Journal of Scientific & Industrial Research
Vol. 85, April 2026, pp. 301-311
DOI: 10.56042/jsir.v85i4.9160

Optimization Studies on Fenugreek Seed Extract via Spray-Freeze Drying

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Received 29 February 2024; revised 28 February 2025; accepted 05 January 2026

The fenugreek seed extract utilized in this study was encapsulated using Spray Freeze Drying (SFD), a novel encapsulation technique, using a unique extraction technique called Pulsed Electric Field (PEF) aided extraction. SFD has several significant industrial benefits, including enhanced bioavailability of components, production of high-quality and stable powder formulations, protection of thermolabile compounds, ease of handling and application, and prolonged shelf life. PEF extraction minimizes thermal degradation and reduces solvent usage, thereby enhancing extraction efficiency and promoting environmental sustainability. By PEF-assisted extraction, ethanol solvent produced 10.89 ± 0.09%extract yield with highest overall polyphenol content (148.0 ± 3.15 mg GAE/g) at sample to solvent ratio of 1:12.The encapsulated powder made from gum acacia binder, with a 1:5 extract to binder ratio and a 20h lyophilization duration was optimized with yield (87.11 ± 1.36%), particle size (42.67 ± 1.28 µm) and polyphenol EE (74.50 ± 0.94%). Physical characteristics of encapsulated powders were measured. Bulk density, true density, porosity and Carr index of spray freeze-dried powder was 0.54 ± 0.01 g/cm^3, 0.57 ± 0.05 g/cm^3, 4.11 ± 0.32% and 10.0 ± 0.24respectively. The solubility, wettability, hygrscopicity, dispersibility and water activity values of spray freeze dried powder were 87.6 ± 1.72%, 5:50 ± 0.00 min:sec, 3.12 ± 0.06 g/kg dry solids, 71 ± 1.56% and 0.36 ± 0.01. This successful extraction and encapsulation technology may be used by industrialists as a suitable extraction method for high value products, as well as to add substantial value to fenugreek seeds and open the door to the creation of new applications in a variety of sectors.

Keywords: Encapsulation, Polyphenols, Powder, Pulsed electric field-assisted extraction, Spray freeze drying

Introduction

The demand for natural, functional, and nutraceutical formulations and foods is increasing day by day. In phytotherapy, or herbal medicine plant components are considered as a GRAS (Generally Considered As Safe) for treating a variety of illnesses. In the modern food and pharmaceutical segments, hazardous chemical based solvent extraction methods are replaced with effective, eco-friendly and natural technologies such as pulsed electric field extraction method and for drying of liquid food samples the spray freeze-drying-based technologies are becoming more popular because of their efficiencies of retention of phytochemicals.[1] Pulsed Electric Field (PEF) system is an effective extraction technique[2], uses short, high-intensity electric pulses (up to 30 kV) over a very little period of time (µs)[3] to extract biological compounds by electroporation of cell membranes.[4] Spray Freeze Drying (SFD) is a low temperature drying technique, in which biomolecules are encapsulated (uniform coating) with suitable coating materials.[5] In the SFD process, an emulsion containing the bioactive compound and coating material is atomized into a freezing medium and subsequently dried using a freeze dryer. Owing to its low-temperature operation, uniform particle size, high porosity, rapid reconstitution, protection of sensitive ingredients, enhanced structural integrity, superior product quality, and improved shelf stability, SFD is considered highly suitable for industrial applications.[6] Mechanical, thermal, and chemical stressors (temperature, light, oxygen, pH, enzymes, and their interactions with other compounds) can all have an impact on the activity of bioactive substances throughout their extraction, processing, and storage.[7] It is crucial to choose an appropriate encapsulation technique and binding material in order to shield bioactive chemicals from these stressors. The aim of this research was to extract bioactive compounds from fenugreek seeds utilizing a pulsed electric field, and subsequently preserve them through optimized spray freeze drying encapsulation.

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Materials and Methods

The experiment was conducted at College of Food Processing Technology & Bioenergy, Anand Agricultural University, Gujarat, India and an overview of present investigation is presented in the (Fig. 1).

Sample Collection and Preparation

One kilogram of fenugreek seeds cv. Gujarat Methi-2, procured from the local market, was soaked in water for 12 h and subsequently germinated in a seed germinator at 25°C and 90% relative humidity for 3 days. The germinated seeds were dried in a hot air oven at 55°C until the moisture content reached 7% (w.b.), as determined by the gravimetric method. The dried sample was then ground and sieved through a 60-mesh screen.

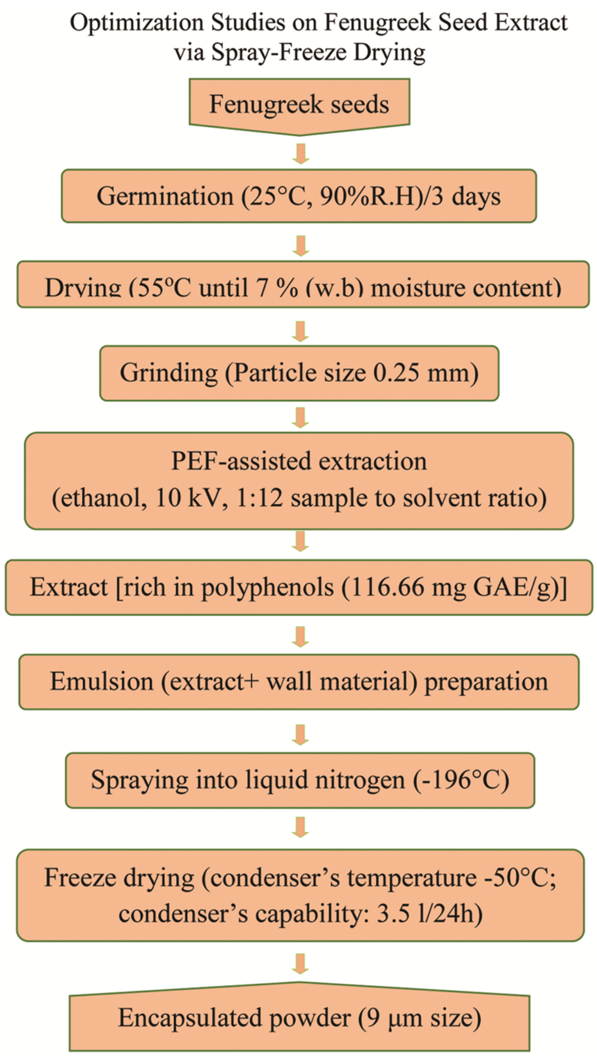

Fig. 1 — The experimental overview of encapsulation of fenugreek seed cv. Gujarat Methi-2 bioactive compounds by spray freeze-drying

Estimation of Fatty Acid Profile

About 2 ml of fat sample added to 5 ml of 0.5 N methanolic sodium methoxide and boiled for 10 min. After cooling 0.5 ml of boron triflouride (14% BF_3) was added for methylation and fatty acid profile was determination of fatty acid profile of fenugreek seeds, gas chromatography.[8]

Estimation of Total Polyphenolics (TPP)

The TPP was estimated as Gallic Acid Equivalents (mg GAE/g extract) by the standard method[9] and standard curve obtained is presented in Fig. 2. In 50ml ethanol 500 mg sample was dissolved in and kept at 80°C for 3h, supernatant was filtered, adjusted to 50 ml. A fraction of the extract (0.5 ml) was added in Folin-Ciocalteu with Na_2CO_3 solution and allowed for 30 min then absorbance was measured at 765 nm.

Yield of Extract

Extract taken from treatment was evaporated in vacuum rotary evaporator. The weights of extract before and after evaporation were taken and the following formula was used to calculate yield of extract.[10]

$$Yield\ (\%) = \frac{W_1}{W_2} \times 100 \qquad \ldots (1)$$

where, W_1 refer to weight (g) of extract residue obtained after solvent removal and W_2 refer to weight (g) of fenugreek seed powder.

Determination of Minerals

Approximately, 1 g of the sample was accurately weighed and transferred into a 250 mL conical flask for further analysis. The sample was digested by adding 30 millilitres of a diacid mixture. A powerful oxidising agent, acid mixture is composed of a 2:1 ratio of Nitric acid (HNO_3) to perchloric acid ($HClO_4$). White vapours appeared once the contents were heated to 200°C. Mineral elements are left in a

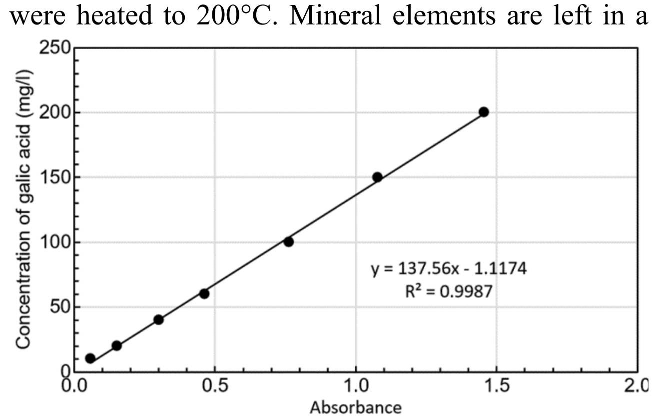

Fig. 2 — Standard curve of gallic acid

colourless solution after organic compounds are broken down by an acid combination into carbon dioxide and water. The mixture poured into a volumetric flask after being filtered via filter paper (Whatman No. 1). In order to prevent any remaining mineral residue from the conical flask, the flask rinsed three times throughout the solution transfer process. Double-distilled water was added to bring the solution's final volume to 50 millilitres then analyzed using ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy) (Model Number 7000 DV, Perkin Elmer USA).[11]

$$Mineral\ content\left(\frac{mg}{100g}\right) = \frac{Reading-Blank}{Sample\ weigt} \times Dilutionfactor \quad \ldots (2)$$

$$Dilution\ factor = \frac{Final\ volume\ of diluted\ sample}{Volume\ of\ aliquot\ taken\ for\ dilution} \quad \ldots (3)$$

Experimental Procedure of PEF-assisted Extraction

The PEF system (ENERGY PULSE SYSTEM - PORTUGAL) was used in multi pulses mode with pulsed electric voltages (5, 10 and 15 kV) throughout the experiment, the 10 number of pulses, 10 µspositive pulse, 10 µs negative pulse, 10 µs relax time and 50 Hz frequency were mainained. The distance between two parallel electrode plates was maintained at 10 cm. Interactions between independent variables at actual levels and its responses were studied using Factorial Completely Randomized Design (FCRD) in software Design Expert® (version 11). ANOVA was conducted on the experimental data and the significance of pulsed electric voltage (5 kV, 10 kV and 15 kV), types of solvent (33% ethanol and 33% methanol), sample to solvent ratio (1:8, 1:10 and 1:12) on yield of extract (%) and an amount of TPP (mg GAE/g).

Encapsulation

Screening of Binding Material for Encapsulation

The binding materials, Guar Gum (GG), Whey Protein Isolate (WPI), Gum Acacia (GA), and Soy Protein Isolate (SPI) were employed for this study. The stability of each binding material was evaluated by dissolving 10 g of the respective material in 50 mL of distilled water separately in test tubes and allowed for three hours and phase separation in test tubes was visually assessed. The most stable binding materials were selected for encapsulation.

Encapsulation of Fenugreek Seed Extract by SFD

In SFD, spraying the sample solution in freezing medium (liquid nitrogen) make formation of small and spherical shape particles with high surface area.[10] Two fluid glass nozzle with nozzle diameter of 0.8 mm (internal diameter of opening) was used. Length, width and thickness of the glass layer of the nozzle were 165, 25 and 1 mm respectively (Fig. 3a). In the SFD process, the extract was freeze-dried (Fig. 3) at three different drying durations (20, 25, and 30 h) as shown in Fig. 3b. The atomizer pressure was maintained at 2.0 kg/cm², while a feed flow rate of 0.6 mL/min and stirrer speed of 72 rpm were consistently maintained throughout the process. Process optimization was carried out using a factorial completely randomized design with Design-Expert software.

Characterization of Spray Freeze Dried Powder

The microencapsulated powder (Fig. 3c) produced under optimized processing conditions was further analyzed for moisture content, total polyphenol content, encapsulation efficiency, and particle size distribution.

Moisture Content

Moisture content of microencapsulated powders determined by gravimetric method.[11]

Particle Size Analysis

Approximately 2 mg of powder sample was added to 5 ml of glycerin solution and vortexed for 1 minute. The suspension was spread on glass slide and covered with cover slip and images were taken and analyzed using image analysis software (Biovis system: PSA2000).

Encapsulation Efficiency

The bioactive compounds were measured at their respective absorbance and results were expressed as amount of Surface Bioactive Compounds (SBC).[12] The surface bioactive compound percentage (SB) and Encapsulation Efficiency (EE) were calculated by using the following equations.

$$SB(\%) = \frac{Surface\ bioactive\ compounds}{Total\ bioactive\ compounds} \quad \ldots (4)$$

$$EE(\%) = 100 - SB \quad \ldots (5)$$

Characterization and Morphology of Microencapsulated Powder

Microencapsulated powder obtained under optimized processing conditions was further evaluated for physical properties.

Water solubility index

Water Solubility Index (WSI) is determined[13] by using the following formula.

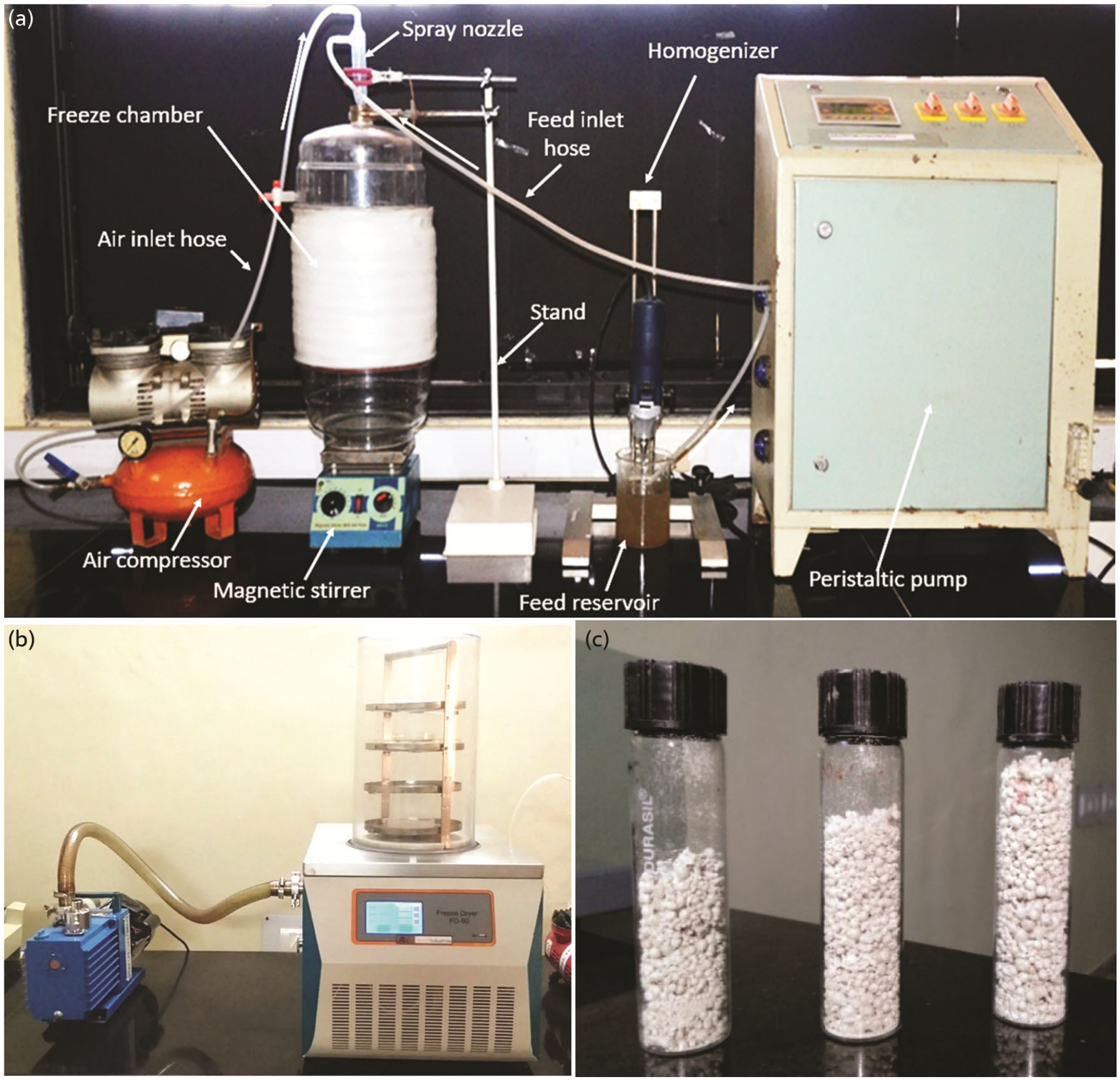

Fig. 3 — Spray freeze drying: (a) Laboratory scale spray freeze drying assembly, (b) Standard freeze dryer, (c) Spray freeze dried powder of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2

$$WSI\ (\%) = \frac{Weigt\ of\ dissolved\ solid\ in\ supernant}{Weigt\ of\ dry\ solid} \times 100 \qquad \ldots (6)$$

Bulk density, Tapped density, Hygroscopicity, Solubility and Wettability

The bulk density (ρ_b) and tapped density (ρ_t), hygroscopicity, solubility and wettability of encapsulated powder were determined by standard methods.[14]

Dispersibility

The dispersibility (D) of the encapsulated powder was calculated[14] as per the following equation.

$$Dispersibility\ (\%) = \frac{(10+W) \times \%DM}{W \times [(100-MC)/100]} \qquad \ldots (7)$$

where, 'W' refer to amount of powder (g) being used, MC refer to moisture content of the powder and DM refer to dry matter in percentage in the reconstituted encapsulated powder after it has been passed through the sieve.

Morphology

Morphology of encapsulated powders analyzed by Scanning Electron Microscope (XL 30 ESEM with EDAX: Resolution: upto 2; Accelerating voltage: 30 kV; Magnification: upto 2,50,000x).

Results and Discussion

Gas Chromatography (GC) analysis of fenugreek seed revealed that linoleic acid, linolenic acid, oleic acid, and palmitic acid were the predominant fatty acids present (Table 1 and Fig. 4). These findings are in agreement with previously reported studies.[15–17]

Effect of PEF-Assisted Extraction on Extract Yield (%)

Polyphenol rich extract yield was increased when PEF-voltage increased (Table 2). The solvent, ethanol showed maximum extract yield than methanol. Maximum extract yield (10.89 ± 0.09%) observed in treatment of 10 kV voltage with ethanol and 1:12 sample to solvent ratio. PEF is energy efficient extraction method compared to conventional methods.[18] Increase in extract yield using PEF process might be due to electroporation effect and electro plasmolys is of cell membranes.[19] The results showed that voltage had no significant effect ($p < 0.05$) on extract yield with C.V value of 14.7, whereas solvent and sample to solvent ratio was found to be significant.

Effect of PEF-Assisted Extraction on TPP (mg GAE/g)

From Table 2 it can be seen that voltage, solvent and sample to solvent ratio had significant effect on total polyphenolics at 5% level of significance with C.V value of 0.874. With an increase in PEF voltage, TPP rose noticeably. In PEF-assisted extraction and enhanced cell permeability allows for better penetration of the extraction solvent into the plant tissue and results in a higher extraction yield of polyphenols. Ethanol exhibited a higher Total Polyphenol Content (TPC) than methanol, and the polyphenol concentration increased with an increase in the sample-to-solvent ratio. Highest TPP was observed in the treatment of 10 kV voltage, ethanol with 1:10 sample to solvent ratio. Similar results were found other reports.[20–22]

Table 1 — Fatty acid composition of fenugreek seeds cv. Gujarat Methi-2

Fatty acid	Value (%)
Myristic acid	0.14 ± 0.01
Palmitic acid	10.01± 0.04
Palmitolic acid	0.12± 0.02
Stearic acid	5.95± 0.05
Oleic acid	12.29± 0.03
Linoleic acid	40.53± 0.09
Linolinic acid	27.73± 0.05

Standardization of Extraction Process

Main goal of extraction process was maximum extract yield hence the treatment P_8 (ethanol as the solvent, PEF-assisted extraction method with 10 kV voltage, and a 1:12 sample to solvent ratio) was selected due to its maximum extract yield (10.89 ± 0.09%).

Encapsulation of Bioactive Compounds from Fenugreek Seeds by SFD

Effect of SFD conditions i.e. type of binder, binder to solvent ratio and lyophilization time on encapsulation of FSE on dependent parameters yield, particle size, polyphenol EE, and TPP were studied and results are tabulated in Table 3.

Effect of SFD on Encapsulated Powder Yield (%)

It was found that encapsulated powder yield increased when extract to binder ratio increased in case of gum acacia and GA with GG as binders.

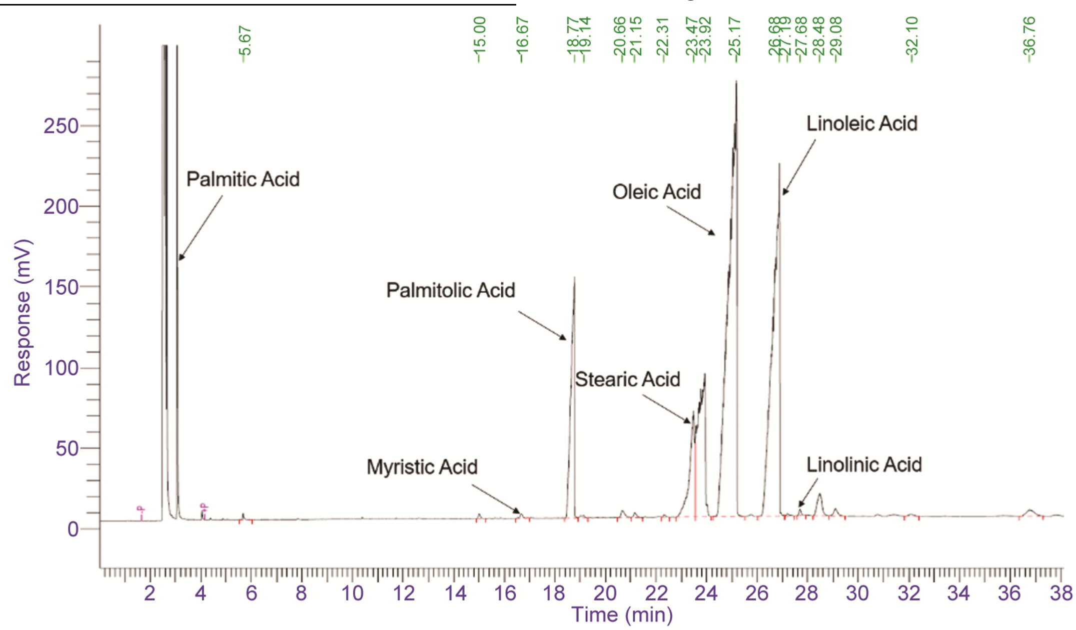

Fig. 4 — GC Chromatogram of fatty acid profile of fenugreek seed cv. Gujarat Methi-2

Table 2 — Effect of pulsed electric field-assisted extraction parameters on extract yield and recovery of total polyphenols

Treatment	Voltage (kV)	Solvent	Sample to solvent ratio	Extract yield (%)	TPP (mg GAE/g)
P_1	5	Ethanol	1:8	6.94 ± 0.21	118.00 ± 3.95
P_2	10	Ethanol	1:8	9.53 ± 0.65	158.00 ± 2.28
P_3	15	Ethanol	1:8	8.93 ± 0.04	97.66 ± 1.97
P_4	5	Ethanol	1:10	7.49 ± 0.08	134.33 ± 3.64
P_5	10	Ethanol	1:10	8.47 ± 0.18	166.66 ± 1.19
P_6	15	Ethanol	1:10	9.97 ± 0.97	93.66 ± 3.42
P_7	5	Ethanol	1:12	6.94 ± 0.65	115.00 ± 2.61
P_8	10	Ethanol	1:12	10.89 ± 0.09	148.00 ± 3.15
P_9	15	Ethanol	1:12	8.86 ± 0.88	140.00 ± 1.42
P_{10}	5	Methanol	1:8	7.04 ± 0.64	118.33 ± 1.65
P_{11}	10	Methanol	1:8	7.82 ± 0.19	146.66 ± 2.58
P_{12}	15	Methanol	1:8	9.39 ± 0.55	124.66 ± 2.55
P_{13}	5	Methanol	1:10	7.10 ± 0.91	126.00 ± 1.46
P_{14}	10	Methanol	1:10	8.47 ± 0.57	154.66 ± 1.85
P_{15}	15	Methanol	1:10	8.27 ± 0.85	88.33 ± 1.36
P_{16}	5	Methanol	1:12	6.94 ± 0.15	123.33 ± 3.65
P_{17}	10	Methanol	1:12	8.03 ± 0.86	138.00 ± 3.12
P_{18}	15	Methanol	1:12	8.61 ± 0.09	89.66 ± 2.42
C.V (%)	—	—	—	14.70	0.874

Table 3 — Effect of spray freeze drying on encapsulation characteristics of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2

Treatment	Binder	Extract: Binder	Lyophilization time (h)	Yield (%)	Moisture (%)	Particle size (µm)	Polyphenols EE (%)	Total polyphenols (mg GAE/g)
T_1	GA	1:5	20	87.11 ± 1.36	4.44 ± 0.15	42.67 ± 1.28	74.50 ± 0.94	72.41 ± 0.15
T_2	GA	1:5	25	87.16 ± 1.65	4.37 ± 0.28	46.03 ± 3.66	76.93 ± 1.69	50.62 ± 0.44
T_3	GA	1:5	30	84.91 ± 2.35	3.96 ± 0.54	44.36 ± 1.46	78.48 ± 3.48	79.08 ± 0.61
T_4	GA	1:4	30	82.18 ± 1.31	4.15 ± 0.38	46.89 ± 3.89	78.26 ± 1.97	80.63 ± 0.99
T_5	GA	1:6	20	86.32 ± 3.56	4.49 ± 0.58	43.80 ± 1.44	75.52 ± 1.46	72.28 ± 0.54
T_6	GA	1:4	20	84.39 ± 4.03	4.42 ± 0.62	41.67 ± 2.56	74.64 ± 2.98	71.44 ± 2.86
T_7	GA	1:4	25	87.11 ± 1.55	4.51 ± 0.46	45.59 ± 1.78	47.91 ± 0.74	52.69 ± 1.49
T_8	GA	1:6	25	85.97 ± 3.34	4.45 ± 0.87	44.07 ± 2.65	46.51 ± 1.66	48.89 ± 2.65
T_9	GA+GG	1:6	25	84.51 ± 1.11	4.78 ± 0.59	51.86 ± 1.49	78.33 ± 2.54	73.15 ± 1.54
T_{10}	GA+GG	1:4	30	88.29 ± 3.36	4.43 ± 0.64	52.08 ± 2.67	73.62 ± 1.46	65.17 ± 0.79
T_{11}	GA+GG	1:4	20	84.08 ± 2.56	4.95 ± 0.45	48.45 ± 1.55	81.60 ± 2.86	74.49 ± 1.65
T_{12}	GA+GG	1:5	30	82.47 ± 2.01	4.42 +± 0.56	52.84 ± 2.54	75.35 ± 0.56	64.06 ± 1.57
T_{13}	GA+GG	1:4	25	81.91 ± 2.52	4.81 ± 0.64	50.44 ± 1.68	77.86 ± 1.86	72.49 ± 0.66
T_{14}	GA+GG	1:5	20	82.45 ± 1.35	4.80 ± 0.77	47.88 ± 3.96	83.42 ± 0.75	74.44 ± 0.69
T_{15}	GA+GG	1:6	20	86.45 ± 3.56	4.87 ± 0.25	48.66 ± 3.48	81.99 ± 1.68	74.96 ± 0.57
T_{16}	GA+GG	1:6	30	84.94 ± 1.05	4.58 ± 0.56	50.91 ± 1.64	73.26 ± 2.94	64.19 ± 2.94
T_{17}	GA+GG	1:5	25	79.71 ± 2.54	4.50 ± 0.89	51.62 ± 1.58	79.14 ± 0.46	72.96 ± 1.79
T_{18}	WPI	1:5	30	72.57 ± 1.66	5.44 ± 0.67	50.32 ± 2.89	27.70 ± 0.96	53.00 ± 2.45
T_{19}	WPI	1:6	30	70.58 ± 1.44	5.33 ± 0.56	47.43 ± 1.65	28.20 ± 0.84	53.65 ± 1.95
T_{20}	WPI	1:6	25	78.66 ± 3.33	5.92 ± 0.47	48.76 ± 0.89	21.66 ± 1.96	53.84 ± 0.48
T_{21}	WPI	1:4	20	78.75 ± 2.45	5.53 ± 0.89	47.73 ± 1.65	24.46 ± 1.56	58.27 ± 0.55
T_{22}	WPI	1:5	25	81.36 ± 2.58	5.73 ± 0.65	48.59 ± 0.68	21.93 ± 1.46	54.16 ± 0.89
T_{23}	WPI	1:5	20	74.91 ± 2.29	5.68 ± 0.49	44.82 ± 1.84	22.50 ± 0.88	56.52 ± 1.88
T_{24}	WPI	1:4	25	75.05 ± 1.24	5.66 ± 0.64	51.45 ± 2.99	22.22 ± 0.96	54.10 ± 1.54

where, G A = Gum acacia, GG = Guar gum, WPI = Whey protein isolate

Table 4 — Statistical analysis (ANOVA) for effect of spray freeze drying on encapsulation characteristics of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2

ANOVA	Powder yield (%)			Moisture (%)			Particle size (µm)			Polyphenols EE			Total polyphenols (mg GAE/g)		
Source of variation	Sig	SE(m)	C.D (0.05)	Sig	SE(m)	C.D (0.05)	Sig	SE(m)	C.D (0.05)	Sig	SE(m)	C.D (0.05)	Sig	SE(m)	C.D (0.05)
Factor (B)	*	0.33	0.947	*	0.05	0.14	*	0.25	0.71	*	0.22	0.63	*	0.41	1.17
Factor (R)	NS	0.33	N/A	NS	0.05	N/A	NS	0.25	N/A	*	0.22	0.63	NS	0.41	N/A
Interaction B × R	*	0.57	1.64	NS	0.08	N/A	*	0.43	1.24	*	0.38	1.10	NS	0.71	N/A
Factor (H)	*	0.33	0.94	*	0.05	0.14	*	0.25	0.71	*	0.22	0.63	*	0.41	1.17
Interaction B × H	*	0.57	1.64	NS	0.08	N/A	NS	0.43	N/A	*	0.38	1.10	*	0.71	2.03
Interaction R × H	*	0.57	1.64	NS	0.08	N/A	NS	0.43	N/A	*	0.38	1.10	NS	0.71	N/A
Interaction B × R × H	*	1.00	2.84	NS	0.15	N/A	*	0.76	2.15	*	0.67	1.90	NS	1.24	N/A
C.V (%)		2.12			5.44			2.75			2.02			3.34	

(* = Significant, NS = Non significant) (Sig = Significance, B = Binder, R = Extract to binder ratio, H = Lyophilization time)

Among 20, 25 and 30 h lyophilization times, 30 h yielded maximum encapsulated powder. Yield was high in gum acacia binder than GA+GG (gum acacia with guar gum) binder followed by WPI. When extract to binder ratio increased, the lyophylization time also increased[23] in protein powder by using both SFD. From Table 4 it could be observed that both lyophilization time and binder had significant effect on powder yield at 5% level of significance, whereas extract to binder ratio was found to be non-significant. All the linear and interactive factors had significant effect ($p< 0.05$) on the encapsulated powder yield (%) at 5% level of significance with C.V value of 2.12.

Effect of SFD on Moisture of Encapsulated Powder

The moisture content was maximum in the encapsulated powder prepared from whey protein isolate binding material. Whey protein isolate has strong hydrogen bonding capacity to interact with a large number of water molecules (Table 3). The viscosity of the binding materials increased with thermal conductivity.[23] The encapsulated powder made using gum acacia binder, a 1:5 extract to binder ratio, and a 30-hours lyophilization duration in a freeze dryer had the lowest moisture content. The findings indicated that, with the exception of binder and lyophylization time, which had significant effects at the 5% level of significance, all linear and interaction components had non-significant effects ($p<0.05$) on the moisture (%) at the 5% level of significance with a C.V value of 5.44%.

Effect of SFD on Particle Size of Powder

Particle size is inversely proportional to the hygroscopicity which in turn causes agglomeration (lumps formation) under humid conditions (Table 3). It was found that when extract to binder ratio (from 1:4 to 1:6) increased, the particle size also increased. Particle size increased when lyophilization time (from 20 h to 30 h) increased. Compared to gum acacia and WPI binders, gum acacia with GG binder was found with larger particle size. In the present investigation lowest particle size was observed in treatment of gum acacia powder, 1:4 extract to binder ratio and 20 h lyophilization time. Lower sample size will drastically reduce the drying time because SFD time varies with the square of particle thickness.[24,25]

Effect of SFD on EE of Polyphenols

High polyphenol EE gives high amount of bioactive compounds retention (Table 3). Highest polyphenol EE was observed in treatment of gum acacia with GG binder, 1:5 extract to binder ratio and 20 h lyophilization time. The gum acacia with GG binder gave maximum polyphenol EE compared to gum acacia and WPI binders. EE is inversely proportional to extract to binder ratio. Polyphenols were not much affected by lyophilization time.[26] However, as lyophilization time increased, the EE decreased in WPI binders.

Effect of SFD on TPP Content

Among binders applied, GA was found with maximum TPP than other binders. When extract to binder ratio increased the TPP were decreased in WPI encapsulated powder. Highest TPP was observed in gum acacia with 1:4 extract to binder ratio lyophilized for 30 h.

Optimization of SFD Encapsulation Process Parameters

The independent parameters i.e. lyophilization time, binder, extract to binder ratio affected the

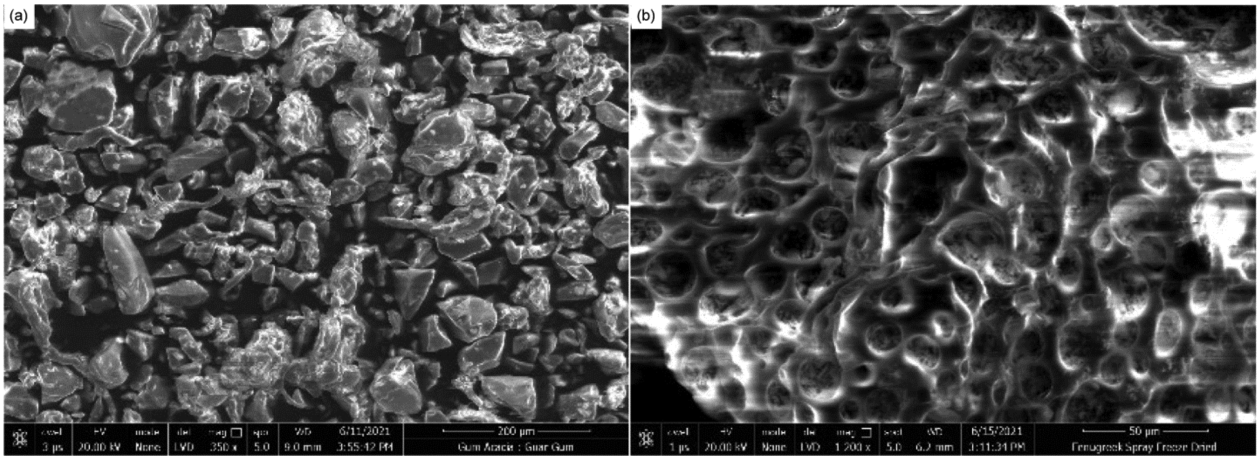

Fig. 5 — SEM images of powders: (a) Gum acacia, (b) Spray freeze dried powder of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2

extraction process. The dependent parameters, yield, moisture content, polyphenols encapsulation efficiency were taken into consideration for optimization of the spray freeze drying encapsulation process. The optimization was done by using statistical analysis by three factorial CRD and the Design-Expert@ 7.0.0 software. The optimized encapsulation process found was with gum acacia as binder, 1:5 extract to binder ratio, 20 h lyophilization time in which maximum yield (87.11 ± 1.36%), minimum moisture content (4.44 ± 0.15% w.b) and maximum polyphenols encapsulation efficiency (74.50 ± 0.94%) were observed. Optimized conditions for SFD are shown in Table 5. The results were validated by conducting the experimental optimized conditions wherein the results showed a marginal variation of 2.1%.

Table 5 — Standardized conditions for spray freeze dried encapsulated powder of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2

Parameter	Value (Mean)
Binder	Gum acacia
Extract to binder ratio	01:05
Processing condition (lyophilization time h.)	20.00
Moisture (%)	4.44 ± 0.15
Yield (%)	87.11 ± 1.36
Polyphenols Encapsulation efficiency (%)	74.50 ± 0.94

Characterization of Encapsulated Powders

The encapsulated powders were characterized for morphological characteristics.

Morphology of Encapsulated Powders

Gum acacia powder exhibited sharp corner surfaces without porous nature whereas the spray freeze dried microcapsules exhibited irregular flake like structures with a porous nature (Fig. 5). Flake like porous structures might be due to the direct sublimation of ice into water vapor during freeze-drying operation. The spray freeze-dried microcapsules were spherical and uniform in size, which was similar to the morphology study of whey protein microcapsules reported by other researchers.[24]

Physical Characteristics of Encapsulated Powders

The data related to characteristics of spray freeze dried encapsulated powder is depicted in Table 6.

Table 6 — Characteristics of standardized spray freeze dried encapsulated powder of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2

Character	Value
Bulk density (g/cm^3)	0.54 ± 0.01
True density (g/cm^3)	0.57 ± 0.05
Porosity (%)	4.11 ± 0.32
Carr Index	10.0 ± 0.24
Solubility (%)	87.6 ± 1.72
Wettability (min:sec)	5:50 ± 0.00
Hygrscopicity (g/kg dry solids)	3.12 ± 0.06
Dispersibility (%)	71.0 ± 1.56
Water activity	0.36 ± 0.01

Bulk density, true density, porosity and Carr index of spray freeze-dried powder was 0.54 ± 0.01 g/cm^3, 0.57 ± 0.05 g/cm^3, 4.11 ± 0.32% and 10.0 ± 0.24 respectively. The solubility, wettability, hygrscopicity, dispersibility and water activity values of spray freeze dried powder were 87.6 ± 1.72%, 5:50 ± 0.00 min:sec, 3.12 ± 0.06 g/kg dry solids, 71 ± 1.56% and 0.36 ± 0.01. Similar results of physico-chemical properties were reported[27,28] in spray dried fruit juices. From the results, it can be interpreted that the higher wettability and dispersibility can be obtained in SFD fenugreek seed

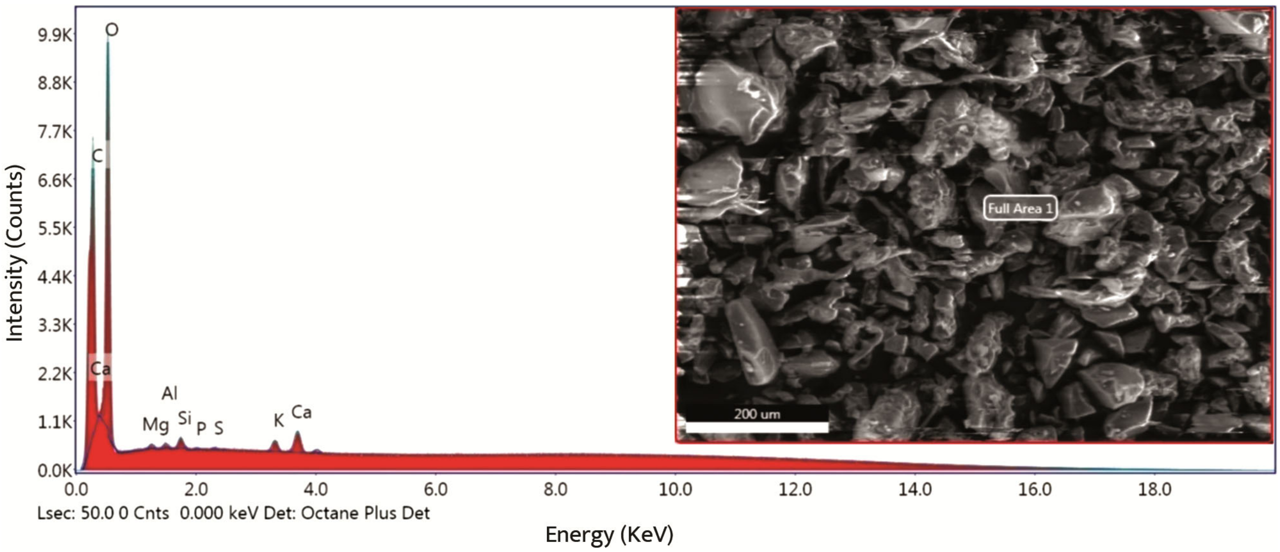

Fig. 6 — Energy Dispersive X-ray Analysis (EDAX) spectrum of wall material (Gum acacia) sample. Scale bar: 200 µm

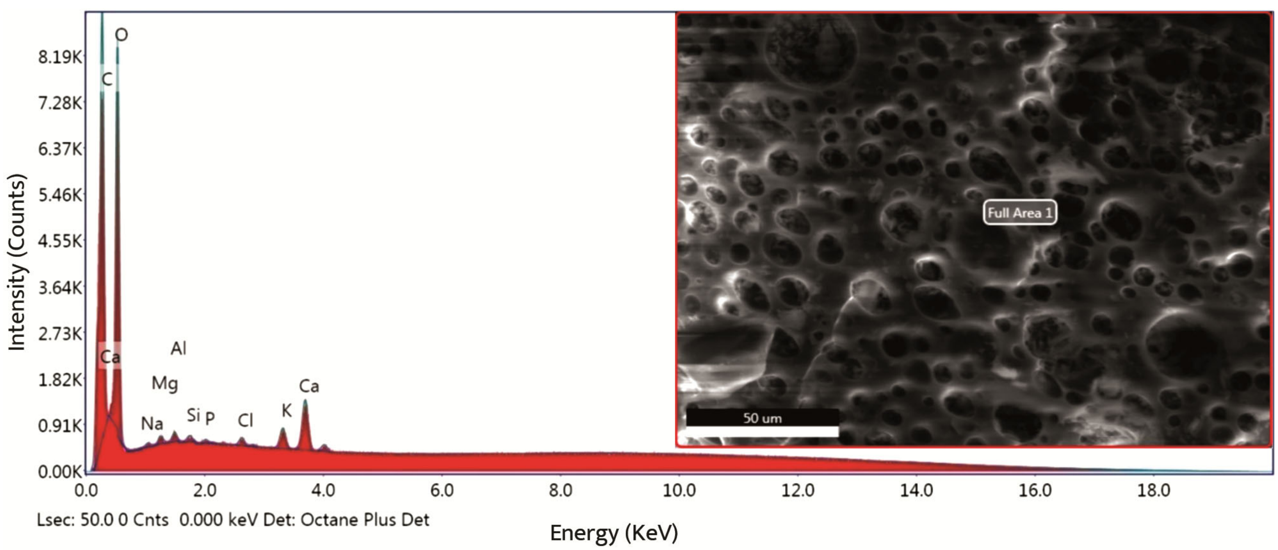

Fig. 7 — Energy Dispersive X-ray Analysis (EDAX) spectrum of spray freeze dried sample of bioactive compounds from fenugreek seeds cv. Gujarat Methi-2 (Scale bar: 50 µm)

Table 7 — EDAX analysis of wall material and spray freeze dried samples of bioactive compounds from fenugreek seeds cv Gujarat Methi-2

Element	Wall material (GA) sample		Spray freeze dried sample	
	Weight%	Atomic%	Weight%	Atomic%
C	36.04	44.21	33.78	41.61
O	57.84	53.27	60.82	56.25
Mg	0.24	0.15	0.13	0.08
Al	0.33	0.20	0.11	0.06
Si	0.32	0.18	0.38	0.20
P	0.17	0.09	0.18	0.09
Cl	0.06	0.03	0.20	0.08
K	0.11	0.05	1.23	0.46
Ca	0.32	0.13	3.18	1.17

where, GA = Gum acacia, C = Carbon, O = Oxygen, Mg = Magnesium, Al = Aluminium, Si = Silicon, P = Phosphorous, Cl = Chlorine, K = Potassium, Ca = Calcium

extract. From the EDAX analysis (Figs 6 & 7; Table 7) it is observed that the toxic metal compounds such as Cu, Pb, Cd, Hg and As are absent and hence this encapsulated powder is considered as safe for consumption.

Conclusions

The highest extract yield (10.89 ± 0.09%) in the PEF-assisted extraction procedure was seen at a voltage of 10 kV and a sample to solvent ratio of 1:12. The encapsulated powder made from gum acacia binder, with a 1:5 extract to binder ratio and a 20 h lyophilization duration was optimized with yield (87.11 ± 1.36%), particle size (42.67 ± 1.28 µm) and polyphenol EE (74.50 ± 0.94%). Therefore, spray freeze drying may be used by industrialists as a suitable extraction method for high value products, as well as to add substantial value to fenugreek seeds and open the door to the creation of new applications in a variety of sectors and may be further investigated to provide novel, creative ideas for the effective distribution of bioactive chemicals in development of functional and nutraceutical food formulations in food industries.

Conflicts of Interest

The authors do not have any conflicts of interest to declare.

Acknowledgement

The authors are extremely thankful to the Indian Council of Agricultural Research for their support.

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