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Optimization of ultrasound probe assisted extraction for PKM1 moringa seed oil

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Abstract

Moringa seed oil (MSO) was extracted from moringa seed powder (MSP) using Soxhlet and ultrasound probe-assisted extraction (UPAE) methods. The process conditions for maximizing oil extraction were optimized using response surface methodology (RSM). The independent variables were in the range of 40-60% ultrasound amplitude, 25-35 min of extraction time, and 1:10-1:20 of solvent-solute ratio for UPAE. The maximum oil yield obtained from the two extraction methods was 40.3 ± 0.79% for Soxhlet extraction (SE) and 36.32 ± 0.44% for UPAE. The process conditions for maximum extraction yield were 60% ultrasound amplitude, 29 min extraction time, and 1:19 of solvent-solute ratio for UPAE and at 60 °C of temperature, 6 h extraction time, and 300 mL of solvent for SE. The optimized extraction process has an oil recovery rate of 90.12% of the total oil content, with an iodine value of 69.18 ± 0.56 g I_2/100 g, an acid value of 2.55 ± 0.02 mg KOH/g, and a peroxide value of 1.07 ± 0.02 meq O_2/Kg. The physical characteristics of MSO included a refractive index of 1.467±0.03, a specific gravity of 0.92 ± 0.01, a pH of 6.12 ± 0.09, and colour values of 65.77 ± 0.34, 0.34 ± 0.01, and 61.2 ± 0.55 for L*, a* and b* respectively. The antioxidant activity and total phenol content (TPC) of UPAE oil were 33.6 ± 0.26% and 40.1 ± 0.67 µg/mL, respectively.

Keywords

DPPH; FTIR; moringa seed oil; PKM1 moringa; total phenol content; ultrasonic probe -assisted extraction

Introduction

About 2.2 million tonnes of moringa seeds were produced from a cultivated area of 43,600 ha in India in 2018, and India remains the leading producer of moringa globally (1). The demand for processed moringa products is increasing worldwide, and the market for moringa seed oil (MSO) is expected to grow from its present value of $291.1 million in 2024 to $504.4 million in 2032 with a compound annual growth rate (CAGR) of 6.3% (2). The MSO is rich in oleic acid (>70%) and contains significant antioxidant levels. It has many important medicinal properties, such as anti-inflammatory, antimicrobial, and anticancer effects. The high demand for MSO is due to its potential application in the pharmaceutical and cosmetic industries (3).

The MSO is presently extracted mainly using traditional methods, such as hydraulic press, screw press, and cold press. The quality of cold-pressed oil is superior due to the low temperature of the extraction process,

which preserves essential nutrients and bioactive compounds (4). However, the main disadvantages of the traditional methods are lower oil yield and longer extraction times. The efficiency of extraction in traditional cold-press methods is very low. Only 18-25% of the available oil content is extracted, leaving a significant portion of oil in the oil cake. The edible oil industries need advanced extraction techniques to overcome the limitations of traditional ones. Traditional methods often require more labour and time, increasing overall costs and reducing competitiveness against modern extraction technologies that can process larger quantities more efficiently (5). Therefore, edible oil industries prefer extraction techniques that are more efficient and cost-effective. Advanced extraction methods, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction, and supercritical fluid extraction, are increasingly used to address the limitations of traditional extraction techniques. The controlled temperature during UAE prevents the thermal degradation of sensitive compounds, maintaining their integrity and enhancing the quality of the final extract (6).

UAE is a non-thermal extraction method that uses ultrasonic waves of high frequency (20–100 kHz) to enhance the extraction of oil and bioactive compounds from various plant materials. The two primary methods of UAE are the ultrasound bath and ultrasonic probe methods. The ultrasonic probe delivers concentrated energy directly into the sample, resulting in a more intense cavitation effect that disrupts cell walls. This leads to higher extraction yields compared to the ultrasound bath, where energy is distributed more uniformly and less effectively throughout the medium (6, 7).

The UAE method was adopted by some researchers to extract MSO from moringa seeds. Ladjouze, ben Hamiche (8), Thirugnanasambandham (9) and Zhong, Wang (10) extracted the MSO through the ultrasound bath method, while Mohammadpour, Sadrameli (11) extracted Moringa peregrina seed oil using the ultrasonic probe. These investigations focused primarily on the effect of process parameters on oil recovery from moringa seeds. The PKM1 moringa seeds have a high oil content of 33-45%, which is greater than that of many other varieties of moringa (12). The oil extracted from PKM1 seeds has an excellent fatty acid composition and a low saturated fat percentage, making it very stable and resistant to oxidation (13). However, there is limited research on the optimization of the oil extraction conditions using ultrasound probes for the maximum oil recovery from the PKM1 moringa seeds.

The main aim of the present study was to extract the maximum oil from PKM1 moringa seeds and to study the effects of oil extraction conditions on the quality of extracted oil. The experimental design adopted was based on Response surface methodology (RSM) and accordingly, a Box-Behnken design (BBD) was used to investigate the effects of interactions between the independent and dependent variables (14). BBD was used when independent variables were three or more than three. The independent variables opted for the study were extraction time, ultrasound amplitude levels, and solvent-solute ratio. The dependent variables measured were oil yield, iodine value, acid value, and peroxide value of the extracted oil. The physio-chemical properties, antioxidant activity, and total phenolic content of the MSO were compared with crude oils to evaluate its better oxidation stability and nutritional value. The functional groups in the MSO obtained from UPAE were identified and characterized using FTIR spectroscopy.

Materials and Methods

Raw material collection and preparation

Moringa seeds were purchased from the Department of Vegetable Sciences, TNAU, Coimbatore, India in 2024. Seeds were cleaned to remove the foreign materials before the dehulling, and seeds were dehulled using laboratory moringa dehuller. The moringa kernels and husk were separated manually. The kernels were ground using a laboratory ball mill and sieved with the sieve shaker to maintain a uniform particle size of 500 µm-700 µm. The moringa seed powder (MSP) was stored in the refrigerator at 4 ± 2°C until further use. Before extraction, the MSP was dried in the hot air oven at 50 °C for 30 min and the moisture content was determined to be 6.4% (w.b.) (15). The chemicals used for the extraction, physicochemical analysis, antioxidant activity, and total phenol contents were of analytical grade. The gallic acid standard was purchased from Sigma Aldrich (St. Louis, MO, USA).

Soxhlet extraction (SE)

The total oil content in MSP was determined using a laboratory Soxhlet extractor (SOXO45, LabQuest Borosil, India), following the standard AOAC method (16). About 50 g of ground MSP was weighed and transferred to a thimble. The oil was extracted with 350 mL of n-hexane for 6 h at 60 °C using the Soxhlet extractor. The extracted oil was placed in an oven at 50 °C to remove residual moisture and was kept in the oven until it reached constant mass (11). The extraction yield of oil and extraction efficiency was calculated using the following Eqn. 1 and 2, respectively.

$$\text{Oil yield (\%)} = \frac{\text{Mass of oil. g}}{\text{Mass of sample. g}} \text{ X } 100 \quad \text{.....(1)}$$

$$\text{Extraction efficiency (\%)} = \frac{\text{Mass of oil recovered. g}}{\text{Total oil content in sample. g}} \text{ X } 100 \quad \text{.....(2)}$$

Ultrasonic probe-assisted extraction (UPAE)

Ultrasonic probe-assisted extraction **(**UPAE) was conducted using the ultrasound probe sonicator (Model: PRO650, Labman) with an output power of 650 W and an auto-adjusted frequency range of 20 to 25 kHz. The probe is made of titanium alloy and has a tip diameter of 6 mm (1/4 inch). The pretrials were conducted to find the limits same as followed in the coriander oil extraction and followed the same procedure (17). About 30 g of MSP was

taken into a 1L glass container. The sample was then mixed with the extraction solvent, n-hexane, and subjected to extraction experimentation for the variables as detailed in Table 1. The ultrasonic probe was inserted directly into the mixture. The input range of selected variables was determined from preliminary experiments. The ultrasound amplitude level can be changed from 0 to 100% and a continuous cycle was applied for the extraction process. After the completion of extraction, the mixture was kept for settlement of solute particles for 30 min. The solvent mixture was separated from solute particles using the rotary vane vacuum pump. Using a rotary evaporator, the oil and solvent were separated from the mixture under vacuum conditions at 50 °C. The oil yield and extraction efficiency were determined in the same way as in the SE.

Table 1. Coded levels for independent variables.

Independent variables	Coded symbol	Coded levels		
		-1	0	1
Extraction time (min)	A	25	30	35
Ultrasound amplitude (%)	B	40	50	60
Solvent-solute ratio (mL/g)	C	10 (1:10)	15 (1:15)	20 (1:20)

Physicochemical analysis

The physical properties of MSO, such as pH, specific gravity, relative density, and colour values (L*, a*, b*) were determined according to AOAC (16). The chemical properties of MSO, such as acid value (AV), iodine value (IV), and saponification value (SV) were determined according to AOAC (18). The peroxide value (PV) was determined (19).

Determination of antioxidant activity

The antioxidant activity of MSO was investigated by estimating its DPPH (1,1-diphenyl-2-picrylhydrazy) free radical scavenging activity. The DPPH free radical scavenging activity of MSO was found with some modifications in the procedure used for moringa oil (10). About 0.2 mL of oil was mixed with 10 mL methanol for 1 min. The mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, the supernatant was separated from the mixture. Then 0.6 mL of supernatant was mixed with 0.0634 mM of DPPH solution prepared in 95% methanol and shaken vigorously. The changes in absorbance of the extracts were measured at 517 nm for 30 min using a UV-vis spectrophotometer (UV-1800, Shimadzu, Japan) until the absorbance reached a steady state. The blank used was methanol (95%). The percentage inhibition of DPPH of the extracts was determined by the following Eqn. 3.

$$\% \text{ inhibition} = 100 \times (A_0 - A) / A_0 \quad \text{.....(3)}$$

where A_0 is the absorbance of the DPPH solution without the sample at 517 nm, and A is the absorbance of the DPPH solution with the sample at 517 nm.

Determination of total phenolic content (TPC)

The total phenolic content of MSO was determined spectrophotometrically using the Folin–Ciocalteu method with some modifications in the procedure (20). About 0.1 mL of oil was mixed with 2.9 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent and the mixture was allowed to react for 3 min. After 3 min, 2 mL of 7.5% sodium carbonate was added and the mixture was then incubated at 40 °C for 30 min. Finally, the absorption was measured at 765 nm in UV-vis spectrophotometer (UV-1800, Shimadzu, Japan). Distilled water was used as blank. Results were expressed as gallic acid equivalent (mg GAE/g).

FTIR analysis of functional groups

Fourier Transform Infrared (FTIR) Spectrophotometer analysis was used to identify the presence of functional groups and impurities in UPAE extracted MSO (21). FTIR spectroscopy identifies functional groups by measuring the absorption of infrared light at specific wavenumbers corresponding to the vibrational modes of chemical bonds. The Miracle10 FTIR (Shimadzu, Japan) was used to record the spectra of MSO in the range of 4000–400 cm^{-1}. The obtained values were analysed with the spectral software (OriginPro 2024b).

Experimental design and statistical analysis

Response surface methodology (RSM) was used to illustrate the effect of independent variables *viz.*, extraction time, ultrasound amplitude, and solvent-solute ratio on the dependent variables such as oil yield, IV, AV, and PV. The statistical software Design Expert (Version 13.0.5.0) was used for the statistical analysis of experimental data. Specifically, each factor was tested at three levels (-1, 0, 1), as presented in Table 1. The quadratic response surface model used to describe the relationships between variables is expressed in Eqn. 4.

$$Y = \beta_0 + \sum\beta_i x_i + \sum\beta_{ii} x_i^2 + \sum\sum\beta_{ij} x_i x_j + \epsilon \quad \text{.....(4)}$$

where *Y* represents the response variable, β_0 is the intercept, β_i are the coefficients for the linear terms, β_{ii} are the coefficients for the quadratic terms, and β_{ij} are the coefficients for the interaction terms between the independent variables x_i and x_j. A total of 17 experimentak runs were conducted and presented in a randomized pattern (Table 2). The least squares multiple regression method was employed to optimize treatment conditions and analyse the influence of independent variables in the extraction process. The model was fitted based on the values of the coefficient of determination (R^2), which should be above 0.85, and the adjusted coefficient of determination (adjusted R^2). The effect of ultrasound amplitude, extraction time, and solvent-solute ratio on oil yield, IV, AV, and PV was evaluated by method of analysis of variance (ANOVA).

Results and Discussion

Total oil yield

The total oil content of PKM1 moringa seed obtained through Soxhlet extraction was 40.3 ± 0.79%. This result was consistent with the 40.1% oil yield from M. *oleifera* seeds in Western Australia (15) and slightly lower than the

41.03% oil yield of PKM1 moringa seeds extracted (22) under the same extraction conditions. There was no significant difference ($p > 0.05$) among these values, indicating the consistency of oil yield across the studies. However, this result was significantly higher than the 33.1% oil yield from PKM1 moringa seeds extracted using n-hexane at 56 °C temperature for 7 h extraction time (23). The lower oil yield obtained in their study was due to the longer extraction time compared to this study. The variations in oil content can be attributed to several factors, including the extraction method, the variety of seed used, the geographical origin of the seeds, and the specific conditions under which the seeds are grown and harvested (24).

Table 2. Experimental factors and responses for UPAE PKM-1 moringa seed oil using Box – Behnken design (BBD).

Run	Extraction Time (min)	Ultrasound amplitude (%)	Solvent-solute ratio (mL/g)	Oil yield (%)	Iodine value (g I_2/100 g)	Acid value (mg KOH/ g)	Peroxide value (meq O_2/ kg)
1	35	50	10	28.5	60.5	0.95	2.54
2	25	60	15	34.7	68.3	2.13	1.34
3	30	50	15	33.7	64.6	1.27	0.90
4	30	50	10	28.3	61.1	1.02	2.32
5	30	50	15	32.5	65.3	1.35	0.87
6	25	40	15	30.9	58.6	1.04	0.63
7	30	40	20	32.1	60.8	1.34	0.57
8	30	50	20	33.9	66.7	1.89	0.72
9	30	60	20	36.8	69.4	2.62	1.21
10	35	50	20	34.2	68.3	1.95	0.78
11	25	50	10	27.8	62.3	1.02	2.08
12	35	40	15	31.2	60.6	1.02	0.72
13	30	60	10	29.6	59.8	1.78	2.57
14	25	50	20	33.2	65.6	1.93	0.53
15	35	60	15	33.9	67.4	2.15	1.50
16	30	40	10	27.5	57.3	0.78	1.83
17	35	40	20	32.5	61.2	1.26	0.61

Effects of extraction time, ultrasound amplitude, and solvent-solute ratio on the oil yield

The response surface plots of the recorded oil yield are shown in Fig. 1 and Table 2. The highest oil yield of 36.8% was obtained at a 60% ultrasound amplitude, 30 min extraction time, and a solvent-solute ratio of 20 mL/g (1:20). In contrast, a lower oil yield of 27.5% was extracted at 40% ultrasound amplitude, 30 min extraction time, and a solvent-solute ratio of 10 mL/g (1:10). This difference was due to the increased ultrasound amplitude and solvent-solute ratio.

Ultrasound amplitude and solvent-solute ratio both had significant effects on the oil yield of extracts ($p < 0.05$), while extraction time showed no significant effect on oil yield ($p > 0.05$), as shown in Table 3. An increase in oil yield from 27.5% to 36.8% was observed with an increase in ultrasound amplitude levels from 40% to 60%. As the ultrasonic amplitude increases, the temperature and pressure within the bubbles rise to 5500°C and 50 MPa, respectively (25), causing them to break down rapidly (26). This breakdown leads to the permeation of the solvent into the cell tissues and accelerates the discharge of the intracellular products into the solvent (27). Similar behaviour was in pumpkin seed oil from the UAE, where an increase in oil yield of 62.46% was observed with an increased amplitude level to 62.50% (28). In this study, at more than 60% ultrasound amplitude level, the oil yield decreased. At higher amplitude levels, cavitation bubbles may become larger and less effective at collapsing, thereby reducing the energy transfer required for efficient extraction (29). Similar results were observed in coriander seed oil extraction, where increasing the amplitude level to 80% during the UAE of coriander seed oil led to an increase in oil yield up to 29.98%. However, at the 90% amplitude level, both the oil yield and DPPH% activity decreased (17).

Similarly, the oil yield increased from 27.5% to 36.8% as the solvent-solute ratio increased from 10 mL/g (1:10) to 20 mL/g (1:20). This variation was correlated with the increasing ultrasound amplitude from 40% to 60%. A higher solvent-solute ratio of 20 mL/g improved the solvent's ability to dissolve oil. This created a steeper concentration gradient which is responsible for diffusion and enhanced mass transfer rate of oil by increasing the contact area between the solvent and seed particles (30). The same variation in oil yield was obtained during UAE of shelled and unshelled M. *oleifera* seed oil at solvent concentrations of 61.56 mL/g and 58.53 mL/g respectively (8). A similar result was reported during nigella seed oil extraction, where oil yield increased from 27 to 30% upon increasing the solvent-solute ratio from 10 to 30 mL/ 5g (31). In this study, at ultrasound amplitude levels greater than 60%, the oil yield decreased due to the concentration gradient becoming zero when the solvent is fully saturated

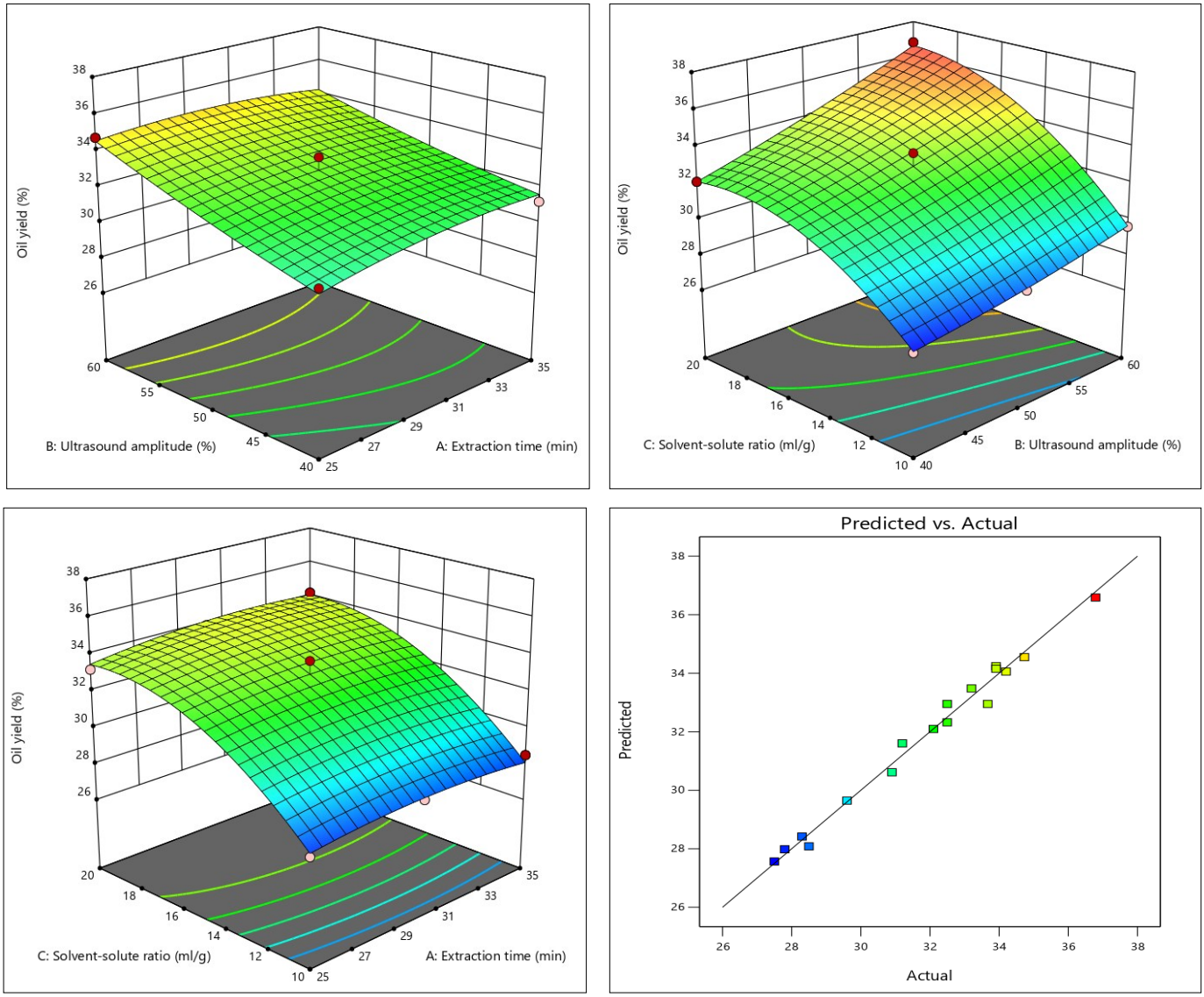

Fig. 1. Response surface graphs for the oil yield of PKM1 during UPAE.

Table 3. Analysis of variance (ANOVA) for the effects of different combinations of extraction time, ultrasound amplitude, and solvent-solute ratio on oil yield, IV, AV, and PV.

Source	Oil yield		Iodine value		Acid value		Peroxide value	
	F - value	*p* - value	F - value	*p* - value	F - value	*p* - value	F - value	*p* - value
Model - Quadratic	58.05	< 0.0001	32.66	< 0.0001	60.05	< 0.0001	62.39	< 0.0001
A – Extraction time	1.09	0.3318	0.3400	0.5781	0.3006	0.6005	8.62	0.0218
B – Ultrasound amplitude	102.10	< 0.0001	137.25	< 0.0001	332.17	< 0.0001	71.83	< 0.0001
C – Solvent-solute ratio	384.06	< 0.0001	117.11	< 0.0001	209.09	< 0.0001	394.90	< 0.0001
AB	2.10	0.1905	2.06	0.1942	0.5473	0.4835	0.0425	0.8426
AC	0.2843	0.6104	5.93	0.0451	0.0001	0.9912	0.6457	0.4481
BC	7.27	0.0308	16.13	0.0051	4.52	0.0710	0.2901	0.6069
A^2	2.58	0.1521	0.8800	0.3794	0.3613	0.5667	0.3065	0.5971
B^2	0.6133	0.4592	20.15	0.0028	17.78	0.0040	2.45	0.1617
C^2	42.36	0.0003	8.98	0.0201	2.98	0.1277	71.74	< 0.0001
Lack of Fit	0.2141	0.9260	2.82	0.4264	2.88	0.4228	38.42	0.1229

with the extracted oil (17). A similar trend was reported in the UAE for various vegetable oils, including papaya seed oil (26), nigella seed oil (31), and black cumin oil (32).

UPAE had a significant effect ($p < 0.05$) on the oil yield. The determination coefficient (R^2) value of 0.9868 and an adjusted determination coefficient (R^2) value of 0.9698 indicated a strong fit for the model. The model for extraction of oil yield is given in Eqn. 5.

$$\text{Oil yield} = 32.95+0.1696A+1.64B+2.87C-0.3234AB + 0.1193AC +0.6032BC-0.3849A^2+0.1876B^2-1.66C^2$$

..... (5)

where A is extraction time (min), B is ultrasound amplitude (%), and C is a solvent-solute ratio (mL/g).

Effects of extraction time, ultrasound amplitude, and solvent-solute ratio on the iodine value (IV) of the oil

The response surface plots of the recorded iodine value are shown in Fig. 2 and Table 2. The highest iodine value of 69.4 g I_2/100 g was obtained at 60% ultrasound amplitude, 30 min extraction time, and a solvent-solute ratio of 20 mL/g (1:20). In contrast, a lower iodine value of 57.3 g I_2/100 g was obtained at 40% ultrasound amplitude, 30 min extraction time and a solvent-solute ratio of 10 mL/g (1:10).

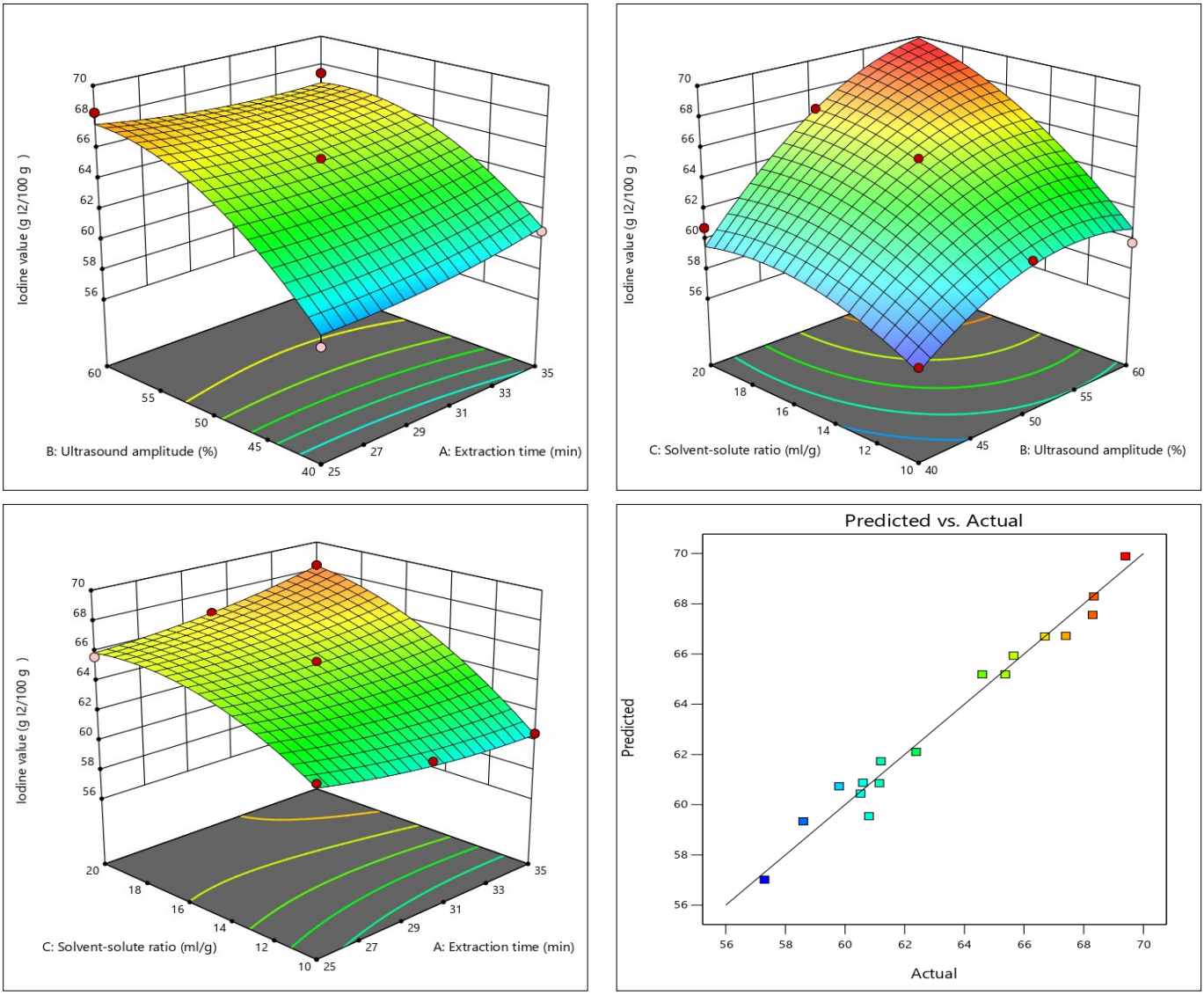

Fig. 2. Response surface graphs for the iodine value of PKM1 during UPAE.

Ultrasound amplitude and solvent-solute ratio both had significant effects on IV of extracts ($p < 0.05$), while extraction time had no significant effect on IV ($p > 0.05$), as shown in Table 3. An increase in the IV from 57.3 to 69.4 g I_2/100 g was observed as the ultrasound amplitude level increased from 40% to 60%. The rise in ultrasound amplitude increased the IV due to fatty acid breakdown caused by excessive heat generated at higher amplitudes. This breakdown resulted in the release of more unsaturated fatty acids, indicating a higher degree of unsaturation in the oil (33, 34). During the flaxseed oil extraction, the IV increased from 184 to 192 g I_2/100 g after being enriched with carotenoids from sea buckthorn pomace at higher amplitude levels (35). However, prolonged exposure to high ultrasound power during sunflower oil extraction resulted in a decrease in IV (36). Meanwhile, some studies have shown that the IV is positively correlated with the extraction duration, as longer extraction times facilitate the extraction of more unsaturated components from the oil matrix (37). Initially, increased ultrasound power increases extraction efficiency and may increase the IV, but excessive power can lead to reduced IV due to the oil droplet coalescence effect (38).

Similarly, the IV increased from 57.3 to 69.4 g I_2/100 g as the solvent-solute ratio increased from 10 mL/g to 20 mL/g. At a higher solvent-solute ratio of 20 mL/g, an increased concentration of the solvent can facilitate better interactions between iodine and the double bonds in the unsaturated fatty acids. This improved interaction may increase the kinetics of the reaction, allowing more iodine to react with the available double bonds to complete the reaction and thus resulting in a higher iodine value (39).

UPAE had a significant effect ($p < 0.05$) on the IV. The determination coefficient (R^2) of 0.9767 and an adjusted determination coefficient (R^2) value of 0.9468 indicated a strong fit for the model. The model for the IV of MSO is given in Eqn. 6.

$$\text{Iodine value} = 65.19 + 0.1750A + 3.52B + 2.92C - 0.5925AB + 1.01AC + 1.66BC + 0.4145A^2 - 1.98B^2 - 1.41C^2 \quad \text{.....(6)}$$

where A is extraction time (min), B is ultrasound amplitude (%), and C is solvent-solute ratio (mL/g).

Effects of extraction time, ultrasound amplitude, and solvent-solute ratio on the acid value (AV) of the oil

The response surface plots of the recorded acid value are shown in Fig. 3 and Table 2. The highest AV of 2.62 mg KOH/g was obtained at 60% ultrasound amplitude, 30 min extraction time, and a solvent-solute ratio of 20 mL/g (1:20). In contrast, a lower AV of 0.78 mg KOH/g was obtained at 40% ultrasound amplitude, 30 min extraction time and a solvent-solute ratio of 10 mL/g (1:10).

Ultrasound amplitude and solvent-solute ratio both had significant effects on the acid value of extracts ($p < 0.05$), while extraction time had no significant effect on acid value ($p > 0.05$), as shown in Table 3. An increase in AV from 0.78 to 2.62 mg KOH/g was observed as the ultrasound amplitude level increased from 40% to 60%. The higher ultrasound power provided a greater opportunity to disrupt oil-bearing structures and hydrolyse triglycerides into free fatty acids, thereby increasing the acid value of the oil (34, 36). The AV of black cumin oil was increased from 10.20 to 12.09 (oleic acid%) as the ultrasonic power increased from 30 to 90 W during UAE (32). An increase in the AV was observed as the solvent-solute ratio increased from 10 mL/g to 20 mL/g due to the enhanced solubilization of free fatty acids in the solvent. This allowed for a more accurate measurement of the acid value because more acids were available for titration (40). Similar results were reported from the rice bran oil, where AV increased from 0.8 to 1.2 mg KOH/g upon increasing the solvent-solute ratio from 10 to 20 mL/g (41).

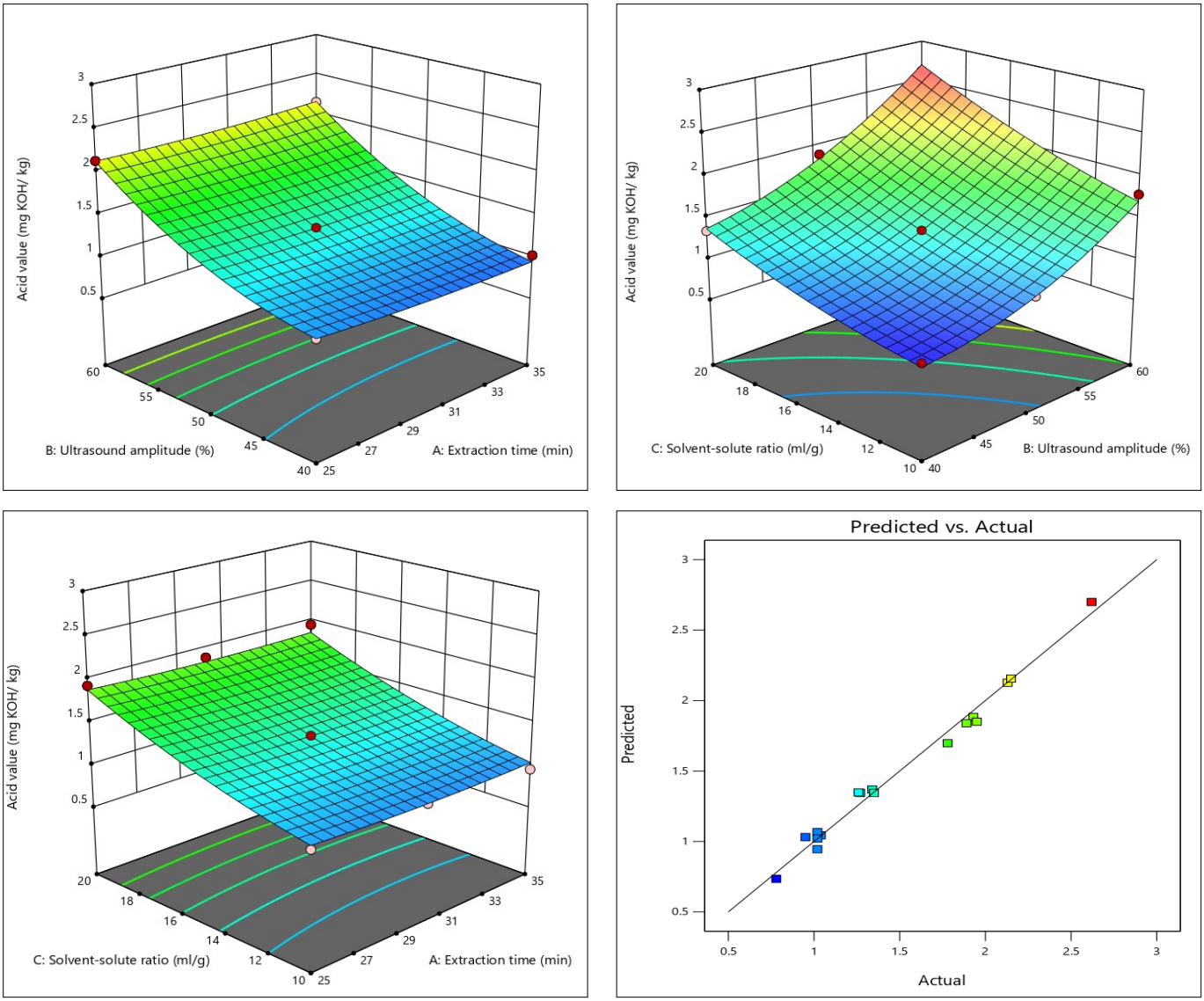

Fig. 3. Response surface graphs for Acid value of PKM1 during UPAE.

UPAE had a significant effect ($p < 0.05$) on the AV. The determination coefficient (R^2) of 0.9872 and an adjusted determination coefficient (R^2) value of 0.9708 indicated a strong fit for the model. The model for the AV of MSO is given in Eqn. 7.

$$\text{Acid value} = 1.34 - 0.0173A + 0.5735B + 0.4092C + 0.0320AB + 0.005AC + 0.0920BC + 0.0278A^2 + 0.1953B^2 + 0.0854C^2 \quad \text{.....(7)}$$

where A is extraction time (min), B is ultrasound amplitude (%), and C is solvent-solute ratio (mL/g).

Effects of extraction time, ultrasound amplitude, and solvent-solute ratio on the peroxide value (PV) of the oil

The response surface plots of the recorded peroxide value are shown in Fig. 4 and Table 2. The highest PV of 2.57 meq O_2/kg was obtained at 60% ultrasound amplitude, 30 min extraction time, and a solvent-solute ratio of 10 mL/g (1:10). In contrast, a lower peroxide value of 0.57 meq O_2/kg was obtained at 40% ultrasound amplitude, 30 min extraction time and a solvent-solute ratio of 20 mL/g (1:20).

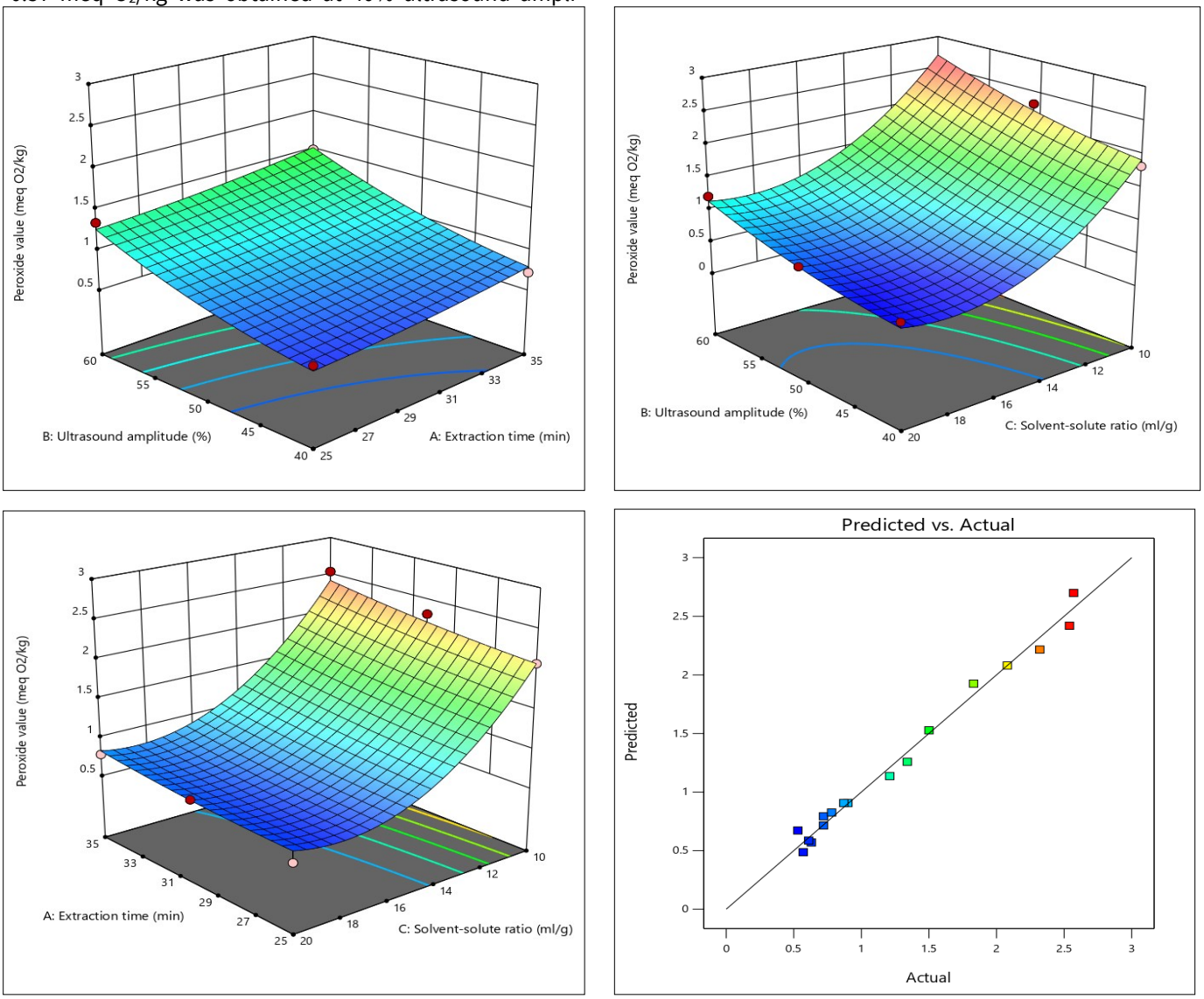

Fig. 4. Response surface graphs for the peroxide value of PKM1 during UPAE.

Ultrasound amplitude, solvent-solute ratio, and extraction time all had significant effects on the PV of oil ($p < 0.05$), as shown in Table 3. An increase in the PV from 0.53 to 2.57 meq O_2/Kg was observed as the ultrasound amplitude level increased from 40% to 60%. At higher ultrasound amplitude (60%), free radicals generated during cavitation break the double bonds in unsaturated fatty acids which leads to increased lipid oxidation and thus increased PV (42). An increase in extraction time with increased ultrasound amplitude led to a higher PV due to prolonged exposure of the sample to ultrasound, which increased heat and resulted in the thermal oxidation of oils. This phenomenon was responsible for the formation of peroxides and hydroperoxides, which were primary products of lipid oxidation (43). Specifically, studies showed that increased ultrasound power from 30 to 90 W resulted in a 12.14% increase in the PV, while extended extraction time from 30 to 60 min could lead to a 54.55% increase in the PV for black cumin seed oil (32). A decreased PV was observed as the solvent-solute ratio increased from 10 mL/g to 20 mL/g due to increased solubility of peroxides formed during the oxidation of unsaturated fatty acids. This solubilization reduced the concentration of peroxides in the oil phase, resulting in a lower peroxide concentration. Similar findings were observed during the palm oil extraction, where a solute-solvent ratio of 1:4 w/v was used (44). An optimal solvent-solute ratio was responsible for the effective extraction and a lower PV of less than 10 meq/kg. The PV less than 10 meq/kg is acceptable for edible purpose (45). This suggested that an optimal solvent ratio could mitigate the oxidative effects during extraction and preserve oil quality (44).

UPAE had a significant effect ($p < 0.05$) on the PV. The determination coefficient (R^2) of 0.9877 and an adjusted determination coefficient (R^2) of 0.9718 indicated a strong fit for the model. The model for the peroxide value of MSO is given in Eqn. 8.

$$\text{Peroxide value} = 0.9063 + 0.1230A + 0.3557B - 0.7506C + 0.0114AB - 0.0464AC - 0.0311BC + 0.0340A^2 + 0.0965B^2 + 0.5589C^2 \quad \text{.....(8)}$$

where A is extraction time (min), B is ultrasound amplitude (%), and C is a solvent-solute ratio (mL/g).

Optimization

The optimized oil extraction conditions for UPAE from the PKM1 moringa seeds were determined using the numerical optimization method in BBD as shown in Table 4. The extraction conditions were optimized to get higher oil extraction yield and lower peroxide value of oil. The optimization conditions obtained by design were 60% ultrasound amplitude, 29 min of extraction time, and 19 mL/g of a solvent -solute ratio with the highest predicted desirability value of 0.983. The predicted and actual values of optimized UPAE are shown in Table 4. The experimental values deviated from the predicted values of 0.44% for oil yield, 0.31% for iodine value, 0.39% for acid value, and 0.46% for peroxide value. This indicated that the actual values were very close to the predicted values with an error of 2% (Table 4).

Physicochemical properties of MSO

The physicochemical properties of the Soxhlet extracted oil (46) and optimized UPAE oil were given in Table 6. The refractive index was the same for both oils. The colour parameters (L*, a*, b*) of UPAE oil were significantly higher ($p < 0.05$) than those of SEO due to the prolonged exposure of the sample to a higher temperature, which degraded the sensitive colour compounds. The peroxide value of SEO (2.03 ± 0.04 meq O_2/kg) was significantly ($p < 0.05$) higher than UPAE oil (1.07 ± 0.03 meq O_2/kg) due to increased oxidation reaction at higher temperatures during SE. The IV, AV, and viscosity of SEO and UPAE oil were 71.3 ± 0.34 g I_2/100 g and 69.18 ± 0.89 g I_2/100 g; 3.16 ± 0.04 mg KOH/g and 2.55 ± 0.02 mg KOH/g; and 0.47 ± 0.01 Pa.s and 0.44 ± 0.01 Pa.s, respectively. The IV and viscosity of SEO were non-significantly higher ($p > 0.05$) than those of UPAE oil. There was no significant difference ($p > 0.05$) in the specific gravity, relative density,

Table 4. Optimized extraction process parameters using Box-Behnken design (BBD).

Parameters	Predicted value	Actual value	*Error %
Extraction time (min)	29	29	
Ultrasound amplitude (%)	60	60	
Solvent-solute ratio (mL/g)	19	19	
Oil yield (%)	36.482	36.32 ± 0.44	0.44
Iodine value (g I2/100 g)	69.40	69.18 ± 0.89	0.31
Acid value (mg KOH/g)	2.560	2.55 ± 0.02	0.39
Peroxide value (meq O2/Kg)	1.073	1.07 ± 0.03	0.27

Table 5. Properties of the absorption peaks detected in the FTIR spectra of the UPAE oil sample.

Sl. No.	Range (cm^{-1})	Peak number (cm^{-1})	Assignment of bonds	Mode of vibration	Intensity	Functional groups
1	4000-3500	3888.49	–O–H–	stretching	very weak	alcohol
2	3700-3584	3718.76	–O–H–	stretching	very weak	alcohol
3	2840-3000	2924.09	–C–H– (CH_2)	stretching (asymmetric)	very strong	alkane
4	2840-3000	2854.65	–C–H– (CH_2)	stretching (symmetric)	very strong	alkane
5	1818-1750	1851.66	–C=O–	stretching	very weak	esters, anhydride
6	1815-1785	1813.09	–C=O–	stretching	very weak	esters, acid halide
7	1750-1735	1743.65	–C=O–	stretching	very strong	esters
8	1550-1500	1550.77	–N–O–	stretching	weak	nitro compounds
9	1550-1500	1527.62	–N–O–	stretching	weak	nitro compounds
10	1486-1446	1465.90	–C–H– (CH_2)	bending (scissoring)	medium	alkane
11	1440-1395	1442.75	–O–H–	bending	medium	carboxylic acid
12	1420-1330	1411.89	–O–H–	bending	weak	alcohol
13	1390-1380	1381.03	–C–H– (CH_3)	bending (symmetric)	very weak	alkane
14	1350-1300	1350.17	–S=O	stretching	very weak	sulfone
15	1250-1020	1234.44	–C–O–, –CH_2–	stretching, bending (out-of-plane)	very weak	carboxylic, alkane
16	1205-1124	1157.29	–C–O– –CH_2–	stretching, bending	strong	esters, alkane
17	754–701	725.23	–(CH_2)n–, –HC=CH–	rocking, bending (out-of-plane)	medium	methylene (polymer chain), ethylene (cis)

Table 6. Physicochemical properties of SE and optimized UPAE moringa seed oils.

Parameters	Soxhlet extraction	Optimized UPAE
Specific gravity (25 °C)	0.94 ± 0.02	0.92 ± 0.01
Relative density (g/cc)	0.81 ± 0.01	0.87 ± 0.01
Refractive index (40 °C)	1.467 ± 0.03	1.47 ± 0.03
pH	5.64 ± 0.02	6.12 ± 0.09
Colour parameters		
L*	56.41 ± 0.34	65.77 ± 0.34
a*	2.17 ± 0.03	0.34 ± 0.01
b*	67.34 ± 0.98	61.2 ± 0.55
Viscosity at 20 °C (Pa.s)	0.47 ± 0.01	0.44 ± 0.01
Acid value (mg KOH/g oil)	3.16 ± 0.04	2.55 ± 0.02
Iodine value (g I2/100 g oil)	71.3 ± 0.34	69.18 ± 0.56
Peroxide value (meq O2/kg oil)	2.03 ± 0.04	1.07 ± 0.03
Saponification value (mg KOH/g oil)	176.5 ± 0.9	178.3 ± 0.99

IV, and viscosity of SE and UPAE oil. However, there was a significant difference in the AV and saponification values between SEO and UPAE oil ($p < 0.05$). SEO exhibited higher AV due to the presence of more free fatty acids, which resulted from partial hydrolysis and lipid oxidation. The high viscosity of SEO was due to its rich composition of long-chain fatty acids and complex phytochemicals (47). The saponification value of SEO was 176.5 ± 0.9 mg KOH/g and that of UPAE oil was 178.3 ± 0.99 mg KOH/g. Crude oils contained more impurities and a wider range of fatty acids resulting in a lower saponification value. The results were consistent with PKM1 moringa oil (48) and M. *oleifera* oil from Nigeria (49).

The antioxidant activity of MSO

The antioxidant activity of SE oil and UPAE oils was evaluated according to their percentage of inhibition. The decreased DPPH absorption revealed the free-radical scavenging ability of moringa oil. The antiradical activity of SE oil and UPAE oil were 30.87 ± 0.04% and 34.4 ± 0.11%, respectively. The UPAE oil showed significantly greater free radical scavenging activity than the SEO ($p < 0.05$). The obtained results were consistent with DPPH values of shelled (33.67%) and unshelled (10.20%) M. *oleifera* seed oils (8). The difference could be due to the geographical origin of the seeds, the analytical methods used and the concentration of standard used.

The total phenolic content of MSO

The TPC of PKM1 moringa oil extracted using SE and UPAE were found to be 40.92 ± 0.75 mg/GAE g and 46.17 ± 0.85 mg/GAE g, respectively. The UPAE oil exhibited significantly greater TPC than the SE oil ($p < 0.05$). The higher TPC of UPAE oil was due to ultrasound treatment which disrupted the cell walls of the sample and released phenolic compounds into the solution (32). Prolonged exposure of the sample to high temperatures during SE degraded the phenolic compounds, resulting in low TPC (11). The results obtained were consistent with 40.18 mg GAE/g of PKM1 MSO (48) and 40.55 mg GAE/g of M. *oleifera* seed oil (50) extracted using SE. Similarly, the TPC in *Moringa peregrina* oil extracted using SE and UAE was 49.11 mg GAE/g and 63.13 mg GAE/g, respectively (11). Variation in TPC values of MSO was due to different varieties of moringa seeds used for the extraction.

FTIR data

The FTIR spectra of MSO are presented in Fig. 5. Table 5 describes the peak characteristics observed in the spectra. The very weak peaks of alcohol and water (O–H) groups were present at 3888.49 cm^{-1} and 3718.76 cm^{-1} which indicated that a small amount of moisture was present in the oil, which had no significant effect on the oil quality. The presence of alkane groups in fatty acids was confirmed by strong peaks at 2924.09 cm^{-1} and 2854.65 cm^{-1}, corresponding to asymmetric and the symmetric stretching of –C–H– (CH_2) respectively (4). The strong peaks confirmed a

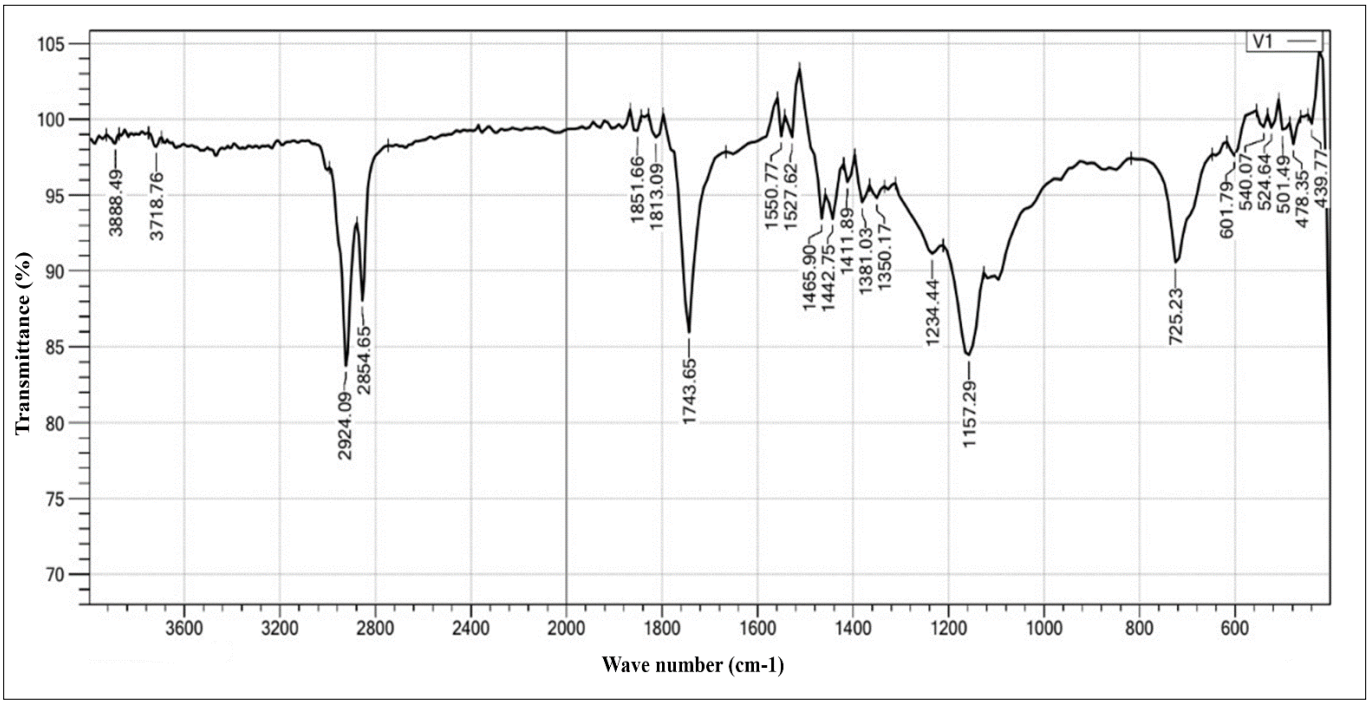

Fig. 5. FT-IR spectra of the UPAE PKM-1 moringa seed oil.

significant amount of methylene groups in the long hydrocarbon chains of fatty acids such as oleic acid and the aliphatic nature of the fatty acid composition of the oil (51). The strong C=O stretching peak at 1743.65 cm^{-1} in the FTIR spectrum of MSO confirmed the presence of ester linkages, which were characteristic of triglycerides found in oils. A stronger peak indicated a higher concentration of fatty acid esters in the oil. Furthermore, the peak at 1381.03 cm^{-1} was attributed to the C–H bending (symmetric) of the CH_2 group, which further supported the presence of aliphatic compounds in the oil. Additionally, the peaks at 1350.17 cm^{-1} corresponded to the stretching of the S=O sulfone group, suggesting that sulphur-containing compounds, such as glucosinolates and isothiocyanates were present in the oil. The peaks at 1234.44 cm^{-1} and 1157.29 cm^{-1}were attributed to the simultaneous stretching and bending of the C–O (ester), and C–H (alkane) groups respectively. The peak at 725.23 cm^{-1} was attributed to –(CH_2) n–, HC=CH (cis) rocking, and bending of the alkane groups. This peak is characteristic of the rocking motions of methylene (–CH_2–) groups found in long-chain hydrocarbons. The obtained results were consistent with the FTIR analysis of moringa seed oil (21)and the analysis of moringa oil extracted by solvent-assisted extraction (52).

Conclusion

In the present study, the UPAE and SE methods were used for the extraction of oil from PKM1 *Moringa oleifera* seeds. The ultrasound amplitude and solvent-solute ratio had a significant effect on oil yield, IV, AV, and PV. The primary objectives of the optimization process to maximize the oil yield and simultaneously minimize the peroxide value of the MSO, were achieved. The optimized conditions obtained from the Box-Behnken Design (BBD) were 60% ultrasound amplitude, 19 mL/g solvent-solute ratio, and 27 min extraction time with desirability of 0.983. UPAE successfully recovered 90.12% of the oil from the total oil content of Moringa seeds. Compared with Soxhlet extraction, UPAE presented a 3.53% increase in antioxidant activity and a 5.25% increase in total phenol content. The presence of oleic acid in oil was identified and analysed with FTIR analysis.

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Authors' contributions

BKM carried out conceptualization, data Curation, formal analysis, and writing the original draft. SG contributed significantly to the development of the methodology, supervised data collection, and also provided critical revisions to the manuscript. TS provided critical resources and equipment necessary for the study. KG contributed by reviewing and editing the manuscript. GA validated the experimental findings and reviewed the manuscript for scientific accuracy and clarity. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

AI Declaration

During the preparation of this work, the author(s) used CHATGPT 4.0 to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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