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# Effect of novel extraction methodologies on quality aspects of bitter melon seeds (*Momardica charantia* L.) oil

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## Abstract
Food industries are always keen to search and adopt novel extraction methodologies to replace the traditional extraction method. Bitter melon leaves, bark, roots, stem and seeds were used in traditional medicine to cure various health associated diseases from ancient years. Bitter melon seeds one such byproduct obtained after processing of Bitter melons. Nutritionally and therapeutically, it is rich in various phytochemicals and active ingredients. Hence, in the present study, an effort has been made to utilize the Bitter melon seeds to extract oil using novel extraction methodologies (under per optimized extraction conditions), namely, microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and ohmic heat-assisted extraction (OAE). The bio-based solvent p-cymene was used for extraction. The quality aspect of extracted oil was assessed and compared with other extraction methods. The obtained results indicate that there is a significant change in the quality parameter in all the samples. The highest FFA of 8.37 (% oleic acid) for MAE followed by OAE (5.57 % oleic acid), CP (4.48 % oleic acid), SXE (4.37 % oleic acid), and lowest of 4.01 for UAE was found. A similar trend in changes in color, smoke point and acid value was seen. From the obtained analysed data, it can be inferred that ultrasound-assisted extraction can produce good quality of Bitter melon seeds oil than the other extraction method. Hence, UAE could serve as a better process intensification tool to extract high-quality, stable oil from biological materials. In addition, the quality of oil has a great influence on the method of extraction and processing conditions.

**Keywords:** Novel extraction methodologies, Bitter melon seeds oil, quality

## Introduction
Bitter melon (*Momardica charantia* L.) is commonly grown as a vegetable crop in the tropical and subtropical region of the world. It is widely known as Bitter gourd or karela and belongs to the family Cucurbitaceae [1]. The fruit, leaves, roots, bark and seeds are used in traditional medicine to cure various health-related problems such as cardiovascular and liver diseases, worms, rheumatism [2]. The presence of various biologically active compounds like terpenes, alkaloids, fatty acids, proteins and steroids have shown anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, hypoglycemic and anti-carcinogenic properties [3]. The seeds of Bitter melons are byproduct obtained from its processing, which is rich in oil, protein and fiber. The seed oil is a potential source of polyunsaturated fatty acids; hence, it has gained special in the scientific field of research. The oil contains α-eleostearic acid (63-72%), which provides potential health benefits, including lowering blood sugar level, anti-oxidant, antitumor and anti-atherosclerotic activities [2].

Extraction and separation of high standard viable compounds from bioresources is one of the vigorous processes in the production process. From the last two decades, evolution in extraction and sample preparation processes towards green analytical chemistry efforts has made towards enhancing the process efficiency and reducing the environmental impact [4]. Generally, oil extraction methods are influenced by physical and chemical characteristics, and their selection greatly influences the yield of oil. Improving the extraction efficiency of traditional extraction methods is a special area of interest in the extraction process. Conventional mechanical pressing or screw pressing is one of the age-old practice in the oil extraction process. Compliances like lower oil recovery percentage, considerable more time for extraction, and energy consumption have encouraged searching novel or green extraction methodologies [5].

Nowadays, traditional oil extraction methods are replaced by chemical or solvent, enzymatic and hybrid methods for industrial applications. Novel extraction methodologies like ultrasound-assisted extraction, microwave-assisted extraction, ohmic heat-assisted extraction, supercritical fluid extraction, radio-frequency assisted extraction, accelerated solvent extraction, cold plasma-assisted extraction have shown greater industrial potential with improved quality and higher yield. In addition, these methodologies considerably cause lower environmental pollution and $CO_2$ footprint [6, 7, 8, 9, 10]. Solvent extraction using n-hexane, acetone, petroleum ether, ethanol, and other solvent is being used. The solvent extraction process considers being effective; however, limitations like solvent residue, generation of volatile organic compounds to the environment, and higher specific energy consumption encourage green solvents' usage [11]. The Innovative Medicines Initiative-CHEM21 have been recognized as bio-based solvents such as p-cymene, d-limonene, α-pinene, β-pinene, and terpineol are safe and can be used as a solvent for extraction of valuable compounds with the specific safety measures in pilot production or kilo labs [12].

The present study aimed to utilize Bitter melon seeds to extract oil under pre-optimized extraction condition using novel extraction methodologies like microwave-assisted extraction, ultrasound-assisted extraction, and ohmic heat-assisted extraction with p-cymene as a green solvent. The quality parameters like color, viscosity, specific gravity, moisture content, refractive index, smoke point and free fatty acids level were evaluated and compared.

## Materials and methods

### Collection of raw material and chemicals

The matured Bitter melon seeds (moisture: 7±0.6 % w.b) were directly collected from a farmer's field, Shikaripura, Shivamogga District (India). Cold-pressed Bitter melon seeds oil was purchased from the local market, Chennai (India). The chemical reagents like p-cymene, sodium hydroxide, phenolphthalein indicator, ethyl alcohol, hydrochloric acids were purchased from Sigma-Aldrich, Bengaluru (India).

### Extraction of oil using novel extraction methodologies

The oil from Bitter melon seeds was extracted as per the pre-optimized process conditions. The optimized extraction condition for microwave-assisted extraction (MAE) is power level:630W, Solid/Liquid ratio: 1:10, and extraction time: 25 min; for ultrasound-assisted extraction (UAE) is ultrasound power level:700W, Solid/Liquid ratio: 1:10, and extraction time: 30 min and; for ohmic heat-assisted extraction (OAE) is electric field strength:35V, Solid/Liquid ratio: 1:10, and extraction time: 25 min [11].

For MAE, a household microwave system (900W) was used for extraction. UAE was performed using a horn-type probe (Sonic CV334, 220 V, 50/60 Hz) with the pulsation of 30 s "on" time and "off" time. A laboratory model ohmic heating apparatus (maximum sample volume: 250 ml) equipped with two rectangular stainless steel electrode (L=1.00mm, W=80.00mm, H=50.00mm) was used for OAE. The test was performed using p-cymene as a green solvent.

### Assessment of quality parameters of extracted oil

- **Color value**

Hunter Lab colorimeter (Model: ColorFlex EZ, USA) was used to measure the color of extracted oil. The values were expressed in terms of L* (lighter/darker), a*(redder/greener) and b* (yellower/bluer) [13]. Color index (CI) and hue angle (H*) was calculated as per the below-given equations.

$$H^* = 180 + \tan^{-1}\frac{b^*}{a^*} \quad (1)$$

- **Specific gravity**

The specific gravity of oils was measured according to the methodology described by AOCS Official Method no. Cc 10 a-25 at 30±2ºC [13].

- **Moisture content**

The moisture content of oil samples was measured using a digital moisture meter (Model: Mettler Toledo HE53, US).

- **Refractive index**

The refractive index of oil was measured by a refractometer (Model: RX-75000CX, Japan) at 25 °C according to the released approach by AOCS Cc 7-25 [15].

- **Viscosity**

The viscosity of extracted oils was analysed using rapid visco-analyser (Model: Anton-Paar MCR 52, Austria). The viscosity was measured at varying shear rate from 0 to 100/s, and a constant temperature of 25±0.50 ºC was monitored using a water bath. Approximately 1.0 g of oil was placed on the surface of the measuring platform, and a P/50 plate probe was used for measurement. The mean viscosity was calculated and expressed as mPa.s.

- **Smoke point**

The smoke point of Bitter melon seed oil was determined as described in AOCS official method Cc 9a-48 with little modification in the procedure [15]. The known amount (5 ml) of oil was poured into the porcelain cup, and the cup was heated rapidly at 150 °C. The k-type thermocouple was suspended at the centre of the cup. The final temperature was recorded when the oil starts producing continuous wisps of bluish smoke.

- **Free fatty acid value**

The free fatty acid level of extracted oil was determined by the titrimetric method described in AOAC 940.28 with little modification in the extraction procedure [14]. For estimation, 1 g of oil in freshly neutralised hot ethyl alcohol and about 1 ml of 0.1% phenolphthalein indicator solution. The mixture was boiled at 65 °C for 5 minutes and immediately titrated with 0.1 N of NaOH solution. The free fatty acid (as oleic acid) was calculated by using the following formula:

$$\text{FFA(as oleic acid)} = \frac{\text{ml of 0.1 NaOH} \times 0.0282 \times 10}{\text{weight of the sample}} \quad (2)$$

- **Acid value**

The acid value was determined as per the methodology described by the AOAC official method Cd 3a63 [14]. Approximately 2.0 g of oil sample was dissolved in 50 mL of neutralized methanol and then titrated with KOH (0.1 mol/L).

- **Statistical analysis**

One way analysis of variance (ANOVA) was performed using Minitab statistical software (version: 17). The analysis was performed in triplicates, and the obtained results were represented as mean value with standard deviation. The differences were considered statistically significant at a level of $p < 0.05$.

## Results and Discussion

### Color value

Color is considered one of the important characteristics of oil, and it reflects the overall quality and process undergone. During extraction, the cell wall gets disintegrate, and the color pigments get dissolved in a solvent. Generally, Bitter melon seeds oil resembles light/dark green in color. The significant variations ($p < 0.05$) in color value was noticed in extracted oils (Table 1). The L* was observed to be highest for CP (16.14) and lowest for SXE (2.69). Similarly, the highest a* of -2.45 for CP and lowest of -0.004 for MAE was found. It indicates that color pigments were degraded due to the effect of microwave power level and holding time. CP has shown the highest b* value of 7.74, which is comparatively higher than the other extraction methods. The results of H* indicates that there is a slight difference in CP and UAE, which highlights the lower degradation of color in UAE because the experiment was conducted at a lower temperature. From H* value, it has proven that the Bitter melon oil resembles green in color because it falls the hue angle around 180°. The color changes in case of OAE is due to electrochemical reactions, similarly in SXE, which may because of higher extraction temperature and process time [16].

### Moisture content

The moisture content indicates the stability of oils and fats. Higher moisture content may lead to hydrolysis of fatty acids. The rate of oxidation and rancidity is dependent on the percentage of moisture content. Hence, it is desirable to keep moisture as low as possible before storage [17]. The highest moisture content of 0.450% for CP and lowest of 0.203 for MAE, followed by UAE (0.206) was found. Our results are in accordance with the findings of Nagesh *et al.*, (2019) [18]. The higher moisture in the case of CP may because the oil was extracted by cold pressing. In addition, Bitter melon seeds may with higher moisture at the time of extraction. The lower moisture in MAE, UAE, SXE and OAE due to the heat/temperature may remove the moisture from the oil during treatment.

### Specific gravity

The significant differences in specific gravity of extracted oil were seen ($p< 0.05$). From table 2, it was observed that MAE had shown the highest specific gravity of 0.922, followed by SXE (0.910), CP (0.904), OAE (0.888) and UAE (0.884). The higher value of specific gravity in MAE maybe because of the microwave's detrimental effect, which has led to degradation of oil and formation of secondary oxidative products. On the other hand, due to the non-thermal effect of ultrasound, lesser oil degradation was found; hence, the specific gravity of oil was eventually lower than that of other oils. Abiodun *et al.*, (2020) [19] have reported that higher specific gravity may because of the $\pi$ bonds make the bonds more rigid and vigorous rotation between C-C chains. The reactive oxidative products like free fatty acids and peroxides get breakdown into secondary oxidative products like aldehydes, ketones and alcohols.

### Refractive index (RI)

The determination of the refractive index of oils and fats helps to distinguish the nature of samples. It gives useful information on saturation, conjugation, presence of hydroxyl substituted and chain length of fatty acids. Measurement of RI plays a major role in the hydrogenation process of oils and fats [20]. The oil obtained from Bitter melon seeds exhibited a different degree of refractive index (Table 2). It was observed that SXE extracted oil had the highest RI value of 1.503. Whereas for MAE, OAE, UAE and CP were found to be 1.499, 1.498, 1.483 and 1.477, respectively, at 25°C. It has been reported that RI directly relates to unsaturation [19]. The thermal decomposition of targeted compounds may be degraded in SXE; hence, RI was higher than other oil samples. Whereas in MAE, a sudden increase in temperature and internal pressure resulted in slight higher RI.

### Free fatty acids value (FFA)

Measurement of FFA level in fats and oils indicates the level deterioration; hence it is one of the most important quality characteristics in edible oil processing industries. From table 2, it was observed to be higher FFA of 8.37 (% oleic acid) for MAE and lowest of 4.01 (% oleic acid) for UAE, followed by SXE (4.37 % oleic acid), CP (4.48 % oleic acid) and OAE (5.57 % oleic acid). Higher FFA in case of MAE implies that oil has a higher tendency to become rancid than the oils extracted from other extraction methodologies. Furthermore, the microwave power level (630W) and longer time of extraction (25 min) may be reinforced and causes hydrolysis of fatty acids [21]. Similarly, OAE has shown the highest FFA value that may be due to hydrolysis of triglycerols with higher temperature. In addition, 0.5M NaCl aqueous solution was added to the p-cymene solvent at the time of extraction to improve the electrical conductivity; this may encourage to increase in the FFA content [11]. The lowest FFA was found because the lower temperature was used in UAE. The longer time of exposure and higher temperature may cause an increase in FFA in SXE.

### Smoke point (SP)

SP value is one most important quality parameter of oils and fats. Frying at high temperatures (>180°C) is a common processing method used for food preparation. In general, the oil used for frying purposes should have a higher SP value. At higher temperature, significant change in various physico-chemical quality aspect of oil occurs due to hydrolysis, isomerization and polymerization. Smoke point indicates the chemical breakdown of oils or fats into glycerol and free fatty acids [22]. The differences in SP value of oil extracted from various methodologies were seen (Table 2). The lower smoke point indicates, the temperature at which oils or fats begins to smoke continuously; thus, bluish smoke will produce. The bluish smoke results from the breakdown of glycerol into acrolein [22]. It was found that the highest SP value for MAE (160.58°C) <OAE (163.86°C) <SXE (164.57°C) <CP (164.71°C) < UAE (165.30 °C) were found. The value of SP was greatly dependent on FFA. In our findings, the SP values were consistent with the FFA values. The highest SP value in MAE shows a higher degradation of fatty acids due to hydrolysis and polymerization [11].

### Acid value (AV)

The acid value is an important sign of oils or fats quality, and it is widely used to measure the amount of FFA (AOCS, 1998). The acid value of MAE extracted oil was found to be around 10.67 (mg KOH/g), which was comparatively higher than the oils were extracted from other methodologies (Table

2). It indicates that the MAE extracted oil higher prone to oxidation with lower storage stability. OAE has shown AV of 6.09 (mg KOH/g) followed by 5.88 (mg KOH/g) for SXE, 4.430 (mg KOH/g) for UAE and 3.76 (mg KOH/g) for CP. There is no significant difference ($p > 0.05$) in PV of UAE and CP, it indicating that UAE resembles the same quality as CP. The WHO/FAO has established the scientific quality standard of permissible level of AV for edible is about 0.60 mg KOH or NaoH/g of oil [23]. However, in our case, the AV values were higher than that of WHO/FAO standards because extracted oils were unrefined.

## Viscosity

The viscosity of oils and fats mainly depends on shear stress and temperature. The universal fact that oil and fats are mainly composed of triglycerides (TAG); hence, the viscosity is strongly dependent on TAG's nature [19]. Figure 1, indicates that the viscosity of oils was decreased with the increase in shear rate. Hence, the viscosity of Bitter melon seeds oil can be categorized as a non-newtonian liquid. On the other hand, the viscosity of MAE found to be much higher than other oil samples. It indicates that the extracted oil may contain a higher percentage of FFA and secondary oxidative products due to oxidation. Our results are in accordance with the findings of Hasan and Khan (2020) [24] one who found a decrease in viscosity of canola-olive and canola-sesame blends. The changes in viscosity imply the difference in the arrangement of the fatty acids on the glycerol backbone of the TAG. Hence, the viscosity is associated with the changes in chemical properties of the oils/fats, such as chain length and saturation or unsaturation [19].

**Table 1:** Color value (L*, a*, b* and H*) and moisture content of oil samples

| Oil samples | L* | a* | b* | H* | Moisture content (%) |
|---|---|---|---|---|---|
| CP[1] | 16.140±1.019[a] | -2.450±0.095[a] | 7.740±0.484[a] | 178.736±0.007[a] | 0.450±0.020[a] |
| SXE[2] | 2.690±0.105[d] | -0.360±0.026[c] | 2.186±0.050[d] | 178.593±0.015[b] | 0.310±0.055[bc] |
| MAE[3] | 6.956±0.101[b] | -0.004± 0.062[e] | 4.206±0.097[c] | 178.432±0.002[d] | 0.203±0.066[c] |
| OAE[4] | 4.643± 0.107[c] | -0.056±0.032[d] | 2.510±0.095[d] | 178.469±0.009[c] | 0.330±0.020[b] |
| UAE[5] | 7.423±0.145[b] | -0.740± 0.062[b] | 4.660± 0.060[b] | 178.587±0.011[b] | 0.206±0.025[c] |
| F-value | 365.58 | 1813.22 | 284.54 | 452.59 | 17.38 |
| P-value | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| R-square value (%) | 99.32% | 99.86% | 99.13% | 99.45 | 87.42 |

**Table 2:** Quality parameters of oil extracted from novel extraction methodologies

| Oi l samples | Sp. Gravity | Refractive index (ºBrix) | FFA (% oleic acid) | Smoke point (ºC) | Acid value (mgKOH/g) |
|---|---|---|---|---|---|
| CP[1] | 0.905±0.012[b] | 1.477±0.004[d] | 4.487±0.283[c] | 164.713±1.376[a] | 3.760±0.040[d] |
| SXE[2] | 0.910± 0.000[b] | 1.503±0.001[ab] | 4.370±0.425[c] | 164.570±0.668[a] | 5.880±0.650[c] |
| MAE[3] | 0.922±0.002[a] | 1.499±0.000[bc] | 8.370±0.436[a] | 160.587± 0.839[b] | 10.670±1.070[a] |
| OAE[4] | 0.888±0.001[c] | 1.498±0.000[c] | 5.573±0.193[b] | 163.860±1.392[a] | 6.093±0.090[b] |
| UAE[5] | 0.884±0.002[c] | 1.483±0.001[d] | 4.013±0.42[c] | 165.303±0.825[a] | 4.430±0.450[d] |
| F-value | 24.21 | 82.42 | 65.11 | 8.77 | 62.62 |
| P-value | 0.00 | 0.00 | 0.00 | 0.003 | 0.00 |
| R-square value (%) | 90.64 | 97.06 | 91.68 | 77.82 | 91.86 |

[1]Cold press extraction; [2]Soxhlet extraction; [3]Microwave assisted extraction; [4]Ohmic heat assisted extraction and; Ultrasound assisted extraction

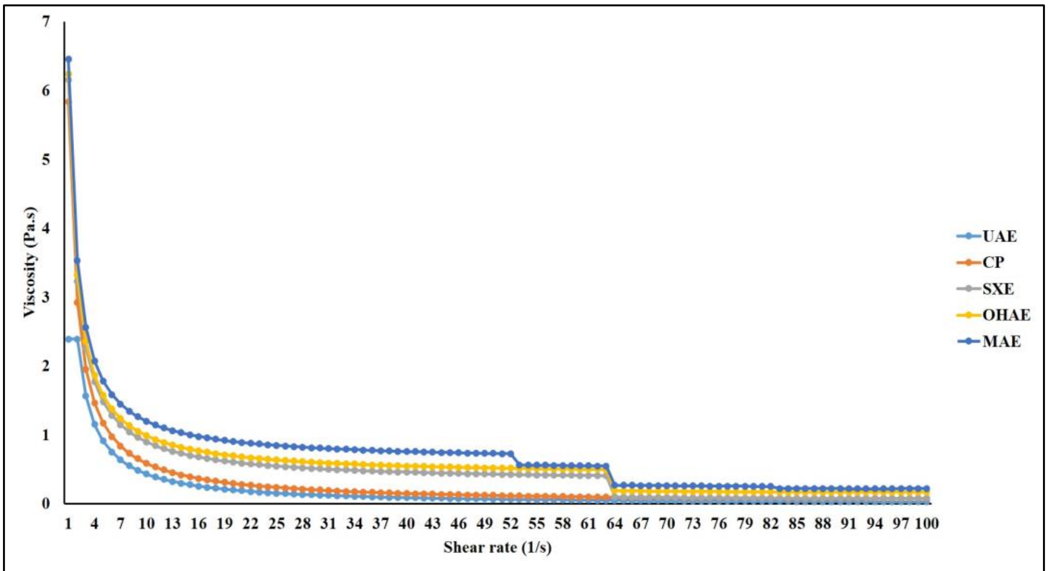


**Fig 1:** Changes in viscosity of Bitter melon seeds oil extracted from various methodologies

## Conclusion

The obtained results indicated that ultrasound-assisted extraction can produce high-quality Bitter melon seeds oil with greater stability and improved quality characteristics.

The significant differences or changes in color, moisture content, viscosity, free fatty acids level, acid value, and smoke point were found due to the method of extraction and processing conditions. The present study provides valuable information on the quality aspects of Bitter melon seeds oil extracted from various methodologies, which is useful for selection of appropriate method for extraction of oil. The quality and stability of edible oil play a prime role in acceptability of fats and oils. Hence, utmost care must be taken while choosing an appropriate method of extraction.

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