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Exploring the effects of traditional pretreatment techniques on Mahua (*Madhuca Longifolia*) seed oil extraction

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Mahua seeds are a potential source of oil with health benefits and industrial applications. This study investigated the effects of pretreatment heating (100, 110, 120, and 130°C for 10 min), steaming (5, 10, and 15 min at 1.05 kg/cm²), and moisture conditioning (6, 9, and 12%) on the quality characteristics of Mahua seed oil. The acid value (AV), free fatty acid (FFA), peroxide value (PV), total phenolic content (TPC), and radical scavenging activity (RSA) were determined as responses to the pretreatments. The highest oil yield of 40.00 ± 0.50% was obtained with heat treatment at 120°C, followed by steaming for 10 min and moisture conditioning at 9%, which resulted in oil yields of 38.56 ± 1.35% and 35.24 ± 1.06%, respectively, compared with 29.66 ± 1.98% for the control. The results revealed that AV, FFA, TPC, and RSA increased, whereas PV decreased with increasing heat-treatment temperature, steaming time, and moisture content. Heat treatment at 120°C resulted in the highest oil yield but also produced higher AV and PV. However, the quality characteristics of the 10min steaming treatment were comparatively better, providing a remarkable oil yield and recovery with moderate AV and PV, along with favourable RSA and TPC. The findings demonstrate the practical potential of steaming as a simple and effective pretreatment for improving Mahua seed oil recovery while maintaining desirable quality characteristics, which may support its application in small-scale and commercial oil processing. However, the study was limited to the selected pretreatment conditions and laboratory-scale extraction, and further pilot-scale validation and storage studies are required to confirm its industrial applicability and oil stability.

Keywords: Acid value, Mahua seed, Oil yield, Oil extraction, Pretreatment, Peroxide value

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Mahua (*Madhuca longifolia*) is an indigenous multipurpose tree of the family Sapotaceae, widely distributed across northern, central, and peninsular India. This fast-growing deciduous species is well adapted to arid and semi-arid regions and supports the livelihoods of tribal and rural communities in several Indian states, including Madhya Pradesh, Uttar Pradesh, Odisha, Jharkhand, Chhattisgarh, Maharashtra, Bihar, and Karnataka[1–3]. Often regarded as a Kalpavriksha (tree of life), its flowers, fruits, seeds, and oil have nutritional, medicinal, and commercial applications. Mahua flowers are consumed and used in fermented beverages, while the seeds provide oil traditionally used for cooking, sweets, and as a ghee substitute[4-7]. Mahua oil is also used in traditional medicine, cosmetics, soaps, and detergents, while its press cake has applications in aquaculture. Its favourable fatty acid composition further supports its potential as a biodiesel feedstock[5,8–10].

Mahua seeds are particularly valuable because they contain approximately 40–50% oil, making them a promising non-conventional oilseed for food, cosmetic, pharmaceutical, and bioenergy applications[11]. However, their commercial utilization remains limited by inadequate processing technologies, insufficient quality standardization, and limited understanding of processing-induced changes in oil quality[12]. Efficient extraction methods that maximize oil recovery while maintaining quality are therefore required to enhance the value of this indigenous resource[13].

Traditionally, mahua seeds are sun-dried, roasted or mildly heated, and manually pounded or pressed to release oil[2,5,8]. Similar principles are applied in modern oil processing through pretreatments such as heating, steaming, and moisture conditioning, which modify cellular structure, oil viscosity, protein characteristics, and enzyme activity to improve oil release[14].

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Mechanical screw pressing is particularly attractive because it is solvent-free, relatively inexpensive, environmentally favourable, and suitable for decentralized processing. However, incomplete rupture of oil-bearing cells can limit oil recovery compared with solvent extraction[15]. Appropriate heat treatment can promote protein coagulation, enzyme inactivation, moisture redistribution, and reduced oil viscosity, while steaming and moisture conditioning can facilitate cellular disruption and oil flow[16,17]. Conversely, excessive pretreatment severity may increase lipid deterioration and adversely affect oil quality[17,18].

Despite extensive research on mahua oil composition, fatty acids, and biodiesel potential, systematic studies on conventional pretreatments for mechanical screw-press extraction remain limited. In particular, information is scarce on the effects of heat treatment, steaming, and moisture conditioning on oil recovery together with acid value, free fatty acids (FFA), peroxide value (PV), total phenolic content (TPC), and antioxidant activity. The lack of optimized pretreatment conditions therefore remains a barrier to efficient utilization of mahua seed oil.

Accordingly, this study evaluated heat treatment, steaming, and moisture conditioning as pretreatments for mechanical screw-press extraction of *M. longifolia* seed oil. The objective was to identify suitable pretreatment conditions for maximizing oil recovery while maintaining desirable physicochemical and antioxidant properties, thereby supporting process standardization and the sustainable utilization of mahua seed oil for food and industrial applications.

Materials and Methods

Source of material

Fresh, mature, and healthy mahua seeds were obtained from Raipur (CG, India).Mature and dehulled (split) seeds were used in this study for all experiments. The seeds were stored in clean, airtight bags to protect them from insect pests and deterioration until further use. Mahua seeds (250 g) were measured using a weighing balance (Aczel, CTG3). All experiments and treatments were performed in triplicates.

Selection of variables and experimental design

The pretreatment ranges were selected based on previous studies on mechanical oil extraction from oil-bearing seeds. Heat treatment at 100-130°C was selected to promote cell disruption, reduce oil viscosity, coagulate storage proteins, and enhance oil release while limiting thermal deterioration[16,18,19]. Steaming for 5-15 min was chosen to facilitate enzyme inactivation and tissue softening while minimizing prolonged thermal exposure and lipid oxidation[17,20,21]. Moisture conditioning at 6-12% was selected to improve seed plasticity, facilitate oil-cell rupture, and enhance mechanical oil expression[17,22]. These ranges also reflect practical conditions used in small- and medium-scale mechanical oil extraction. The overall experimental methodology adopted in this study, including the major processing steps and analytical procedures, is illustrated in (Supplementary Fig. S1).

A one-factor-at-a-time (OFAT) design was employed, with one pretreatment variable varied while other conditions remained constant, enabling assessment of its independent effect on oil yield and physicochemical quality. Given the limited information on Mahua seed pretreatment, OFAT was considered suitable for establishing baseline conditions. Although Response Surface Methodology (RSM) can assess variable interactions and optimize processing conditions, its application was beyond the present scope and is recommended for future optimization studies.

Pretreatments

Mahua seed samples were subjected to three conventional pretreatment methods: heat treatment, steaming, and moisture conditioning. For heat treatment, seeds were heated in an oven (CQ138S, Samsung Electronics Pvt. Ltd., India) at 100, 110, 120, and 130°C for 10 min, following a slightly modified method of Suri *et al.*[23]. The heated seeds were immediately subjected to oil extraction to minimize moisture variation and oxidative changes. Untreated seeds served as the control. For steaming, seeds were treated in an autoclave at 110°C and 1.05 kg/cm² pressure for 5, 10, and 15 min, based on a modified method of Mizutani *et al.*[24]. After steaming, the samples were allowed to stabilize with respect to temperature and moisture distribution before screw pressing. For moisture conditioning, the seed moisture content was adjusted to 6, 9, and 12% (wb) using the moisture adjustment equation described by Coskun *et al.*[25]. The calculated amount of distilled water was thoroughly mixed with the seeds, which were sealed in airtight containers and equilibrated overnight at 26–28°C. The moisture-conditioned seeds were subsequently heated at 70°C for 20 min in a hot-air

oven (MAC, OUD-224, Samarth Electronics, India) following Holser[26], and immediately subjected to screw pressing. The resulting oil was collected, weighed, and analysed for yield and physicochemical quality parameters.

$$Q = W_i \left[\frac{m_f - m_i}{100 - m_f}\right]$$

Where,

Q- the weight of water to be added, (g)

W_i- the initial weight of the mahua seed sample, (g)

m_i- the initial moisture content of the grain sample, (% db)

m_f- the final moisture content of the mahua seed sample (% db).

Oil extraction and yield

Oil extraction was performed using a laboratory-scale single-screw mini oil mill equipped with a 2 HP (3400 W) electric motor (NS15000V, Handy Think Engineering, Coimbatore, India). The expeller had a capacity of 10–15 kg h^{-1}, screw speed of 75 ± 2 rpm, and screw clearance of 0.6 mm. The oil mill was preheated for 5–10 min, and pretreated Mahua seeds were pressed at 80 ± 2°C under identical operating conditions. The expressed crude oil was filtered through a 35-µm stainless-steel mesh and centrifuged at 6000 rpm for 15 min at room temperature (Remi R-8C BL, Remi Elektrotechnik Ltd., Mumbai, India). The clarified oil was collected in amber glass bottles and stored at −20°C until analysis[27].

Oil extraction yield was calculated as the percentage of recovered oil relative to the initial seed sample mass, whereas oil recovery represented the percentage of total seed oil recovered through mechanical extraction. Both parameters were calculated according to Thilakaratna *et al.*[27]

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extracted oil} \times 100}{\text{Initial sample weight}}$$

$$\text{Oil recovery (\%)} = \frac{\text{Oil yield from Mechanical Extraction} \times 100}{\text{Oil yield from soxhlet extraction}}$$

Quality analysis of the extracted oil

Acid value (AV) and free fatty acids (FFA)

Acid value was determined according to Suri *et al.*[23] and expressed as mg KOH g^{-1} oil. Briefly, 2.5 g of oil was accurately weighed, mixed with 50 mL ethanol containing phenolphthalein indicator, and heated at 80°C for 15-20 min in a water bath. The hot solution was titrated against standardized KOH solution with vigorous shaking until a persistent light-pink endpoint was obtained (15 s). Acid value was calculated as:

$$\text{Acid value (mg KOH/g)} = \frac{56.2 \times V \times N}{W}$$

Where, V is the volume of oil sample titrant, (ml), N is the normality of potassium hydroxide solution, (mL)and Wis weight of the oil sample, (g)

FFA content was calculated using equation[23].

$$\text{Acid value} = \text{Free fatty acid \% (as oleic)} \times 1.99$$

Peroxide value (PV)

Peroxide value was determined according to Suri *et al.*[23] and expressed as meq peroxide kg^{-1} oil. Approximately 5 g of oil was dissolved in 30 mL of acetic acid–chloroform mixture (3:2, v/v), followed by addition of 0.5 mL saturated KI. After standing with occasional shaking, 30 mL distilled water was added, and the liberated iodine was titrated with 0.1 N sodium thiosulfate until the yellow colour nearly disappeared. Approximately 2 mL starch indicator was then added, and titration was continued until the blue colour disappeared. PV was calculated as:

$$\text{Peroxide value (meq./kg oil)} = \frac{(S - B) \times N \times 1000}{\text{Mass of sample (g)}}$$

Where, *S* is the sample titration volume (mL), *B* is the blank titration volume (mL), *N* is the normality of sodium thiosulfate, and *W* is the mass of oil sample (g).

DPPH radical scavenging activity

Radical scavenging activity (RSA) was determined using the DPPH assay following Cisneros *et al.*[28] and Muangrat *et al.*[29,28], with slight modifications. 1 g of oil was mixed with 1 mL *n*-hexane and 1 mL of 80% (v/v) methanol, vortexed, and shaken at 150 rpm for 1 h (Remi RS-24BL). The mixture was centrifuged at 6000 rpm for 15 min, and the methanolic phase was collected. Its reaction with freshly prepared DPPH solution was monitored at 515 nm after 30 min incubation under reduced light. RSA was calculated as:

$$\text{Radical Scavanging Activity (\%)} = \left[\frac{A_C - A_{30}}{A_C}\right]$$

Where, A_c is the absorbance of the control and A_{30} is the absorbance of the sample after 30 min.

***Total phenolic content* (*TPC*)**

TPC was determined using the Folin–Ciocalteu method described by Cisneros *et al.*[28]. Briefly, 250 µL methanolic oil extract was diluted with 1750 µL of 80% methanol. Folin–Ciocalteu reagent (1 mL) was added and mixed for 3 min, followed by 1 mL of 10% sodium carbonate. After incubation in the dark for 90 min, absorbance was measured at 725 nm against a reagent blank. TPC was quantified using a gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) kg^{-1} oil.

Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) to determine the differences among treatment means. Statistical significance was set at $p< 0.05$[21,30].

Results

Effect of pretreatment on oil yield and recovery

Pretreatment significantly affected oil yield and recovery ($p < 0.05$; Fig. 1-3). Heat treatment increased oil yield from 29.63 ± 1.98% in the control to a maximum of 40.00 ± 0.50% at 120°C. However, a further increase in temperature to 130°C reduced the yield to 24.33 ± 1.52%. Oil recovery followed a similar trend, indicating that moderate heating improved oil release, whereas excessive heating reduced recovery. Steaming also significantly influenced oil yield and recovery ($p < 0.05$; Supplementary Table S1). The highest oil yield was obtained after 10 min of steaming, reaching 38.56 ± 1.35%, compared with 29.63 ± 1.98% for the control (Fig. 2). Although the 5- and 10-min treatments differed significantly from the control, the 15-min treatment did not show a significant improvement. Oil recovery exhibited a similar response. Moisture conditioning significantly affected oil yield and recovery ($p < 0.05$; Supplementary Table S1). Oil yield increased with moisture content up to 9%, where the maximum yield of 35.24 ± 1.06% was observed. Further moisture addition to 12% reduced the yield to 25.74 ± 1.28%. The corresponding oil recovery followed the same pattern, indicating that an optimum moisture level was required for effective oil release

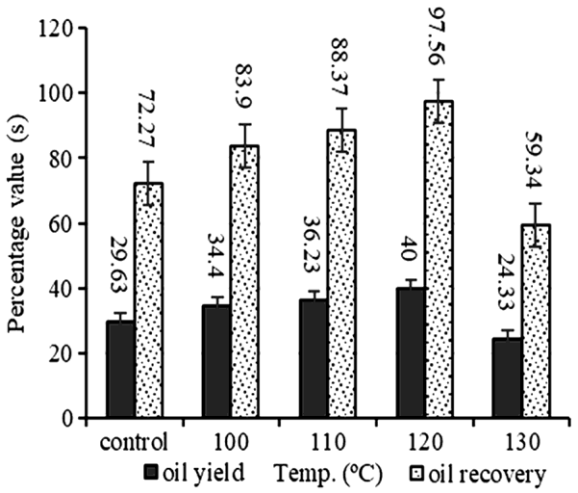


Fig. 1 — Effect of heating on oil yield and recovery

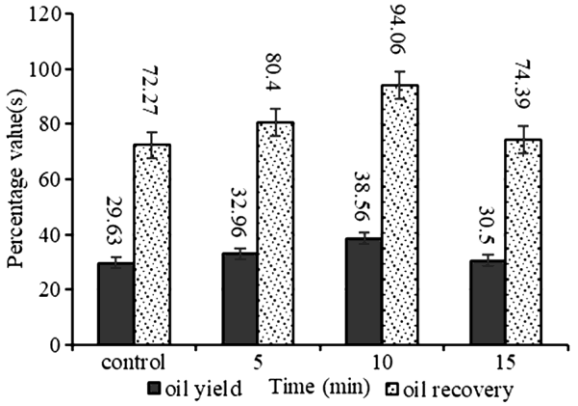


Fig. 2 — Effect of steaming on oil yield and recovery

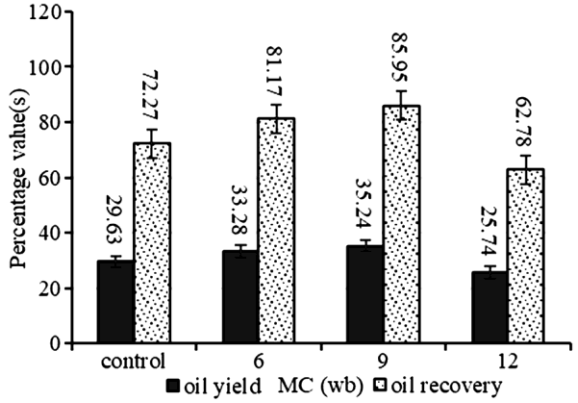


Fig. 3 — Effect of moisture conditioning on oil yield and recovery

Effect of pretreatments on different physicochemical properties of extracted oil

Pretreatment significantly influenced the physicochemical and antioxidant properties of the extracted oil (Supplementary Table S1). The acid value of the control oil was 2.89 ± 0.34 mg KOH g^{-1} oil. Heat treatment increased the acid value progressively with temperature, reaching the highest value at 130°C (Table 1). Steaming and moisture conditioning also increased the acid value, although the increase was lower than that observed after heat treatment. A similar trend was observed for free fatty acid (FFA) content, which increased from 1.45 ± 0.17% in the control to the highest value after heat treatment at 130°C. These results indicate that severe thermal conditions promoted the release of free fatty acids. The peroxide value (PV) was 9.80 ± 0.77 meq kg^{-1} oil in the control (Table 1). Heat treatment initially increased PV at 100°C, followed by a decrease at higher temperatures. Steaming and moisture conditioning generally reduced PV compared with the control, with the lowest value observed after 12% moisture conditioning. The changes in PV indicate that pretreatment influenced the oxidation status of the extracted oil. DPPH radical-scavenging activity was significantly affected by pretreatment ($p = 0.001$). The control oil showed an activity of 54.26 ± 1.82%, while the highest activity was recorded after 15 min of steaming at 69.41 ± 1.37% (Table 1). Heat treatment and moisture conditioning also improved DPPH activity at selected conditions. The total phenolic content (TPC) of the control oil was 18.42 ± 0.83 mg GAE 100 g^{-1} oil. Pretreatment significantly increased TPC ($p = 0.001$, Supplementary Table S1). The highest value, 30.86 ± 1.25 mg GAE 100 g^{-1} oil, was obtained after heat treatment at 130°C. Steaming and moisture conditioning also increased TPC, although their values remained lower than that obtained with the highest heat-treatment condition.

Discussion

Effect of pretreatment on oil yield and recovery

The pretreatments markedly influenced the mechanical extraction of Mahua seed oil by altering seed structure, oil viscosity, moisture distribution, and oil–matrix interactions. Heat treatment increased oil yield up to 120°C, reaching 40.00 ± 0.50% (Fig. 1), compared with 29.63 ± 1.98% for the untreated control, indicating enhanced oil liberation. Moderate heating can promote lipase inactivation, protein coagulation, cell disruption, and reduction in oil viscosity, thereby facilitating oil migration during pressing[23,31,32]. However, the sharp decline at 130°C (24.33 ± 1.52%) suggests that excessive heating may cause moisture loss, matrix hardening, excessive protein denaturation, and thermal deterioration, which can restrict oil movement. Thus, 120°C provided the most favourable balance for oil recovery.

Steaming produced a similar response, with oil yield increasing from 29.63 ± 1.98% in the control to 38.56 ± 1.35% after 10 min, followed by a decline to 30.50 ± 2.93% after 15 min. Figure 2 moderate steaming softens cellular structures, increases tissue permeability, and partially denatures proteins, facilitating rupture of oil-bearing cells during pressing[33,34]. The lower yield after 15 min may be associated with excessive moisture uptake and structural compaction, which can reduce pressability

Table 1 — Quality evaluation of extracted oil

S.no	Treatment		Quality parameters				
			Acid value (mg KOH/g)	Free fatty acid (%)	Peroxide value (meq. /kg oil)	Radical Scavenging% % (DPPH)	Total Phenolic Content (mg GAE/kg oil)
1.	Control		10.4 ±1.08	5.20±0.56	9.8±0.77	42.08 ±1.10	14.38 ±1.60
2.	Heat treatment (ºC)	100	12.59 ±0.72*	6.32±0.66*	11.72±0.41*	45.6 ±1.25**	15.08 ±1.34^{NS}
3.		110	13.10 ±0.97**	6.58±0.29*	8.45±1.02^{NS}	52.05 ±2.12***	16.88 ±0.49*
4.		120	13.67 ±0. 44***	6.86±0.93**	7.23±1.41*	53.67 ±0.56***	22.71 ±0.77***
5.		130	16.80 ±0.99***	8.44±0.32***	6.89±1.3**	56.01 ±0.78***	25.15 ±1.85***
6.	Steaming (min.)	5	11.43 ± 0.53^{NS}	5.74±0.96^{NS}	8.68±0.62^{NS}	66.29 ±0.95***	19.18±0.79***
7.		10	12.19 ± 0.54*	6.12±0.56^{NS}	6.98±1.21**	69.3 ±0.35***	19.63±0.64***
8.		15	12.89 ± 1.05**	6.47±0.73*	7.45±1.26*	70.72 ±1.47***	20.12±0.78***
9.	Moisture conditioning	6	10.83 ± 0.67^{NS}	5.44±0.22^{NS}	7.17±0.48**	51.42 ±0.85***	15.89 ±1.29NS
10.	(MC w.b.)	9	11.68 ±0. 25^{NS}	5.86±0.93^{NS}	6.45±0.94***	57.27 ±0.48***	16.73 ±1.48*
11.		12	12.06 ± 0.51*	6.06±0.31^{NS}	5.98±0.63***	64.55 ±0.78***	19.76 ±0.56***

Signification codes : 0 < *** < 0.001 < ** < 0.01 < * < 0.05, NS-Non-Signifiant

and oil migration[33,34]. Therefore, 10 min steaming was the most effective condition for oil extraction.

Moisture conditioning also improved extraction up to 9% moisture, where oil yield reached 35.24 ± 1.06%, compared with 29.63 ± 1.98% for the control (Fig. 3). Appropriate moisture can increase seed plasticity, soften the cellular matrix, promote oil-droplet coalescence, and facilitate cell rupture during mechanical pressing[22]. In contrast, 12% moisture reduced yield to 25.74 ± 1.28%, probably because excess water promotes oil–water interactions/emulsion formation and decreases effective compressibility and oil migration[35]. Hence, 9% moisture was optimal for mechanical oil recovery.

Effect of pretreatment on oil quality

Pretreatment conditions significantly affected the hydrolytic and oxidative characteristics of the extracted oil. Acid value increased from 2.89 ± 0.34 mg KOH g^{-1} in the control, with the greatest increase observed after heat treatment (5.12 ± 0.18 mg KOH g^{-1} at 130°C; $p = 0.001$). The increase may result from enhanced hydrolysis and thermal degradation of triacylglycerols, particularly under severe heating conditions[21]. Steaming produced a smaller increase (3.15–4.08 mg KOH g^{-1}; $p = 0.001$), whereas moisture conditioning resulted in the lowest increase (3.06–3.82 mg KOH g^{-1}; $p = 0.001$). The corresponding FFA values followed the same pattern, increasing from 1.45 ± 0.17% in the control to 2.56 ± 0.09% after heating at 130°C. These results indicate greater hydrolytic deterioration under severe heat, whereas steaming and moisture conditioning were comparatively less detrimental[22,36,37,21].

Peroxide value showed a different response. Heat treatment initially increased PV to 11.72 ± 0.41 meq kg^{-1} at 100°C, but it decreased at higher temperatures to 6.89 ± 1.30 meq kg^{-1} at 130°C ($p = 0.001$). This decline should not be interpreted as improved oxidative stability because hydroperoxides formed during primary oxidation can decompose at elevated temperatures into secondary oxidation products[23,18]. Steaming and moisture conditioning generally produced lower PVs than the control, with minimum values of 6.98 ± 1.21 meq kg^{-1} and 5.98 ± 0.63 meq kg^{-1}, respectively. The lower PV may reflect reduced peroxide accumulation and, potentially, release of endogenous antioxidant compounds during pretreatment[36,21].

Antioxidant activity and apparent total phenolic content generally increased with pretreatment severity, although the response depended on treatment type. Heat treatment reduced DPPH radical-scavenging activity at 100°C (50.48 ± 1.49%) but increased it to 66.53 ± 1.81% at 130°C. This behaviour may reflect initial degradation of heat-sensitive antioxidants followed by enhanced release of phenolic compounds and formation of reducing Maillard reaction products at higher temperatures[17,28]. Steaming produced progressively higher DPPH activity (58.62–69.41%), while moisture conditioning increased it to 64.18 ± 1.44% at 12%, indicating improved release or extraction of antioxidant compounds[38].

The apparent TPC followed a similar trend, increasing from 18.42 ± 0.83 mg GAE 100 g^{-1} oil in the control to 30.86 ± 1.25 mg GAE 100 g^{-1} oil at 130°C. Steaming increased TPC to 27.18 ± 1.05 mg GAE 100 g^{-1} oil, whereas moisture conditioning increased it to 25.47 ± 0.97 mg GAE 100 g^{-1} oil. Thermal and moisture-induced structural disruption can enhance the release of bound phenolic compounds, while heat-induced Maillard products may also contribute to Folin–Ciocalteu responses[17,28]. The positive association between TPC and DPPH activity supports the contribution of extracted antioxidant compounds to radical-scavenging capacity[39].

Conclusion

The effects of heat treatment, steaming, and moisture conditioning on the oil yield, recovery, and quality characteristics of Mahua seed oil were evaluated. Among the pretreatments, heat treatment at 120°C resulted in the highest oil yield (40.00 ± 0.50%), followed by steaming for 10 min (38.56 ± 1.35%) and moisture conditioning at 9% (35.24 ± 1.06%). Although heat treatment produced the highest oil yield, it was associated with comparatively higher acid and peroxide values. Moisture conditioning resulted in lower AV and PV but required a longer processing time and produced lower oil yield and recovery than heat treatment and steaming. Among the pretreatment methods evaluated, steaming for 10 min emerged as the most suitable option when oil recovery and quality attributes were considered together. Although heat treatment at 120°C achieved the maximum oil yield, steaming offered a more desirable combination of yield, recovery, and chemical quality, with moderate AV and PV and favourable RSA and TPC. The shorter treatment duration and simpler processing requirements further enhance the practical suitability of steaming for Mahua seed oil extraction. Thus, 10-min steaming has potential for application as a pre-

extraction step in Mahua oil processing, particularly where improved oil recovery and preservation of desirable quality attributes are desired. Nevertheless, the present findings are restricted to laboratory-scale conditions and the pretreatment levels investigated; pilot-scale validation, process economics, energy assessment, and storage stability studies are needed before recommending its wider commercial implementation.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_25(10)(2026)1011-1018_SupplData.pdf

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author Contributions

MS: Conceptualization, methodology, formal analysis, and writing, original draft. PSP: Validation, data curation, and writing review and editing. DK: Supervision, analysis, and revision. NKM: Resources, validation, and revision. NT: writing and preparation of manuscript. All authors reviewed and approved the final version of the manuscript.

Use of Artificial Intelligence (AI)

During the preparation of this manuscript, the authors used ChatGPT (OpenAI-GPT 5.6) solely for language editing, grammar correction, and improving the clarity and readability of the manuscript. The AI tool was not used to generate scientific data, perform data analysis, interpret experimental results, or make scientific conclusions. The authors take full responsibility for the originality, and integrity of the content presented in this manuscript.

Data Availability

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

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