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Original Research Article

Isolation and Enrichment of Omega Fatty Acids from *Ocimum basilicum* Seeds

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Abstract: The medicinal plant *CIM-Soumya* (*Ocimum basilicum*) variety has been globally adopted for the extraction of essential oil. This crop is capable of producing 400-500 kg of seeds per hectare. This seed-oil has been explored in the present study for its nutraceutical application. Ocimum, a non-explored seed-oil, contains ω-3 fatty acids (linoleic acid: 24.8%, and linolenic acid: 51.4%). It contains saturated fatty acids (SFA-12.0%), monounsaturated fatty acids (MUFA-10.7%) and polyunsaturated fatty acids (PUFA-76.2%). The process has been optimized for the production of free fatty acids (FFAs) from ocimum oil using aqueous-alkali solution at temperature (78 °C), agitation (180 rpm), time (4 h), and aqueous alkali-FFAs ratio (6:1) with 93% of total FFA yield. The SFAs were separated from unsaturated fatty acids (UFAs) through the urea-complexation process at the optimized conditions such as fatty acid-urea ratio (1:2), crystallization temperature (8 °C), crystallization time (100 min), reflux temperature (75 °C), and reflux time (65 min) with a yield of 75%. The enriched UFAs was composed of ω-6-fatty acid (27%) and ω-3-fatty acid (61%).

Keywords: Ocimum Basilicum, Extraction, Urea Precipitation, Omega Fatty Acids, GC-FID, TGA.

1. INTRODUCTION

Tulsi (Basil), also referred to as the 'Herbe Royale' in French literature, (Tyagi *et al.,* 2025) is a medicinal as well as an aromatic herb/shrub that is used in several forms like, as an ornamental plant, culinary herb, flavoring agent, and as an important bioactive aromatic ingredient in the food and cosmetic industry, as well as in the households. The demand for basil essential oil shows a positive growth trend as a result of which, the cultivation is also ever expanding. Genus *Ocimum* contains about 150 species of herbs and *basilicum* is one of them (Purushothaman *et al*., 2018). Commonly referred to as 'Sweet Basil', *Ocimum basilicum,* is a commercially and culturally important variety of the genus '*Ocimum'* that belongs to the family *Lamiaceae*. This variety was first published by Linnaeus in his book species plantarum (Tyagi *et al*., 2025). The plant is compatible with a hot and humid equatorial climate and thrives abundantly in such countries. Major Asian countries that soar high in the production of this plant are India, Burma, Sri Lanka, Thailand, Pakistan, etc. This species is native to India and is intensely cultivated in the tropical regions of the country (Tyagi *et al*., 2025). The plant is cultivated an annual crop in the fields (Tyagi *et al*., 2025). Water stress has been known to hamper the production of essential oil in this variety. Hence, during the summer season, weekly irrigation is practiced (Tyagi *et al*., 2025). This species has also been favoured a lot by the English and French breeders during the 19th century (Tyagi e*t al*., 2025).

Globally, Sweet Basil has been a major part of the Italian cuisine in the Ligurian province. Pesto, a very famous Italian pasta sauce, uses it as the main ingredient (Tyagi *et al*., 2025). The *basilicum* species generally

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contains 20 chemically important compounds like linalool, methyl eugenol (a major active compound) etc. Monoterpenes like Camphor, limonene, citral etc. are also present. The Sweet Basil flowers have an intense purple colour due to the presence of pigmentation compounds called anthocyanins (Purushothaman *et al.,* 2018).

The *O.basilicum* variety is mostly cultivated with the purpose of extraction of essential oil from the above ground herbs with a recovery of only 0.5-0.7%. The resultant seed-oil contains ω-fatty acids (UFAs). The seed oil of *O. sanctum* (Holy Basil) seed-oil has been reported to be a chemopreventive (Prakash & Gupta, 2000), immunomodulatory (Mediratta *et al*., 2002), anti-inflammatory agent (Singh *et al*., 1996), anti-microbial against resistant strains, an anti-pyretic, an anti-coagulant, etc. (Singh *et al*., 2007).

Several varieties of *O.basilicum* have been developed by the CSIR-CIMAP institution. *The CIM-Soumya* variety has been known for its various commercial applications. The essential oil of this crop, rich in Methyl Chavicol, a major chemotype in several *basilicum* varieties*,* has been explored to great depths with the globally recognised establishment of its commercial value. In the South Asian region, India has been known to be a major producer of this essential oil with an annual production of nearly 1000 tonnes. Apart from this, the crop is capable of producing 400-500 kg of seeds (per hectare). This seed-oil has been explored in the present study for its nutraceutical activities. The demand for edible oil is ever increasing. Hence, palm oil is now being considered as a substitute with the aim of minimizing the demand-supply gap which will help increase the production by 3-times of its previous level. However, palm oil has been known to contain a very high percentage of saturated fatty acids (SFAs), which negatively impact our health 10.

Vegetable oil, rich in omega-fatty acids and has lesser trans-fatty acids (TFAs) as well as lower levels of cholesterol, remains a consistently demanded product amongst the health conscious consumers for good health (Dhaka *et al*., 2011). Primarily, the developing nations' demand for edible oils has been rapidly increasing, primarily by the developing nations. Alternative oil-seed crops that are capable of producing a good quantity of essential oils are now being explored to meet the positive demand growth. (National Research Council, 1981). Hence, the goal of this work is the assessment, characterization, and exploring the potential applications of the ocimum seed-oil extracted from the non-explored plant, *O.basilicum*. The *basilicum* seed-oil has been reported to have a higher percentage of unsaturated fatty acids (UFAs), such as ω-fatty acids. Edible oils, rich in UFAs help reduce the risk of cardiac ailments.

In this project, the extracted ocimum seed-oil has been hydrolysed using alkali in an aqueous-alkali medium to produce FFAs. These FFAs were then used as a substrate for the enrichment of UFAs through the urea-complexation method to get free UFAs from higher SFAs.

2. MATERIALS AND METHODS

2.1 Chemicals Used

Substrates and chemicals like NaOH, NaCl, HCl, urea, anhydrous Na_2SO_4 and organic solvents like hexane, ethyl acetate, ethanol were used, purchased from Merck, India.

2.2 Source for Seed Collection and Extraction Method

Mature seeds of the *O. basilicum* variety '*Soumya'* mature were collected from the CSIR-CIMAP (Lucknow, India) experimental field. The seeds were then dried and their grounded powder was then utilised for isolating the desired fatty oils. Nearly 500 g of the seed powder was then put in the soxhlet apparatus and were extracted with hexane for a duration of 6 h. Post extraction, the solution was filtered and the solvent was evaporated, in a rotatory evaporator (Buchi make) in a vacuum environment of 150 mbar. The oil obtained post evaporation, was stored in a refrigerator with the purpose of analysis and experimentation.

2.3. The Physicochemical Characterization of the Extracted Fatty Oil

For the purpose of measuring the refractive index (RI), the ATAGO refractometer (RX 7000 α) was put to use.

Kem densitometer (DA 500) at a temperature of 20 °C, was also used to determine the specific gravity of the obtained oil.

2.3.1. Acid Value

The titration method was used to determine the acid value (FSSAI, 2015). In simple words, 2 g of the obtained oil transferred into a conical flask, and 25 mL of ethanol was then along with 1 mL of the naphthalene indicator. The developed solution was then stirred occasionally till oil got fully dissolved in ethanolic medium. After this, the solution was slightly heated only to make it seem warm and then it was titrated against a 0.1 N KOH solution. The acid value of the oil (mg KOH/g) was then computed using the following formula: '56.1*V*N/W', where V is the volume of KOH (in mL), 'N' stands for the normality of the KOH solution, and 'W' describes the weight of the oil (g) taken for titration.

2.3.2. Percentage of Free Fatty Acid (FFA)

The percentage of FFA was computed from the acid value and it turned out to be 50% of the acid value.

2.3.3. Saponification Value

The titration method was used for the calculation of the saponification value of the oil. Nearly 2 g of the oil was taken in a conical flask with about 25 ml of ethanolic KOH solution (0.5 N). The obtained

mixture was then placed in a water bath for about an hour for the completion of the saponification process of the oil (Mohanty *et al*., 2021). The amount of the unspent KOH solution was estimated with the help of a titration method that uses a HCl solution of known strength, with phenolphthalein as the indicator for the determination of the end point of titration. The saponification value (mg KOH/g) was computed using the following formula - '56.1*(B − S)*N/W', where 'B' in "ml" stands for the volume of the standard HCl required for blank titration, 'S' (ml) denotes the volume of the standard HCl meant for the titration of the sample, 'N' is the normality of the HCl solution with a known strength, and 'W' denoted the weight (g) of the oil taken for titration.

2.4. Preparation of Free Fatty Acids

The obtained oil was hydrolysed with a 4% aqueous NaOH solution, and then the optimization study of the process parameters was performed. The process has been optimized based on the laboratory optimized protocol (Naziya *et al*., 2025). The ratio of the obtained oil to the aqueous solution as per the protocol was 1:6, the temperature was 75 °C, agitation at 180 rpm and the duration of the hydrolysis time was 4 h. After this, the reaction mixture using HCl was maintained at pH value 5. It was then kept for 2 h to clearly separate the oil from the aqueous layer. The upper layer, the oily component, was then collected and washed with warm water (50 °C) repeatedly, to completely remove the acid-alkali along with the glycerol part. Post removal of the acid, alkali and glycerol, the solution was then dried using anhydrous $MgSO_4$. Finally, the FFAs were stored in a refrigerator for their characterization and purification.

2.5. Urea-Complex Precipitation

For the selective isolation of UFAs from the obtained oil, the stored FFAs were mixed with 95% ethanol (aqueous) and were stirred at higher temperature (not extremely high) to the point at which the mixture transformed into a clear solution. This reaction was carried out as per the laboratory developed protocol (Naziya *et al*., 2025). After this, distinct oily and crystal phases were obtained. The FFAs to urea ratio (1:2) and FFAs to ethanol ratio (1:10) was maintained at 10 °C in 2 h. After this, ethanol was removed from the oily phase. The concentrated liquid was then repeatedly washed with warm water saturated with warm water at 50 °C for getting the UFA-rich part. The released FFAs floated above the aqueous phase, which was later separated. Then, it was repeatedly washed with NaCl saturated warm water (at 50 °C), and dried over anhydrous $MgSO_4$. Mostly, UFAs do not participate in the complex formation process. Hence, they are isolated in the liquid phase very easily.

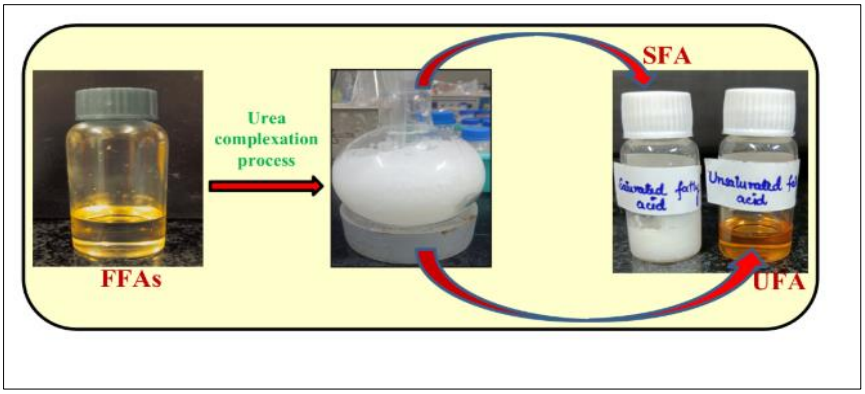


Figure 1: Urea Complexation Process
(Source: Taken in the lab)

2.6. Gas Chromatography (GC) and Mass Spectrometric (GC/MS) Analysis

After the previous steps, the samples were derivatized to Fatty Acid Methyl Ester (FAME) as per the official protocol (Hammond, 1993). The FFA composition was determined using Agilent GC together with a Flame Ionization Detector (FID). The supelcowax capillary column was used with the dimensions, 25m x 0.25 mm x 0.25 mm. Under 42 Kpa, the initial column pressure, H_2 acted as the carrier gas with a flow rate of 1.2 ml/min and 50:1 split ratio. 265 and 275 °C, were the set temperatures of the injector and FID respectively. The temperature of the GC oven was set to 120-180 °C (2 °C/min), and 180-235 °C (4 °C/min). For the confirmation of the compound, PerkinElmer GC/MS, was also used at the same conditions as set in GC-FID. The relative percentage of the area of the compounds was determined with the help of the peak normalization method. The MS parameters were as follows: 1.5 V detector voltage, 70 eV ionization voltage, 2 s peak width, 50-400 amu mass range. Individual peaks were validated through the comparison of the compound mass spectra to mass spectra from NIST-1, NIST-2, Adams computer libraries.

2.7. Characterization of Urea Crystals with FFAs

2.7.1. Thermogravimetric Analysis (TGA)

TGA evaluations of the enriched fractions and urea crystals from the fatty oil were analyzed through the TGA/DSC 1 Star system of Mettler-Toledo make. About 10 mg of the samples for the evaluation were taken in the silica crucible and put in the weighing panel. The

difference in the weight (weight loss) was then recorded in the temperature program of 30-600 °C (10 ºC/ min) that used nitrogen as a purge gas at a flow rate of 40 mL/min. The TGA data was further evaluated by taking the first derivative to obtain differential thermo-gravimetry (DTG).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Analysis of Fatty Oil

The oil extracted from the seeds of O. *basilicum* was pale yellow and had the following properties: The refractive index came out to be 1.4; density, 0.9 g/cm^3; the acid value was 4.1 mg of KOH/g of oil; the percentage of free fatty acids was 0.2%; saponification value, 164 mg KOH/g of oil.

3.2. Preparations of FFA from Ocimum Seed Oil

The oil was hydrolysed with the help of a 4% aqueous NaOH solution, and then the optimization study for the process parameters was performed. The ratio of the oil to aqueous solution was 1:6, temperature 75 ºC, agitation at 180 rpm and the hydrolysis duration was 4 h. The reaction mixture was then maintained at pH 5 using HCl, and then kept for 2 h for the clear separation of the oily and the aqueous layer. The upper o layer of oil was collected and then washed with warm water (50 ºC) repeatedly to ensure the complete removal of the acid-alkali along with glycerol. It was then dried over anhydrous $MgSO_4$. The FFAs were then stored in a refrigerator for their characterization and purification.

3.3. Enrichment of UFA Using Urea Complexation Process

The optimised protocol has been developed considering the variable parameters such as the temperature, time, rpm, and oil-aqueous alkali ratio. The optimized parameters are as follows, FFAs to urea ratio -1:2 and FFAs to ethanol ratio - 1:10 at 10 ºC in 2 h. After carefully following the above mentioned parameters, the oily phase (UFA) and the crystal phase became visible. The upper oily layer of FFAs in the ethanol was then separated and the ethanolic portion was removed using the rotavapour. The oily part was then repeatedly washed with warm water to get the UFA rich part. The UFAs were then dried over anhydrous Na_2SO_4. The lower phase of urea-crystal was then treated with warm water. The aqueous phase was then partitioned using hexane to get the SFA concentrated portion. The hexane layer was then washed repeatedly with water and then dried anhydrous Na_2SO_4. This urea-complexation process has shown to be effective for the full separation of SFAs in the form of urea-complexes. The chemical composition of enriched UFA is given in **Table 1.**

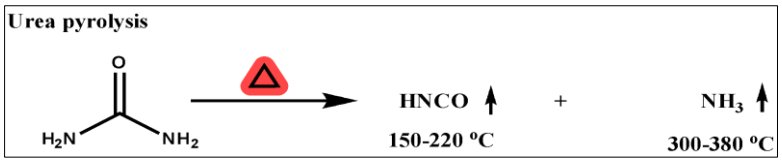
Table 1: Chemical composition of oil, UFA rich-portion and SFA rich-portion

Compound	FFA* (%)	UFA (%) enriched portion	SFA (%) enriched portion
Yield of fatty oil (%)	93.0	75.0	13.9
Palmitic acid (16:0)	8.5	0.4	40.2
Stearic acid (18:0)	3.5	0.3	32.5
Oleic acid (18:1)	10.7	8.5	14.8
Linoleic acid (18:2)	24.8	27.0	6.3
Linolenic acid (18:3)	51.4	61.0	2.9
Total	98.9	97.2	96.7
SFA	12.0	0.7	72.7
MUFA	10.7	8.5	14.8
UFA	76.2	88.0	9.2

***FFA is calculated from 100 G of Oil**

The ocimum oil was composed of SFAs (12.0%), MUFAs (10.7%) and PUFAs (76.2%). From the point of view of nutrition, it showed a higher percentage of oleic acid (10.7%), linoleic acid (24.8%), and linolenic acid (51.4%). It consisted of SFAs like palmitic acid (8.5%), and stearic acid (3.5%). From hydrolysis, 93.0% FFAs were produced. Further, urea-crystallization process produced 75.0% of UFA rich-fractions. These fractions selectively contained 96.5% of UFAs including ω-6-fatty acid (27.0%), and ω-3-fatty acid (61.0%). As a result of their straight molecular structure, these SFAs were tightly bound with urea and got separated as crystals (solid residue). This crystallized portion contained 24.0% of UFAs and had a higher percentage of SFAs (72.7%). The liberated UFAs had oleic, linoleic, and linolenic acids. This optimised process removed the SFAs in the form of SFA-urea crystals with very few percentages of UFA as per the degree of unsaturation.

Pure urea together with the synthesized urea-complex, enriched with UFA and SFA was analyzed on TGA, and their degradation profiles were also compared to that of the extracted ocimum seed oil (Figure 2a). It was observed that the urea crystals started degrading at 150 °C, and generated equimolar HCNO and NH_3 on breaking down (Zhu *et al*., 2021). The percentage of the liberated was 68.89% (Step 1 of degradation 150-220 ˚C) of the total mass loss and 31.53% (the remaining part) (Step 2 of degradation 300-380 ˚C) loss in mass was then observed due to the evolution of NH_3. This fact is also supported by the molecular weight percentages of HCNO (71.66%) and NH_3 (28.33%) in urea.



It has been interpreted that ocimum oil consisting of SFA and UFA begins degrading from 120 ˚C and experiences mass loss up to 500 ˚C of thermal degradation. Two major degradation patterns were observed at 160-300˚C and 320-400˚C in the urea-complex. These are not very different from that of degradation of urea. The formation complexes of SFA in the ocimum seed oil and urea can be visualized through the striking difference in the TGA pattern. Pure urea without fatty acid has been observed to degrade at a higher temperature range as compared to the urea-complex. These patterns have been clearly noted in DTG thermogram (Figure 2b) in which the shifting of maximum weight loss temperatures are observed due to oil impurity, the urea degrades easily to HCNO and NH_3, hence the urea devolatilization pattern has been observed at a lower temperature range in the urea-crystal. Quantitatively, in the urea-crystals, the SFAs participated in the crystallization, hence, the devolatilization of fatty acid was delayed and it devolatilized at 360 °C.

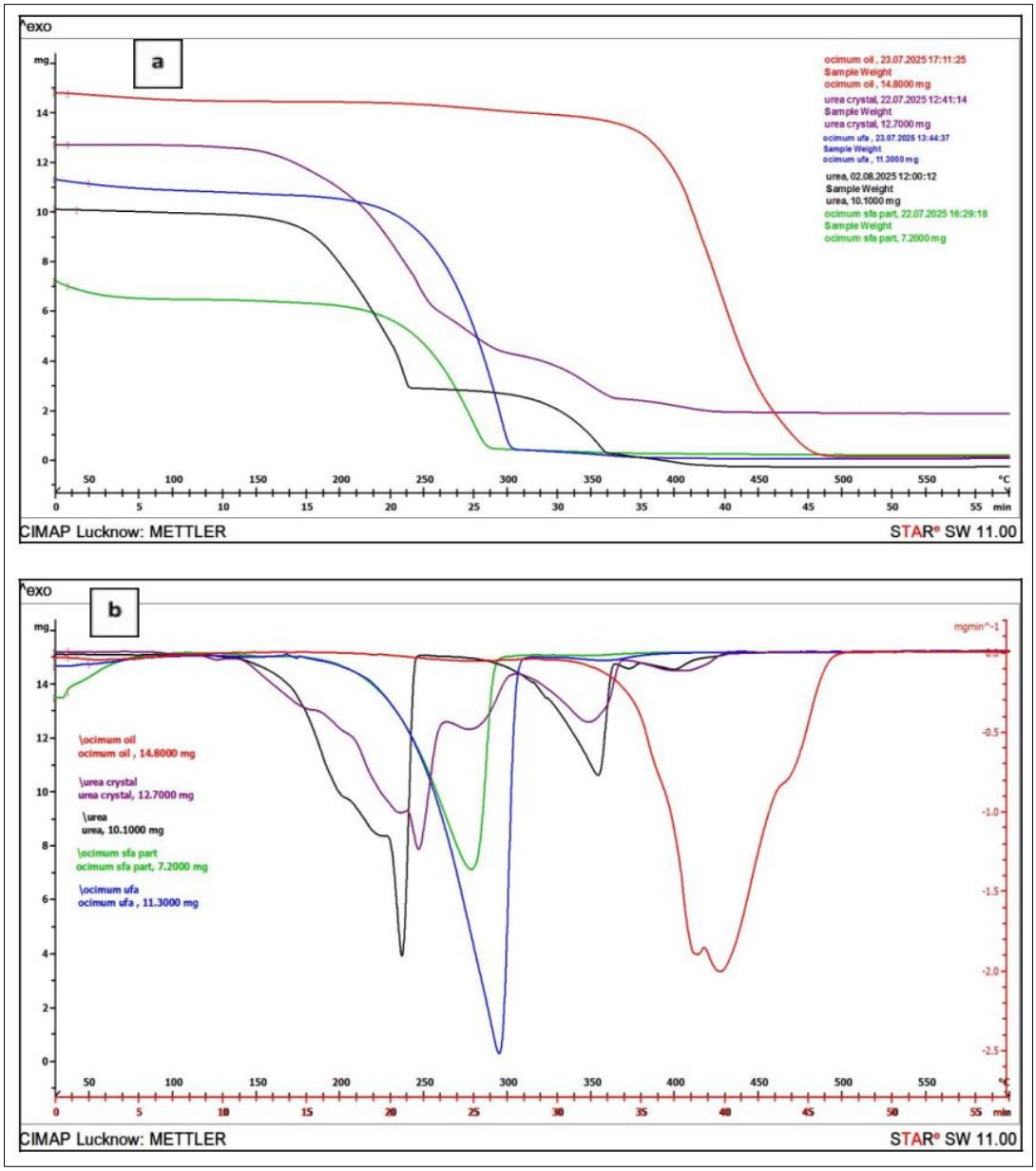


Figure 2: Urea crystals and enriched UFA and SFA, (a)TGA curve, (b) DTG curve

4. CONCLUSION AND FUTURE DIRECTIONS

During the current project, a protocol for the isolation of UFAs from ocimum seed-oil has been developed. The aqueous-alkali hydrolysis of triglyceride is suitable for generation of FFAs. These FFAs are then treated with urea at optimised conditions for the partial formation of urea-FFA complexes, preferably with SFA. The urea-complexation process selectively helps separate the SFAs; hence the purity of UFA was attained up to 96.5%. UFAs, the ω-fatty acids category, is enriched through the urea complexation process, and are then separated as an oily layer. The single stage urea-complexation method is very efficient for the selective isolation of UFAs, which is better used as a food and nutraceutical supplements. The urea inclusion method requires general chemicals like urea and ethanol. This process is operated at low temperatures. The urea-complexation process is a much greener method with lower involvement of volatile solvents. The process is much simpler and attains a higher mass balance and better purity. The enriched UFAs consisted of ω-6-fatty acid (27.0%), ω-3-fatty acid (61.0%) and were free from SFAs. These UFAs, isolated from ocimum seed-oil can be used as a functional food for better health and immunity.

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