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Research Article

Extraction and Quality Assessment of Soybean Oil and Its Application in Fatty Acid Methyl Ester Production

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Abstract: Soybeans are a globally important oilseed crop valued for their nutritional and industrial applications. This study aimed to evaluate the physicochemical properties of soybean oil extracted from seeds collected in Kathmandu, Nepal. Oil was extracted using a Soxhlet apparatus with n-hexane as the solvent. Seed moisture content was determined gravimetrically. The extracted oil was characterized for saponification value, acid value, peroxide value, ester value, free fatty acid content, density, specific gravity, boiling point, and percentage yield. The oil was further converted into fatty acid methyl esters through transesterification, and ester formation was confirmed using thin-layer chromatography. Gas chromatography-mass spectrometry was used to attempt a detailed analysis of the esters. The soybean seeds had a moisture content of 9.25%. Oil extraction yielded 7.8% with a specific gravity of 0.93 at 25°C. Saponification, acid, peroxide, and ester values were 198.80 mg KOH/g, 0.6725%, 3.33 mmol/kg, and 198.17 mg KOH/g, respectively. TLC confirmed the formation of methyl esters, although GC-MS analysis did not provide conclusive results. The extracted soybean oil exhibited physicochemical properties suitable for food or industrial use after refining. Transesterification successfully produced fatty acid methyl esters, demonstrating potential for biodiesel or other industrial applications. This study provides region-specific characterization of soybean oil from Kathmandu, offering baseline data for local industrial and nutritional applications.

Keywords: *biodiesel, characterization, methyl esters, physicochemical, transesterification*

1. Introduction

Vegetable oils are vital components of human nutrition and play a significant role in various industrial applications. They act as a concentrated source of energy, provide essential fatty acids, and transport fat-soluble vitamins like vitamins A, D, E, and K (FAO & WHO, 2010; McWilliams, 2017). Among various oil crops grown across the world, the soybean (*Glycine max*) crop ranks as one of the most significant oilseed crops with high oil and protein content, adaptability, and economic significance (Clemente & Cahoon, 2009).

Soybeans are an annual leguminous crop classified under the family Fabaceae that is native to East Asia (Hymowitz, 2004; Liu, 2008). It is now cultivated extensively in countries such as the United States, Brazil, China, India, and several developing nations. In Nepal, soybean, locally known as *Bhatamas*, is grown mainly in mid-hill and Terai regions and is commonly used as food, cooking oil, and livestock feed (Manandhar, 2021). Globally, soybeans account for a significant share of vegetable oil production, with annual production exceeding 340 million

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metric tons (FAO, 2020). Soybean seeds typically contain about 18–22% oil and 35–40% protein, making them nutritionally superior to many other oilseeds (Rosentrater & Cheng, 2010). Soybean oil is characterized by a high proportion of unsaturated fatty acids, mainly linoleic acid (C18:2), oleic acid (C18:1), and linolenic acid (C18:3). These fatty acids are related to several beneficial health effects, including decreased serum cholesterol levels and reduced risk of cardiovascular diseases (Sacks, 2006). Consequently, soybean oil is used as an edible oil for cooking, frying, and baking.

Soybean oil not only plays a vital role in nutrition but is also used for several industrial applications. For instance, soybean oil is used in soap manufacture, paint production, printing inks, cosmetics, pharmaceuticals, and biodiesel. The drying properties of soybean oil make it particularly useful in oil-based paints and printing inks (Gunstone, 2011; Liu, 2014). Moreover, esters of soybean oil are used in lubricants and surfactants (Baser & Buchbauer, 2009). With the increase in demand for renewable energy resources that are environmentally safe and eco-friendly, soybean oil has been identified as a potential candidate for biodiesel production (Ma & Hanna, 1999). The quality and suitability of vegetable oils for consumption and industrial applications depend mainly on their physicochemical properties. Acid value, peroxide value, saponification value, ester value, density, and specific gravity of oil are important parameters that offer valuable information about oil quality, purity, stability, and extent of degradation (Aremu et al., 2015). For example, acid value and free fatty acid content indicate the extent of hydrolytic rancidity, while peroxide value measures oxidative deterioration of oils during storage (Mohammed & Ali, 2015). Oils with low acid and peroxide values are considered to be of better quality and more suitable for consumption. Oil extraction methods also significantly influence yield and quality. Common methods include mechanical pressing, solvent extraction, supercritical fluid extraction, and aqueous extraction (Gunstone, Harwood, & Dijkstra, 2007; Rosenthal, Pyle, & Niranjan, 1996). Among these, solvent extraction using the Soxhlet apparatus is widely employed in laboratory-scale studies due to its simplicity, efficiency, and high oil recovery (Andrew et al., 2017). *n*-hexane is the most commonly used solvent because of its nonpolar nature, low boiling point, and high selectivity toward lipids (Hammond et al., 2005; Gunstone et al., 2007). However, oil obtained through solvent extraction is usually crude and contains impurities such as phospholipids, free fatty acids, pigments, and moisture, which necessitate further refining (B.M.Z. et al., 2006).

In recent times, the increasing concern about the depletion of fossil fuels and environmental pollution has led to an increase in the search for alternative sources of energy. Biodiesel, which refers to fatty acid alkyl esters produced from vegetable oil or animal fat, has been recognized as an alternative energy source for conventional diesel fuel (Fukuda et al., 2001). Biodiesel is biodegradable, non-toxic, and has lower emissions of carbon monoxide, suspended particles, and sulfur oxides compared to petroleum-based diesel (Namasivayam et al., 2010). Fatty acid methyl esters (FAMEs) are produced by transesterification, a chemical process that involves the reaction of triglycerides with methanol in the presence of an acid or base catalyst to produce methyl esters and glycerol (Ma & Hanna, 1999; Knothe, Van Gerpen, & Krahl, 2005**).** Transesterification lowers the viscosity of oils and makes them more suitable as fuels (Baskar et al., 2019). Analytical techniques such as thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC-MS) are widely used to confirm ester formation and determine fatty acid composition (Bartle & Myers, 2002).

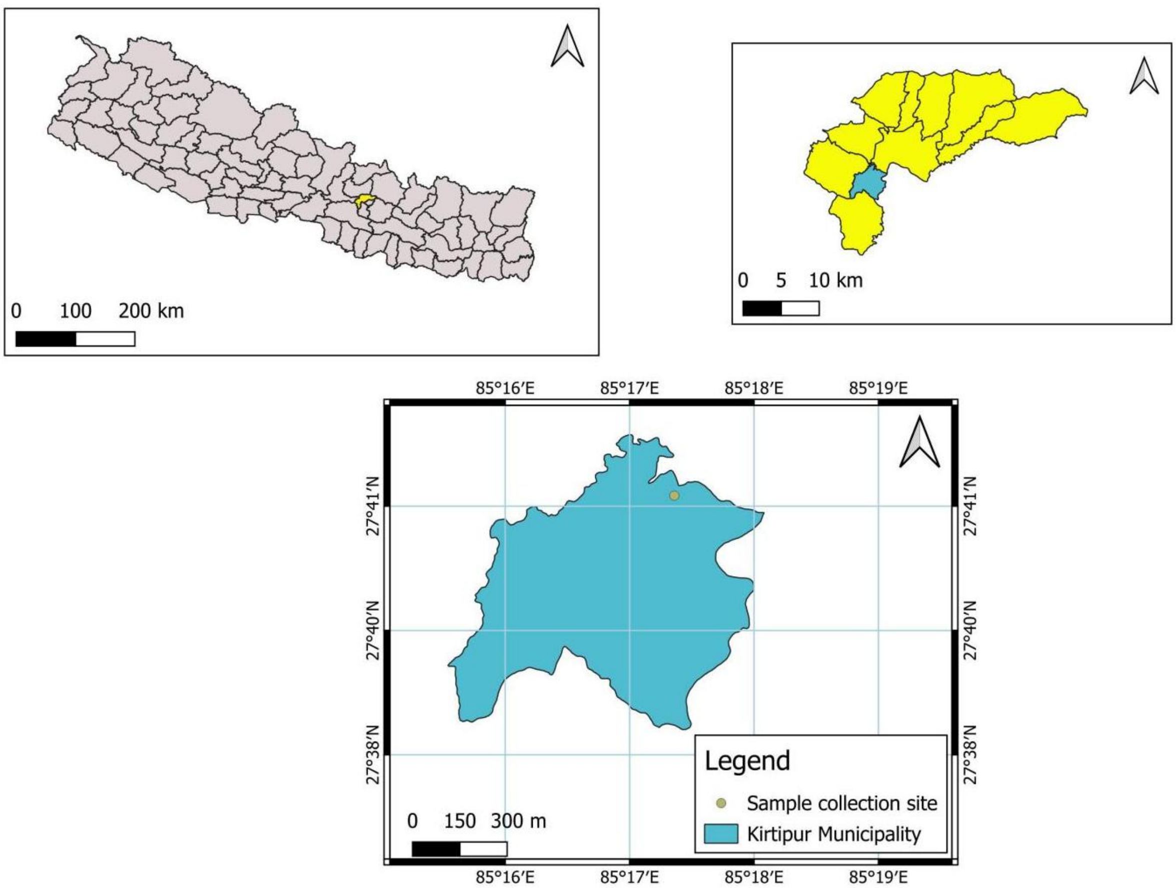
Considering the growing demand for vegetable oils, edible oil quality assessment, and renewable energy sources, systematic studies on the extraction and characterization of soybean oil are essential. The present study aims to extract oil from locally available soybean seeds using Soxhlet extraction and to evaluate its physicochemical properties. Furthermore, the oil extracted is converted into fatty acid methyl esters, and chromatographic analysis is performed for the confirmation of the esters. This research aims to contribute to the knowledge of the quality, usefulness, and utilization of the oil obtained from the seeds of the soybean crop grown in Nepal.

2. Materials and Methods

2.1 Study Area and Sample Collection

Soybean (*Glycine max*) seeds used for the research were obtained from Kathmandu, Nepal (**Figure 1**). Seeds were obtained three months before the analysis. The seeds were free from any defects, insects, and fungal infections. Samples were stored in clean, dry polyethylene bags under laboratory ambient conditions until further analysis (Manandhar, 2021).

Figure 1: Map showing the sample collection site



2.2 Sample Preparation

Soybean seeds were first cleaned manually by removing any foreign matter such as dust, stones, and broken seeds. The cleaned seeds were then dried in a hot air oven (UNITEMP Hot Air Oven, Model: UHC-100, UNITEMP Instruments, New Delhi, India) at 100±3 °C for 8 hours to reduce surface moisture and impurities. After drying, the seeds were cooled at room temperature (25±3 °C) with 50% relative humidity in a desiccator containing $CaCl_2$ as a desiccant. This prevented the moisture absorption by the seeds. Dried soybean seeds were ground into fine powder using a coffee grinder (Philips

Mixer Grinder, *Model: HL7756*, Philips, India) to increase surface area and improve extraction efficiency (Rosentrater & Cheng, 2010). The ground samples were stored in airtight containers before oil extraction.

2.3 Determination of Moisture Content

Moisture content (MC) of the soybean seeds was determined using the oven-drying method. Approximately 40 g of ground soybean sample was weighed accurately (W_1) in an analytical balance (Shimadzu Analytical Balance, Model: AUW220D, Shimadzu Corporation, Kyoto, Japan) and dried in a hot air oven at 100±3 ℃ until a constant weight was obtained (W_2). MC was calculated using the following **Equation (1)** (AOAC, 2016). This method is commonly employed owing to the simplicity and accuracy of the moisture level determination in oilseeds (Lee et al., 2013).

$$\text{Moisture Content (\%)} = \frac{a-b}{a} \times 100 \quad \text{……………………(1)}$$

Where a= Initial weight in grams, and

b = final weight in grams.

2.4 Oil Extraction Using Soxhlet Apparatus

Oil extraction was carried out using the Soxhlet oil extraction equipment according to normal laboratory procedures (**Figure 2**). Approximately 40 g of the ground soybean sample was placed in a cellulose extraction thimble and inserted into the Soxhlet extractor (Borosilicate glass Soxhlet extractor, *Borosil®, Model: Soxhlet-250*, Borosil Glass Works Ltd., Mumbai, India). A round-bottom flask containing 250 mL of n-hexane (≥99.0%, AR grade, Merck, Darmstadt, Germany) was attached to the apparatus. The extraction process was conducted for 8–9 hours at the boiling point of *n*-hexane (69 ±2 °C) in a heating mantle (REMI Heating Mantle, Model: HM-250, REMI Electrotechnik Ltd., Mumbai, India). During extraction, the solvent was continuously refluxed and siphoned, allowing efficient dissolution of lipids from the sample matrix. After completion of extraction, the solvent–oil mixture was concentrated using a rotary evaporator (BUCHI Rotavapor®, *Model: R-300*, Büchi Labortechnik AG, Flawil, Switzerland) to recover *n*-hexane. The recovered solvent was reused, while the residual oil was dried to constant weight and stored in airtight containers for further analysis (Andrew et al., 2017). The oil yield (%) was calculated using the formula mentioned in **Equation (2).**

$$\text{Oil Yield (\%)} = (\%) = \frac{b}{a} \times 100 \quad \text{……………………(2)}$$

Where, b= Weight of extracted oil in grams, and

a= weight of dried sample in grams.

2.5 Physicochemical Characterization of Extracted Oil

The oil extracted using the described procedure was analyzed for various physicochemical characteristics in order to assess the quality, stability, and possible uses of the oil.

2.5.1 Determination of Acid Value

Acid value was determined by titrating the oil sample dissolved in ethanol with standardized potassium hydroxide (KOH) solution (≥85%, AR grade, Merck, Darmstadt, Germany) using phenolphthalein (Merck, Darmstadt, Germany) as an indicator. Acid value was calculated as milligrams of KOH required to neutralize free fatty acids present in one gram of oil as represented by **Equation (3)** (Khadda et al., 2014):

$$\text{Acid value} = \frac{V \times N \times 56.1}{W} \quad \text{……………………(3)}$$

Where, *V* = volume of KOH used in mL,

N = normality of KOH, and

W = weight of oil sample in grams.

2.5.2 Determination of Saponification Value

Saponification value was determined by refluxing a known weight of oil with excess alcoholic KOH solution. The unreacted KOH was titrated against a standardized oxalic acid solution (≥99.5%, AR grade, Merck, Darmstadt, Germany) using phenolphthalein as an indicator. Saponification value was calculated according to standard procedures as represented by **Equation (4)** (Denniston et al., 2004):

$$SV = \frac{(B-S)\times N\times 56.1}{W} \quad (4)$$

where B and S are volumes of acid used in blank and sample titrations, respectively.

2.5.3 Determination of Peroxide Value

Peroxide value was determined by reacting the oil sample with potassium iodide in an acetic acid (≥99.7%, AR grade, Merck, Darmstadt, Germany) and chloroform (≥99.7%, AR grade, Merck, Darmstadt, Germany) mixture. Liberated iodine was titrated with sodium thiosulfate ($Na_2S_2O_3\cdot 5H_2O$, ≥99%, AR grade), Merck, Darmstadt, Germany) using starch (Merck, Darmstadt, Germany)as an indicator. Peroxide value was expressed in milliequivalents of active oxygen per kilogram of oil (Lai Kim & Cher Siang, 1987).

2.5.4 Determination of Ester Value

Ester value was determined as the difference between the saponification value and the acid value, as shown in **Equation (5)**. It is the value of the quantity of the esterified fatty acids present in the oil (Aremu et al., 2015):

$$\text{Ester Value} = SV - AV \quad (5)$$

Density was determined using the relative density bottle with a capacity of 10 mL at room temperature, i.e., 25°C. These parameters serve as indicators of oil purity and fatty acid composition (Tabasum et al., 2012).

2.6 Preparation of Fatty Acid Methyl Esters (FAMEs)

Transesterification of soybean oil was performed using methanol in the presence of an alkaline catalyst. A measured quantity of oil was mixed with methanolic KOH and heated with constant stirring. After completion of the reaction, the mixture was allowed to stand for phase separation. The upper ester layer was separated, washed with warm distilled water to remove residual catalyst and glycerol, and dried over anhydrous sodium sulfate (Ma & Hanna, 1999).

2.7 Qualitative Ester Identification (chemical test)

The presence of ester functional groups in the fatty acid methyl ester (FAME) sample was qualitatively confirmed using the phenolphthalein test and the hydroxamic acid (≥99.7%, AR grade, Merck, Darmstadt, Germany) test, following standard organic analytical procedures. For the phenolphthalein test, approximately 0.1 g of the sample was mixed with distilled water, and two drops of phenolphthalein indicator were added. Dilute sodium hydroxide solution (≥99.7%, AR grade, Merck, Darmstadt, Germany) was then added dropwise until a faint pink color appeared. The subsequent disappearance of the pink color indicated ester hydrolysis under alkaline conditions. For the hydroxamic acid test (≥99.7%, AR grade, Merck, Darmstadt, Germany), approximately 0.4 g of the sample was treated with hydroxylamine hydrochloride in an ethanolic sodium hydroxide solution and gently heated. After cooling, dilute hydrochloric acid (≥99.7%, AR grade, Merck, Darmstadt, Germany) was added to acidify the mixture, followed by the addition of a ferric chloride solution. The formation of a deep red or violet-colored complex confirmed the presence of ester groups through the formation of ferric hydroxamate complexes. These tests are widely employed for the qualitative confirmation of ester functionalities in biodiesel and esterified lipid samples (Shriner et al., 2004; Furniss et al., 2005; Christie, 2010).

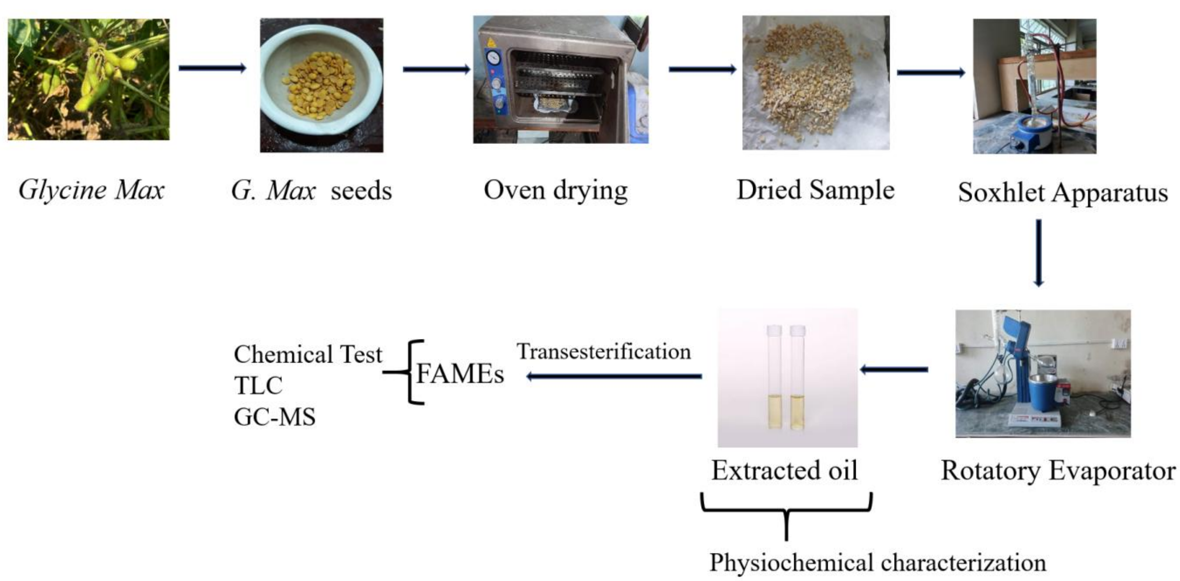
2.8 Thin Layer Chromatography (TLC) Analysis

TLC was employed to confirm the formation of FAMEs. Silica gel TLC plates (Silica gel 60 F_{254} aluminum sheets, Merck, Darmstadt, Germany) were used as the stationary phase, while a mixture of hexane, diethyl ether, and acetic acid in the ratio of 9:2:1 served as the mobile phase. Oil and ester samples were spotted on the plate and developed in a TLC chamber. The separated components were visualized under UV light and in the presence of iodine vapor (≥99.7%, AR grade, Merck, Darmstadt, Germany). Retention factor (Rf) values were calculated to differentiate oil from ester components (Bele & Khale, 2011).

2.9 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analysis (Shimadzu GC-MS-QP2010 Plus, Shimadzu Corporation, Kyoto, Japan) of the prepared FAME samples was conducted at the National Academy of Science and Technology (NAST), Kathmandu, Nepal. The technique was used to identify fatty acid components based on their mass spectral fragmentation patterns and retention times (Bartle & Myers, 2002). Although analysis was performed, conclusive identification was limited due to instrumental and operational constraints.

Figure 2: Schematic representation of soybean oil extraction and processing.



3. Results and Discussion

3.1 Moisture Content of Soybean Seeds

The moisture content of the soybean seeds used in the present study was found to be 9.25% (**Table 1**). Moisture content is an important parameter in the oilseed processing industry, as it has a direct bearing on the oil obtained. Oilseeds with high moisture contents are prone to microbial growth, which might result in the hydrolysis of the oil, thereby deteriorating the quality of the oil obtained (Rosentrater & Cheng, 2010). The observed moisture content lies within the acceptable range for oilseed storage, typically below 10%, and is comparable to values reported for soybean seeds in similar climatic conditions (Aremu et al., 2015) (**Table 1**). Adequate drying before extraction likely contributed to the relatively low acid value obtained for the extracted oil, as excess moisture accelerates hydrolytic rancidity (Gunstone, 2011; Shahidi & Zhong, 2010).

3.2 Oil Yield

The percentage yield of soybean oil obtained by Soxhlet extraction using *n*-hexane was 7.80% (**Table 1**). This yield is lower than the industrial oil content of soybean, which is reported to be approximately 18–22% (Clemente & Cahoon, 2009). The lower yield observed in this study may be attributed to several factors, including seed variety, agronomic conditions, storage duration, particle size, and laboratory-scale extraction limitations (Rosenthal, Pyle, & Niranjan, 1996; Hammond et al., 2005). Low oil yields under laboratory conditions were also observed by Andrew et al. (2017), as the cell walls were not completely disrupted, thereby limiting the oil obtained. Additionally, the lack of oilseed pretreatments such as flaking and enzymatic treatments might also have contributed to the low oil obtained. However, the quality of the oil obtained was satisfactory for further analysis (Rosenthal, Pyle, & Niranjan, 1996; Gunstone et al., 2007).

3.3 Physical Properties of Extracted Soybean Oil

The color of the extracted soybean oil was observed to be yellowish, having an odor (Table 1) , similar to crude vegetable oils. The density of the extracted soybean oil was observed to be 0.93 g/mL at 25°C, which is consistent with the reported values of crude soybean oil, which range from 0.92 to 0.94 g/mL (Tabasum et al., 2012). The boiling point of the extracted soybean oil was observed to be 300±3℃ (Table 1) . The density of the extracted soybean oil was observed to be 0.93 g/mL, indicating that the extracted oil is of high purity, as unsaturated fatty acids have lower density values. The extracted soybean oil is expected to have a high amount of unsaturated fatty acids, as it is consistent with the fatty acid composition of crude soybean oil, which is rich in linoleic acid and oleic acid (Sacks, 2006).

3.4 Acid Value and Free Fatty Acid Content

The acid value of the extracted soybean oil was observed to be 0.67% (**Table 1**), which shows that the concentration of free fatty acids in the extracted soybean oil is low. Acid value is an important factor that determines the extent of hydrolytic degradation of triglycerides during the process of extraction and storage of oils (Gunstone, 2011; Shahidi & Zhong, 2010). Oil samples having an acid value less than 1% are considered safe for use in food products after refining (Mohammed & Ali, 2015). Low acid value in the extracted soybean oil implies that there was a minimum amount of hydrolysis of triglycerides during the process of extraction, probably due to proper drying of the seeds before extraction (Gunstone, 2011; Shahidi & Zhong, 2010). Comparable acid values for soybean oil have been reported by Aremu et al. (2015) and Khadda et al. (2014), who found acid values ranging between 0.5% and 1.2%. Low free fatty acid content is particularly desirable for biodiesel production, as high acid values can interfere with base-catalyzed transesterification reactions (Ma & Hanna, 1999)

3.5 Saponification Value

The saponification value of the soybean oil was determined to be 198.8 mg KOH/g (**Table 1**). Saponification value reflects the average molecular weight of fatty acids present in the oil; higher values indicate shorter-chain fatty acids, while lower values indicate longer-chain fatty acids (Denniston et al., 2004). The obtained value falls within the typical range reported for soybean oil (190–205 mg KOH/g), confirming the presence of predominantly long-chain fatty acids such as linoleic and oleic acids (Aremu et al., 2015). High saponification value suggests that the oil is suitable for soap and cosmetic industries, as well as for esterification and transesterification processes (Gunstone, 2011; Knothe, Van Gerpen, & Krahl, 2005). The success of this experiment further proves that the soybean oil has potential industrial use.

3.6 Peroxide Value

The peroxide value of the extracted soybean oil was found to be 3.33 mmol/kg (**Table 1**).

Peroxide value is a measure of primary oxidation products formed during the initial stages of lipid oxidation and is widely used as an indicator of oxidative rancidity (Lai Kim & Cher Siang, 1987). Fresh edible oils typically exhibit peroxide values below 10 mmol/kg. The low peroxide value indicates that the oil is stable and has not oxidized over the time of extraction and storage. Similar peroxide value results were obtained in previous studies for fresh oil (Mohammed & Ali, 2015). Low peroxide value is desirable, especially when the oil is to be consumed and used as a source of biodiesel fuel because oxidation affects the flavor and performance of the oil.

3.7 Ester Value

The ester value of the soybean oil, calculated as the difference between the saponification value and acid value, was found to be 198.17 mg KOH/g (**Table 1**). The definition of ester value is the amount of esterified fatty acids found in the oil, which is an indirect measure of triglycerides (Aremu et al., 2015). The highest ester value indicates that the majority of the fatty acids were found in the esterified state and not as free fatty acids. This further indicates the quality of the oil, which is desirable and suitable for the production of biodiesel fuel through the process of transesterification. High ester value is desirable when the oil is to be used in the industries because it is stable and can be easily reacted with the catalyst to produce biodiesel fuel (Ma & Hanna, 1999; Knothe, Van Gerpen, & Krahl, 2005).

Table 1: Comparison of Physicochemical Properties of Soybean Oil in the Present Study with WHO/FAO Standards and Reported Literature.

Parameter	Present Study	WHO/FAO / Codex Standard	Reported Literature Range	References
Color	Golden yellow	Pale to golden yellow	Pale to golden yellow	FAO/WHO (2019); Aremu et al. (2015); Rosentrater & Cheng (2010)
Density (g/mL at 25 °C)	0.93	0.91–0.93	0.92-0.94	Codex Alimentarius (1999); Tabasum et al. (2012); Mohammed & Ali (2015)
Moisture content (%)	9.25	≤ 10 (crude oils)	6-10	FAO (2010); Lee et al. (2013); Rosentrater & Cheng (2010)
Saponification value (mg KOH/g)	198.8	189–195	190-205	Codex Alimentarius (1999); Aremu et al. (2015); Khadda et al. (2014)
Acid value (mg KOH/g)	0.67	≤ 4.0	0.5-1.5	FAO/WHO (2019); Mohammed & Ali (2015); Aremu et al. (2015)
Peroxide value (meq O_2/kg)		≤ 10 (fresh oil)	< 10	FAO/WHO (2019); Lai Kim & Cher Siang (1987); Mohammed & Ali (2015)
Ester value (mg KOH/g)	198.17	Not specified	185-200	Aremu et al. (2015); Denniston et al. (2004)
Free fatty acid (%)	0.33	≤ 2.0 (as oleic acid)	< 1.0	FAO/WHO (2019); Ma & Hanna (1999); Fukuda et al. (2001)

Parameter	Present Study	WHO/FAO / Codex Standard	Reported Literature Range	References
Glycerol (%)	10.84	Not specified	9-12	Ma & Hanna (1999); Namasivayam et al. (2010)
Oil yield (%)	7.80	Not specified	6-12 (lab scale)	Andrew et al. (2017); Rosentrater & Cheng (2010)

The physicochemical values of soybean oil, as determined in the present study, were in agreement with WHO/FAO (Codex Alimentarius) standards and literature values (**Table 1**). Important quality characteristics such as acid values, peroxide values, free fatty acids, density, and moisture content were within acceptable limits, suggesting good quality oil as presented in **Table 1**. Small differences in saponification values and ester values may be due to various factors, such as varietal differences, processing, and analysis. It may be concluded that soybean oil, as produced in the present study, meets the requirements for quality standards and is in good agreement with literature values.

3.8 Transesterification and FAME Formation

The extracted soybean oil was successfully converted into fatty acid methyl esters (FAMEs) using base-catalyzed transesterification with methanol. This was evident from the formation of two distinct layers, with one layer being the upper ester layer and the other being the glycerol layer. Transesterification significantly reduces the viscosity of vegetable oils and improves their suitability for use as biodiesel (Fukuda et al., 2001). The low acid value of the oil favored efficient transesterification, as high free fatty acid content can lead to soap formation and reduced ester yield (Ma & Hanna, 1999).

3.9 Qualitative Confirmation of Ester Groups in FAME

The qualitative tests conducted for ester functional groups yielded positive results for the FAME sample. In the phenolphthalein test, the initially developed pink coloration disappeared upon standing, indicating alkaline hydrolysis consistent with the presence of ester linkages. Likewise, the hydroxamic acid test was used, and a clear deep red color was formed upon the addition of ferric chloride, confirming the formation of ferric hydroxamate complexes. These observations unequivocally confirm the presence of ester functional groups in the analyzed FAME sample (**Figure 3**). The positive response of both tests is in agreement with the expected chemical structure of fatty acid methyl esters, which constitute the principal components of biodiesel derived from vegetable oils. Comparable observations have been reported in earlier studies on ester verification in biodiesel and lipid-derived esters (Ma & Hanna, 1999; Fukuda et al., 2001; Christie, 2010).

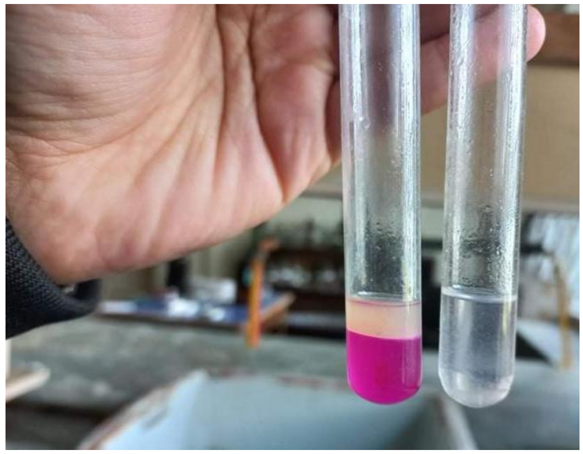


Figure 3: Qualitative Confirmation of Ester Groups in FAME.

3.10 Thin Layer Chromatography (TLC) Analysis

The formation of FAMEs was confirmed using TLC analysis. It was clear from the results that TLC analysis can separate the oil sample from the ester sample. It was also clear that different R_f values were present for different samples. A high Rf value was present for the ester sample as opposed to the crude oil. This is due to the low polarity of the ester. TLC analysis has been shown to work well in previous studies when analyzing biodiesel and fatty acid esters (Bele & Khale, 2011). It can, therefore, be concluded that TLC analysis can be used to analyze FAMEs and that transesterification was successful.

3.11 GC-MS Analysis

The GC-MS analysis was attempted to determine the composition of fatty acids present in the FAMEs synthesized. However, it was not possible to achieve accurate identification of individual fatty acids present in FAMEs. Though it is not possible with this analysis, GC-MS is one of the most accurate techniques for identifying fatty acids with high sensitivity and specificity (Bartle & Myers, 2002). Detailed fatty acid profiling would further enhance understanding of the nutritional and industrial value of locally produced soybean oil. The early-eluting peaks corresponding to cyclohexane and toluene represent low-molecular-weight volatile organic compounds that are not intrinsic constituents of soybean oil. Their occurrence is most plausibly attributed to residual extraction solvents, laboratory-based contamination, or column and system background effects. Such solvent-related peaks are frequently reported in GC-MS analyses of edible oils, particularly when *n*-hexane- or petroleum ether-based extraction and sample preparation protocols are employed (Christie, 2010; de Koning et al., 2012). In contrast, the dominant late-eluting peak observed at a retention time of approximately 56.4 min was identified as terephthalic acid esters (**Figure 4** and **Table 3)**. These high-molecular-weight ester compounds are commonly associated with plasticizers and are not naturally occurring components of soybean oil triglycerides.

Table 2: Typical FAMES in soybean oil and their GC-MS retention characteristics reported in literature.

Expected FAME	Carbon Chain / Unsaturation	Typical Retention Characteristics	Approximate GC–MS Retention Time (min)	Key Literature Sources
Methyl palmitate	C16:0 (saturated)	Shorter carbon chain; elutes earlier than C18 FAMEs	~7-10	Christie (1989); Knothe (2005); Pintathong et al. (2021)
Methyl stearate	C18:0 (saturated)	Longer carbon chain; higher retention than C16:0	~10-15	Knothe (2005); Ramos et al. (2009); Pintathong et al. (2021)
Methyl oleate	C18:1 (monounsaturated)	One double bond reduces retention relative to C18:0	~10-11	Christie (1989); Ramos et al. (2009); Pintathong et al. (2021)
Methyl linoleate	C18:2 (polyunsaturated)	Two double bonds; slightly lower retention than C18:1	~10-11	Knothe (2005); Xu et al. (2018); Pintathong et al. (2021)
Methyl linolenate	C18:3 (polyunsaturated)	Highest unsaturation; elutes slightly later than C18:2	~11-12	Christie (1989); Ramos et al. (2009); Xu et al. (2018)

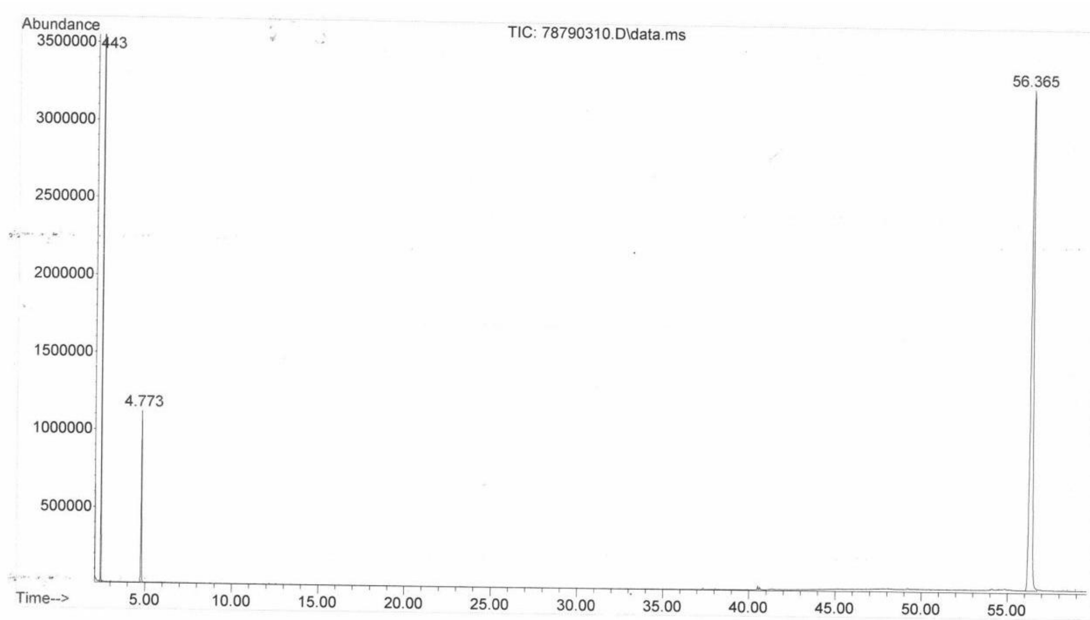
Soybean oil is primarily composed of fatty acids such as linoleic, oleic, palmitic, and stearic acids, esterified within triglyceride structures (Gunstone, Harwood & Dijkstra, 2007; Shahidi, 2005). The detection of terephthalate esters strongly suggests contamination arising from leaching of plastic materials, including sample containers, tubing, septa, or other polymeric components of the analytical system. Additional sources may include contamination during sample storage, handling, or instrumental background contributions. Similar observations of phthalate and terephthalate ester contamination during GC–MS analysis of oils, fats, and environmental samples have been widely documented, emphasizing the susceptibility of such analyses to plastic-derived artifacts (Peñalver et al., 2000; Fierens et al., 2012; Net et al., 2015)

.GC-MS analysis of fatty acid methyl esters derived from soybean oil identified three prominent peaks, namely cyclohexane, toluene, and terephthalic acid esters as shown in **Figure 4** and **Table 3**. Cyclohexane and toluene are commonly identified as solvent residues or laboratory contaminants, and hence, they are not natural fatty acid methyl ester components. The prominent peak with a retention time as high as 56.36 minutes was identified as terephthalic acid esters, which are commonly identified as contaminants from plastic ware, septa, or solvents, but not from vegetable oils.

Table 3: GC-MS Identified Compounds in FAME Prepared from Soybean Oil (NIST08 Library)

Peak No.	Retention Time (min)	Area (%)	Library-Identified Compound	CAS No.	Match Quality (%)
1	2.443	15.83	Cyclohexane	110-82-7	91-95
2	4.773	6.42	Toluene	108-88-3	91
3	56.365	77.75	Terephthalic acid esters (e.g., di-(2-ethylhexyl) terephthalate)	100-32-4	83-91

Figure 4: GC-MS total ion chromatogram of FAME prepared from soybean oil.



In contrast, literature consistently indicates that FAMEs of soybean oil consist mainly of methyl palmitate (C16:0), methyl stearate (C18:0), methyl oleate (C18:1), methyl linoleate (C18:2), and methyl linolenate (C18:3) eluting in the 5-20 minutes range under standard GC-MS conditions (**Table 2**). The absence of these typical FAME components in the current chromatogram may indicate the presence of interfering contaminants that have obscured the FAME components of interest or the use of sub-optimal conditions for the sample handling process (Knothe, 2005; Christie, 1989; Biedermann & Grob, 2010)

4. Conclusion

The present study successfully explored the extraction of soybean oil from readily available soybean (*Glycine max*) seeds and evaluated their physicochemical properties to understand their quality and possible uses. Soxhlet extraction with *n*-hexane solvent was successfully used to extract oil from dried soybean seeds, and 7.8% oil extraction was achieved. Although this yield was lower than industrial values reported in literature, it was sufficient for comprehensive physicochemical characterization and further chemical modification. The moisture content of the soybean seeds was found to be 9.25%, which lies within the acceptable range for oilseed storage and processing. Appropriate management of the moisture content may have been a factor in the desirable quality parameters of the extracted oil. The physical characteristics of the oil, such as density and specific gravity (0.93 at 25 °C), were in agreement with those of crude soybean oil, suggesting the presence of mainly unsaturated fatty acids. Chemical analysis showed that the soybean oil had a low acid value (0.6725%) and a low peroxide value (3.33 mmol/kg), suggesting that the oil was stable and suitable for consumption after appropriate refining. The saponification value of 198.8 mg KOH/g falls within the typical range for soybean oil and confirms the presence of long-chain fatty acids, primarily oleic and linoleic acids (not conclusive in this study). The highest ester value (198.17 mg KOH/g) further indicates that the majority of fatty acids were present in esterified form, reflecting good triglyceride integrity and oil quality. The extracted soybean oil was successfully converted to fatty acid methyl esters through a base-catalyzed transesterification reaction. The formation of esters was also qualitatively confirmed through thin-layer chromatography, which showed a clear separation of triglycerides and methyl esters. The low free fatty acids in the soybean oil also favored the efficient conversion to biodiesel, indicating the potential of the extracted soybean oil as a good source of biodiesel. Though the GCMS results did not conclusively identify the fatty acids, the methodology employed is a good one and can be used to conduct further detailed studies in the future. The results obtained from this study clearly indicate that the soybean oil extracted from soybeans grown in Nepal has good physicochemical characteristics and is of significant potential value to society, especially when considering its nutritional, industrial, and potential energy applications. It also suggest that the various other methods and techniques are necessary to validate the composition of the FAMEs prepared.

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Data availability statement: The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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