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Metabolomics profiling and techno-functional characterization of freeze-dried pumpkin flower (*Cucurbita maxima*) powder for food fortification

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Abstract

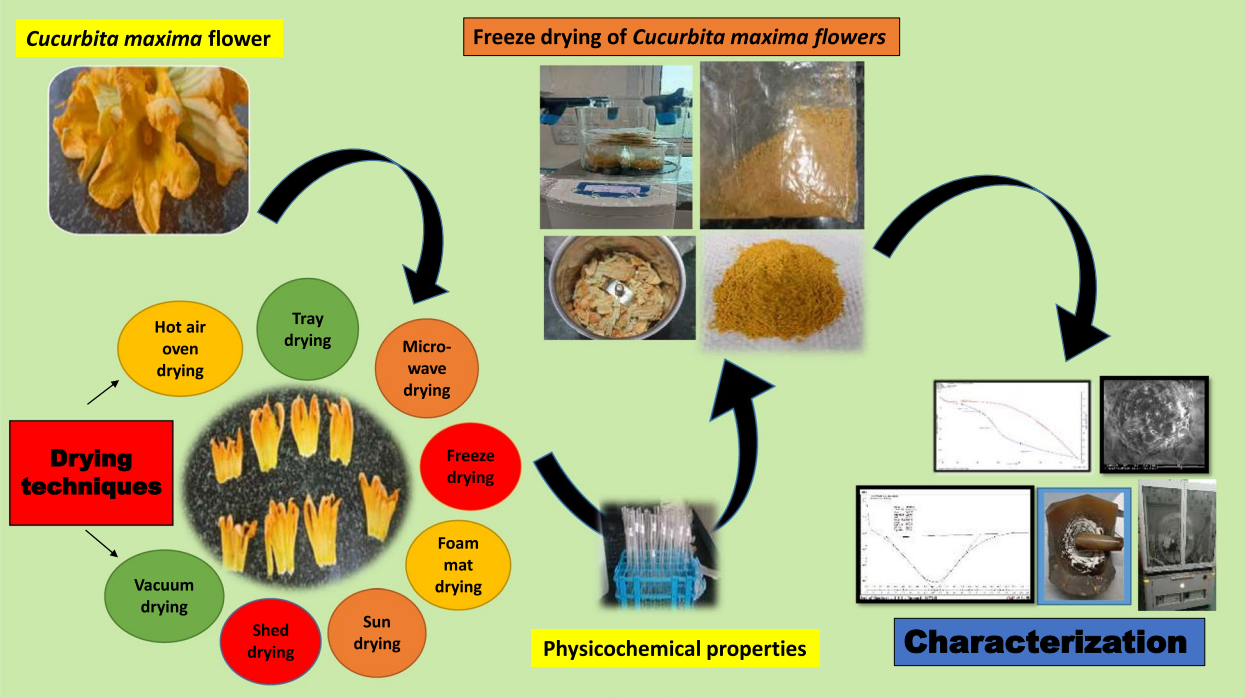
Traditionally valued for their aesthetic and aromatic appeal, edible flowers are now recognized for their nutritional and medicinal benefits. They represent an affordable and sustainable plant-based food source with growing potential in functional food applications. This study aimed to determine the most suitable drying method for maintaining the nutritional quality of pumpkin flowers. Freeze-drying emerged as the optimal technique, effectively preserving vital nutrients. The resulting freeze-dried pumpkin flower powder underwent comprehensive evaluation, including physicochemical properties, proximate composition, bioactive compounds, and techno-functional attributes. Advanced characterization techniques such as X-ray diffraction, differential scanning calorimetry, Fourier-transform infrared spectroscopy, and high-resolution mass spectrometry revealed an amorphous structure in the powder, indicated by a broad peak at $2\theta = 21.57°$. HR-MS analysis showed the presence of different functional compounds such as β-carotene, α-carotene, different types of flavonoids, organic acids, etc. The analysis identified key bioactive compounds, including beta-carotene, alpha-carotene, flavonoids, and organic acids, contributing to its high antioxidant activity (90.52%). Additionally, the powder demonstrated excellent solubility and thermal stability, making it suitable for various food applications. Elemental analysis confirmed a rich composition, with notable proportions of carbon (42.1%), nitrogen (8.1%), oxygen (43.4%), potassium (4.4%), and calcium (1.3%). These findings highlight the potential of freeze-dried pumpkin flower powder as a nutrient-dense, functional ingredient for fortified food products. This research underscores the viability of freeze-drying in enhancing the shelf life and nutritional value of edible flowers, positioning them as a promising ingredient in the health food industry.

Keywords *Cucurbita maxima*, Characterization, HRMS, Bioactive compounds, Antioxidant activity, ^{1}H NMR

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Graphical Abstract



Introduction

Because of their distinctive flavors, vivid colors, and adaptability in the kitchen, edible flowers have become more and more popular in recent years. Pumpkin flowers, sometimes referred to as pumpkin blossoms or squash blossoms, have a specific place in the food business among other delicious flowers. Since ancient times, several varieties of squash and pumpkin plants have produced these delicate, vividly colored flowers, which are utilized in traditional dishes (Pires et al., 2019). These flowers are not only visually appealing but also possess culinary and medicinal significance. Pumpkin flowers are typically large and showy, measuring around 5 to 8 cm (2 to 3 inches) in diameter. They have a distinct trumpet-like shape, with a long, slender tube extending from a wide, flared petal arrangement. The flowers are produced in both male and female forms by the plant (Ahmad & Khan, 2019). The male pumpkin flowers are more abundant than their female counterparts. They grow on long, thin stalks and do not develop into fruit. They have a mild, slightly sweet flavor with subtle vegetal notes. In various cultures, the flowers are harvested and used in a variety of dishes. They can be consumed both raw and cooked. Raw pumpkin flowers make an attractive addition to salads, providing a fresh and visually appealing element. Some cultures use dried pumpkin flowers to prepare herbal tea, which is believed to have soothing and calming properties. Pumpkin flowers are not only enjoyed for their taste but also offer potential health benefits. They are a rich source of vitamins A and C, as well as minerals like potassium, magnesium, and calcium. These flowers also contain antioxidants and dietary fiber. In traditional medicine, pumpkin flowers have been used to alleviate inflammation, aid digestion, and promote overall wellness (Hussain et al., 2022). The pumpkin flower powder as fortificant can serve as a noble ingredient and its dried powder can also be used in different formulations. Pumpkin flower powder serves as a distinctive natural food fortificant, valued for its abundant nutrients, including significant amounts of vitamins (A, C, E, and B-complex), essential minerals (iron, calcium, zinc), antioxidants (flavonoids, carotenoids), and dietary fiber. It provides multiple health benefits, such as immune system support and anti-inflammatory effects. Unlike synthetic fortificants, this whole-food ingredient improves nutrient bioavailability, integrates seamlessly into various recipes without altering flavor, and is often derived sustainably from agricultural byproducts, making it an environmentally friendly and adaptable choice for food enrichment. Some researchers have incorporated

different dried flower powders as a fortificant. Rose flower powder, which is abundant in phenolic compounds and anthocyanins, holds promise for various health benefits. This research investigates how RFP influences the physicochemical, antioxidant, and sensory characteristics of cookies, as well as its role in enhancing storage stability (Hnin et al., 2021). A study optimized freeze-drying parameters through response surface methodology to maintain vitamin C content, antioxidant activity, and hygroscopicity in *moringa* flowers. By evaluating factors such as temperature, vacuum pressure, and drying duration, the research aims to determine the best conditions for preserving nutrients and improving drying efficiency (Pandidurai et al., 2022). Drying is an essential post-harvest process aimed at maintaining the quality, stability, and bioactive profile of plant-based materials. The method of drying employed can greatly influence the physical and chemical properties, nutrient content, and functional attributes of the final dried product (Duc et al., 2019). Conventional methods like sun drying involve exposing materials to direct sunlight, which is economical but can result in nutrient loss due to extended exposure to heat and oxidation. Shade drying, another traditional technique, reduces direct heat exposure, helping to preserve color and volatile components to a certain degree. Modern drying techniques provide better regulation of temperature and moisture extraction, improving the retention of bioactive substances (Hasan et al., 2019). Freeze drying, for instance, operates at very low temperatures (−50 °C) through sublimation, maintaining the sample's structural integrity and bioactive properties (Bhatta et al., 2020). Tray drying and hot air drying employ controlled heat (40 °C) for specific time periods, effectively lowering moisture levels while safeguarding important phytochemicals. Microwave drying uses high-energy waves (900 W) for brief periods, enabling quick moisture removal but with possible risks of thermal damage. Vacuum drying, conducted at moderate temperatures (60 °C) under low pressure, helps prevent oxidation and improves the preservation of compounds sensitive to heat. The selection of a drying method is crucial in defining the ultimate quality of dried plant materials. This research investigates various drying techniques and their effects on the composition of metabolites, aiming to refine preservation approaches for bioactive compounds. Recently metabolomics has been used widely to identify the functional compounds present in plant extracts. The importance of metabolomics as a technique in food science is becoming more widely acknowledged. It provides information about the intricate biochemical characteristics of food components. The nutritional value, safeguarding, and integrity of food can potentially be evaluated by researchers by examining metabolites, which are tiny particles that are the by-products of biological activities. This method makes it possible to identify bioactive substances including antioxidants and anti-inflammatory drugs that enhance flavor, fragrance, and physiological benefits. Metabolomics can also assist in identifying pollutants and adulterants, guaranteeing food safety and adherence to legal requirements (Utpott et al., 2022). In addition to improving our knowledge of food composition, the use of metabolomics methods aids in the establishment of functional foods that are suited to certain dietary requirements and health objective. Although male pumpkin flowers hold significant nutritional and medicinal value, there is a notable lack of research on effective drying methods for them. Current studies predominantly concentrate on pumpkin fruits and seeds, creating a substantial gap in knowledge regarding the best practices for preserving the bioactive compounds and nutrients found in the flowers. The influence of various drying techniques such as sun drying, oven drying, freeze drying, and microwave drying on preserving the essential vitamins, antioxidants, and flavonoids is poorly understood. Moreover, the impact of these drying methods on sensory attributes like color, texture, aroma, and rehydration ability has not been thoroughly investigated. Additionally, there is limited exploration into the commercial potential of dried male pumpkin flowers, including their possible uses in functional foods, herbal products, or as natural food additives. Filling these research gaps would offer crucial insights into refining drying techniques to preserve nutritional quality, enhance shelf stability, and broaden the industrial applications of male pumpkin flowers. This study significantly expands the existing knowledge on pumpkin flowers by employing a multidisciplinary approach that integrates phytochemical profiling, antioxidant assessment, and techno-functional characterization. While prior research has primarily focused on basic compositional analysis, this work delves deeper into their industrial processing potential and stability under varying drying conditions, providing critical insights for practical applications. Furthermore, the research incorporates advanced characterization methodologies (e.g., high-resolution spectroscopy and chromatography, if applicable) to elucidate the functional and nutraceutical significance of bioactive compounds in pumpkin flowers. To our knowledge, this represents one of the first systematic efforts to establish pumpkin flowers as a sustainable bioresource for value-added food ingredient development. By doing so, this study effectively bridges the gap between traditional uses and modern food innovation, paving the way for their integration into functional food formulations. These findings not only enhance scientific understanding but also open new avenues for sustainable

food processing, nutraceutical development, and waste valorization, positioning pumpkin flowers as a promising fortificant for future food and health applications.

Materials and methods

Sample collection and preparation

Fresh male pumpkin flowers (*Cucurbita maxima*) were sourced from local farms in Varanasi. The edible flowers were harvested at the anthesis stage at 7:00 AM. Using scissors, the blossoms were carefully cut from the plant, leaving a stalk of 5 to 8 cm. The freshly collected flowers were placed in perforated plastic containers and processed within two hours. The flowers were cleaned, sorted, and subjected to various drying methods, including freeze drying, microwave drying, foam mat drying, shade drying, tray drying, sun drying, and hot air oven drying under specific conditions. Each flower was separated into petals, stamens, and peduncle, and the petals were gently tapped with an absorbent tissue to remove excess moisture before being rinsed 3–4 times with distilled water. The prepared samples were then treated according to the requirements of each drying technique. For sun drying, the cleaned petals were spread on a muslin cloth and exposed to direct sunlight from 10:00 AM to 4:00 PM for two days (27 ± 2 °C) (Bika et al., 2021). For freeze drying, the sorted petals were placed in perforated LDPE bags and kept in a freeze dryer at −50 °C for 24 h (De et al., 2017). Shade drying involved spreading the petals on a cotton sheet and allowing them to air dry for two days (28 ± 2 °C) (Faust & Dole, 2021). Tray drying was conducted by spreading the petals on trays and applying continuous heat at 40 °C for six hours (Matouk et al., 2016). Microwave drying was performed at 900 W for 5 to 7 min (Quan et al., 2023), while vacuum drying was carried out at 60 °C for 10 h (Ji et al., 2012). For hot air drying, the petals were placed in trays and heated at 40 °C for two hours (Dorozko et al., 2019). Foam mat drying was done according to the method employed by Thuy et al., (2022). Based on preliminary literature, the optimization process was conducted by systematic key input parameters, including albumin concentration (10% w/w), carboxymethyl cellulose content (0.5% w/w), and drying temperature (70 °C). Once dried, the samples were ground in a grinder and then vacuum sealed in LDPE pouches and were stored at 4 °C till further use.

Centesimal composition

The hot air oven drying method was used to calculate the moisture content. Ash content was assessed using a muffle furnace, crude fat content was determined using a Soxhlet apparatus, protein content was analyzed using the Kjeldahl method, crude fiber content was assessed through acid/alkali digestion, and carbohydrate content was calculated. These analytical methods were applied to all the pumpkin blossoms dried using different drying methods (AOAC, 2016). The total phenolic content, antioxidant activity as well as total flavonoid content was measured according to previously cited study with some modification (Shah & Modi, 2015). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to assess antioxidant activity by combining the sample with a DPPH solution and measuring the absorbance at 517 nm after incubation. A reduction in absorbance signified scavenging activity. The total phenolic content (TPC) was measured using the Folin–Ciocalteu reagent, where the sample was mixed with the reagent, followed by the addition of sodium carbonate, and the absorbance was recorded at 765 nm. The results were reported in gallic acid equivalents (GAE). The total flavonoid content (TFC) was evaluated using the aluminum chloride colorimetric method, in which the sample was reacted with aluminum chloride, and the absorbance was measured at 415 nm. The findings were expressed in rutin equivalents (RE).

Flow properties

Each sample of *Cucurbita maxima* had its flow characteristics examined, including bulk density, tapped density, flowability, as well as cohesiveness. Flow characteristics were determined using the method mentioned in previous study (Meghwal & Goswami, 2017).

Bulk density and tapped density

A cylindrical container was used to analyze the bulk density of pumpkin flower powder. The cylinder was filled with the powder sample up to a height of 10 cm. The height and mass of the powder were then recorded. The tapped density was determined by gently tapping a vessel filled with rice milk powder on a cushioned rubber surface. The measurement was taken following 250 ± 15 taps from a height of 3 mm (Padma et al., 2022). The bulk density as well as the tapped density of the powder was calculated using the below given equations.

$$\rho(\mathrm{b}) = \frac{\mathrm{M_p}}{\mathrm{V_P}} \tag{1}$$

$$\rho(\mathrm{t}) = \frac{\mathrm{M_c}}{\mathrm{V}_c} \tag{2}$$

Note: $\rho_{(b)}$ – Porous bulk density, Mp—Porous powder mass, Vp—Porous powder volume, $\rho_{(t)}$—Tapped density, M_c—Compact powder mass, V_c—Compact powder volume.

Cohesiveness and flowability

The flowability and cohesiveness of the powdered sample are represented by the Carr index as well as the Hausner ratio, respectively. The bulk as well as the tapped density of the powdered sample was used to estimate the Hausner ratio, as stated in the equation below.

$$HR = \frac{\rho_t}{\rho_b} \tag{3}$$

$$\mathrm{CI} = 100 - \left(\frac{100}{\mathrm{HR}}\right) \tag{4}$$

where HR is the Hausner ratio and CI (%) is the Carr index.

Functional properties

The relationship between powder as well as water or oil is generally connected to the functional characteristics of powders. The functional properties of the powdered samples were determined according the previous literature with some modifications (Jha et al., 2021).

Foaming capacity and stability

Two grams of *Cucurbita maxima* flower powder, as well as 50 mL of milli-Q water, were combined appropriately in a 100 mL capacity measuring cylinder to test the foaming capacity along with the stability of the mixture. Rapid shaking was used to quickly create foam from the suspension. Foam capacity was measured as the amount of foam that developed after shaking, and foam stability was measured as the amount of foam that remained after 60 min of shaking.

To measure the solubility of the powdered *Cucurbita maxima* flower, one gram of the powder was mixed with 10 mL of distilled water in a centrifuge tube of 50 mL capacity. This mixture was then heated to 80 °C for 30 min. After heating, the tubes were rapidly cooled and centrifuged at 6000 rpm for 15 min. The solubility was determined by evaporating the centrifugation supernatant and recording the weight of the residue (dried). The solubility was calculated using the following formula.

$$(\%)\mathrm{Solubility} = \left(\frac{\text{wt. of final sample after evaporation}}{\text{wt. of the initial sample}}\right) \times 100 \tag{5}$$

Capacity of oil absorption

By blending 1g of the powdered sample with oil (10 mL) in a centrifuge tube holding up to 50 mL, the oil absorption capacity was measured. The mixture was shaken vigorously for an hour. Following this, it was centrifuged at 6000 rpm (20 min). The separated oil was then extracted with a pipette, and the final weight was noted. The amount of oil absorbed per gram of sample was used to determine the substance's absorption capability.

Swelling power

In a centrifuge tube with holding capacity of 50 mL, one gram finely ground substance as well as distilled water (10 mL) was combined. It was then subjected to heat treatment at 80 °C with continuous stirring for 30 min. The tubes were cooled to the ambient temperature. Further, it was centrifuged for 15 min at 6000 rpm. Supernatant was removed and the mass was estimated. The following formula was used to calculate swelling power.

$$Swellingproperties(\%) = \left(\frac{Weightofpaste}{Weightofdrypowder}\right) \tag{6}$$

Characterization of the freeze -dried pumpkin flower

Scanning electron microscopy-Energy dispersive X-ray (SEM–EDX)

Scanning electron microscopy (SEM) (Tescan Mira 3 LMU) equipped with EDX (Energy-dispersive X-ray) spectroscopy with 1 nm resolution at 15 kV was used to measure the morphology as well as the size of the powdered flower petals. EDX was used to analyze the minerals in the powdered sample (Peron et al., 2021).

X-Ray diffraction (XRD)

X-Ray diffraction patterns were generated utilizing X-ray tube containing Copper based anode (Model-X'Pert PRO, Panalytical, Netherlands). The powdered material was firmly placed onto a glass slide with an aluminum holder. Tests were run with angles of diffraction at 4^0 to 40° (2Θ). The scanning rate was 4° per min (30 mA) as well as 40 kV (Suhag et al., 2021).

Thermogravimetric analyzer- Differential scanning calorimetry (TGA–DSC)

A thermogravimetric analyzer (Model: NETZSCH, Libra TGA209, Germany) was used to examine the thermal stability of freeze-dried *Cucurbita maxima* sample. Thermogravimetric analysis (TGA) calculates the change in sample mass as a function of temperature. A sample size of 12 mg was applied to each experiment. Under a nitrogen environment, a running sample measurement was conducted between 25 and 600 °C at a 10 °C/min heating rate. The DSC (Model—Shimadzu DSC-50 system, Japan) was used to conduct a thermal analysis of the dried samples in which 2 mg powdered sample was flattened. Thermograms were recorded in a typical aluminum pan that was ramped up in temperature (30 to 130 °C) at 10 °C per

minute rate, while constantly infusing nitrogen (20 mL/min) (Kumar et al., 2018).

Fourier transform infrared spectroscopy (FTIR)

Five milligrams of KBr and about 5 mg of every specimen were used in the Fourier transform infrared spectroscopy analysis for the functional group. All powdered pumpkin blossoms were run through a KBr beam splitter-equipped Shimadzu 8400S FTIR spectrometer. The spectra of the individual samples were examined in accordance with Stuart's recommendations. FTIR spectrophotometer was utilized with 0.85 cm^{-1} resolution and 400–4000 cm^{-1} range of spectra (Chawla et al., 2020).

^{1}H NMR (Proton Nuclear Magnetic Resonance)

Previously freeze-dried samples of *Cucurbita maxima* flowers were used for this analysis. Freeze-dried materials along with 70% (v/v) deuterated acetone were combined and the mixture was centrifuged for about 20 min at 6000 rpm. Gradient pulses were used for solvent signal attenuation during relaxation and selection during COSY investigations. Software programs Topspin v4.0 as well as MestReNova 9.1 were used to process and analyze the final data. Chemical shifts of the sample were recorded in ppm, using the methyl group 1H resonances of acetone as a standard reference (δ = 2.05 ppm) (Brito et al., 2021).

Sample preparation for High Resolution Mass Spectroscopy (HRMS) and instrumentation

Using an orbital shaker, 500 mg sample of pumpkin flower was mixed with 4 mL of n-Hexane and agitated for 4 h at ambient temperature. The mixture was then centrifuged for 5 min at 25 °C (5000 rpm), and the resultant liquid was extracted. 4 mL of an 8:2 methanol–water solution were added to the remaining pellet, which was then agitated on the orbital shaker for four more hours at room temperature. After centrifuging this mixture once again for five minutes at 4 °C (5000 rpm), the supernatant was taken out for analysis.

Using its high-resolution, precise mass capabilities, the Orbitrap Eclipse Tribrid Mass Spectrometer (Thermo Fisher Scientific) was used in this investigation to analyse tiny molecules. Using a Dionex UltiMate 3000 RSU HPLC System, molecules were separated. The solvent composition for the small molecules was as follows: 100% water and 0.1% formic acid made up Solvent A, 80% acetonitrile and 0.1% formic acid made up Solvent B, and 100% methanol and 0.1% formic acid made up Solvent C. A 100 mm long and 2.1 mm wide Hypersil GOLDTM C18 column with a particle dimension of 1.9 µm was used for chromatographic separation. All analyses were carried out with"Compound Discoverer 3.3.3.200"at its default parameters, and each technical replication of the flower powder underwent three HR-MS analyses.

Statistical analysis

The data was processed as well as analyzed using the software ORIGIN 9.1, USA. All the investigations were carried out in triplicate. SPSS (Version 23) was used to analyze the data at 5% level of significance.

Results and discussion

Centesimal composition

The moisture content of dried pumpkin flower powder critically influences its functional properties, shelf life, and nutritional efficacy. An optimal moisture range (typically 5–8%) ensures desirable physical characteristics such as improved flowability, reduced caking, and enhanced solubility, facilitating uniform blending into food products. Excess moisture (> 10%) promotes microbial growth, enzymatic activity, and oxidative degradation, compromising the powder's stability and bioactive compounds like polyphenols and carotenoids. Conversely, overly low moisture (< 4%) can lead to excessive brittleness, increased dustiness, and reduced rehydration capacity. Properly controlled moisture levels preserve the powder's antioxidant potential, nutrient integrity, and sensory qualities while extending shelf life by minimizing water activity ($A_w < 0.6$). Thus, precise drying techniques are essential to maintain the balance between functionality, stability, and nutritional value in pumpkin flower powder as a fortificant. Table 1 shows the proximate analysis and antioxidant profile of the dried samples. Among the different dried samples, freeze dried sample contained the least amount of moisture content ($p < 0.05$) that is 9.4%. The level of moisture in a substance is largely determined by the temperature and duration of the drying process. Techniques involving higher temperatures, such as hot air drying, generally yield lower moisture levels because they accelerate water evaporation. In contrast, freeze-drying (FD) often maintains the protein's natural structure. The way water is retained within the material depends on the stability of cell membranes and the presence of hydrophilic regions in proteins. When drying processes cause protein denaturation, they weaken water retention by breaking hydrogen bonds and altering hydrophilic amino acid side chains. The dried flower's quality and texture are guaranteed to last for more than 6 months if the moisture content is between 8 and 11.5% (Singh et al., 2004). It is affirmed that the lower the moisture content of the sample, the higher will be the ash content. Freeze dried sample with the lowest moisture content has the higher amount of ash content (16.20%) than the other samples treated with different

Table 1 Physico-chemical and antioxidant profile of *Cucurbita maxima* flower powder

Parameters		Hot air oven drying	Vacuum drying	Freeze drying	Shed drying	Foam mat drying	Microwave drying	Sun drying	Tray trying
Proximate analysis									
Moisture content %	**Wet basis**	13.5 ± 0.30^{d}	14.5 ± 0.30^{e}	9.46 ± 0.05^{a}	13.56 ± 0.04^{d}	13.53 ± 0.35^{d}	11.4 ± 0.14^{b}	12.26 ± 0.25^{c}	14.26 ± 0.35^{e}
	Dry basis	15.61 ± 0.35^{d}	16.96 ± 0.35^{d}	10.45 ± 0.06^{a}	15.69 ± 0.05^{d}	15.65 ± 0.41^{d}	12.87 ± 0.16^{b}	13.98 ± 0.29^{c}	16.63 ± 0.41^{e}
Ash (%)	**Wet basis**	11.73 ± 0.20^{c}	9.08 ± 0.00^{a}	16.2 ± 0.10^{f}	14.63 ± 0.15^{e}	11.8 ± 0.10^{c}	10.24 ± 0.05^{b}	12.3 ± 0.20^{d}	12.06 ± 0.02^{d}
	Dry basis	13.57 ± 0.23^{c}	10.61 ± 0.00^{a}	17.89 ± 0.11^{f}	16.94 ± 0.17^{e}	13.66 ± 0.12^{c}	11.56 ± 0.06^{b}	14.03 ± 0.23^{d}	14.06 ± 0.02^{d}
Crude fat (%)	**Wet basis**	3.28 ± 0.03^{c}	3.29 ± 0.01^{c}	3.07 ± 0.02^{a}	3.21 ± 0.01^{c}	3.24 ± 0.02^{c}	3.18 ± 0.01^{b}	3.27 ± 0.02^{c}	3.24 ± 0.04^{c}
	Dry basis	3.79 ± 0.03^{a}	3.85 ± 0.01^{a}	3.39 ± 0.02^{a}	3.72 ± 0.01^{a}	3.75 ± 0.02^{a}	3.59 ± 0.01^{a}	3.73 ± 0.02^{a}	3.78 ± 0.05^{a}
Crude fiber (%)	**Wet basis**	5.46 ± 0.02^{d}	4.22 ± 0.02^{a}	5.95 ± 0.05^{f}	4.42 ± 0.02^{b}	5.37 ± 0.01^{c}	5.52 ± 0.02^{e}	5.32 ± 0.02^{c}	4.27 ± 0.02^{a}
	Dry basis	6.31 ± 0.02^{c}	4.93 ± 0.02^{a}	6.57 ± 0.06^{c}	5.12 ± 0.02^{b}	6.22 ± 0.01^{c}	6.23 ± 0.02^{c}	6.07 ± 0.02^{c}	4.98 ± 0.02^{a}
Protein (%)	**Wet basis**	2.97 ± 0.02^{a}	3.57 ± 0.030^{d}	3.91 ± 0.06^{f}	3.36 ± 0.01^{c}	3.54 ± 0.15^{d}	3.22 ± 0.02^{b}	3.64 ± 0.02^{e}	3.17 ± 0.02^{a}
	Dry basis	3.43 ± 0.02^{a}	4.17 ± 0.04^{b}	4.32 ± 0.07^{b}	3.89 ± 0.01^{a}	4.10 ± 0.17^{b}	3.63 ± 0.02^{a}	4.15 ± 0.02^{b}	3.70 ± 0.02^{a}
Carbohydrates (%)	**Wet basis**	66.42 ± 0.47^{f}	65.32 ± 0.30^{e}	58.58 ± 0.21^{a}	60.79 ± 0.23^{b}	62.50 ± 0.43^{c}	66.43 ± 0.15^{f}	63.11 ± 0.24^{d}	62.96 ± 0.24^{e}
	Dry basis	76.75 ± 0.54^{g}	76.38 ± 0.35^{g}	64.68 ± 0.23^{a}	70.38 ± 0.27^{b}	72.35 ± 0.50^{d}	74.96 ± 0.17^{f}	71.97 ± 0.27^{c}	73.44 ± 0.28^{e}
Antioxidant profile									
Total phenolic content (mg GAE/g)		17.31 ± 0.0015^{e}	11.708 ± 0.0012^{b}	21.38 ± 0.0002^{f}	12.72 ± 0.0002^{c}	10.13 ± 0.0016^{b}	15.50 ± 0.0015^{d}	9.45 ± 0.0050^{a}	15.07 ± 0.0001^{d}
% inhibition DPPH (2,2-Diphenyl-1-picrylhydrazyl)		53.43 ± 0.0012^{a}	60.35 ± 0.0017^{b}	90.52 ± 0.0012^{f}	75.18 ± 0.0012^{d}	68.87 ± 0.0016^{d}	66.01 ± 0.0012^{e}	69.77 ± 0.0008^{d}	70.37 ± 0.0017^{e}
Total flavonoid content (mg RE/g)		65.28 ± 0.0012^{a}	70.65 ± 0.0015^{c}	81.98 ± 0.0017^{e}	68.39 ± 0.0012^{b}	63.75 ± 0.0017^{a}	67.58 ± 0.0012^{b}	71.98 ± 0.0081^{c}	73.58 ± 0.0015^{d}

All the data are represented as mean ± standard deviation ($n = 3$)

[a –g]Means with the same superscript in a row vary significantly ($p < 0.05$) from each other

drying techniques. Our results are consistent with earlier studies (Alakali et al., 2015). The researchers observed a greater percentage of 4.55 ± 0.06% compared to the fresh leaves, which had an ash content of 2% in leaves of *Moringa oleifera*, that were dried in the shade at 30 °C that is ambient temperature. Despite all drying techniques, there is a noticeable rise in fiber content because the blooms'nutritional density increases with the loss of moisture content. It helps to manage the cholesterol and glycemic index and is advantageous for a number of intestinal bacteria and digestive problems. As a result, the fiber content of the freeze -dried sample i.e. 5.95% was the highest among all the samples. In the current study, the hot air oven dried sample had the greatest crude fat content, at 3.28%, which was substantially higher. The crude fat levels ranging between 0.82 and 0.87% were not significantly different from the one present in *Euphorbia hirta L.* plant (Mathad et al., 2019). In respect to all the drying techniques, the freeze-dried sample (3.91%) showed the highest amount of crude protein. It revealed a substantially ($p < 0.05$) increased content of the crude protein. Both the samples that were vacuumed that is 18.60% as well as freeze-dried that is 18.72% of the dried *Ocimum basilicum* leaves showed the same level of protein content (Siti Mahirah et al., 2018). In comparison to all the other drying techniques, hot air oven (66.24%) revealed considerably ($p < 0.05$) increased carbohydrate content. As the sugars are subjected to elevated heat levels, quick caramelization takes place. Because of this, there is a chance of losing carbs with of low molecular mass, which is why the minimal variation of carbohydrate amount within drying processes is taken into consideration. DPPH represents an organic chemical compound comprising stable free radicals. DPPH radical scavenging activity was the highest in the freeze-dried sample (90.52%) when compared to other drying methods. Freeze dried sample of *Ocimum basilicum* leaves showed similar results that is the DPPH activity of 92.60% (Siti Mahirah et al., 2018). In the current investigation, TPC of the freeze-dried sample was considerably ($p < 0.05$) greater than that of the other dried samples (21.38 mg GAE/g). The optimal preservation and stability result from the effective deactivation of polyphenol oxidase enzymes, which prevents cell contents from rupturing and phenolic compounds from breaking down. Lower temperature exposure results in increased flavonoid retention. Freeze dried sample also showed the highest amount of total flavonoid content (81.98 mg RE/g) when compared to other drying techniques. Compared to methods such as freeze drying and vacuum-microwave freeze drying (which result in a flavonoid content of 43.62–88.33 mg RE/g), *Rosa rugosa* exhibited a more significant reduction in flavonoid content when subjected to microwave-assisted vacuum drying that is 11.54 ± 0.01 mg/100 g (Qiu et al., 2020).

Functional and flow parameters

Bulk density

The powder's bulk density, which is determined by factors like particle size, inter particle pulls, and the strength of contact points, is a crucial determinant of the compatibility with convenience of packaging and transportation of food product. The freeze-dried *Cucurbita maxima* flowers demonstrated the highest amount of bulk density (0.40 g/cm^3). Low moisture content in the powder sample might be used to describe this. Increased bulk density of the sample may also be caused by structural changes made during the blanching pretreatment that results in a most compact/closed structure after drying as well as tighter arrangement of particles that reduces empty spaces (Tze, 2012).

Tapped density

The powdered sample's volume acquired with tight packing of components by tapping is referred to as tapped density. The volume of the inter particle space can change due to pretreatments and packaging techniques, so the appropriate density needs to be established. A powder sample's tapped density, which allows for maximum packaging of the sample under the impact of outside pressures, is one of its main quality characteristics. The average density of samples ranged from 0.40 to 0.52 g/cm^3 (Table 2). According to this analysis, the freeze-dried sample showed lower tapped density that is 0.42 g/cm^3, indicating that it is more porous than the other samples.

Carr index as well as Hausner's ratio

Carr Index, which considers both the powder's bulk as well as tapped density, is a measure of flowability. Carr index of the samples ranged from 4.76% to 31.91%. Table 2 demonstrates that the freeze-dried sample had the minimum Carr index and thus represents very good flowability characteristics, when compared to other samples. The Hausner's ratio of the dried samples varied from 1.05 to 1.30. It can be seen that the freeze -dried sample showed the minimum Hausner's ratio that may attribute to the freeze -dried sample being the most cohesive one than the others.

Solubility

The water solubility of *Cucurbita maxima* (pumpkin) flower powder is a crucial functional attribute influencing its effectiveness in food applications, particularly in beverages, soups, and instant fortified products. In this study, solubility indices varied from 12.09%

Table 2 Techno-functional properties of *Cucurbita maxima* flower powder

Parameters	Hot air oven drying	Vacuum drying	Freeze drying	Shed drying	Foam mat drying	Microwave drying	Sun drying	Tray trying
Flow properties								
Bulk density (g/cm^3)	0.40 ± 0.0001^{e}	0.39 ± 0.0004^{d}	0.40 ± 0.0001^{f}	0.39 ± 0.0007^{d}	0.38 ± 0.0003^{e}	0.36 ± 0.0003^{a}	0.37 ± 0.0005^{b}	0.39 ± 0.0002^{c}
Tapped density (g/cm^3)	0.52 ± 0.0001^{f}	0.47 ± 0.0004^{e}	0.42 ± 0.0003^{a}	0.45 ± 0.0008^{e}	0.45 ± 0.0004^{c}	0.46 ± 0.0001^{d}	0.43 ± 0.0005^{b}	0.45 ± 0.0002^{d}
Carr index (%)	23.76	17.02	4.76	13.33	15.55	21.73	13.79	13.33
Haunser's ratio	1.30	1.20	1.05	1.15	1.18	1.27	1.16	1.15
Functional properties								
Swelling properties (g/g)	15.38 ± 0.0023^{a}	20.89 ± 0.0018^{c}	30.51 ± 0.0015^{e}	25.28 ± 0.0010^{d}	19.87 ± 0.0020^{c}	17.98 ± 0.0098^{b}	23.28 ± 0.0018^{d}	21.85 ± 0.0023^{c}
Oil absorption capacity (g/g)	1.11 ± 0.0087^{a}	1.45 ± 0.0010^{d}	1.67 ± 0.0022^{f}	1.62 ± 0.0048^{f}	1.46 ± 0.0025^{d}	1.22 ± 0.0074^{d}	1.35 ± 0.0014^{c}	1.58 ± 0.0057^{e}
Foaming properties (mL)	4.20 ± 0.0053^{f}	2.11 ± 0.0055^{b}	1.45 ± 0.0057^{a}	2.22 ± 0.0052^{b}	3.67 ± 0.0059^{d}	3.84 ± 0.0054^{e}	3.55 ± 0.0058^{c}	3.33 ± 0.0055^{c}
Solubility (%)	12.09 ± 0.0005^{a}	13.55 ± 0.0009^{b}	17.05 ± 0.0001^{d}	15.98 ± 0.0006^{c}	12.88 ± 0.0006^{a}	12.98 ± 0.0008^{a}	13.45 ± 0.0004^{a}	13.89 ± 0.0005^{b}

All the data are represented as mean ± standard deviation ($n = 3$)

[a] [−g]Means with the same superscript in a row vary significantly ($p < 0.05$) from each other

(freeze-dried) to 17.05% (oven-dried), demonstrating that drying methods significantly ($p < 0.05$) affect dissolution properties. The higher solubility observed in oven-dried powder is likely due to structural changes induced by heat, enhancing water interaction, whereas freeze-drying may preserve cellular integrity but reduce dispersibility. Protein solubility is strongly influenced by the preservation of its structural conformation. In pumpkin flower powder, the gentle freeze-drying process helps retain the protein's natural folding, enhancing solubility by keeping polar groups accessible. On the other hand, harsher methods like hot air drying and microwave drying can trigger protein aggregation or Maillard reactions, resulting in insoluble protein clusters. Heat-induced denaturation often decreases solubility by promoting disulfide cross-linking or hydrophobic interactions, which block water-binding sites. Similarly, banana blossom powder (*Musa paradisiaca*) exhibited solubility levels between 13.90% and 16.53%, reinforcing the impact of pretreatment and drying techniques on solubility, which is crucial for digestibility and nutrient absorption (Elaveniya & Jayamuthunagai, 2014). In food fortification, increased solubility ensures even distribution in liquid formulations, improving both sensory qualities and nutrient availability. Therefore, optimizing drying conditions, such as moderate oven-drying, could enhance the usability of pumpkin flower powder in ready-to-mix supplements, health beverages, and fortified sauces, where rapid dissolution is essential for functionality and consumer appeal.

Foaming properties

Processing techniques, pH level, as well as surface tension, are frequently thought to have an impact on foaming capacity. The scattered foam between the samples will aid in keeping the whip from falling apart even if the powder with limited foam-forming ability is thought to be a poor foaming ingredient in food preparations. The *Cucurbita maxima* flower sample had a substantially variable foaming capacity that varied between 1.45 and 4.20 mL ($p < 0.05$). The appropriate foaming ability is associated with the rapid absorption of the protein at the junction of air as well as water and the protein's structural rearrangement at this interface. In contrast to other dried samples, the freeze-dried sample proved to have the greatest foaming capacity. It could be because the drying technique minimizes damage to the protein structure as little as possible, protecting the protein fraction's capacity to foam.

Swelling properties

The swelling capacity of *Cucurbita maxima* (pumpkin) flower powder, ranging from 15.38 g/g to 30.51 g/g, is a key functional attribute shaped by protein-starch interactions and drying techniques. A higher protein content can create a rigid matrix around starch granules, limiting water absorption and reducing swelling ability. However, drying processes such as oven-drying or freeze-drying can alter this structure, potentially increasing swelling

by freeing starch for hydration. In general, the swelling behavior of a material is determined by its capacity to absorb water and expand, influenced by hydrophilic polysaccharides (such as pectins and cellulose) and the degree of protein unfolding. Freeze-drying maintains a porous structure and high surface area, facilitating greater water penetration and enhanced swelling. In contrast, high temperatures in hot air drying can cause cell wall collapse, reducing matrix porosity and diminishing swelling ability. While mild protein denaturation may initially improve swelling, excessive thermal cross-linking during drying ultimately restricts it. Similar trends have been observed in cardaba banana flour, where pretreatment methods enhanced swelling power (Iliyasu & Ayo-Omogie, 2019). In food applications, greater swelling capacity (e.g., 30.51 g/g) enhances thickening, gelation, and water retention, making pumpkin flower powder ideal for soups, baked products, and meat substitutes. Conversely, lower swelling (15.38 g/g) is better suited for low-viscosity formulations like beverages. Adjusting drying methods to optimize protein-starch interactions can help tailor its functionality for specific food applications.

Oil absorption capacity

The oil absorption capacity (OAC) of *Cucurbita maxima* (pumpkin) flower powder is a crucial functional characteristic influenced by factors such as particle hydrophobicity, size, surface charge, chemical composition, and drying techniques. The oil absorption capacity (OAC) of proteins is largely governed by the availability of non-polar side chains that interact with lipids through hydrophobic forces. Moderate protein denaturation—such as that induced by microwave drying can increase OAC by revealing these hydrophobic regions. However, excessive heat treatment promotes excessive protein aggregation, leading to a more compact structure with reduced surface area and diminished OAC. In freeze-dried, the OAC may remain relatively low due to the preservation of tightly folded protein conformations, unless partial denaturation occurs during prior processing steps. In this study, freeze-dried (1.67 g/g) and shade-dried (1.62 g/g) samples demonstrated higher OAC compared to other drying methods, likely due to structural modifications that enhance hydrophobic interactions, including protein denaturation or the exposure of non-polar groups during drying. This property plays a significant role in food applications by improving flavor retention, mouthfeel, and texture, particularly in bakery products, sausages, and soups, where it helps create a spongy, fat-retaining matrix. Similar trends have been reported in *Beta vulgaris* L. (beetroot) powder, where pretreatments influenced OAC (Chaudhary & Kumar, 2020), reinforcing that processing techniques are key determinants of lipid-binding efficiency. For food manufacturers, optimizing drying methods such as freeze-drying for premium formulations can enhance the sensory and functional qualities of pumpkin flower powder in high-fat food applications.

Characterization of freeze- dried pumpkin flower powder

X-ray diffraction analysis (XRD)

Figure 1 depicts the XRD profile of the freeze-dried pumpkin flower powder. The XRD evaluation revealed two unique peaks at $2\Theta = 21.57^{o}$ and $2\Theta = 35.75^{o}$ which correspond to reflection angle of 100 and 200, respectively. The presence of cellulose type I becomes evident through the appearance of a peak at a 2 Theta angle of 21.85°, indicating its affiliation with the α-cellulose crystallographic group. This claim is corroborated by the pattern of diffraction of pumpkin blossoms, which suggests that cellulose may be a significant factor in the increased fiber content. The results have been validated from JCPDS: 05–0661. Similar results were seen in the study mentioned by Patil et al.(2018). Silver in the cubic phase is seen in the diffraction pattern; FCC that is face centered cubic structure plane of (111) may be identified by the intense peaks at 2Θ value of 38.15^{o} in the eco-friendly synthesis of silver nanoparticles by utilizing *M. longifolia* flower extract. Another study was conducted in which the $2\Theta = 22.85^{o}$ corresponding to reflection angle of 141 was observed (Gangaram et al., 2023). The researchers concluded that this type of XRD pattern can be due to the amorphous behavior of the flower material. The instability and stretching of the peaks may be caused by the presence of amorphous macromolecular structures in plant components such as hemicellulose, lignin, etc.

Functional group analysis

Cucurbita maxima freeze-dried flower powder was analyzed for the presence of different functional groups by FTIR depicted in Fig. 2. Table 3 lists several functional groups that are present in the freeze-dried pumpkin flower powder along with the bonding details that pertain to them. The FTIR analysis of pumpkin flower powder identified several functional groups linked to bioactive compounds, which contribute to its biological properties. Broad O–H stretching peaks at 3777.74 cm^{-1} (free alcohol) and 3235.80 cm^{-1} (intermolecular bonded alcohol) suggest the presence of hydroxyl-containing compounds, such as phenolics and flavonoids, known for their potent antioxidant and anti-inflammatory effects. C-H stretching bands at 2918.02 cm^{-1} (alkene) and 2474.15 cm^{-1} (aldehyde doublet) indicate

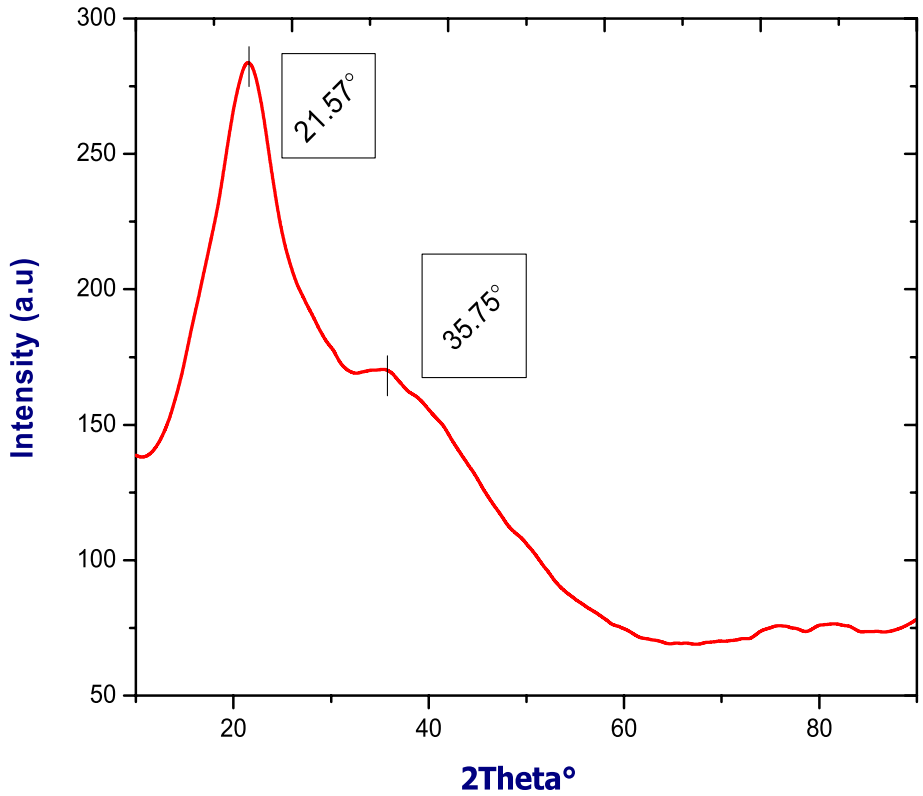


Fig. 1 XRD graph depiction of the Cucurbita maxima freeze dried powder

unsaturated hydrocarbons and carbonyl-containing compounds, which may provide antimicrobial and antifungal benefits. A notable peak at 2073.58 cm^{-1}, corresponding to N=C=S stretching, points to the presence of isothiocyanates, recognized for their anticancer and detoxifying properties. The C=O stretching observed at 1629.71 cm^{-1} indicates conjugated ketones and monosubstituted alkenes, associated with anti-diabetic and anti-inflammatory activities. Additionally, O–H bending at 1431.21 cm^{-1} confirms the presence of carboxylic acids, which may support wound healing and antimicrobial functions. C-O stretching at 1234.24 cm^{-1} and 1131.71 cm^{-1} suggests ethers and secondary alcohols, commonly found in glycosides and flavonoids, known for their cardioprotective and neuroprotective effects. Peaks at 1004.98 cm^{-1} and 677.65 cm^{-1} hint at minor fluoro and halo compounds, which could have antiviral and antimicrobial potential. These functional groups confirm the presence of phytochemicals like polyphenols, flavonoids, aldehydes, ketones, and glucosinolates, underscoring the nutritional, medicinal, and therapeutic value of pumpkin flower powder. A study shows that FTIR is commonly used to tentatively identify functional groups. The oils from *H. sabdariffa* flower, *S. maydis*, and *C. odorata* leaf exhibited peaks indicating hydroxyl, carbonyl, and polysaccharide groups, confirming the presence of fatty acids, esters, proteins, lipids, and nucleic acids. Bioactive compounds such as phenolics, flavonoids, and polysaccharides were identified, which contribute to antioxidant, antimicrobial, and anti-inflammatory activities (Alara & Abdurahman, 2019). Another study used FT-IR spectroscopy to analyze functional groups in freeze-dried *Cucurbita maxima* seed. Peaks at 3450–3250 cm^{-1} indicated hydroxyl groups, and 3120–3030 cm^{-1} showed amide (N–H) groups. C–H stretching was observed at 2950–2850 cm^{-1}, and CH_3/CH_2 deformations around 1450 cm^{-1}. Bands at 1105–1010 cm^{-1} indicated lipids, confirming the presence of key bioactive compounds (Andronie et al., 2022). Another study demonstrated the utility of FTIR in identifying functional groups in plant samples. In the present research, shade-dried pumpkin flower powder showed peaks at 1738.33 cm^{-1} (C=O), 1607.89 cm^{-1} and 1404.20 cm^{-1} (COO^-), 1045 cm^{-1} (C–O), 3284.19 cm^{-1} (O–H), and 2920.79 cm^{-1} (C–H). The absence of peaks between 2000–2200 cm^{-1} was observed (Gargi et al., 2023).

Microstructure and elemental analysis

Scanning electron microscopy was done to analyze the morphology of the *Cucurbita maxima* freeze dried

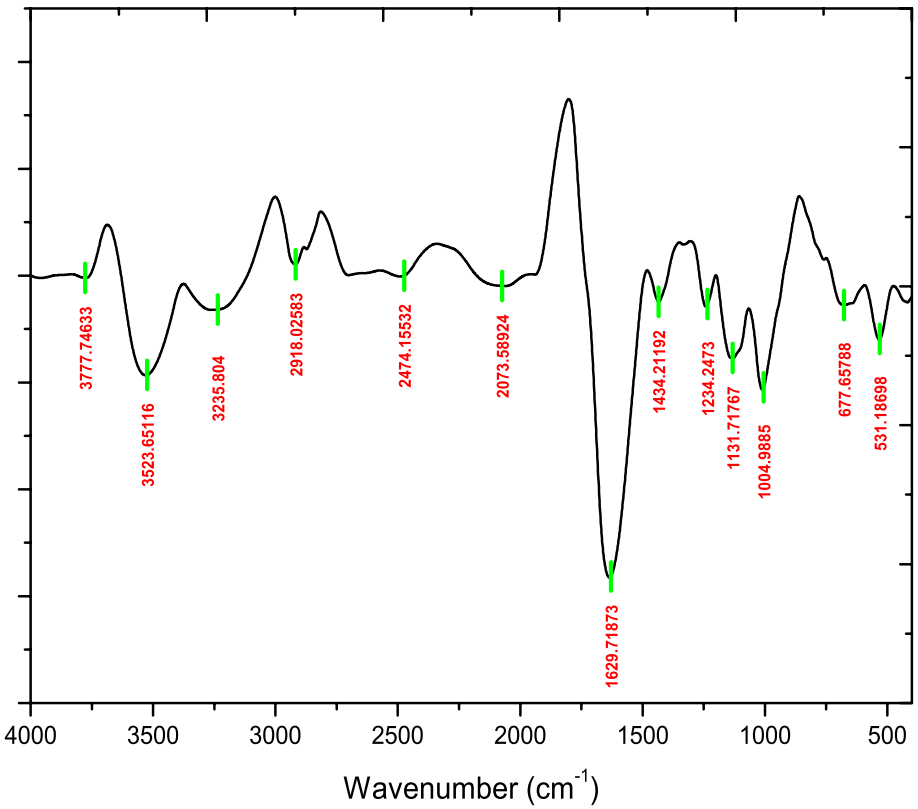


Fig. 2 FTIR spectra of Freeze dried powder of Cucurbita maxima flower

Table 3 Elemental analysis of the freeze- dried *Cucurbita maxima flower* powder

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	R	A
Carbon	6.1	50.3	900.3	6.2	0.2389	1.0323	0.9837	0.5498
Nitrogen	8.1	8.4	36.6	50.2	0.0090	1.0079	0.9938	0.1090
Oxygen	5.4	38.9	645.4	10.1	0.0694	0.9868	1.0029	0.1621
Phosphorus	14.4	0.2	41.0	15.2	0.0030	0.8599	1.0501	0.9008
Sodium	10.5	0.1	36.9	11.3	0.0029	0.8337	1.0600	0.9892
Potassium	23.4	1.6	400.2	3.2	0.0387	0.8294	1.0686	1.0209
Calcium	17.2	0.5	96.2	9.3	0.0112	0.8444	1.0724	1.0059

Note: Here, Net Int is Net Intensity

K ratio is Ratio of the X-ray intensity of the unknown sample to the intensity from a standard sampl

Z-Atomic Number Correction (Z)

R-Absorption Correction (Rho)

A-Fluorescence Correction (A)

powder. The flower powder showed a fibrous structure which was previously confirmed from the XRD analysis. Similar results were seen in another study (Ayoub et al., 2016). Furthermore, in order to analyze the elemental composition of the freeze-dried powder of *Cucurbita maxima flower,* energy dispersive X-ray spectroscopy was used for elemental analysis. Figure 3 depicts the SEM and EDX of freeze-dried powder of pumpkin flower. The graph and the Table 4 obtained from the analysis showed different elemental composition namely, carbon (6.1%), nitrogen (8.1%), oxygen (5.4%), phosphorus (14.4%), potassium (23.4%) and calcium (17.2%).

Thermographic analysis

Thermal stability of the freeze -dried powder of *Cucurbita maxima* flower was investigated by

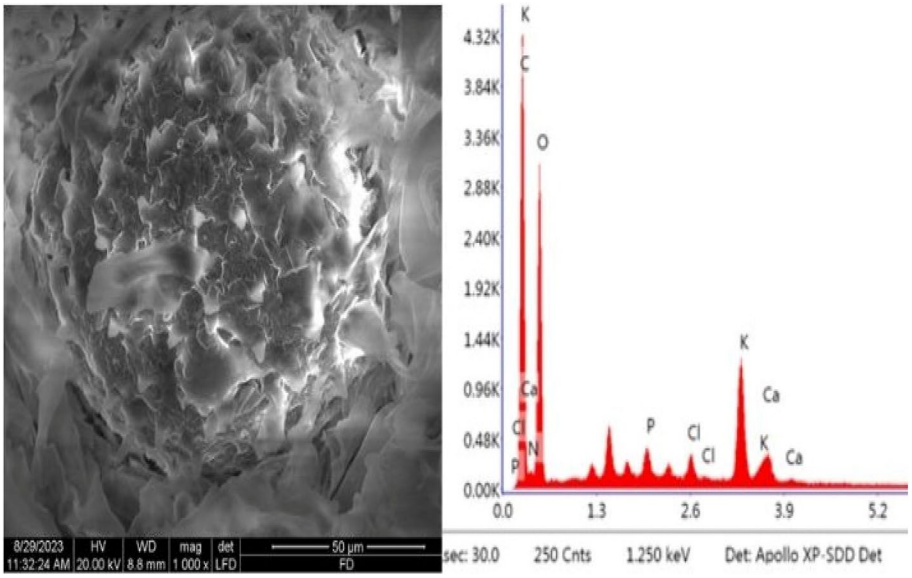


Fig. 3 Microstructure and elemental analysis of Freeze -dried powder of Cucurbita maxima flower

Table 4 Functional groups depiction in *Cucurbita maxima* freeze dried powder

Wavenumber (cm^{-1})	Specific wavenumber (cm^{-1})	Bond	Functional group
4000-3584	3777.74	O-H stretching	Free alcohol
	3573.65		
3550-3200, 3333-3267	3235.80	O-H stretching, C-H stretching	Intermolecular bonded alcohol, alkyne
3100-3000	2918.02	C-H stretching	alkene
2830-2695	2474.15	C-H stretching	Doublet aldehyde
2140-1990	2073.58	N=C=S stretching	Isothiocyanate
1685-1666	1629.71	C=O stretching,	Conjugated ketone, monosubstituted alkene
1440-1395,	1431.21	O-H bending	carboxylic acid
1225-1200	1234.24	C-O stretching	vinyl ether
1150-1085	1131.71	C-O stretching	aliphatic ether, secondary alcohol
1400-1000	1004.98	C-F stretching	Fluoro compound
690-515	677.65	C-Br stretching	halo compound
	531.18		

Thermogravimetric analysis and Differential Scanning Calorimetry (TGA–DSC) from 30 °C to 700 °C at a heating rate of 10 °C/min. The onset temperature for DSC was (T_{onset}) = 34.02 °C, (T_{endset}) = 97.36 °C and denaturation peak (T_{peak}) = 66.75 °C. The energy required for denaturation ΔH = 51.650 J/g. Glass transition temperature was absent in the analysis. Nearly 6.54 wt% weight loss was observed between 30 °C and 150 °C due to water evaporation. About 46.09 wt% weight loss was observed between 200 °C and 410 °C, due to phase transition or organic matter decomposition. The investigation indicated that alterations in enthalpy and the breakdown of organic matter at different temperatures indicated that the freeze-dried substance from *Cucurbita maxima* flowers lacks thermal stability.

Metabolomic profiling of *Cucurbita maxima* freeze dried powder

Numerous analytical techniques have been applied recently to detect and measure flavonoids, bioactive compounds, polyphenols, and other substances. When compared to alternative detection methods, the

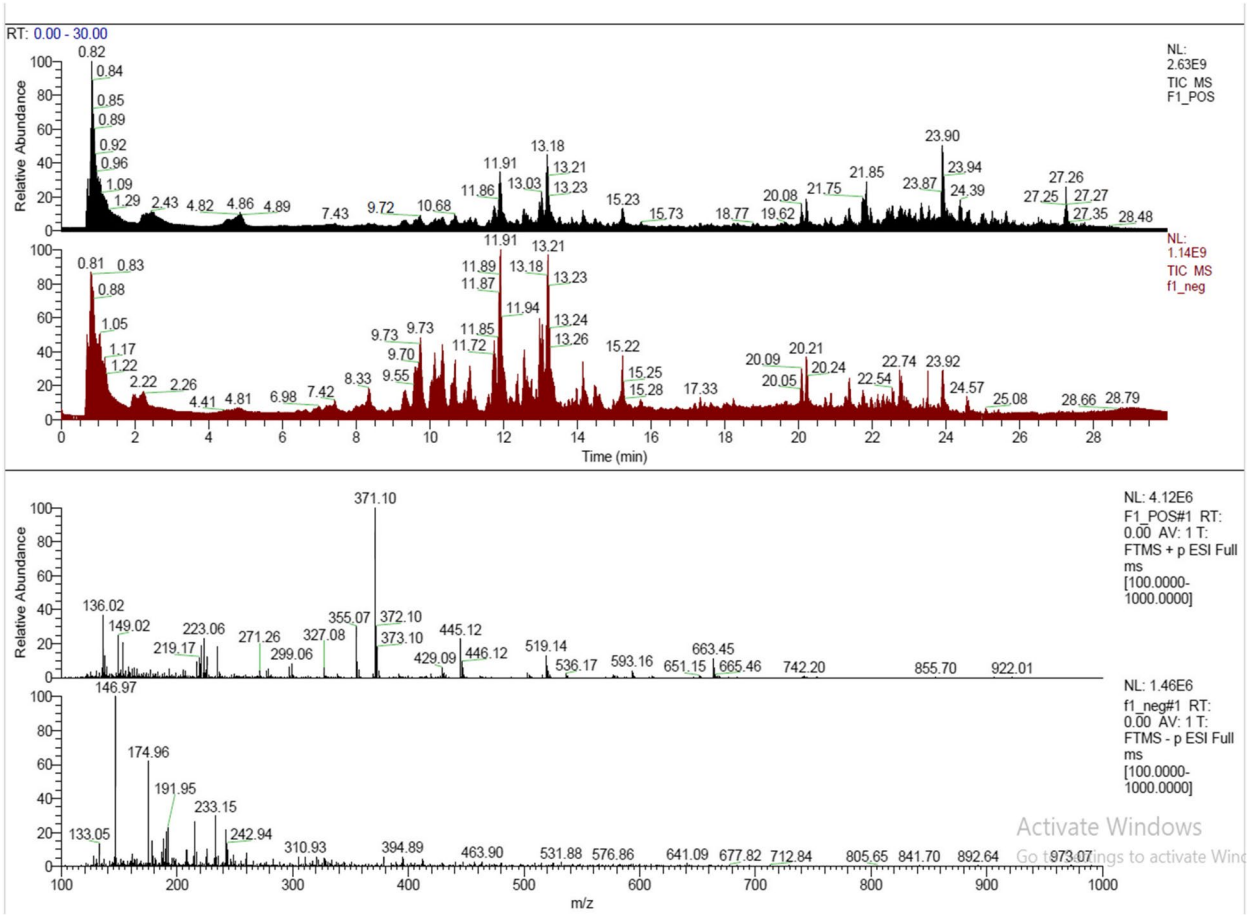


Fig. 4 **a** TIC MS chromatograms and FTMS ESI (**b**) chromatograms for metabolomics profiling of freeze-dried Cucurbita maxima flower powder

HR-MS technique is growing in popularity. An advanced UHPLC-Q Orbitrap HRMS technique was used in this investigation for accurate and high-resolution analysis of the sample's constituent parts. Both positive and negative modes show spectral peaks in the chromatograms. The sample's TIC MS chromatogram, showing relative abundance with time, has been shown in Fig. 4a. Figure 4b also displays the FTMS ESI chromatogram, showing highlights and relation between the m/z ratio and relative abundance. Approximately 1098 compounds from various classes were found using this technique. Forty-five of the annotated peaks were recognized and recorded among all compounds. Table 5 provides a thorough explanation of these substances, encompassing their chemical makeup and biological roles.

Several compounds falling in different categories such as flavonoids, organic acids, fatty acids, carotenoids etc., were identified. While studying different pumpkin cultivars, Singh and Kumar (2022) found compounds such as chlolorogenic acid, quercetin, luteolin, ferulic acid, etc. which aligns with the present findings. A broad class of plant metabolites called flavonoids is well-known for its antioxidant qualities. They contribute to pollinator attractiveness, plant colors, and a host of other health advantages, including as anti-inflammatory and anticancer properties. Compounds such as rutin, kaempferol, luteolin, isorhamnetin, quercetin-3β-D-glucoside, gentisic acid, caffeine, and sinapinic acid were identified in the freeze-dried powder. Carboxylic acids with lengthy aliphatic chains make up fatty acids and they are essential for many biological functions, such as cellular structure and energy storage. This category also includes essential fatty acids, which must be received through food. Compounds such as carnosic acid, abietic acid, tetracosatetraenoic acid, oleic acid, α-linolenic acid, and pinolenic acid fall in this category and are found in pumpkin flower powder (Table 4). Some of the fatty acids such as oleic acid, linoleic acid etc. were found by Ghosh et al. (2021) using GC–MS. Proteins are made up of organic substances called amino acids and play an important

Table 5 HRMS analysis for functional compounds identification in freeze dried *Cucurbita maxima* flower powder

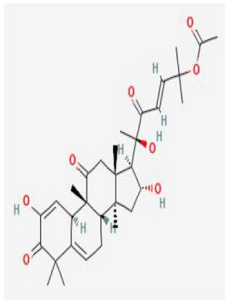
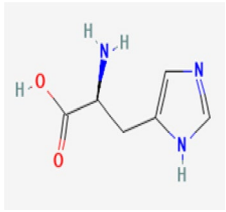
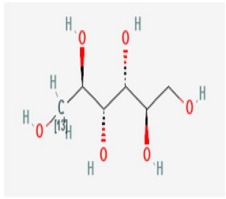
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Cucurbitacin E		$C_{32}H_{44}O_8$	556.68	554.58	0.52	Antioxidant, anti-cancer and its analgesic properties helps to alleviate pain and discomfort. Effective against certain parasitic infections. Helps regulate blood sugar levels, potentially benefiting individuals with diabetes.	(Dos Reis et al., 2018; Din et al., 2023; Aramabasic Jovanovic et al., (Aramaba 2023); Esmeeta et al., 2022)
L-Histidine		$C_6H_9N_3O_2$	155.15	154.06	0.77	Essential for the synthesis of proteins and histamine, a neurotransmitter that is important for immunological responses and the regulation of physiological processes. Antioxidant qualities	(Dos Reis et al., 2018)
D-(-)-Mannitol		$C_6H_{14}O_6$	182.17	181.07	0.79	It is well-known for its biological qualities, especially as a diuretic that lowers blood pressure and encourages the generation of urine. Antioxidant properties	(Dos Reis et al., 2018; Din et al., 2023)

Table 5 (continued)

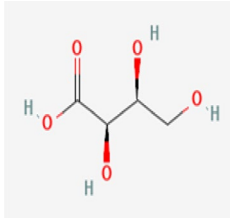
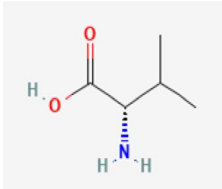
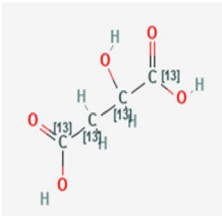
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
L-Threonic acid		$C_4H_8O_5$	136.10	195.05	0.80	Involved in the metabolism of carbohydrates, among other metabolic activities and has potential antioxidant properties	(Dos Reis et al., 2018)
Valine		$C_5H_{11}NO_2$	117.14	118.08	0.81	Necessary for protein synthesis, muscle development, muscle repair, and the generation of energy during exercise.	(Nikawa et al., 2021)
DL-Malic acid		$C_4H_6O_5$	134.08	133.01	0.84	It may improve general health and wellness by shielding cells from oxidative stress due to its antioxidant properties	(Dos Reis et al., 2018)

Table 5 (continued)

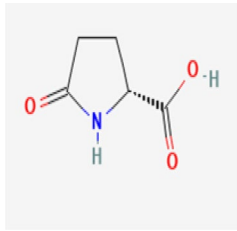
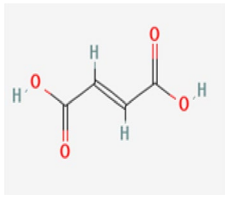
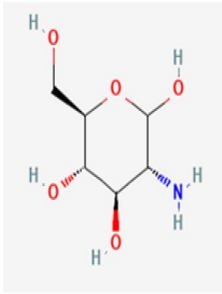
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
D-(+)-Pyroglutamic Acid		$C_5H_7NO_3$	129.14	130.04	0.84	It may help cognitive processes including learning and retention and plays a part in the creation of neurotransmitters. Antioxidant properties	(Dos Reis et al., 2018)
Fumaric acid		$C_4H_4O_4$	116.07	115.00	0.84	Reduce inflammation in the gut and other parts of the body and inhibits the growth of cancer cells. Lowers blood sugar levels	(Davinelli et al., 2018; Aramabasic Jovanovic et al., (Aramaba 2023); Esmeeta et al., 2022; Salinas et al., 2021)
D-glucosamine		$C_6H_{13}NO_5$	179.17	180.08	0.94	May also aid in cartilage regeneration and repair, reducing joint discomfort and increasing mobility. Additionally, because it can support the structural integrity of the intestinal lining, it has being investigated for its possible involvement in improving gut health.	(Salinas et al., 2021)

Table 5 (continued)

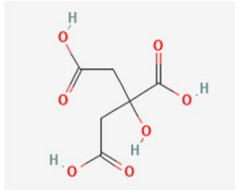
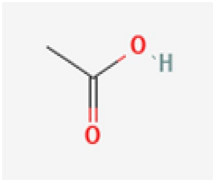
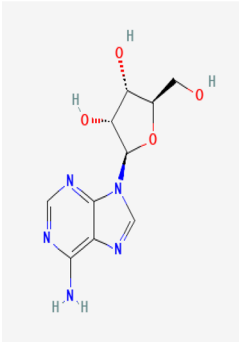
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Citric acid		$C_6H_8O_7$	192.12	191.02	1.05	Antioxidant, preventing oxidative damage to cells and assisting in the neutralization of free radicals.	(Dos Reis et al., 2018)
Acetic acid		$C_2H_4O_2$	60.05	192.06	1.06	Essential for the synthesis of acetyl-CoA and synthesis of fatty acids and energy. Antibacterial properties.	(Vaou et al., 2022)
D-Adenosine		$C_{10}H_{13}N_5O_4$	267.24	268.10	1.18	Crucial part of ATP, which is necessary for cells to store and distribute energy. Essential for several physiological functions, such as blood flow control, neurotransmission, and anti-inflammatory reactions.	(Davinelli et al., 2018)

Table 5 (continued)

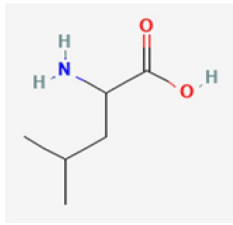
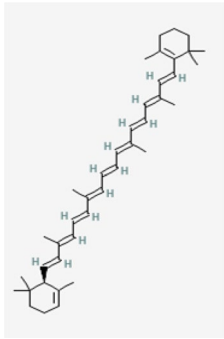
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
DL-β-Leucine		$C_6H_{13}NO_2$	131.17	130.08	1.45	It is a necessary amino acid that is vital for muscle repair and protein synthesis, which makes it vital for general development and recuperation	(Nikawa et al., 2021)
α - carotene		$C_{40}H_{56}$	536.87	532.98	1.56	Boosts the immune system, aiding in the body's defense against infections and illnesses. Decreases risk of chronic conditions like heart disease and certain types of cancer. Helps maintain healthy skin and may protect against UV-induced skin damage.	(Erkelens, & Mebius., 2017); Esmeeta et al., 2022)

Table 5 (continued)

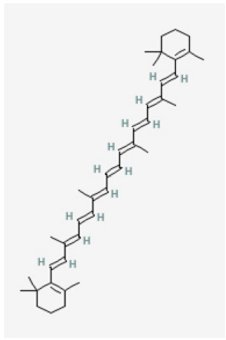
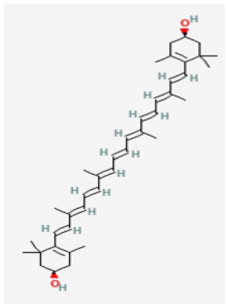
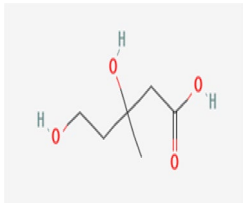
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
β - carotene		$C_{40}H_{56}$	536.87	535.82	1.58	Supports the immune system by promoting the production of immune cells and enhancing overall immune function. Contributes to healthy skin and may provide some protection against sunburn. Reduces the risk of certain cancers	(Erkelens, & Mebius., 2017); Esmeeta et al., 2022)
Zeaxanthin		$C_{40}H_{56}O_2$	568.87	566.58	1.89	Acts as an antioxidant, combating oxidative stress and reducing cell damage. Supports the immune system due to its antioxidant properties.	(Erkelens, & Mebius., 2017); Dos Reis et al., 2018)
Mevalonic acid		$C_6H_{12}O_4$	148.15	147.06	2.23	Essential precursor in the manufacture of cholesterol along with other sterols, which affects lipid metabolism. Synthesis of isoprenoids that are necessary for cellular functions such as growth as well as signaling	(Noce et al., 2021)

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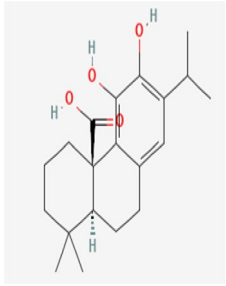
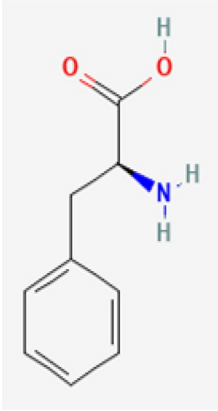
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Carnosic acid		$C_{20}H_{28}O_4$	332.43	331.85	2.45	Powerful antioxidant, anti-inflammatory and antimicrobial properties. Supports brain health and protect against neurodegenerative diseases like Alzheimer's and Parkinson's. Regulates blood sugar levels and support weight management.	(Dos Reis et al., 2018; Sindhu et al., 2021; Aramabasic Jovanovic et al., 2023)
L-Phenylalanine		$C_9H_{11}NO_2$	165.18	164.07	2.49	Precursor to neurotransmitters including dopamine, norepinephrine, and epinephrine. It is essential for the body's synthesis of proteins, which supports both muscle development and repair as well as general metabolic functions.	(Nikawa et al., 2021)

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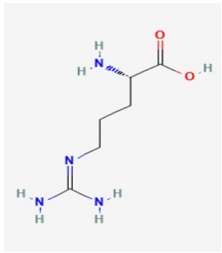
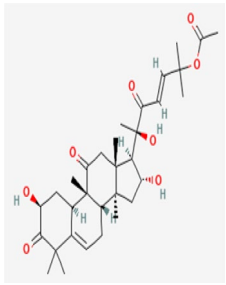
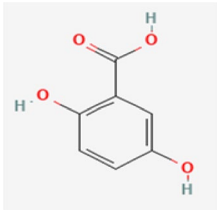
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
L-(+)-Arginine		$C_6H_{14}N_4O_2$	174.20	164.07	2.49	It contributes to immunological response and might improve immune system performance, which would benefit general health.	(Erkelens & Mebius., 2017)
Cucurbitacin B		$C_{32}H_{46}O_8$	558.70	556.54	2.58	Anti-inflammatory properties. Anti-cancer and plays a role in managing blood sugar levels and improving insulin sensitivity. Beneficial for skin health, potentially addressing conditions like psoriasis.	(Davinelli et al., 2018; Din et al., 2023; Aramabasic Jovanovic et al., 2023; Esmeeta et al., 2022)
Gentisic acid		$C_7H_6O_4$	154.12	153.01	2.99	Antioxidant and anti-inflammatory properties.	(Davinelli et al., 2018; Dos Reis et al., 2018)

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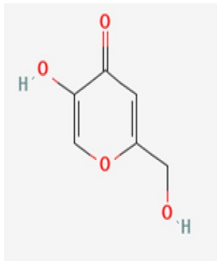
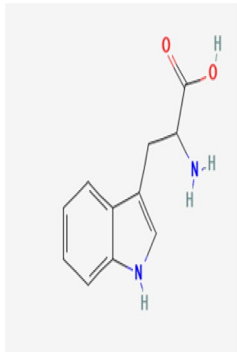
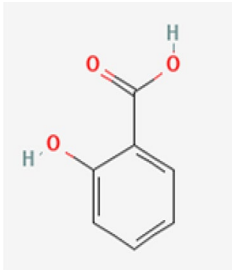
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Kojic acid		$C_6H_6O_4$	142.10	143.03	4.00	It is well known for its ability to lighten skin because it prevents the creation of melanin by inhibiting the tyrosinase enzyme, which lessens hyperpigmentation. Because of its antioxidant qualities, which shield the skin against oxidative stress, and potential antibacterial benefits.	(Vaou et al., 2022)
DL-Tryptophan		$C_{11}H_{12}N_2O_2$	204.22	188.07	4.86	The neurotransmitter serotonin, which controls mood, hunger, and sleep, is derived from the important amino acid DL-tryptophan. By raising serotonin levels in the brain, it could potentially enhance the quality of sleep and lessen the symptoms of depression as well as anxiety	(Dos Reis et al., 2018)
Salicylic acid		$C_7H_6O_3$	138.12	137.02	5.34	Excellent in acne treatment because it functions as a keratolytic substance which breaks down the connections between dead skin cells to exfoliate the skin. Anti-inflammatory qualities	(Davinelli et al., 2018)

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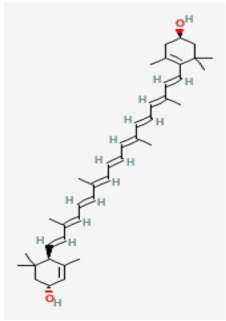
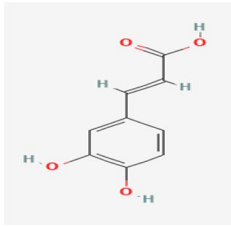
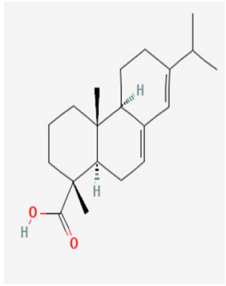
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Lutein		$C_{40}H_{56}O_2$	568.87	566.54	5.65	Promotes eye health by reducing the risk of age-related macular degeneration and cataracts. Reduces risk of cardiovascular diseases. Supports cognitive function and reduce the risk of cognitive decline	(Sindhu et al., 2021)
Caffeic acid		$C_9H_8O_4$	180.15	179.03	7.31	Potent antioxidants and anti-inflammatory qualities	(Davinelli et al., 2018; Dos Reis et al., 2018)
Abietic acid		$C_{20}H_{30}O_2$	302.45	301.58	8.97	Reduces inflammation in the body, potentially benefiting conditions like arthritis and inflammatory disorders. Promotes digestive health and alleviate symptoms like indigestion and bloating.	(Davinelli et al., 2018)

Table 5 (continued)

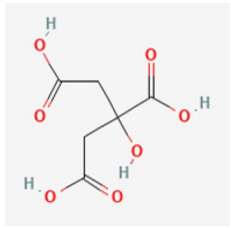
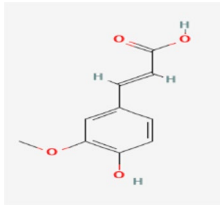
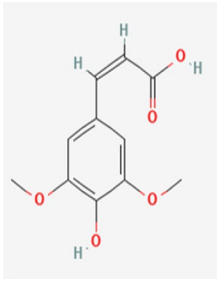
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Coumarin		$C_9H_6O_2$	146.14	147.04	9.71	Prevents blood clotting, coumarin can help avoid thrombosis. Anti-inflammatory, antioxidant qualities that help shield cells against oxidative stress and may also lessen inflammation in the body	(Davinelli et al., 2018; Dos Reis et al., 2018; Sindhu et al., 2021)
Ferulic acid		$C_{10}H_{10}O_4$	194.18	177.05	10.25	Potent antioxidant and anti-inflammatory qualities which might potentially treat inflammatory disorders including cardiovascular diseases along with arthritis.	(Dos Reis et al., 2018; Sindhu et al., 2021)
Sinapinic acid		$C_{11}H_{12}O_5$	224.21	283.08	11.17	Due to its ability to lower cholesterol and stop the production of arterial plaque, it has been linked to possible cardioprotective benefits. It has also demonstrated promise in promoting metabolic health, which may help with insulin sensitivity and glucose management.	(Sindhu et al., 2021; Noce et al., 2021)

Table 5 (continued)

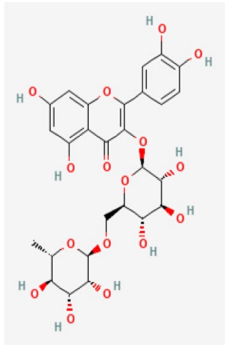
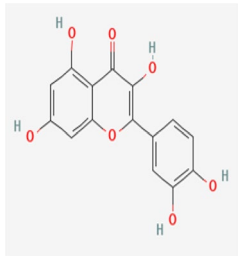
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Rutin		$C_{27}H_{30}O_{16}$	610.52	609.14	11.91	Reduces inflammation, Boost the immune system, helps in lowering of cholesterol levels, reduces blood pressure and helps in improving blood sugar control	(Davinelli et al., 2018; Erkelens, & Mebius., 2017; Din et al., 2023, Aramabasic Jovanovic et al., 2023; Noce et al., 2021)
Quercetin		$C_{15}H_{10}O_7$	302.23	303.04	11.91	Potent antioxidant that lowers the risk of chronic illnesses by preventing oxidative stress. Alleviate allergic responses and respiratory disorders by acting as a natural antihistamine and reducing inflammation	(Dos Reis et al., 2018)

Table 5 (continued)

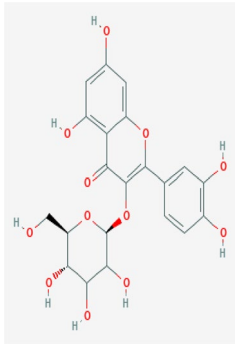
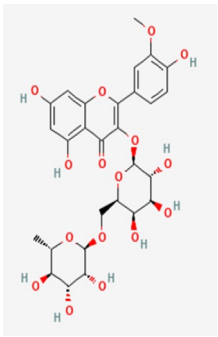
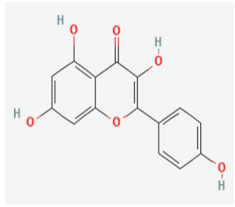
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Quercetin-3β-D-glucoside		$C_{21}H_{20}O_{12}$	464.379	463.08	11.92	By scavenging free radicals, this substance lowers oxidative stress and shields cells from harm. By reducing inflammatory reactions, it may help to treat ailments like allergies and persistent inflammation	(Davinelli et al., 2018; Dos Reis et al., 2018)
Isorhamnetin		$C_{16}H_{12}O_7$	316.26	317.06	11.92	By enhancing blood circulation and lowering the risk of atherosclerosis. Additionally, it could contribute to brain health and perhaps lower the possibility of neurodegenerative illnesses through neuroprotective actions	(Sindhu et al., 2021)
Kaempferol		$C_{15}H_{10}O_6$	286.23	287.05	12.98	Potent antioxidant and anti-inflammatory diseases including allergies and arthritis.	(Davinelli et al., 2018; Dos Reis et al., 2018)

Table 5 (continued)

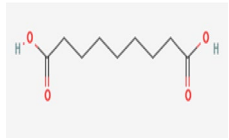
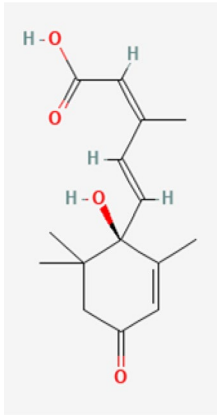
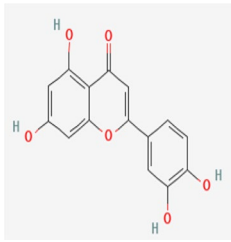
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Azelaic acid		$C_9H_{18}N_2O_5$	234.24	187.09	13.27	Antibacterial properties. Anti-inflammatory properties that support healthy skin by lowering redness and inflammation.	(Vaou et al., 2022)
(±)-Abscisic acid		$C_{15}H_{20}O_4$	264.31	247.13	13.93	Well-known for controlling how plants grow and develop, especially in response to stressful environmental factors like salt and drought. It is of importance in both agriculture as well as medical research since it has also been investigated for possible health impacts on humans, such as anti-inflammatory qualities.	(Davinelli et al., 2018)
Luteolin		$C_{15}H_{10}O_6$	286.23	285.04	15.73	Strong antioxidant qualities. It also has anti-inflammatory properties, which helps lessen the severity of asthma and allergy symptoms.	(Dos Reis et al., 2018)

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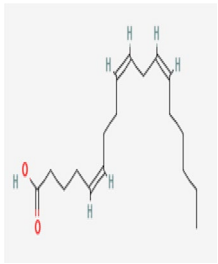
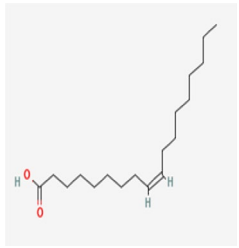
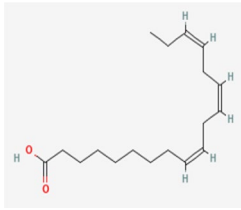
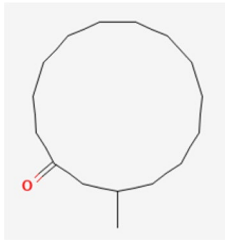
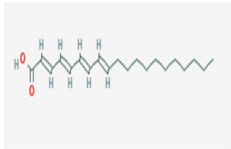
Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Pinolenic acid		$C_{18}H_{30}O_2$	278.42	279.23	23.33	Helps in promoting metabolic health in addition to its appetite-regulating along with anti-inflammatory properties. By lowering dangerous cholesterol levels and enhancing blood vessel health, it may enhance lipid profiles. Antioxidant properties	(Dos Reis et al., 2018; Sindhu et al., 2021; Din et al., 2023; Noce et al., 2021)
Oleic acid		$C_{18}H_{34}O_2$	282.46	279.23	23.91	Reduce the risk of cardiovascular illnesses by raising HDL and decreasing LDL. Its anti-inflammatory properties have been demonstrated, which can help with chronic inflammatory disorders and promote general health.	(Sindhu et al., 2021; Noce et al., 2021)
α-Linolenic acid		$C_{18}H_{30}O_2$	278.42	279.23	24.03	Vital for heart health and brain function. Anti-inflammatory properties	(Davinelli et al., 2018)

Table 5 (continued)

Identified compound	Structure	Molecular formula	Molecular weight	m/z	Retention time	Key function	References
Muscone		$C_{16}H_{30}O$	238.41	239.23	24.40	Used in perfumery because of its pleasant, musky fragrance and is known to have possible pheromonal effects. It could also have analgesic and anti-inflammatory qualities.	(Davinelli et al., 2018)
Tetracosatetraenoic acid		$C_{24}H_{40}O_2$	360.58	361.31	27.94	Essential for cellular communication and contributes to the production of eicosanoids that are critical for controlling immunological responses and inflammation	(Davinelli et al., 2018)

role in various biological processes, such as metabolism, neurotransmitter production, and muscle regeneration. Compounds namely, D-glucosamine, L-(+)-arginine, L-tryptophan, L-phenylalanine, DL-β-leucine, and L-histidine were also identified in *Cucurbita maxima* flower powder. Vidhya et al. (2022) while investigating various fatty acids and amino acid content found amino acids such as leucine, arginine, histidine etc. in the two different species of pumpkins. Compound such as L-Threonic acid, DL-Malic acid, Citric acid, Azelaic acid, Salicylic acid, Acetic acid, and fumaric acid were also identified in the flower powder as shown in Table 4. Basically, compounds having acidic characteristics that are frequently engaged in metabolic processes are called organic acids and offer several health advantages, including strong antioxidants properties. One of the researchers found similar results while investigating the yield as well as antioxidant properties of different varieties of pumpkins (Oloyede et al., 2022). Nucleotides and related compounds are essential for cellular communication and metabolism and are the building components of the nucleic acids. They play a role in the chemical breakdown of genetic material and the transport of energy and D-adenosine, D-(+)-pyroglutamic acid, and mevalonic acid are a few of them which were identified. Some compounds such as D-(-)-Mannitol, β-Carotene, Lutein, Zeaxanthin, Muscone, Horminone, Coumarin, and Kojic acid were also found in pumpkin flower powder. Numerous phytochemicals as well as metabolites that don't cleanly fit into the aforementioned categories are included in this category. They could play particular functions in plants and offer possible health advantages. Similar results were found while studying the effect of airflow and temperature on the *dried Cucurbita maxima* flowers in which the carotenoids content was analyzed (Niño et al., 2024). In nutshell metabolomic profiling of freeze-dried *Cucurbita maxima* (pumpkin) flower powder highlights a diverse range of bioactive compounds, including carotenoids, polyphenols, vitamins, and essential amino acids, which contribute to its significant nutritional and functional benefits. The presence of key antioxidants such as β-carotene, lutein, and α-tocopherol, along with bioactive metabolites with anti-inflammatory and hypoglycemic properties, reinforces its potential for food fortification aimed at addressing micronutrient deficiencies and chronic diseases. Freeze-drying effectively preserves these heat-sensitive nutrients, making the powder a valuable fortificant for improving the nutritional profile of staple foods like baked products, soups, and infant formulas, especially in regions with limited dietary diversity. Consequently, *Cucurbita maxima* powder represents a sustainable, nutrient-rich ingredient for enhancing public health through strategic food fortification initiatives.

^{1}H NMR of *Cucurbita maxima* freeze dried powder

Proton NMR tests were also carried out to determine the principal phenolic chemical groups found in the *Cucurbita maxima* freeze-dried sample. Phenolic acids frequently show paired (d) peaks at 6.98 ppm, which are linked to the ortho- as well as meta-protons of the hydroxyl-benzoic part {at chemical shift (dd), multiplicity (J = 7.9 Hz), and vicinal coupling constants, (2.0 Hz), which were used to identify many groups of chemicals, including carotenoids, and phenolic acids, as well as flavonoids. As a result, in addition to the NMR signals at 6.91 ppm that are typical of hydroxybenzoic moieties, the methoxy group was also found to have a strong signal at 4.28 ppm. Other peaks were also seen at 2.37, 1.25, 1.62, etc. which showed bioactive components. Similar results were seen in an experiment conducted on the edible flowers of *Helichrysum italicum*. The study found that besides other indications at 6.45 ppm (d, J = 17.0 Hz) and 6.79 ppm (d, J = 17.0 Hz) displaying significant coupling values and interactions in the 2D 1H-1H COSY spectrum, confirm the existence of these types of substances in the flower extracts (Primitivo et al., 2022).

Conclusion

Analyzing pumpkin flowers drying under different conditions has given important knowledge about the basic makeup, chemical composition, antioxidant effects, and general traits of the dried powder. Examining freeze-dried powder from *Cucurbita maxima* flowers also unveiled its superb ability to dissolve, its colloidal properties with great dispersal, and the existence of intricate carbohydrate formations such as lignin and hemicellulose as spotted through XRD testing. This freeze-dried powder displayed notably high levels of phenolic compounds, antioxidants, and flavonoids. The freeze -dried sample was found to have the highest nutrient retention. From the above mention parameters, the *Cucurbita maxima* flower powder has the potential to be used in fortification and can be added to make value added food products. The freeze-dried sample though rich in nutrients and solubility, can be used in the field of nanotechnology for the synthesis of nanoparticles as well in the nanocomposite formation for food packaging. It can be commercially utilized, as it is cheap, easily available and can be found throughout the year.

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Authors'contributions
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