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# Comparative evaluation of sesame oil obtained with microwave-assisted enzymatic extraction and conventional solvent extraction

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**Abstract**
This research investigates the impact of extraction methods on sesame seed oil, specifically comparing traditional Soxhlet extraction (SE) with the relatively newer Microwave-Assisted Enzymatic Extraction (MAEE). While SE exhibited a higher oil yield (46.13%) compared to MAEE (45.20%), the difference was not statistically significant, suggesting MAEE as a viable alternative to conventional methods. These findings align with prior research, emphasizing SE's efficiency in oil extraction. SE, involving solvent circulation, yielded a darker oil due to increased pigment extraction. Specific gravity values showed a slight decrease in MAEE (0.915) compared to SE (0.917), yet the difference was not significant. The refractive index (RI) of MAEE-extracted oil (1.463) was significantly higher, indicating potential advantages in oil quality. MAEE's transparency to monochromatic light suggests a preference for obtaining high-quality oil from sesame seeds. Free Fatty Acid (FFA) values were higher in MAEE (1.33%) than SE (1.19%), signifying potential benefits in oil quality and stability. The use of enzymes in MAEE may mitigate oxidation and degradation during extraction, contributing to lower FFA values. Saponification values were significantly higher in SE (197.34) compared to MAEE (192 mg KOH/g), attributed to solvents extracting a wider range of fatty acids. The degree of saturation, determined by iodine value, was significantly higher in MAEE (111.19) than SE (107.6), possibly due to enzymatic hydrolysis resulting in a higher yield of unsaturated fatty acids. Peroxide values were non-significantly different between MAEE (1.79 meq$O_2$/kg) and SE (1.72 meq$O_2$/kg), suggesting oxidation in sesame oil is influenced more by intrinsic factors than extraction technique. MAEE's potential advantage lies in improved shelf-life and stability, attributed to its higher antioxidant content. MAEE presented as a promising alternative with potential benefits in terms of oil quality, transparency, and stability.

**Keywords:** Sesame seed, microwave, enzyme, green solvent, physicochemical properties, extraction efficiency

## Introduction

India is the largest agrarian subcontinent supporting 26% world's agricultural population on 12% arable land. India has emerged as the world's fifth-largest vegetable oil economy, accounting for 7.4% of oilseed production, 5.8% of oil production, and 6.1% of oil meal production. Additionally, India consumes 9.3% of the world's edible oil. Oilseeds are the second most important crop in India after cereals, and their production has grown at a rate of 4.1% per year over the past three decades (Jat *et al*., 2019) [6]. India is the 4$^{th}$ largest oilseeds producer in the world. It has 20.8% of the total area under cultivation globally, but accounting for only 10% of global production. The country produces groundnut, soybean, sunflower, Sesamum, Niger seed, mustard, and safflower oilseed. The production of oilseeds in India has been increasing for the last five years. In 2020-21, the production of oilseeds, was 365.65 lakh tonnes which was a 10% increase over the preceding year. During the year 2015-16 to 2020-21, the compound annual growth rate (CAGR) of oil seed production was 7.7% (National food security mission, 2022). Despite significant progress achieved in the oilseeds production in India, vegetable oils import, during 2017-18 was around 14.59 MT (about 60% of the total need) thus, India became the third-largest edible oil importer. India also imports soybean oil from Argentina and Brazil, as well as palm oil from Malaysia and Indonesia (Commodity Profile of Edible Oil, 2019) [3].

Due to increasing prices, India's expenditure on imported edible oils rose by 34.18% to Rs. 1.57 lakh crore by the end of October 2022. However, the volume of imports increased by 6.85%, reaching a total of 14.03 million tonnes. There has been great emphasis on edible oil production to make India self-sustainable, as a result, during the last 20 years, the production of edible oils from all sources increased (+84%) from 5.5 MT in 2000-2001 to 10.1 MT in 2016–2017. With the rising economy and changing food habits, from 2009-2010 to 2016-2017, the per capita consumption of edible oils in India increased by 41%, rising from 13.3 kg to 18.7 kg per head per year (Agricultural statistics at a glance, 2017). Over the past twenty years, the total consumption of vegetable oils and fats has risen from 9.7 million tonnes in 2000-2001 to 22.4 million tonnes in 2021-2022 (Statista 2023). Despite being the world's largest producer of oilseeds, India continues to be a net importer of these oilseeds. In India, the largest source of oil has been the seeds of annual plants such as ground nut, soybean, mustard/rapeseed, sesame seed and sunflower. Sesame seed oil is a popular edible oil, widely used in cooking, food preparation, and traditional medicine due to its high nutritional value, antioxidant properties, and health benefits (Uzun *et al*., 2008) [20]. However, conventional methods of extracting sesame seed oil involve the use of hazardous organic solvents, such as hexane, which raises environmental and health concerns (Prat *et al*., 2015) [13]. In recent years, the development of green solvent technology has gained considerable attention in the field of oil extraction as a sustainable and eco-friendly alternative to conventional solvent extraction methods. Microwave-assisted enzymatic green solvent extraction is a technique that has been used successfully in extracting various bioactive compounds from plants (Kuo *et al*., 2012) [8]. In recent years, this extraction technology has gained increasing interest in oil extraction, as it offers several advantages over conventional methods, such as faster extraction times, lower solvent consumption, and higher extraction yields (Zhang *et al*., 2018) [24]. Combining MAE with green solvents and enzymes has been shown to enhance extraction efficiency while reducing the environmental impact of the extraction process. In this study, we aim to investigate the use of microwave-assisted enzymatic extraction technology to extract and characterise sesame seed oil and compare with the conventional solvent extraction in terms of its physicochemical properties including iodine value, refractive index, Specific gravity, free fatty acids (FFA), peroxide value (PV), and saponification values of the extracted oils. The selection of appropriate enzymes, soaking time, and amount of solvent are crucial for maximizing oil yield in extraction processes. These all three-parameter selected by the preliminary experiment.

## Materials and Methods

In the current study, a comparative evaluation was conducted to understand the MAEE system performance conventional solvent extraction technique (Soxhlet extraction) based on physical and chemical parameters of the extracted sesame oil.

### Materials and Preparation of sample

Sesame seeds, sourced from ICAR-Indian Institute of Oilseeds Research Hyderabad, underwent meticulous cleaning via a specific gravity separator to eliminate foreign material. The study focused on selecting undamaged and bold kernels. Subsequently, the cleaned sesame seeds were dried in a hot air oven, maintaining up to 10% moisture content (Dry basis) as per the method outlined by Sahay and Singh in 2003 [15]. To enhance oil extraction efficiency, it is crucial to increase the surface area of contact between the material and the extracting solvent. This necessitates rupturing the cell wall of the seeds for optimal oil yield. The dried sesame seeds were ground for 30 seconds using a laboratory mixer-grinder, and the resulting sample was sieved to meet the specified particle size (<0.4 mm) during the experiment.

To prevent rancidity and the formation of free fatty acids, the dehulled ground powder was carefully packed and sealed in airtight polythene bags. This preserved material was stored in a refrigerator at 4 ˚C until utilized for subsequent experiments, following the guidelines presented by Kanitkar *et al*. in 2011 [36].

### The microwave-assisted enzymatic extraction procedure

The extraction process involved a flat-bottom flask containing ground sesame seeds and one of the five selected enzymes and solvents. Each experiment used approximately 50 g of ground sesame seeds (Particle size < 0.4 mm) placed in a 500 ml flat-bottom flask. Subsequently, 150 ml of solvent was added, and the mixture was thoroughly shaken for 40 minutes to ensure proper solvent-solid interaction. After the soaking time, the solvent-seed mixture was exposed to a microwave at a power level of 350 watts for a treatment duration of 180 seconds to extract oil from the sesame seeds.

Following the microwave treatment, an overhead glass condenser was attached and sealed on the extraction flask. Upon completion of the designated treatment times, defatted meal separation from the miscella was achieved through vacuum filtration using Wattaman No. 1 filter paper. The filtrate obtained was then transferred to a rotary vacuum evaporator for oil and solvent separation. The rotary evaporator functioned by heating the mixture under reduced pressure, causing solvent evaporation, and leaving the oil behind. After solvent removal, the oil was collected in a clean glass vial or bottle under reduced pressure, as described by Terigar *et al.* in 2011 [18].

Extraction oil yield is the ratio of the amount of oil extracted to the amount of a given sample and expressed in percentage. (Li *et al*., 2004; Kwiatkowski and Cheryan, 2002) [9, 25].

$$\text{Oil yield (\%)} = \frac{\text{Amount of oil extracted(g)}}{\text{Weight of sample (g)}} \text{x100}$$

### Soxhlet (Hexane) method of oil extraction

The oil present in the ground sesame seed sample was determined by the standard Soxhlet extraction method (AOAC, 1984). Five grams fresh ground sesame seed sample was taken in a thimble and plugged with fat-free cotton. This filled thimble was fitted in the extraction tube of the apparatus. A 250 ml extraction flask was set on the water bath heater for constant and uniform heating. The extraction tube was then fitted in the neck of the flask. Approximately 100 ml of hexane solvent was filled in the extraction tube in such a way that it could be syphoned in the extraction flask through the sample. After fitting the overhead condenser on the mouth of the extraction tube, heating was started. Thus, the sample was extracted in a repeatable manner for 14 hours. After completion of extraction time, extraction assembly was detached. For the separation of the solvent (n-hexane), extraction flask was kept in vacuum oven at 105 ºC for 2 hours. Then, weighing of the cooled flask with oil was done and oil content in the sample was estimated by using equation.

$$\text{Oil yield (\%)} = \frac{\text{Amount of oil extracted(g)}}{\text{Weight of sample (g)}} \times 100$$

**Physical and Chemical Analysis of the Extracted Oil**

The physicochemical properties of sesame oil, extracted through microwave-assisted enzymatic extraction and soxhlet (Hexane) method of oil extraction. The obtained oil underwent assessment based on physical and chemical parameters, including iodine value, refractive index, specific gravity, free fatty acids (FFA), peroxide value (PV), and saponification values. Standard AOCS methods from 1997 were employed to determine these properties. The refractive index was measured using a refractometer.

**Statistical analysis**

The data underwent statistical analysis through analysis of variance (ANOVA) using IBM SPSS Statistics 26.0. All experiments were conducted in triplicate. Statistical significance was established at a probability value of 0.05. The presented data represent mean values and standard deviations derived from determinations made in triplicate.

**Results and Discussion**

The effect of extraction methods on oil extraction from sesame seeds were analyzed. The results reveal that oil yield obtained through Soxhlet extraction was higher (46.13%) compare to the MAEE (45.20%). The oil extracted through MAEE was lower than that obtained through SE, but not significantly. These findings suggested that MAEE could be a viable alternative to conventional methods of oil extraction for sesame seeds. The obtained results are in line with the research conducted by Savoire *et al*., 2013 [16], which concluded that SE is a highly efficient method for extracting oil with a greater yield in comparison to other methods. Soxhlet extraction is a widely used method for the extraction of lipids from various sources. It involves the use of a solvent, usually hexane, which is circulated through the sample multiple times to extract the oil. This method is known to produce a high yield of oil but is time-consuming and requires the use of large amounts of solvent. Microwave-assisted enzymatic extraction (MAEE) is a relatively newer method of oil extraction that involves the use of microwave radiation to heat the sample, combined with the use of enzymes to break down the cell walls and release the oil. This method is known to be efficient and can result in a high yield of oil while also reducing the extraction time and the amount of solvent required.

Oil color is a crucial quality parameter that affects the appearance and acceptability of extracted oil. In the case of sesame seed extracted oil, the color was significantly higher in SE (86.5) as compared to MAEE (66.5). The color of the extracted oil by SE was 20% darker than MAEE respectively. An analysis of variance was performed to investigate the effect of extraction techniques on oil yield, and it was observed that these techniques had a significant effect on oil yield ($p$<.0001). The color of the extracted oil is due to the difference in the amount and type of pigments that were extracted by each method. Soxhlet extraction (SE) is a time-tested method that has been used extensively for the extraction of oil from various sources. This method involves heating the plant material with a solvent, which dissolves the oil and then evaporating the solvent to obtain the oil. This process is likely to extract a higher amount of pigments from the plant material, resulting in a darker colour of the extracted oil.

The oil extracted by the MAEE method had the lower specific gravity value (0.915) compare to the SE (0.917). However, the specific gravity values of oils extracted through these methods did not significantly differ from each other. To further investigate the effect of extraction methods on the specific gravity of sesame oil, ANOVA was conducted. The results revealed that the extraction methods did not have a significant effect on the specific gravity of the oil at both the 1% and 5% levels of significance.

The MAEE method resulted in the lower RI (1.463) compare to the SE (1.468). The analysis of the RI of extracted oil obtained by different methods. The results indicated that the RI of extracted oil using the MAEE method was significantly higher than the other methods used for oil extraction at a 5% level of significance. The findings suggested that the MAEE method of oil extraction could be a preferred option for obtaining high-quality oil from sesame seeds. This could be due to the reason that MAEE extracted oil is free from suspended particles and more transparent to the incident monochromatic light of the refractometer. The RI of the extracted oil is influenced by factors such as the degree of saturation, molecular weight, and presence of impurities.

The Free Fatty Acid (FFA) value is an essential parameter that indicates the quality and stability of the extracted oil. The MAEE method yielded the higher FFA value (1.33) compare to the se method (1.19%). The FFA values of the oils extracted by SE and MAEE methods were significantly different from each other at a 5% level of significance. For instance, the use of enzymes in MAEE can help break down the cell walls of the plant material and facilitate the release of oil without generating high temperatures, which could reduce the degree of oxidation and degradation of the oil. Similarly, SE uses solvents to extract oil under mild conditions, which can also result in lower FFA values. A similar trend was found by Uquiche *et al*. (2008) [19] for chilian hazelnut oil.

The saponification value of SE-extracted oil was 197.34, which was significantly higher than that of the MAEE-extracted oils were 192 mg KOH/g. The higher saponification value observed for the SE-extracted oil in comparison to the other methods could be due to the use of solvents, which can extract a wider range of fatty acids from the oilseeds, including shorter chain fatty acids. This could result in a higher average molecular weight and a higher degree of unsaturation in the extracted oil, leading to a higher saponification value This result is similar to the findings of Terigar *et al*. (2011) [18].

The degree of saturation of sesame oil can be determined by its iodine value. The extracted oil using MAEE showed a significantly higher iodine value (111.19) compared to the values obtained from the SE methods (107.6). The iodine value of oil obtained by the SE and MAEE methods varied significantly at a 5% level of significance. For example, it has been reported that enzymatic hydrolysis can result in a higher yield of unsaturated fatty acids compared to other extraction methods. This can result in higher purity and concentration of the oil in the extract, which may contribute to the higher iodine value (Terigar *et al*., 2011) [18]. In addition to these factors, the chemical composition of the oil itself can also contribute to the higher iodine value.

The peroxide values of the extracted oil obtained through the MAEE and SE methods were found to be non - significantly different with values of 1.79and 1.72 meq$O_2$/kg, respectively. The non-significant differences in peroxide values among the extracted oils using different techniques could be attributed to the fact that the oxidation of sesame oil is mainly influenced by the presence of unsaturated fatty acids and antioxidants, rather than the extraction technique. In general, sesame oil is known to contain high levels of unsaturated fatty acids and natural antioxidants, such as tocopherols and lignans, which can protect the oil against oxidation. However, the MAEE technique may be advantageous in producing sesame oil with improved shelf-life and stability. Higher oxidative stability of the MAEE oil may arise from the high antioxidant content in extracted oil (Aguilera and Stanley, 1999) [2].

## Conclusion

Microwave-assisted enzymatic green solvent extraction can be an effective alternative to the conventional solvent extraction method for the extraction of oil from sesame seeds. In conclusion, the study investigated the impact of different extraction methods on oil extraction from sesame seeds, focusing on Soxhlet extraction (SE) and Microwave-Assisted Enzymatic Extraction (MAEE). The results demonstrated that Soxhlet extraction yielded a higher oil percentage compared to MAEE, albeit not significantly different. The findings suggest that MAEE could serve as a viable alternative to traditional methods, providing a high oil yield with reduced extraction time and solvent usage. The color analysis revealed that SE produced significantly darker oil compared to MAEE, with a 20% difference. This discrepancy is attributed to the higher extraction of pigments by the Soxhlet method. Specific gravity values indicated a slightly lower value for MAEE, though not statistically significant. The refractive index (RI) of MAEE-extracted oil was lower, suggesting greater transparency, possibly due to the absence of suspended particles. Free Fatty Acid (FFA) values were higher in MAEE, indicating potential benefits in terms of oil quality and stability. The use of enzymes in MAEE may contribute to lower oxidation and degradation during extraction, leading to lower FFA values. Saponification values were higher in SE-extracted oil, potentially due to solvents extracting a broader range of fatty acids, resulting in higher molecular weight and unsaturation. The iodine value, a measure of the degree of saturation, was significantly higher in MAEE-extracted oil, suggesting a higher concentration of unsaturated fatty acids. Peroxide values showed non-significant differences between MAEE and SE, indicating that oxidation was influenced more by the oil's intrinsic composition than the extraction technique. However, the MAEE method may offer advantages in terms of shelf-life and stability due to its higher antioxidant content. MAEE showed promise as an efficient and potentially superior alternative, offering advantages in terms of oil quality, transparency, and stability. The choice between extraction methods should consider the specific requirements for oil properties and production efficiency. The findings can be useful for the development of new methods for oil extraction that utilize enzyme and solvent combinations to increase efficiency and yield.

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