}$, a constant temperature of 25°C , and detection at 403 nm . The runtime per sample was 20 min. The content of HSYA in the determination solution was quantified using a standard curve of HSYA standard solution ($0.052\text{--}0.208\text{ }\mu\text{g}/\mu\text{L}$). The standard curve was expressed by the equation: $y = 21,206.79x - 18.395$, with an R^2 value of 0.9999. Where y represents the peak area and x represents the content of HSYA in the sample solution (mg/mL). HSYA content was expressed as $\text{mg HSYA/g dry safflower powder}$. The content of HSYA in the sample solution was calculated using the specified formula:

$$\text{The content of HSYA (mg/g)} = (C \times V)/M \quad (10)$$

where C represents the HSYA content of the sample solution (mg/mL); V is the volume of the sample solution (mL); M is the mass of the sample (g).

Total flavonoid content

To measure total flavonoid content (TFC), 1.0 g of safflower powder was thoroughly mixed with 50 mL of 70% ethanol, then the mixture was sonicated at 70°C for 30 min and then centrifuged at $10,000\text{ rpm}$ for 10 min at 4°C , followed by filtration through a $0.22\text{ }\mu\text{m}$ microporous membrane filter to obtain the sample solution.

A 4 mL aliquot of the sample solution was mixed with 0.3 mL of 5% NaNO_2 solution, thoroughly homogenized, and subsequently allowed to stand for 6 min. Subsequently, 0.3 mL of 10% AlCl_3 was added, mixed well, and the solution was allowed to stand for another 6 min. Afterwards, 4 mL of 4% NaOH solution was added, followed by dilution to 10 mL with 70% ethanol. After a 15 min standing period, the absorbance was measured by a UV spectrophotometer (UV-1800, Shimadzu Management (China) Co., Ltd., Shanghai, China) at 510 nm [13]. A 70% ethanol solution was used as the solvent blank for absorbance correction. All tests were conducted under consistent experimental conditions. The TFC in the sample solution was quantified using a calibration curve prepared from rutin standard solutions ($0.02\text{--}0.06\text{ mg/mL}$). Results were expressed as $\text{mg rutin equivalent (RE)/g DW}$. The standard curve was expressed by the equation: $y = 12.68x + 0.0284$, with an R^2 value of 0.998. Where y represents the absorbance and x represents the TFC in the determination solution (mg/mL). The TFC of the sample solution was calculated according to the equation:

$$\text{TFC (mg/g)} = (C \times V \times N)/M \quad (11)$$

where C represents the flavonoid content of the determination solution (mg/mL); N is the dilution factor; V is the volume of the determination solution (mL); M is the mass of the sample (g).

Total polysaccharide content

To determine total polysaccharide content (TPC), 2.0 g of sample powder were thoroughly mixed with 50 mL of purified water. The mixture was subjected to ultrasonic extraction at 65 °C for 55 min and then filtered through a 0.22 µm microporous membrane filter to collect the filtrate. The filtrate was then subjected to protein removal using the Sevag method. A 2 mL aliquot of the deproteinized filtrate was mixed with five times its volume of absolute ethanol and maintained at 4 °C for 24 h. The mixed solution was centrifuged at 5,000 rpm for 10 min at 4 °C, and the supernatant was removed to collect the precipitate. The precipitate was washed with anhydrous ethanol and subsequently dried at 30 °C to a constant weight. The dried precipitate was dissolved in 20 mL of purified water to obtain the sample solution [14].

A 1.0 mL aliquot of the sample solution was transferred to a test tube, diluted to 3 mL with purified water, and thoroughly mixed with 1 mL of 5% phenol solution

Determination of DPPH radical scavenging activity of safflower

To measure the radical scavenging activity, 0.3 g of safflower powder was thoroughly mixed with 50 mL of anhydrous ethanol. Ultrasonic extraction was performed for 30 min, after which the extract was filtered through a 0.22 µm microporous membrane to obtain the sample solution. During the extraction process, the samples were protected from light [16]. Five aliquots of the sample solution (5, 4, 3, 2, and 1 mL) were each diluted with anhydrous ethanol to a final volume of 6 mL, resulting solutions at concentrations of 6, 5, 4, 3, 2, and 1 mg/mL, respectively. A 100 µL aliquot of each sample solution was mixed with 100 µL of a 0.1 mmol/L DPPH solution. The DPPH stock solution was freshly prepared on a daily basis and stored under dark conditions. The mixtures were kept in darkness for 30 min, after which the absorbance values were measured at 517 nm using a microplate reader (Spark 10 M, Diken (Shanghai) Laboratory Equipment Co., Ltd., Shanghai, China). The antioxidant activity was expressed as the percentage inhibition of DPPH radicals, which was calculated at each tested concentration. The DPPH radical scavenging activity was calculated according to the specified formula [17].

$$\text{DPPH radical scavenging activity (\%)} = [A_0 - (A_1 - A_2)]/A_0 \times 100 \quad (13)$$

and 5 mL of concentrated sulfuric acid. The mixture was heated in a 40 °C water bath for 15 min, cooled in an ice bath for 5 min, and the absorbance was subsequently measured at 490 nm using a UV spectrophotometer (UV-1800, Shimadzu Management (China) Co., Ltd., Shanghai, China) [15]. All tests were carried out under consistent experimental conditions at room temperature. The TPC of determination solution was determined using a calibration curve prepared from standard glucose solutions (0.2–1.0 mg/mL). Results were expressed as mg glucose equivalent (GE)/g DW. The standard curve was expressed by the equation: $y = 7.67x - 0.011$, with an R^2 value of 0.9991. Where y represents the absorbance, x represents the TPC in the determination solution (mg/mL). The TPC of the sample solution was calculated according to the equation:

$$\text{TFC (mg/g)} = (C \times V \times N)/M \quad (12)$$

where C represents the concentration of glucose in the determination solution (mg/mL); N is the dilution factor; V is the volume of the determination solution (mL); M is the mass of the sample (g).

where A_0 represents the absorbance of the mixture of 100 µL 0.1 mmol/L DPPH solution and 100 µL anhydrous ethanol; A_1 represents the absorbance of the mixture of 100 µL sample solutions and 100 µL of 0.1 mmol/L DPPH solution; A_2 represents the absorbance of the mixture of 100 µL sample solutions and 100 µL anhydrous ethanol.

Determination of anticoagulant ability

The anticoagulant activity of safflower extracts was evaluated using activated partial thromboplastin time (APTT), thrombin time (TT), and prothrombin time (PT) assays. The experimental procedures followed standard protocols as described in the Supplementary Material (Section S1.2). All measurements were conducted at 37 °C under consistent conditions, and the clotting times were expressed in seconds (s).

Statistical analysis

Each experiment was repeated three times. Statistical significance was assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's HSD test. Significant differences between groups were indicated by different lowercase letters (a, b, c, etc.), where groups sharing

the same letter were considered not significantly different ($P \geq 0.05$), whereas groups with different letters were considered significantly different ($P < 0.05$). The results are expressed as the mean $\pm$ standard deviation ($n=3$). Graphs were generated using GraphPad Prism 9.5 and Origin 2022. Statistical significance was analyzed using SPSS 27.

Results and discussion

Particle size

Particle size distribution, typically represented by D_{10} , D_{50} , and D_{90} , is a standard indicator for characterizing particle size, with D_{50} corresponding to the median size. The particle size distribution curves of the powders are presented in Fig. 1B, with the corresponding data summarized in Table 1. As shown in Table 1, D_{10} values decreased from 142 μm to 6.24 μm , D_{50} values decreased from 375.33 μm to 27.8 μm , and D_{90} values decreased from 674 μm to 75.23 μm . This substantial reduction in particle size indicates that superfine grinding is significantly more effective than conventional milling, likely due to the combined effects of shear, collision, and impact forces that efficiently break down particles. Correspondingly, the specific surface area of the powders increased markedly from 46.15 m^2/kg to 427.20 m^2/kg , which enhances the contact area for solvent interaction and may improve the dissolution and extraction efficiency of intracellular bioactive compounds.

The span value reflects the uniformity of particle size distribution, which is critical for powder solubility and bioavailability. As summarized in Table 1, the span values of safflower powders increased from 1.42 to 2.48 with increasing grinding intensity. SF325 exhibited a larger span value compared to SF50 and SF100, but was not significantly different from SF200. This suggests that superfine grinding produces a slightly broader size distribution, which may enhance dissolution rates while maintaining functional bioactivity, consistent with previous observations in water dropwort powders and *Lycium ruthenicum* Murray powders [18, 19].

Cell wall breakage ratio (Φ), representing the percentage of cells with damaged walls, increased dramatically

from 7.78% in coarser powders to 73.75% in SF325 (Table 1). This substantial increase is consistent with the findings of Xu et al. [20], and indicates that superfine grinding effectively disrupts cell walls, reducing mass transfer resistance and facilitating the release of intracellular compounds such as flavonoids and polysaccharides. Crucially, this extensive structural disruption also played a pivotal role in modulating the volatile profile. The breakdown of the cellular matrix likely facilitated two key processes: first, the direct release of volatile oils from ruptured oil cells; and second, the promotion of contact between glycosidases and glycosidic precursors. This enhanced enzymatic interaction accelerated the hydrolysis and subsequent formation of esters and terpenes, directly contributing to the enriched fruity and floral notes observed in superfine powders. Collectively, the combination of reduced particle size, increased specific surface area, and enhanced cell wall breakage contributes synergistically to the improved extraction efficiency and bioavailability of bioactive components in functional plant powders.

Microscopic morphology

Figure 2 presents the actual and SEM images of safflower powder particles with different sizes. From the SEM images, it is evident that the superfine grinding makes the particle size and shape of the safflower powders change significantly. As shown in Fig. 2 A2, A3, B2, and B3, the conventionally crushed coarse powders exhibited relatively large particles, with diameters greater than 100 μm , with irregular shapes that included both spherical and fragmented forms. In contrast, Fig. 2 C2, C3, D2, and D3 illustrates that superfine grinding reduced particle size to below 50 μm , producing more regular morphologies. Furthermore, as the particle size decreased, partial aggregation phenomena were observed in the superfine powder. This aggregation is likely a result of the mechanical forces generated during superfine grinding, which not only reduced particle size but also increased surface energy. The resultant instability of the particles increased interparticle adhesion, leading to agglomeration as the particles sought a more stable state [18].

Table 1 Particle size distribution of the safflower powders

Sample	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)	Specific surface area (m^2/kg)	Span	Φ (%)
SF50	142.00 $\pm$ 8.19 ^a	375.33 $\pm$ 3.21 ^a	674.00 $\pm$ 9.54 ^a	46.15 $\pm$ 1.22 ^d	1.42 $\pm$ 0.01 ^c	7.78 $\pm$ 0.06 ^d
SF100	31.67 $\pm$ 0.15 ^b	120.33 $\pm$ 0.58 ^b	301.33 $\pm$ 2.31 ^b	128.83 $\pm$ 0.55 ^c	2.24 $\pm$ 0.01 ^b	22.92 $\pm$ 0.10 ^c
SF200	7.55 $\pm$ 0.02 ^c	40.12 $\pm$ 0.12 ^c	107.33 $\pm$ 0.58 ^c	341.37 $\pm$ 0.59 ^b	2.48 $\pm$ 0.02 ^a	57.64 $\pm$ 0.12 ^b
SF325	6.24 $\pm$ 0.05 ^c	27.80 $\pm$ 0.26 ^d	75.23 $\pm$ 2.08 ^d	427.20 $\pm$ 3.46 ^a	2.48 $\pm$ 0.05 ^a	73.75 $\pm$ 0.42 ^a

The values were expressed as the mean $\pm$ standard deviation of triplicate experiments. Values in same column with different letter indicate significant difference ($P < 0.05$)

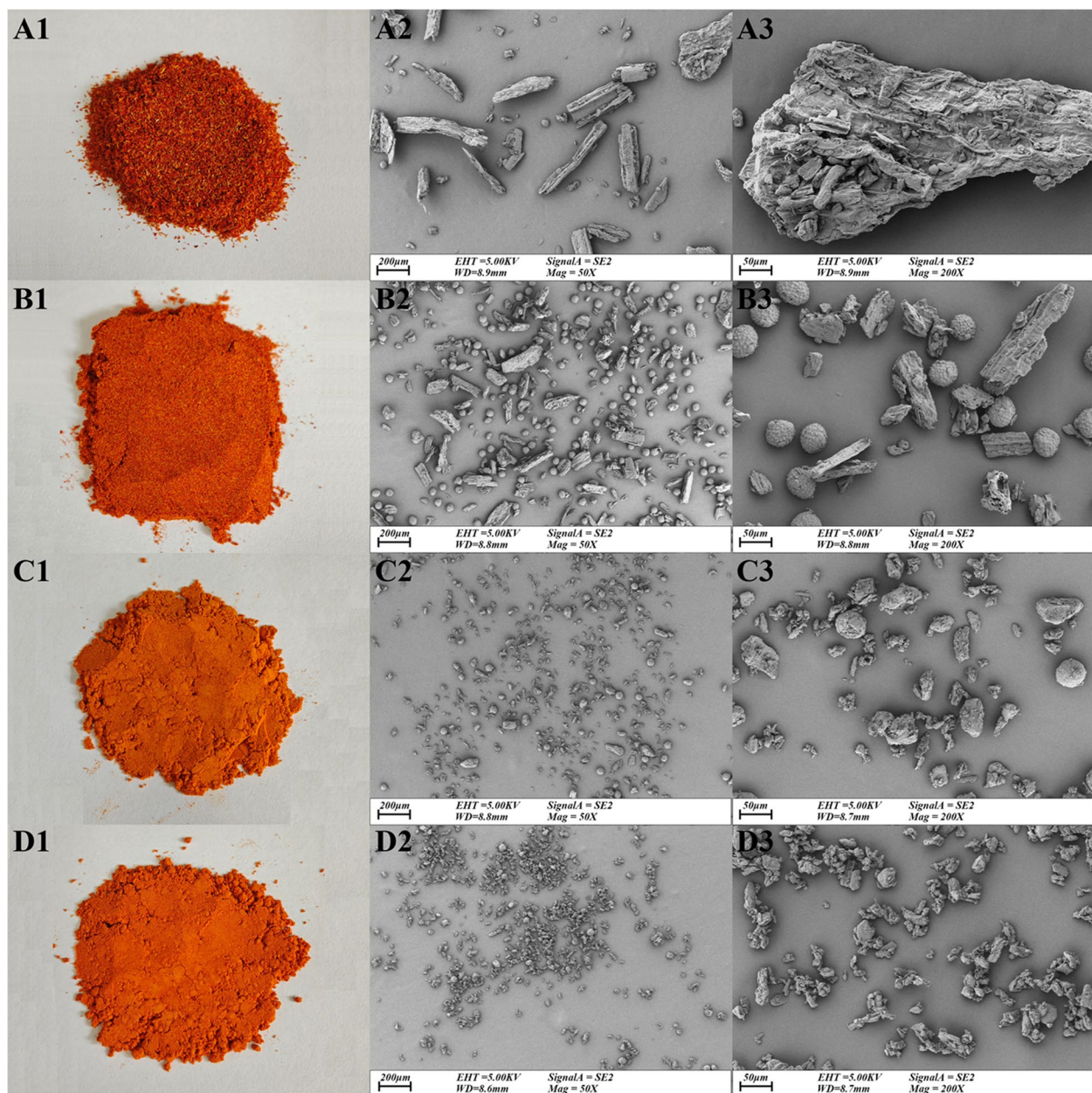


Fig. 2 Actual and SEM images of the safflower powders. Actual images: **A1** SF50 powder; **B1** SF100 powder; **C1** SF200 powder; **D1** SF325 powder. SEM images: **A2** SF50 powder, 50X; **A3** SF50 powder, 200X; **B2** SF100 powder, 50X; **B3** SF100 powder, 200X; **C2** SF200 powder, 50X; **C3** SF200 powder, 200X; **D2** SF325 powder, 50X; **D3** SF325 powder, 200X

The morphological changes observed by SEM are closely related to the functional properties of safflower powders. The reduced particle size and increased specific surface area enhanced water solubility, antioxidant capacity, and facilitated the release of intracellular compounds. Conversely, the weakening of water-holding capacity suggests that superfine powders absorbed water less effectively due to structural disruption. These results

are consistent with previous studies demonstrating that superfine grinding enhances both solubility and bioactivity across a range of powdered materials [13, 21, 22].

Color values

The main pigments responsible for the visual color of safflower include flavonoids, anthocyanins, carotenoids, and chlorophyll. The color parameters of powders with

different particle sizes are presented in Table 2. As particle size decreased, the L^* value (brightness) of safflower powders increased significantly from 50.71 to 61.49. Similarly, the a^* value (red–green axis) increased from 20.45 to 23.91, while the b^* value (yellow–blue axis) increased markedly from 18.59 to 30.06. Correspondingly, the overall color difference (ΔE^*) increased significantly with decreasing particle size, ranging from 0 to 16.12. The significant increase in the L^* value of safflower powders can be explained by the Kubelka–Munk theory, as reducing particle size increases the specific surface area, thereby enhancing light scattering and reflectivity. This led to a physical brightening of the finer powders. Meanwhile, the alterations in a^* and b^* values are likely due to the improved exposure of intracellular pigments caused by cell wall disruption and potential surface oxidation [23]. Similar findings have been reported in superfine-ground green tea [24], downy rose-myrtle [25], and hawthorn fruit powders [26], where a smaller particle size increased chromaticity due to greater pigment availability. From a functional perspective, improved brightness and chromaticity can enhance the visual appeal of safflower powders in food and nutraceutical applications, as color strongly influences consumer perception of quality and freshness.

Flow property

Tap density is a critical parameter for evaluating the filling properties of powders, particularly in tablet and capsule filling processes, where a higher tap density facilitates product formation. As shown in Table 3, the tap density of safflower powders increased significantly with decreasing particle size, ranging from 391.65 to 663.62 mg/mL, with significant differences observed among the groups. This trend is consistent with the findings of Huang et al. [27]. This demonstrates that superfine grinding can effectively reduce interparticle gaps in *Carthamus tinctorius* powder, leading to a more compact internal structure between particles. This process increases interparticle friction and induces electrostatic adsorption between particles, thereby enhancing the tapped density. Additionally, for particulate systems, intermolecular forces (primarily van der Waals forces

Table 2 Color values of safflower powders

Sample	L^*	a^*	b^*	ΔE^*
SF50	50.71 ± 0.16 ^d	20.45 ± 0.88 ^b	18.59 ± 0.49 ^d	0
SF100	52.25 ± 0.17 ^c	23.98 ± 0.20 ^a	21.76 ± 0.20 ^c	5.00 ± 0.03 ^b
SF200	59.64 ± 0.18 ^b	23.42 ± 0.31 ^a	29.59 ± 0.63 ^b	14.49 ± 0.34 ^a
SF325	61.49 ± 0.10 ^a	23.91 ± 0.44 ^a	30.06 ± 1.16 ^a	16.13 ± 0.95 ^a

The results were expressed as the means of three determinations ± standard deviations. Data marked with different lowercase superscripts in same column are significantly different ($P < 0.05$)

Table 3 Flow property of safflower powders

Sample	Tap density (mg/mL)	Angle of repose (°)	Angle of slide (°)
SF50	391.65 ± 0.89 ^d	50.66 ± 0.21 ^a	52.79 ± 0.76 ^a
SF100	552.19 ± 5.83 ^c	49.40 ± 0.34 ^b	32.35 ± 0.11 ^b
SF200	608.62 ± 2.53 ^b	46.43 ± 0.37 ^c	23.54 ± 0.40 ^c
SF325	663.62 ± 5.28 ^a	44.70 ± 0.27 ^d	18.59 ± 0.15 ^d

The results were expressed as the means of three determinations ± standard deviations. Data marked with different lowercase superscripts in same column are significantly different ($P < 0.05$)

and cohesive forces) are inversely proportional to particle size. After pulverization, the reduced particle size consequently strengthens intermolecular interactions, making the powder more prone to agglomeration. These findings align with the results from microscopic morphology analysis.

The angles of repose and slide are important indicators for evaluating powder flowability, as they are related to the friction coefficient and the properties of raw material particles. Smaller angles of repose and sliding angles indicate better powder flowability. As shown in Table 3, the angles of repose and sliding angles of safflower powders decreased significantly ($P < 0.05$) with decreasing particle size. Specifically, the angle of repose decreased from 50.66° to 44.70°, while the sliding angle dropped markedly from 52.79° to 18.59°. Xu et al. reported similar changes during the grinding process of tartary buckwheat bran [28]. This phenomenon may be attributed to the reduced particle size, which facilitates easier aggregation among powder particles, allowing them to slide collectively under gravitational force, thereby enhancing flowability.

Hydration characteristics

The main indicators reflecting the hydration characteristics of powdered materials are WSI, WHC, and SC. WSI, which reflects edible quality and is related to the digestion and absorption of food components, primarily depends on the contact area between the powder and aqueous solution. As shown in Table S1, the WSI of safflower powders significantly increased with decreasing particle size, rising from 36.67% to 46.53%. Similar trends have been observed in *Dendrobium officinale* and bitter melon powders [21, 29]. This phenomenon may be attributed to the increased specific surface area and porosity of the powder at smaller particle sizes, which expose more polar group-containing molecules, thereby enhancing the contact area and binding sites with water molecules and improving hydrophilicity. Although SEM images revealed partial aggregation in superfine powders due to high surface energy, such aggregation is likely reversible

upon hydration, allowing the high specific surface area to facilitate rapid wetting. Furthermore, the disruption of the cellular matrix directly exposes soluble intracellular compounds to the solvent. This release mechanism, rather than surface area expansion alone, is considered the primary driver for the observed increase in the WSI.

WHC, a critical indicator for evaluating the hydrophilic properties of powders, refers to the ability of hydrated materials to retain moisture under external forces, reflecting the binding strength between the material and water molecules. The WHC of safflower powders significantly decreased with reduced particle size ($P < 0.05$), dropping from 7.78 g/g to 4.59 g/g. Similar trends have been reported in *Quercus salicina* (Blume) leaf powders, superfine-ground oat bran polysaccharides and *Rosa rugosa* cv. plena powders, where superfine grinding led to comparable changes in hydration properties, characterized by increased solubility (higher WSI) but reduced WHC [30–32]. The decrease in WHC suggests the structural collapse of the porous fiber matrix. This disruption essentially eliminates the interstitial voids responsible for water retention in coarse powders, thereby diminishing the powder's ability to mechanically entrap water [33]. Furthermore, from a practical application perspective, the decreased WHC of superfine powders suggests they are less likely to form swollen sediment. This property is advantageous for developing clear safflower beverages (e.g., tea or functional wine) with improved mouthfeel and texture. Additionally, the reduced bound water in the residue may help inhibit microbial growth, potentially extending shelf-life, although oxidative stability should be managed due to the high exposure of active compounds.

SC is related to water absorption, particle swelling, interparticle porosity, and the powder's water-binding capacity. It exhibits a positive correlation with the stability of powders in water, where powders with higher SC tend to dissolve more easily and demonstrate better stability and suspension properties. Table S1 shows that the SC of safflower powders increased from 2.67 mL/g to 7.20 mL/g as particle size decreased, with significant differences observed between SF100 and SF200 ($P < 0.05$). These results demonstrate that superfine grinding induces distinct hydration property changes, including increased WSI, decreased WHC, and increased SC. Similar patterns have been reported in soybean residue powders and inonotus obliquus mushroom powders [34, 35]. The underlying mechanism may be attributed to the enlarged specific surface area and enhanced porosity of the powders, which facilitate water penetration and solubility but simultaneously disrupt the structural polysaccharide matrix responsible for water retention.

Consequently, superfine grinding improves the solubility and dispersibility of safflower powders while weakening their moisture-holding capacity. These changes are particularly relevant for the processing, quality control, and functional utilization of safflower-based products.

Fourier transform infrared spectroscopy analysis

The FTIR results of safflower powders are shown in Fig. 1C. There were no significant changes in peak shapes among the four powders, suggesting that no new compounds were formed. This suggests that the grinding process mainly influenced physical properties such as particle size and surface area rather than chemical composition.

According to the transmittance of the absorption peak, all the samples were at approximately 2923 cm^{-1} , 1730 cm^{-1} , 1517 cm^{-1} and 1240 cm^{-1} with obvious absorption peaks. The absorption peak at 2923 cm^{-1} was attributed to the C-H asymmetric stretching. The absorption peak at 1730 cm^{-1} corresponds to the C=O stretching vibration [36]. The absorption peak at 1517 cm^{-1} was related to C=C stretching of the aromatic ring [37]. The absorption peak at 1240 cm^{-1} was related to the C–O–C stretching vibration [8].

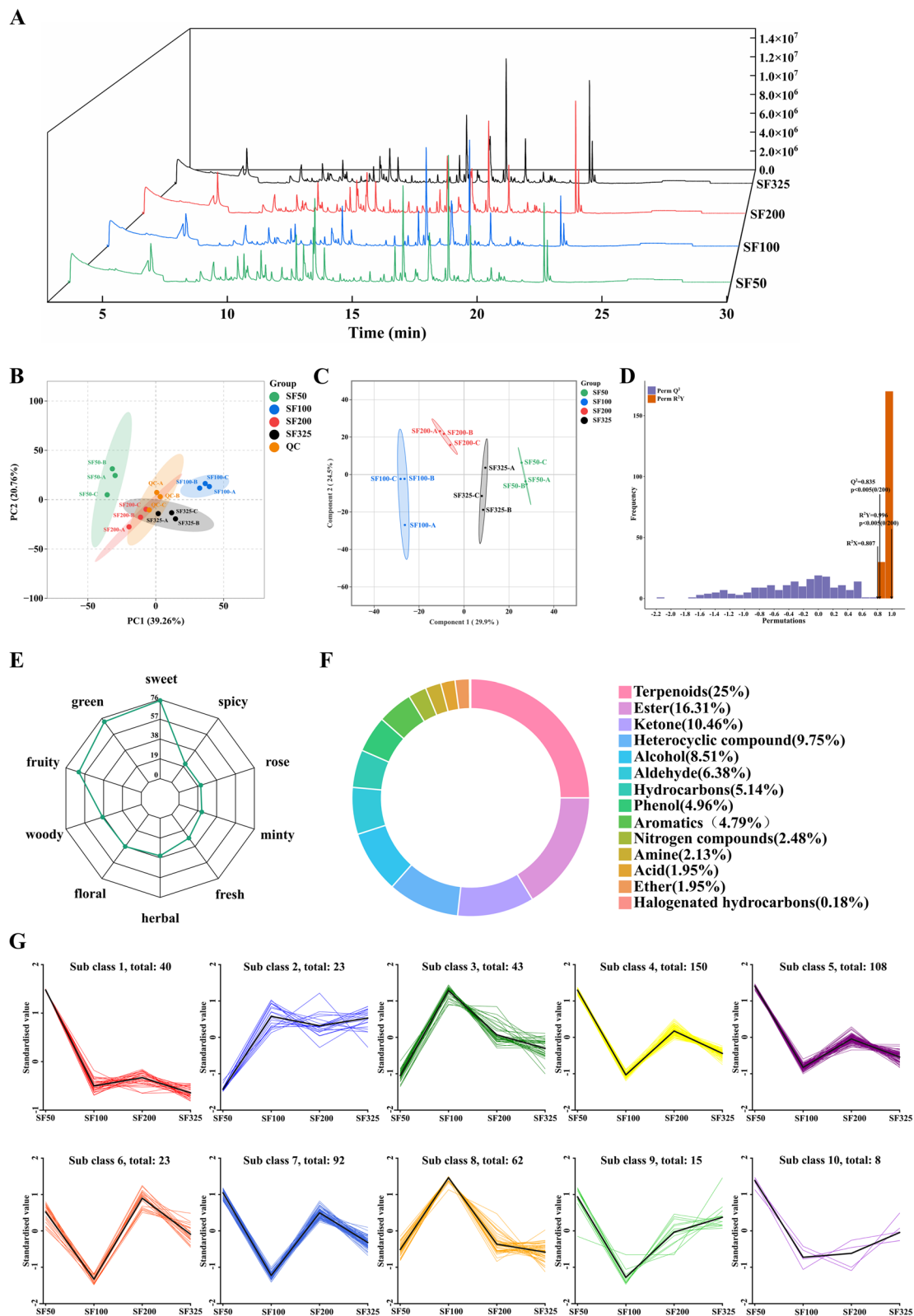
Interestingly, while peak positions remained unchanged, slight differences in peak intensity were noted, particularly in the C=O and C–O–C regions. These variations may be related to increased exposure of functional groups after cell wall disruption by superfine grinding, which could facilitate stronger interactions with water molecules, thereby improving solubility and swelling capacity, as observed in [hydration characteristics](#).

Furthermore, the crystallinity index (CrI) of cellulose, estimated from FTIR absorbance ratios, showed a slight decrease with decreasing particle size. This suggests partial disruption of crystalline cellulose structures during superfine grinding, which could enhance the release of bound bioactive compounds. Similar trends have been reported for superfine-ground ginseng and wheat bran powders, where lower cellulose crystallinity improved extractability of phytochemicals [38, 39]. Overall, the FTIR analysis confirms that superfine grinding preserves the primary chemical structures of safflower while promoting subtle modifications in hydrogen bonding and crystallinity. These structural changes likely contribute to the enhanced hydration and bioactive release properties observed in superfine powders.

Volatile components analysis

Principal component analysis

GC–MS was employed to characterize the volatile components of safflower powders, and the corresponding spectra are presented in Fig. 3A. Analysis of the



three-dimensional spectra from the four safflower sample groups revealed variations in peak signal intensities among different grinding methods, indicating corresponding differences in volatile component contents. Principal component analysis (PCA) was conducted on all samples based on these metabolic signals. As shown in Fig. 3B, PC1 and PC2 explained 39.26% and 20.76% of the total variance, respectively. A greater distance between sample clusters indicates more pronounced differences in volatile components, whereas closer clustering suggests smaller differences. As shown in Fig. 3B, the SF200 and SF325 groups cluster closely with the Quality Control (QC) samples, in marked contrast to the coarse powders. Given that QC samples represent the aggregate metabolic profile of the entire dataset, this spatial proximity implies that superfine grinding facilitates a more exhaustive and stable extraction, effectively releasing the comprehensive spectrum of metabolites. Conversely, the significant deviation of coarse powders from this 'total' profile indicates incomplete extraction, likely attributed to the mass transfer resistance imposed by intact cell walls.

These findings are consistent with previous reports that superfine grinding tends to preserve volatile compounds by reducing processing-induced thermal and mechanical degradation [40]. The closer clustering of superfine-ground powders (SF200, SF325) implies greater retention of the original safflower aroma profile, while conventional grinding (SF50, SF100) may induce secondary chemical changes such as lipid oxidation or breakdown of glycosidically bound volatiles, leading to more pronounced compositional shifts. Overall, PCA results demonstrated that particle size and grinding method exert differential effects on the volatile composition of safflower powders.

Orthogonal projections to latent structures discriminant analysis

This study used orthogonal partial least squares discriminant analysis (OPLS-DA) to discern between groups based on their metabolic profiles. OPLS-DA can visualize the differences between samples through the model's score plots, effectively differentiate between observations in different groups, and find the significant variables that contribute to the differences between groups. As can be seen from Fig. 3C, the SF50, SF100, SF200, and SF325 do not intersect with other samples, are clearly distinguished and have significant differences.

The OPLS-DA model was evaluated using R^2X , R^2Y , and Q^2 . R^2X and R^2Y indicate the proportion of variance explained by the X and Y matrices, respectively, whereas Q^2 reflects the model's predictive ability. Values approaching 1 for these three metrics signify a stable and reliable model. Figure 3D presents the OPLS-DA

validation plot, with $Q^2=0.835$, $R^2X=0.807$, and $R^2Y=0.996$, indicating that the established model is statistically valid.

Furthermore, the variable importance in projection (VIP) scores derived from the OPLS-DA model can be used to pinpoint the specific volatile compounds that most significantly differentiate the safflower powder groups. Such metabolites may include aldehydes, alcohols, esters, or terpenoids, which are known to influence safflower's aroma and functional properties. Identification of these key VOCs would provide deeper insight into how grinding alters the sensory and potentially bioactive qualities of safflower powders. In summary, OPLS-DA analysis corroborates the PCA findings, demonstrating that particle size reduction through different grinding processes leads to distinct volatile compositions. The model also highlights specific metabolites as potential biomarkers for differentiating safflower powders processed under varying grinding conditions.

Analysis of different metabolites

Liminary screening of differential metabolites between sample groups was performed based on the Variable Importance in Projection (VIP) derived from the OPLS-DA model, combined with p-values. The screening criteria applied in this study were ($VIP>1.0$) and ($P<0.05$). A total of 564 differential metabolites were identified as common to SF50, SF100, SF200, and SF325.

Of these, differential metabolites have 14 different classes (Fig. 3F). Among these, terpenoids (25%), esters (16.3%), ketones (10.46%), and heterocyclic (9.75%) compounds were predominant. Terpenoids are particularly important contributors to aroma and potential bioactivity, esters are often linked to fruity or floral notes, ketones influence oxidative stability and flavor quality, while heterocyclic compounds can impart complex sensory attributes. The relative abundance of these classes suggests that particle size reduction not only affects metabolite diversity but may also modulate the sensory and functional qualities of safflower powders. Overall, it provides a complementary visualization to PCA and OPLS-DA, confirming the distinct metabolic profiles generated by different grinding methods and highlighting specific metabolite classes as potential biomarkers for distinguishing safflower powder processing strategies.

Sensory and flavor characteristic analysis

Based on the annotated sensory-flavor characteristics of metabolites, the top 10 largest quantity sensory-flavor attributes were selected for radar chart visualization. The outermost ring labels denote the sensory-flavor characteristics, with the green-labeled numerical values

indicating the quantity of metabolites possessing each corresponding flavor characteristic. As shown in Fig. 3E, the top 10 largest quantity sensory-flavor attributes were: sweet (75), green (72), fruity (63), woody (39), floral (38), herbal (36), fresh (28), minty (23), rose (22), and spicy (22). The radar chart visualization of the top 10 sensory-flavor attributes provides insight into the chemical basis of safflower's flavor profile. Sweet, green, and fruity notes were the most abundant, with metabolite counts of 75, 72, and 63, respectively, suggesting that these flavors dominate the overall sensory perception. These flavor attributes are likely associated with specific bioactive compounds, including flavonoids, phenolic acids, and volatile terpenoids, which contribute not only to taste but also to antioxidant and other functional properties. Woody, floral, and herbal notes further reflect the diverse secondary metabolite composition, while lower-abundance flavors such as fresh, minty, rose, and spicy may add subtle complexity and balance to the overall profile. The correspondence between sensory-flavor characteristics and metabolite abundance indicates that the bioactive compounds responsible for functional benefits may simultaneously influence flavor perception. These findings are valuable for guiding the targeted extraction, processing, and formulation of safflower-based products, allowing optimization of both sensory quality and functional efficacy.

K-means clustering analysis

To investigate variation trends in metabolite relative content across different groups, the relative content data for all differential metabolites were first normalized using unit variance scaling (UV). Subsequently, K-means clustering analysis was performed to classify metabolites with similar abundance patterns; the result is shown in Fig. 3G. To further identify key markers reflecting the degree of processing, we focused on 9 specific metabolites that exhibited a continuous up-regulation or down-regulation trend from SF50 to SF325 (Table S2), including esters (5), terpenoids (2), and ketone (1), phenol (1). These compounds showed a consistent gradient change with decreasing particle size, suggesting they are strictly modulated by the grinding intensity.

In this study, five volatile compounds — isobutyl isovalerate, 3-hexen-1-ol acetate (E), 4'-hydroxyacetophenone, butanoic acid, 2-methyl-, 2-methylpropyl ester, and 3-hexen-1-ol acetate (Z) — were up-regulated in processed safflower powder. These metabolites are primarily derived from fatty acid or phenylpropanoid metabolism and are commonly associated with sweet, fruity, green, and floral aromas. Their accumulation suggests enhanced enzymatic activity during processing, particularly involving lipid oxidation, esterification, and secondary

metabolite biosynthesis [41]. These changes may contribute to the development of a more complex and desirable aroma profile in safflower powder products.

Conversely, four volatile compounds — D-verbenone, 3-methyl-4-nitrophenol, 3-nonenoic acid methyl ester, and 5-methyl-2-(1-methylethyl)-cyclohexanone — were down-regulated. These compounds are typically associated with pungent odor notes, and their reduction may result from suppressed terpene and phenolic metabolism or degradation induced by mechanical processing [42]. Although sensory panel evaluation was not conducted, the enrichment of these volatile compounds indicates a significant modification in the volatile profile of the superfine powders. The increased relative content of compounds chemically associated with fruity and floral notes suggests a potential optimization of the aroma characteristics, which could theoretically contribute to a more favorable olfactory profile.

Bioactive chemical components content

Previous research on safflower indicated that HSYA was the primary active component responsible for its pharmacological effects. HSYA exhibits antioxidant activity, inhibits thrombus formation, and suppresses platelet aggregation [43]. Figure 4A shows the HPLC chromatograms of HSYA standard and sample solutions. As illustrated in Fig. 4B, the HSYA content increased significantly ($P < 0.05$) from 12.92 to 18.96 mg/g as particle size decreased. As shown in Fig. 4C,D, the TFC and TPC from powders with different particle sizes were measured. The TFC and TPC increased significantly ($P < 0.05$) with decreasing particle size, with TFC rising from 5.38 to 6.98 mg/g and TPC from 9.02 to 14.48 mg/g (Fig. 4C,D), consistent with previous findings [21]. This indicated that superfine grinding disrupted plant cell walls, enabling more active ingredients to be released or exposed [18]. Additionally, smaller particle sizes provided a higher specific surface area and a higher WSI value (see above), which allowed greater and more efficient contact with the extraction solvent, thereby improving the dissolving capacity of phytochemical compounds.

DPPH radical scavenging activity

The enhanced content of HSYA, flavonoids, and polysaccharides was reflected in the improved antioxidant activity of the safflower powders, as evaluated by DPPH radical scavenging assays (Fig. 5A). At a concentration of 1 mg/mL, the scavenging rate increased from 24.31% to 34.54%, and at 2 mg/mL, from 36.38% to 44.47%. Further increases were observed at 3 mg/mL (42.81% to 59.37%), 4 mg/mL (58.19% to 71.00%), 5 mg/mL (67.66% to 81.12%), and 6 mg/mL (78.02% to 93.24%), demonstrating

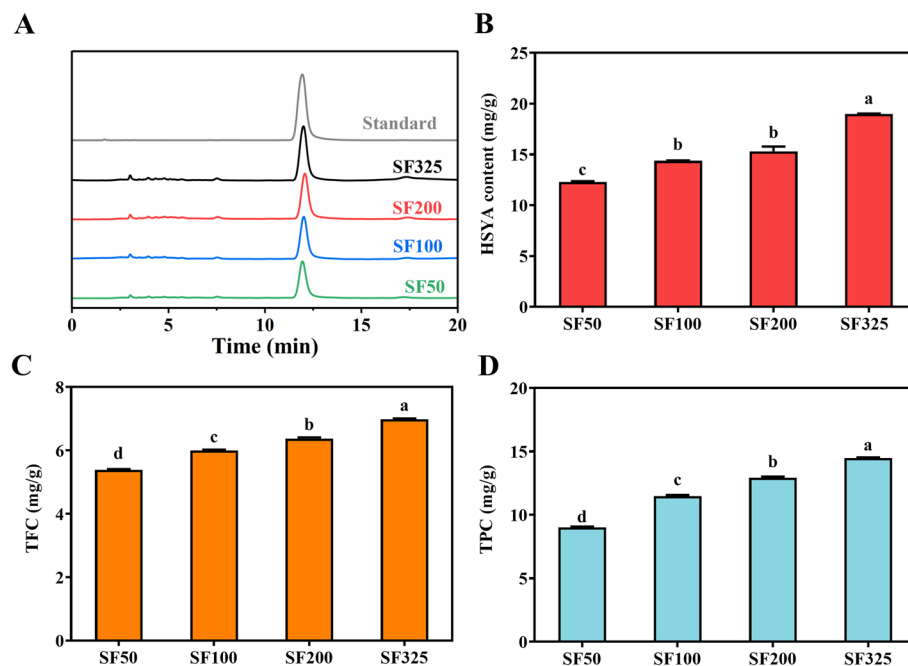


Fig. 4 Bioactive chemical components of safflower powders. **A** HPLC chromatograms of safflower powders; **B** HSYA content of safflower powders. **C** TFC of safflower powders; **D** TPC of safflower powders. Values with different superscript letters in the same graph are significantly different ($P < 0.05$)

a clear positive correlation between concentration, particle size reduction, and antioxidant capacity. These results suggest that superfine grinding not only promotes the release of bioactive compounds but also minimizes their loss, thereby enhancing the overall antioxidant potential of safflower powder, in agreement with previous reports [33, 44, 45]. Collectively, these findings indicate that controlling particle size through superfine grinding is an effective strategy to improve both the chemical composition and functional bioactivity of safflower powder.

Anticoagulant activity

As shown in Fig. 5B–D, at a concentration of 2 mg/mL, APTT was significantly prolonged ($P < 0.05$), increasing from 21.67 s to 36.37 s, and PT also extended from 11.65 s to 13.25 s. However, TT showed no significant changes, likely due to the low concentration, which resulted in insufficient active components to affect TT. At a concentration of 10 mg/mL, APTT was markedly prolonged ($P < 0.05$), rising from 26.01 s to 45.29 s. Both TT and PT were also significantly prolonged, increasing from 33.56 s and 15.80 s to 42.78 s and 30.38 s,

respectively. At a concentration of 50 mg/mL, APTT was further significantly prolonged ($P < 0.05$), increasing from 36.25 s to 64.11 s. TT and PT likewise showed significant extensions, increasing from 40.69 s and 22.46 s to 54.53 s and 40.33 s, respectively. These results indicate that the anticoagulant activity increased with higher concentrations. Additionally, as the particle size decreased, the anticoagulant activity significantly increased, likely due to cell rupture during grinding, which released more phytochemicals, and the reduced particle size also enhanced the specific surface area, improving the contact area and interaction efficiency with extraction solvents, which facilitated the extraction of phytochemicals [46]. The prolongation of clotting times (APTT, PT, TT) in superfine powders exhibited a significant linear correlation with the concentration of HSYA (Fig. 5E). This strong correlation suggests that the improvement in anticoagulant activity is concentration-dependent. FTIR analysis confirmed that superfine grinding decreased cellulose crystallinity, signifying the disruption of the cell wall matrix. This structural breakdown eliminates the physical barrier to solvent extraction, leading to a higher yield of

(See figure on next page.)

Fig. 5 Bioactivities and the correlation heatmap of safflower powders. **A** DPPH radical scavenging rate of safflower powders; **B** APTT of safflower powders; **C** PT of safflower powders; **D** TT of safflower powders; **E** Heatmap of the Pearson correlation coefficients and significance levels between bioactive chemical components and volatile components and bioactivities and color value. Values with different superscript letters in the same graph are significantly different ($P < 0.05$). The asterisks (*) and double-asterisk (**) in heatmap indicating statistically significant correlations ($P < 0.05$ and $P < 0.01$)

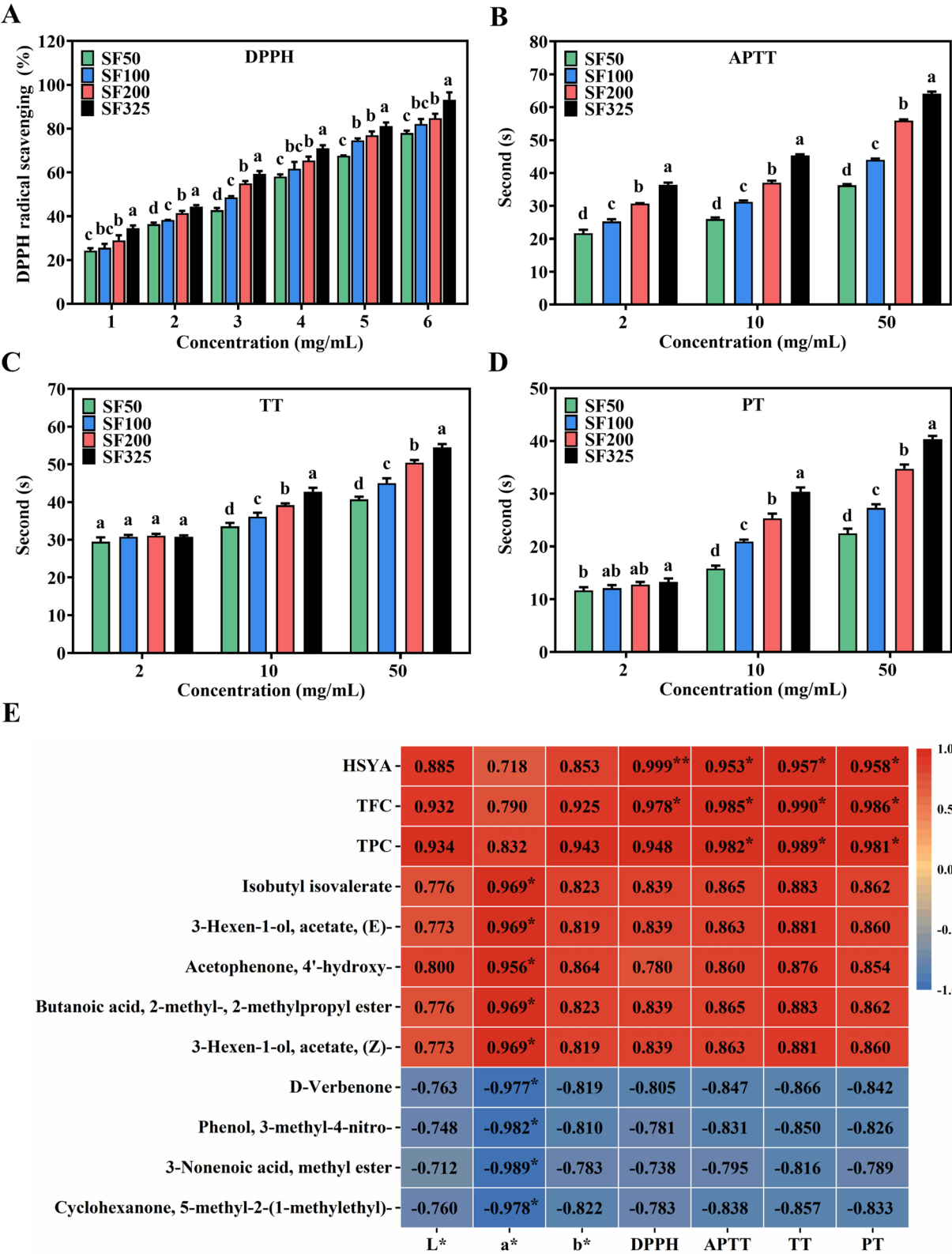


Fig. 5 (See legend on previous page.)

dissolved HSYA. Consequently, the enhanced bioactivity is attributed to the improved bioaccessibility and release of active ingredients facilitated by the physical destruction of the safflower matrix, rather than alterations in the intrinsic biological potency of the compounds.

Correlation analysis

To investigate the interdependencies among different characteristics, Pearson correlation coefficients were used to measure the linear relationship between bioactive chemical components and bioactivities and color value to evaluate the significance level. The r value represents both the strength and direction of the correlation. The closer the absolute value of r is to 1, the stronger the correlation becomes. A positive r value indicates a positive correlation, whereas a negative r value indicates a negative correlation.

As shown in Fig. 5E, the content of HSYA is significantly and positively correlated to DPPH, APTT, TT, and PT, and is positively correlated with L^* , a^* and b^* . TFC is significantly and positively related to DPPH, APTT, TT, and PT, and is positively correlated with L^* , a^* , and b^* . TPC is significantly and positively related to APTT, TT, and PT and is positively correlated with DPPH, L^* , a^* , and b^* . These phenomena indicate that increasing the content of HSYA, TFC and TPC can significantly increase bioactivities of safflower powder, and affect the color of safflower powder, increasing its brightness and bringing it closer to red and yellow. The analysis of Fig. 5E reveals that the content of HSYA has the most significant influence on the DPPH radical scavenging activity, as indicated by the highest correlation coefficients ($r=0.999$). It is indicated that HSYA plays a major role in the antioxidant effect of safflower.

Among the volatile compounds of interest, several esters, including isobutyl isovalerate and 3-hexen-1-ol acetate (E and Z isomers), exhibited strong positive correlations with both bioactivities and color parameters, particularly the b^* value, with statistically significant relationships ($P<0.05$). These results suggest that these compounds may contribute to enhanced antioxidant and anticoagulant activities, as well as to a more yellowish appearance of the powder.

In contrast, compounds such as D-verbenone and 3-methyl-4-nitrophenol showed strong negative correlations with these parameters, especially b^* , indicating their potential role in diminishing both the functional properties and the color attributes of the powder.

Conclusion

This study demonstrates the impact of superfine grinding on the properties of safflower powder. The results demonstrated that superfine grinding obtained a finer

particle size powder, and improved the brightness of the powder, shifting its color towards red and yellow hues, which could promote its use in dyes. The reductions in the angles of repose and slide, along with the increase in tap density, indicate that superfine powder exhibits improved flowability and filling properties. This makes safflower powder mixtures less prone to separation after blending, resulting in a more stable quality in processed pharmaceuticals. Superfine grinding significantly improved the extraction efficiency of bioactive compounds (HSYA, TFC), leading to higher antioxidant and anticoagulant activities in the dispersion. The observed increase in bioactivity (Fig. 5) strongly correlated with the elevated concentration of active ingredients (Fig. 4). This suggests that the process enhances the bioaccessibility and release of phytochemicals from the disrupted cell matrix, rather than altering their intrinsic chemical potency. This study provides new insights into the effects of superfine grinding on safflower powder properties and offers theoretical support for its applications in food, health products, and pharmaceuticals.

Glossary

FTIR	Fourier transform infrared spectroscopy
SSA	Specific surface area
SEM	Scanning electron microscopy
WSI	Water solubility index
WHC	Water-holding capacity
SC	Swelling capacity
GC-MS	Gas chromatography-mass spectrometry
HSYA	Hydroxysafflor yellow A
TFC	Total flavonoid content
TPC	Total polysaccharide content
APTT	Activated partial thromboplastin time
PT	Prothrombin time
TT	Thrombin time

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44403-026-00054-3>.

Supplementary Material 1.

Authors' contributions

Tianjian Guo: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. Yu Li: Methodology, Investigation, Formal analysis, Conceptualization, Data curation. Jun Li: Supervision, Investigation, Funding acquisition, Conceptualization. Xiao Chen: Project administration, Supervision, Funding acquisition. Ghulam Murtaza: Writing – review & editing, Supervision, Conceptualization, Methodology, Investigation. Han Cheng: Supervision, Funding acquisition, Validation. Xincai Xiao: Supervision, Investigation, Conceptualization. Leiling Shi: Project administration, Resources, Funding acquisition. Ruixi Gao: Writing – review & editing, Supervision, Conceptualization. Xianju Huang: Writing – review & editing, Supervision, Project administration, Conceptualization, Methodology, Funding acquisition.

Funding

This work was supported by Key Research and Development Special Project of Hubei Provincial Science and Technology Plan (2024BBB033), National Key

R&D Program of China (2024YFC3506704 & 2024YFC3506705), the International Cooperation Project of Science and Technology of Hubei Province (2024EHA054) and the Fund for Academic Innovation Teams of South Central Minzu University (XTZ24025).

Data availability

Data in this report are available on request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

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.

Author details

¹School of Pharmaceutical Sciences, South-Central Minzu University, Wuhan 430079, China. ²Hubei Yaosheng Traditional Chinese Medicine Technology Co. Ltd, Zaoyang 441200, China. ³Department of Pharmacy, COMSATS University Islamabad, Lahore Campus, Lahore 54000, Pakistan. ⁴Xinjiang Institute of Chinese and Ethnic Medicine, Urumqi 830002, China.

Received: 14 January 2026 Revised: 2 February 2026 Accepted: 26 February 2026

Published online: 13 April 2026

References

- Li J-X, Xu D-Q, Cui D-X, Fu R-J, Niu Z-C, Liu W-J, et al. Exploring the structure-activity relationship of Safflower polysaccharides: from the structural characteristics to biological function and therapeutic applications. *J Ethnopharmacol.* 2025;339:119131. <https://doi.org/10.1016/j.jep.2024.119131>.
- Tang N, Xu X, Guo Z, Meng X, Qian G, Li H. Preparation of safflower fermentation solution and study on its biological activity. *Front Microbiol.* 2024;15:1472992. <https://doi.org/10.3389/fmicb.2024.1472992>.
- Yao D, Wang Z, Miao L, Wang LY. Effects of extracts and isolated compounds from safflower on some index of promoting blood circulation and regulating menstruation. *J Ethnopharmacol.* 2016;191:264–72. <https://doi.org/10.1016/j.jep.2016.06.009>.
- Kim HW, Hwang KE, Song DH, Kim YJ, Ham YK, Lim YB, et al. Wheat fiber colored with a safflower (*Carthamus tinctorius* L.) red pigment as a natural colorant and antioxidant in cooked sausages. *LWT Food Sci Technol.* 2015;64(1):350–5. <https://doi.org/10.1016/j.lwt.2015.05.064>.
- Li CX, Liu YY, Feng HS, Ma SZ. Effect of superfine grinding on the physicochemical properties of bulbs of *Fritillaria unibracteata* Hsiao et K.C. Hsia powder. *Food Sci Nutr.* 2019;7(11):3527–37. <https://doi.org/10.1002/fsn3.1203>.
- Liang K, Liang S, Zhu H. The physicochemical characteristics and phenolic bioaccessibility of defatted millet bran powder prepared using superfine grinding. *LWT Food Sci Technol.* 2024;201:116173. <https://doi.org/10.1016/j.lwt.2024.116173>.
- Huang X, Liang K-H, Liu Q, Qiu J, Wang J, Zhu H. Superfine grinding affects physicochemical, thermal and structural properties of *Moringa Oleifera* leaf powders. *Ind Crops Prod.* 2020;151:112472. <https://doi.org/10.1016/j.indcrop.2020.112472>.
- Zhang Z-Q, Chen S-C, Wang Q-L, Liu C-Q, Xiao J-H, Huang D-W. Effects of traditional grinding and superfine grinding technologies on the properties and volatile components of *Protaetia brevitarsis* larvae powder. *LWT.* 2023;173:114307. <https://doi.org/10.1016/j.lwt.2022.114307>.
- Yi K, Wu L, Wu L, Song X, Xia Q, Cao Y, et al. Effects of superfine grinding *Tremella fuciformis* stem on the texture and quality of dough for steamed bun. *Food Chemistry: X.* 2024;23:101529. <https://doi.org/10.1016/j.fochx.2024.101529>.
- Weng D, Zha S-H, Zhu Y, Li H, Hou S-B, Zhao Q-S, et al. Effect of particle size on the physicochemical and antioxidant properties of *Forsythia suspensa* (Thunb.) Vahl leaf powders. *Powder Technol.* 2022;410:117866. <https://doi.org/10.1016/j.powtec.2022.117866>.
- Zhao D, Yang F, Dai Y, Tao F, Shen Y, Duan W, et al. Exploring crystalline structural variations of cellulose during pulp beating of tobacco stems. *Carbohydr Polym.* 2017;174:146–53. <https://doi.org/10.1016/j.carbpol.2017.06.060>.
- Committee N. Pharmacopoeia of the People's Republic of China. Beijing: China Pharmaceutical Science and Technology Press; 2020.
- Zhao YY, Wu X, Wang YJ, Jing RJ, Yue FL. Comparing physicochemical properties of hawthorn superfine and fine powders. *J Food Process Preserv.* 2017;41(2):8. <https://doi.org/10.1111/jfpp.12834>.
- Wang QQ, Liu SQ, Xu L, Du B, Song LJ. Purification, characterization and bioactivities of polysaccharides extracted from safflower (*Carthamus tinctorius* L.). *Molecules.* 2023;28(2):15. <https://doi.org/10.3390/molecules28020596>.
- Chen L, Huang GL. Extraction, characterization and antioxidant activities of pumpkin polysaccharide. *Int J Biol Macromol.* 2018;118:770–4. <https://doi.org/10.1016/j.jbiomac.2018.06.148>.
- Ozkan K, Bekiroglu H, Bayram Y, Sagdic O, Erbas S. In vitro bioaccessibility, antioxidant and antibacterial activities of three different safflower (*Carthamus tinctorius* L.) genotypes. *Food Sci Technol.* 2022;42:7. <https://doi.org/10.1590/fst.08921>.
- Zhang Y, Yu L, Jin W, Li C, Wang Y, Wan H, et al. Simultaneous optimization of the ultrasonic extraction method and determination of the antioxidant activities of Hydroxysafflor Yellow A and Anhydrosafflor Yellow B from Safflower using a response surface methodology. *Molecules.* 2020;25(5):1226.
- He SD, Tang MM, Sun HJ, Ye YK, Cao XD, Wang JH. Potential of water dropwort (*Oenanthe javanica* DC.) powder as an ingredient in beverage: functional, thermal, dissolution and dispersion properties after superfine grinding. *Powder Technol.* 2019;353:516–25. <https://doi.org/10.1016/j.powtec.2019.05.048>.
- Zhang J, Dong Y, Nisar T, Fang Z, Wang Z-C, Guo Y. Effect of superfine-grinding on the physicochemical and antioxidant properties of *Lycium ruthenicum* Murray powders. *Powder Technol.* 2020;372:68–75. <https://doi.org/10.1016/j.powtec.2020.05.097>.
- Xu J, Liu Y, Zhang N, Xiong S, Zhang L, Wang J. Effect of superfine grinding on the physicochemical properties of straw mushroom (*Volvariella volvacea*) powders rich in vitamin D2. *J Food Process Preserv.* 2022;46(12):e17192. <https://doi.org/10.1111/jfpp.17192>.
- Meng Q, Fan H, Xu D, Aboshora W, Tang Y, Xiao T, et al. Superfine grinding improves the bioaccessibility and antioxidant properties of *Dendrobium officinale* powders. *Int J Food Sci Technol.* 2017;52(6):1440–51. <https://doi.org/10.1111/ijfs.13405>.
- Lu M, Yan L, Wang B, Tian S. Effect of vibrating-type ultrafine grinding on the physicochemical and antioxidant properties of Turkish galls in Uyghur medicine. *Powder Technol.* 2018;339:560–8. <https://doi.org/10.1016/j.powtec.2018.07.095>.
- Yan Z, Alimu R, Wan JW, Liao XW, Lin SM, Dai SJ, et al. Composition of major quinoxalene hydroxysafflor yellow A and anhydrosafflor yellow B is associated with colour of safflower (*Carthamus tinctorius*) during colour-transition but not with overall antioxidant capacity: A study on 144 cultivars. *Food Res Int.* 2022;162:11. <https://doi.org/10.1016/j.foodres.2022.112098>.
- Zhao Z, Dai Z, Jiang X, Yu L, Hu M, Peng J, et al. Influence and optimization of long-time superfine grinding on the physicochemical features of green tea powder. *J Food Compos Anal.* 2023;117:105124. <https://doi.org/10.1016/j.jfca.2022.105124>.
- Gao X, Zhu D, Liu Y, Zha L, Chen D, Guo H. Physicochemical properties and anthocyanin bioaccessibility of downy rose-myrtle powder prepared by superfine grinding. *Int J Food Prop.* 2019;22(1):2022–32. <https://doi.org/10.1080/10942912.2019.1702999>.
- Liu S, Yu J, Zou J, Yang Y, Cui L, Chang X. Effects of different drying and milling methods on the physicochemical properties and phenolic content of hawthorn fruit powders. *J Food Process Preserv.* 2020;44(6):e14460. <https://doi.org/10.1111/jfpp.14460>.
- Huang X, Dou JY, Li D, Wang LJ. Effects of superfine grinding on properties of sugar beet pulp powders. *LWT Food Sci Technol.* 2018;87:203–9. <https://doi.org/10.1016/j.lwt.2017.08.067>.
- Xu Q, Huang R, Yang P, Wang L, Xing Y, Liu H, et al. Effect of different superfine grinding technologies on the physicochemical and antioxidant properties of tartary buckwheat bran powder. *RSC Adv.* 2021;11(49):30898–910. <https://doi.org/10.1039/D1RA05093A>.

29. Zhu Y, Bai J, Qian X, Yang X, Zhou X, Zhao Y, et al. Effect of superfine grinding on physical properties, bioaccessibility, and anti-obesity activities of bitter melon powders. *J Sci Food Agric*. 2022;102(11):4473–83. <https://doi.org/10.1002/jsfa.11802>.
30. Hong S-J, Das PR, Eun J-B. Effects of superfine grinding using ball-milling on the physical properties, chemical composition, and antioxidant properties of *Quercus salicina* (Blume) leaf powders. *J Sci Food Agric*. 2021;101(8):3123–31. <https://doi.org/10.1002/jsfa.10941>.
31. Qiu L, Zhang M, Xu B, Wang B. Effects of superfine grinding on the physicochemical properties, antioxidant capacity, and hygroscopicity of *Rosa rugosa* cv. Plena powders. *J Sci Food Agric*. 2022;102(10):4192–9. <https://doi.org/10.1002/jsfa.11768>.
32. Liu R, Zhu T, Li J, Wu T, Li Q, Meng Y, et al. Physicochemical and antioxidative properties of superfine-ground oat bran polysaccharides. *Food Sci Technol Res*. 2016;22(1):101–9. <https://doi.org/10.3136/fstr.22.101>.
33. Gao WJ, Chen F, Wang X, Meng QR. Recent advances in processing food powders by using superfine grinding techniques: a review. *Compr Rev Food Sci Food Saf*. 2020;19(4):2222–55. <https://doi.org/10.1111/1541-4337.12580>.
34. Li GH, Guo WY, Gao XL, Wang YH, Sun SS. Effect of superfine grinding on physicochemical and antioxidant properties of soybean residue powder. *Food Sci Nutr*. 2020;8(2):1208–14. <https://doi.org/10.1002/fsn3.1409>.
35. Wang J, Wang C, Li W, Pan Y, Yuan G, Chen H. Ball milling improves extractability and antioxidant properties of the active constituents of mushroom *Inonotus obliquus* powders. *Int J Food Sci Technol*. 2016;51(10):2193–200. <https://doi.org/10.1111/ijfs.13180>.
36. Li C, Wang Y, Ge S, Liu Y, Jiang X, Wang X. Identification of cigarettes with different grades by using FTIR microspectroscopy. *Spectrochim Acta A Mol Biomol Spectrosc*. 2025;326:125249. <https://doi.org/10.1016/j.saa.2024.125249>.
37. Dias AF, de Oliveira RN, Deglise X, de Souza ND, Brito JO. Infrared spectroscopy analysis on charcoal generated by the pyrolysis of *Corymbia citriodora* wood. *Materia*. 2019;24(3):7. <https://doi.org/10.1590/s1517-707620190003.0700>.
38. Jiang G, Wu Z, Ameer K, Li S, Ramachandraiah K. Particle size of ginseng (*Panax ginseng* Meyer) insoluble dietary fiber and its effect on physicochemical properties and antioxidant activities. *Appl Biol Chem*. 2020;63(1):70. <https://doi.org/10.1186/s13765-020-00558-2>.
39. Zhang Y, Zhang M, Guo X, Bai X, Zhang J, Huo R, et al. Improving the adsorption characteristics and antioxidant activity of oat bran by superfine grinding. *Food Sci Nutr*. 2023;11(1):216–27. <https://doi.org/10.1002/fsn3.3054>.
40. Liu A, Li H, Xu W, Zhu L, Ye S, Li T, et al. Modification of black wheat bran by superfine grinding and *Neurospora crassa* fermentation: physicochemical properties, mixed flour quality, steamed bread quality, and flavor. *LWT*. 2024;213:117049. <https://doi.org/10.1016/j.lwt.2024.117049>.
41. Dudareva N, Klempien A, Muhlemann JK, Kaplan I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol*. 2013;198(1):16–32. <https://doi.org/10.1111/nph.12145>.
42. Sharma LK, Agarwal D, Rathore SS, Malhotra SK, Saxena SN. Effect of cryogenic grinding on volatile and fatty oil constituents of cumin (*Cuminum cyminum* L.) genotypes. *J Food Sci Technol*. 2016;53(6):2827–34. <https://doi.org/10.1007/s13197-016-2258-0>.
43. Bacchetti T, Morresi C, Bellachioma L, Ferretti G. Antioxidant and pro-oxidant properties of *Carthamus tinctorius*, hydroxy safflor yellow A, and safflor yellow A. *Antioxidants*. 2020;9(2):10. <https://doi.org/10.3390/antiox9020119>.
44. Meng Q, Chen F, Xiao T, Zhang L. Superfine grinding of *Dendrobium officinale*: the finer the better? *Int J Food Sci Technol*. 2019;54(6):2199–208.
45. Zhao G, Zhang R, Dong L, Huang F, Tang X, Wei Z, et al. Particle size of insoluble dietary fiber from rice bran affects its phenolic profile, bioaccessibility and functional properties. *LWT Food Sci Technol*. 2018;87:450–6. <https://doi.org/10.1016/j.lwt.2017.09.016>.
46. Wang KH, Li SF, Zhao Y, Li HX, Zhang LW. In vitro anticoagulant activity and active components of safflower injection. *Molecules*. 2018;23(1):8. <https://doi.org/10.3390/molecules23010170>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.