



Study on the Yield and Physicochemical Properties of Oils from Ethiopian Castor Seed (*Ricinus communis* L.) Varieties and Biodiesel Production

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Abstract

Castor seed is one of the seeds with an untapped potential for its oil for biofuel production. The aim of this study was to compare the yield and physicochemical characteristics of oils produced from two varieties of Ethiopian castor seed varieties (HIRUY and ABARO) and to evaluate the methyl ester profile of these oils for the production of biofuels. Soxhlet and screw press methods were used with a factorial of 2X2 for the evaluation of the oil yield and the physicochemical properties. This finding showed that the oil yield is significantly influenced by the interaction between the variety and the method of extraction. A higher oil yield (56.693%) was obtained from the HIRUY variety by the Soxhlet extraction method and a lower oil yield (42.06%) was obtained from the ABARO variety by the mechanical extraction method. The GC-MS analysis of the biodiesel showed that methyl ricinoleate is produced by the acid and base transesterification method, yielding yields of 89 and 87 per cent biodiesel from HIRUY and ABARO oil, respectively. The GC-MS analysis of the biodiesel showed that methyl ricinoleate is the maximum composition of methyl ester of fatty acids. For biodiesel produced from oil of the HIRUY and ABARO seed varieties, the yields were 91.58% and 90.92%, respectively. The biodiesel produced from both varieties of castor seeds has a higher content of unsaturated fatty acid methyl esters. The findings of this study showed that the use of HIRUY castor seed oil may be preferable to ABARO castor seed oil for the production of biodiesel in terms of relatively higher oil and biodiesel yields, of acceptable oil quality and of high unsaturated fatty acid methyl ester content.

Keywords: ABARO, Castor seed oil, Biofuel Energy, Extraction method, HIRUY, Oil yield, Trans-esterification.

Introduction

The growth in human populations and the industrialization of the world economy are the main drivers of today's rapid increase in energy demand¹. Reports also show that fossil fuels are the world's primary energy sources². This widespread use of such energy sources has created a number of problems. This includes environmental pollution (climate change and global warming) which is causing the melting of polar ice. This in turn leads to biodiversity loss and species extinctions, food shortages, deteriorating human health and millions of people to poverty around the world^{3,4}.

In Ethiopia, more than 5 percent of total energy consumption comes from energy sources such as oil⁵. These imported oil products represent a major part of total import expenditure and absorb a large proportion of total export earnings⁶. Reports also indicate that Ethiopia spends more than 50 percent of its total export earnings to meet its fuel demand, and that around 80 percent of its oil imports come from Sudan⁷. The transport sector in general accounts for about 52 per cent of imported oil, which is the majority of the consumer of oil and a major source of carbon di oxide (CO₂) emissions in the environment⁸. The

challenges of fossil fuels and their use have prompted humanity to seek alternative and renewable energy sources for growing economic activity worldwide, to meet the growing need for reliable and environmentally friendly energy and to reduce pollution⁹⁻¹¹.

Biodiesel is a type of biofuel which is becoming increasingly important for direct use as a substitute for conventional (fossil) fuels^{12,13}. Mono-alkyl fatty acid esters¹⁴ are a type of long-chain fatty acid. These are used, for example, in all sectors of society: in electricity generation, heating, cooling, industrial processes and, in particular, in transport as an alternative to fossil fuels^{15,16}. The use of biofuels also minimises dependence on imported fossil fuels, promotes a climate-friendly energy source and reduces greenhouse gas emissions. Reports have shown that using 1 kg of biodiesel results in a reduction of around 3 kg of CO₂ emissions. These figures represent a significant reduction of 65-90 percent¹⁷⁻¹⁹. Biodiesel produced from vegetable oil esters is a quality and clean diesel fuel¹¹. It also has improved lubrication properties, biodegradability and recyclability²⁰. Non-edible vegetable or fruit oils are preferred because they are toxic for human consumption and grown on land where it is not possible to grow edible oil crops. It is sustainable, renewable, affordable and cost-effective^{21,22}.

Castor (*Ricinus communis* L.) is believed to be native to the Ethiopian region of tropical East Africa, where it undergoes great diversity²³. It is a potential source of biodiesel because of its rapid growth, it can grow on any soil, it is drought-resistant and is known for producing high quality oil²⁴. Its oil is the only commercial source of ricinoleic acid²³. This exclusively fatty acid makes up approximately 90 per cent of castor oil. Furthermore, ricinoleic acid is not significantly influenced by environmental conditions and is highly soluble in alcohol, which is beneficial for biodiesel quality²⁵. Recently, Melkasa Agricultural Research Centre in the Central Rift Valley of Ethiopia, released two varieties of castor seed, HIRUY and ABARO, for improved yields²⁶. However, there are no adequate reports comparing the oil yields using the various extraction methods and the quality of the oil for biodiesel from these newly released varieties of castor seeds. A study was therefore launched to evaluate the oil yield from the Soxhlet and Screw-on extraction methods, the physicochemical properties of the oil obtained from the seeds of these newly released varieties of castor beans and their methyl ester profile.

Materials and Methods

Collection of plant material (seed): Two castor bean varieties released from the Melkassa Agricultural Research Centre (MARC) in Ethiopia were used in this study. Both varieties were called HIRUY and ABARO. They were released from the central Rift Valley of Ethiopia in 2007 and 2011, respectively²⁷⁻²⁹. The experimental activities were carried out in the Food Science laboratory, the College of Agriculture, Hawassa University, and the Department of Food Process Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia.

Experimental treatments and design: The experiment was structured in a completely randomized design (CRD) with three replicates. For the extraction of oil, two varieties of castor beans (HIRUYA and ABARO) were used. Two extraction methods, namely the Soxhlet and the mechanical screw-out extractions (Scheme-1) were used. Thus, the factorial combination of 2 x 2 was used. For the physical and chemical properties of the oils, biodiesel yield, and fatty acid methyl ester profile of biodiesel, the experiment was considered only one factor i.e. variety.

Experimental setup and procedures: Characteristics of the castor seed samples: Seed weight and seed color: Approximately 100 seed samples of both the HIRUY and the ABARO castor varieties were weighed using an analytical weighing scale. Average seed weights were considered for discussion in accordance with the literature report³⁰. The colors of the seed of the castor seed varieties were also used as parameters.

Seed moisture content: About 100g of the purified seed sample was weighed and dried at 105°C degrees Celsius. After every two hours the sample was taken out of the furnace and placed in

the dryers for 30 minutes for cooling and then weighed. The procedure was repeated until a steady weight was obtained after 7 hours. The moisture content of the seed has been calculated by the method described in the literature^{31,32} and the following formula has been used to determine the moisture content (equation-1).

$$\text{Moisture content of seeds} = \frac{(W_1 - W_2)}{W_2} * 100 \quad (1)$$

Where, W1 = Original weight of the sample before drying (g);
W2 = Weight of the sample after drying (g).

Oil extraction: For the extraction of oil from castor bean varieties, two different extraction methods (Soxhlet and mechanical screw press) were used. Details of the methods are given below.

Plant material preparation: For the Soxhlet extraction: The castor seeds were cleaned manually and weighed using analytical balance to obtain the initial weight. Then the seeds were dried in a drying furnace at 105°C for 7 hours to remove moisture and volatile material. The dried sample was then cooked in a frying pan to coagulate the protein and to liquefy the oil, which makes the extraction process easier. The cooked sample was dehulled (separating the husks from the seeds) manually in order to obtain a high yield of oil. The dehulled kernel was then crushed to a fine powder with a mortar and a pestle to weaken or break the cell walls and release the castor oil. The crushed castor seed was stored in the desiccator until used for the extraction³³.

For mechanical screw press extraction: Castor seeds were cleaned manually and weighed using a standard weighing scale to obtain the initial weight. Then the seeds were dried in a drying furnace at 105°C for 7 hours to remove moisture and volatile material. The dried sample was then cooked in a fryer to coagulate the protein and to liquefy the oil, which makes the extraction process easier. The cooked castor seed was stored in the desiccator until used for extraction.

Oil extraction methods: Soxhlet extraction: 100g of the prepared sample was packed in filter paper and charged in a thimble. The apparatus was then mounted on a round bottom flask, connected to a condenser. Petroleum ether was poured from the top of the apparatus into a round bottom flask. The apparatus used was then heated to 60°C to boil the solvent. The steam from the boiling solvent rose up the vertical pipe into the condenser. Then the steam condensed and dripped into the thimble in the center. The extracted oil was then seeped through the pores of the thimble into a filled siphon tube, which was then transferred to a round bottom flask³¹. The extraction process took twelve hours. The resulting mixture was concentrated in a flask and the solvent from the extracted oil is extracted by means of a rotary evaporator. The extracted oil was then removed from the pipe, dried in an oven and cooled in a

desiccators. The oil yield has been weighed and calculated using the following formula (equation-2)³³⁻³⁵.

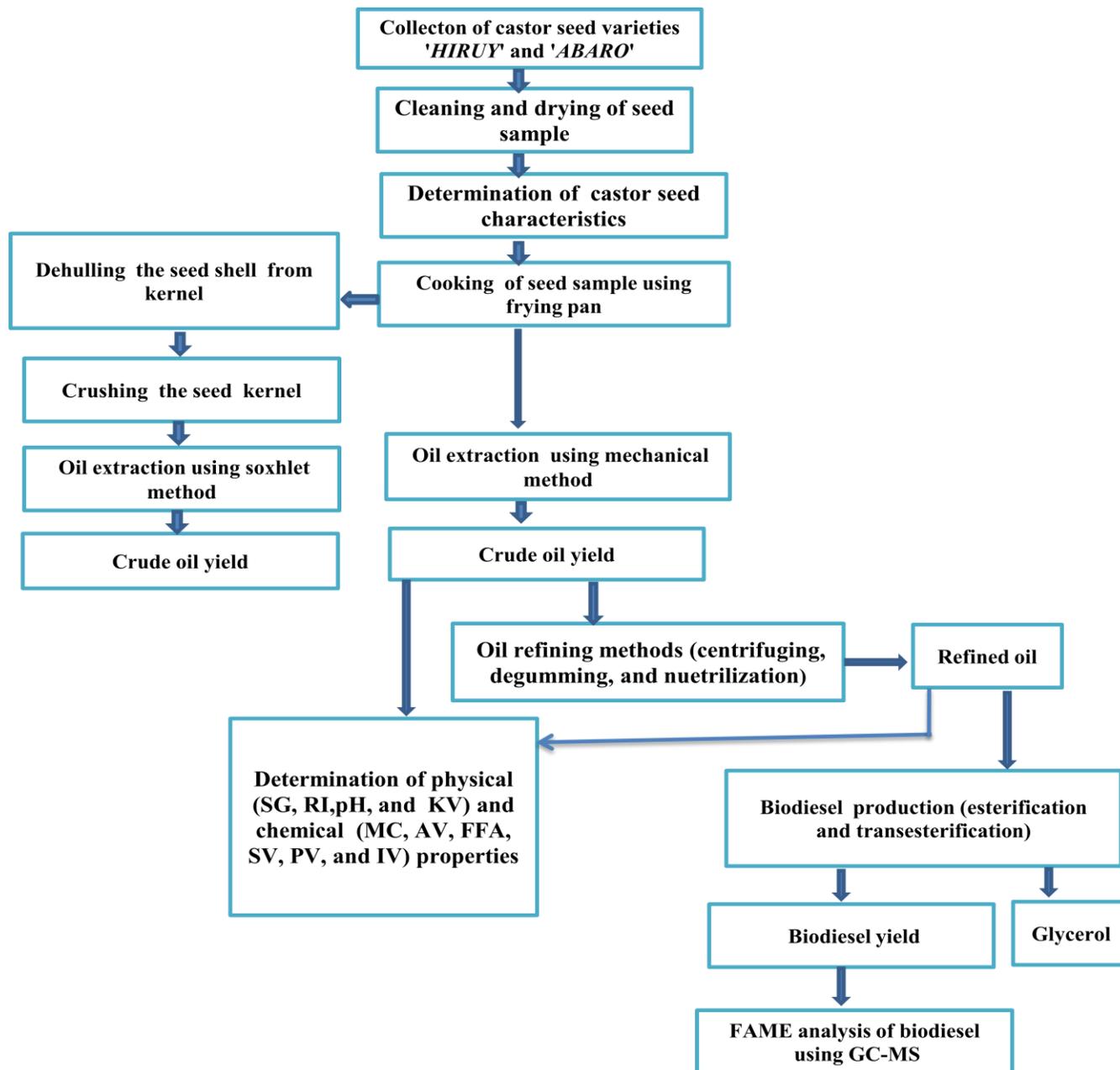
$$\text{oil yield \%} = \frac{\text{weight of castor oil} * 100}{\text{weight of castor seed extracted}} \quad (2)$$

Mechanical screw press extraction: The screw press was cleaned and made ready for use. The temperature was set at 60°C and increased to 70°C. The extractor was adjusted for low speed and small outlet holes for cake residue. Then 100 g of cooked castor seed samples were poured into the inlet hopper.

The extracted castor oil was collected and weighed and calculated by the formula given above (equation-2).

Pretreatment of the crude castor oil: Physical and chemical refining methods were used to refine the extracted crude oil. The detailed steps are presented the following sub-sections.

Centrifuging: Crude castor oil was poured into a clean. The speed was set at 1,000 centrifugal forces and the time was 40 minutes. Finally, crude oil was filtered using filter paper and purified^{34,36}.



Scheme-1: The Schematic diagram for castor seed collection, oil extraction, physicochemical property, and biodiesel production processes.

Degumming: The centrifuged oil was weighed in a 250 ml flask placed on a magnetic stirrer on a hot plate, stirred at 600 revolutions per second and heated to 85°C. Approximately 60 ml of 0.1 M of phosphoric acid was added into the mixture and allowed to settle for 30 minutes. Then 100 ml of distilled water was added and allowed to stand for a further 10 minutes. The mixture was transferred to a separate funnel and allowed to settle for 15 minutes. The lower layer was drained to separate the oil in the upper layer from the lower layer. The oil was washed three times repeatedly to ensure the purity of the degummed oil³². The acid pretreatment loss was calculated using following formula (equation-3)³³:

$$\text{Acid pretreatment loss} = \frac{\text{weight of crude oil} - \text{weight of pretreated oil}}{\text{Weight of crude oil}} \quad (3)$$

Neutralization: The degummed oil was weighed in a 250 ml flask and mixed with 25 ml of 0.5N sodium hydroxide. The mixture was then heated to 80 °C with a magnetic stirrer on a hot plate, stirring at 600 rpm to break up any emulsions that may have formed during the neutralization. Then sodium chloride (10% of the oil content) was added into the mixture to dissolve the soap formed. The mixture was then transferred to a separatory funnel and allowed to stand for one hour to remove a soap formed in the process. Distilled hot water was added repeatedly to the mixture until any soap remaining in the solution has been removed completely. The neutralised oil in the upper layer was separated and poured into a flask. The oil was dried in an oven at 105 °C for 2 hours to remove moisture. Finally, the neutralisation pretreatment loss was calculated using the following formula (equation-4)³⁹⁻⁴¹.

$$\text{Neutralization loss} = \frac{\text{Weight of degummed oil} - \text{weight of neutralized oil}}{\text{weight of degummed oil}} \quad (4)$$

Physicochemical properties of the extracted castor oil: The physicochemical properties of the oil have a direct or indirect influence on the quality of the biodiesel³⁷. Some important physical and chemical characteristics of the extracted oils were determined in order to assess their suitability for biodiesel production.

Moisture content: The moisture content of the extracted castor oil was determined by means of a drying furnace. Approximately 10 g of the oil sample were weighed and dried in a furnace at 105°C for 2 hours. The sample of oil was removed from the furnace, cooled for 30 minutes in a desiccator and weighed. The procedure was repeated until a steady weight was obtained. The oil's moisture content was then calculated using the following formula (equation-5)^{31,32,38}.

$$\text{Moisture content of oils}\% = \frac{(W1 - W2)}{W2} * 100 \quad (5)$$

Where W1= Original weight of sample before drying (g); W2 =Weight of sample after drying (g).

Refractive index: The refractive index of castor oil was determined by means of a refractometer. The glass prism of the

refractometer was cleaned with alcohol to remove dust. Then a few drops of the oil sample were placed on the lower prism and rubbed and sealed with a second covering prism. Then the light source of the meter was turned on. Through the eye piece of the meter, the dark part of the image was adjusted to match the cross-intersection. The scale pointer was pointing to the refractive index without any parallax error. Then, oil readings at room temperature were recorded^{31,38,39}.

Specific gravity: The crude oil was poured into a pre-weighed flask (W0). Then the flask was filled with the oil sample and weighed using a weighing device (W1). A similar procedure was used to determine the weight of the same volume of distilled water. Finally, the specific gravity of the oil was calculated using the following formula (equation-6)^{31,32,40}.

$$\text{Specific gravity \%} = \frac{W1 - W0}{W2 - W0} = \frac{\text{Mass of the substance}}{\text{Mass of an equal volume of water}} \quad (6)$$

Where, W0 = Weight of empty bottle (g). W1 = Weight of the bottle and oil content (g). W2 = Weight of bottle and water content (g).

Kinematic viscosity: The kinetic viscosity of the oil was determined by means of a digital viscometer. The spindle is turned through the calibrated viscometer spring in a 50 ml sample of oil in a 100 ml beaker. The number of the coil used was 02. The viscous drag of the liquid on the coil is measured by the spring displacement. The viscosity of the oil at room temperature was shown on the screen of the visco-meter. This was then observed in centipoises^{32,33}.

Saponification value: A 5 g oil sample was weighed into a 250 ml conical flask and mixed with 25 ml of 0.1N ethanolic potassium hydroxide. 25 ml of the blank solution was measured in a separate conical flask. The two samples were then continuously agitated and permitted to boil gently for one hour. The reflux condenser was positioned on the flask holding the mixture. A small number of phenolphthalein indicator drops were added to the warm solution. The mixture was then titrated with 0.5M HCl until the endpoint was reached, at which point the pink color of the indicator faded. The same procedure was used for the blank solution. The saponification value was determined by applying the following formula (equation-7)^{31,32,41}.

$$\text{Saponification Value} = \frac{56.1N (V0 - V1)}{M} \quad (7)$$

Where, V0 = the volume of the solution used for blank test; V1 = the volume of the solution used for determination. N = Actual normality of the HCl used. M = Mass of the sample.

Free fatty acids and acid value: Approximately five grams of oil sample were weighed in a 250 ml conical flask. Next, 50 ml of newly neutralized hot ethyl alcohol and 1 ml of phenolphthalein indicator were added to the solution. The

mixture was then heated until the oil was fully dissolved. The solution was subsequently titrated with 0.01N potassium hydroxide while being constantly shaken until the pink colour persisted for 15 seconds. The free fatty acids and the acid value were calculated using the following formulas (equation-8 and 9)^{31,33,41}.

$$\text{Free fatty acid} = \frac{28.2 \times V \times N}{W} \quad (8)$$

$$\text{Acid value} = \frac{56.1 \times V \times N}{W} \quad (9)$$

Where V = Volume in ml of standard potassium hydroxide. N = Normality of the potassium hydroxide solution. W = Weight in g of the sample.

Iodine value: Approximately 5 grams of oil were weighed in a 250 milliliter conical flask and dissolved in 25 milliliters of carbon tetrachloride. Next, 25 ml of Dam's reagent (Wij's solution) was added to the mixture using a safety pipette within a fume chamber. The stopper was then inserted, and the flask contents were vigorously swirled. Next, the flask was left in the dark for 2.5 hours. Subsequently, 20 ml of a 10% aqueous potassium iodide solution and 125 ml of distilled water were added using a measuring cylinder. The solution was titrated with 0.1M sodium thiosulphate solutions until the yellow colour was almost gone. A small amount of 1% starch solution indicator was added, and titration was then continued by slowly adding sodium thiosulphate while vigorously shaking until the blue coloration was no longer visible. The same procedure was used for the blank test. The iodine value of the oil sample was determined by applying the formula outlined in equation 10^{31,32,40}.

$$\text{Iodine value} = \frac{12.69C(V1-V2)}{M} \quad (10)$$

Where C = Concentration of sodium thiosulphate used, V1 = Volume of sodium thiosulphate used for blank (ml), V2 = Volume of sodium thiosulphate used for determination (ml), M = Mass of the sample (g), Iodine factor = 12.69.

pH value: The pH value of the oil sample was determined using a pH meter. The pH electrode was standardized with a buffer solution. Then about 100 ml of oil was taken in a conical flask. The electrode was then immersed into the sample and the pH reading was taken³¹.

Peroxide value: About 5g of oil sample was weighed in a 250 ml conical flask and mixed with 30ml of acetic acid chloroform solution. Then the flask was swirled to dissolve the sample. 0.5 ml of the potassium iodide solution was added into the flask containing the mixture. The content of the flask was swirled vigorously and allowed to stand for 1 minute and then 30ml of distilled water was added to it. The mixture was titrated with standard thiosulphate solution using a 1% starch as an indicator. Similar procedure was applied for the blank test. Finally, the peroxide value was calculated using the formula (equation-11)^{31,40,42}.

$$\text{Peroxide value} = \frac{(T-B) \times N \times 1000}{W} \quad (11)$$

Where T = titration volume for sample (ml), B = titration volume for blank (ml), N = normality of thiosulphate used, W = Weight of sample (g).

Biodiesel production: Castor oil refined using the screw-press method was used to prepare biodiesel through a two-step process. The steps consisted of acid-catalyzed esterification and subsequently base-catalyzed transesterification for Castor oil with higher FFA content. The method was chosen for the complete conversion of FFA and TG (triglyceride) into fatty acid methyl esters or for boosting the yield of biodiesel.

Esterification process: The initial step involved in the preparation of fatty acid methyl esters from refined castor oil was esterification. Boron trifluoride catalysed the reaction in methanol. The oil sample was contained in a 250 ml conical flask. Subsequently, 12% boron trifluoride in methanol was prepared and subsequently transferred into the reaction mixture. The flask was subsequently placed on the hot plate, which was equipped with a magnetic stirrer. Next, a reflux condenser placed at the top of the flask was used to condense the methanol vapors. The reaction was performed for approximately one hour at 60 °C. The cooled solution was then transferred to the separatory funnel at room temperature. The mixture was left to settle under gravity for 24 hours at room temperature. The esterified oil was collected and dried in an oven at 105 °C for 30 minutes^{33,43}.

Trans-esterification process: The process of transesterification is the second phase in the generation of biodiesel from the esterified sample. In this reaction, sodium hydroxide acted as a catalyst alongside methanol. The previously collected and dried esterified oil from the initial step was placed in a 250 ml conical flask. Subsequently, a freshly prepared 0.5N sodium hydroxide solution in methanol was introduced into the oil-containing reaction medium. To ensure complete conversion of triglycerides to biodiesel, a methanol-to-oil molar ratio of 6:1 was maintained. The transesterification reaction took place under reflux conditions, with the mixture heated for 1 hour at 60 °C while being stirred continuously. Once the reflux was concluded, the solution was left to cool down to room temperature. The cooled reaction mixture was then poured into a separatory funnel, shaken for 10 minutes, and permitted to stand for 24 hours at room temperature to allow for gravitational separation. After settling, the upper layer (biodiesel) was extracted from the lower layer (glycerin) and underwent gentle washing with an equal volume of warm distilled water (in a separatory funnel) along with 1-2 drops of acetic acid. In the final steps, the biodiesel was collected and dried in an oven at 105°C for 30 minutes. The percentage yield was calculated using the formula given below (equation-12)^{31,33,43}.

$$\text{Yield of biodiesel}(\%) = \frac{\text{weight of biodiesel}}{\text{weight of sample oil}} * 100 \quad (12)$$

GC-MS analysis of the prepared biodiesel: The prepared biodiesel, a fatty acid methyl ester, was determined using gas chromatography-mass spectrometry (GC-MS). The GC-MS experiments were conducted at the Food Process Engineering Department of Addis Ababa Science and Technology University, Ethiopia. The experimental details are outlined below. A small volume of approximately 1 micro-liter of purified biodiesel was carefully introduced into a 1.5 milliliter GC sample vial with a Teflon cap. The protocol optimization employed for the biodiesel analysis using GC MS comprised a capillary column with dimensions of 30m×0.25 mm i.d., and a film thickness of 0.25µm. The ratio of the injected 1-µL FAMES was split 1:10. The carrier gas used was helium, flowing at a rate of 1.5 ml/min. The injector and detector temperatures were set at 280°C and 250°C respectively. The oven was initially set to 100°C for 2 minutes and then ramped at a rate of 10°C/min to 125°C over 1 minute, next it was heated at a rate of 5°C/min to 220°C, held at 220°C for 5 minutes, and then increased to 300°C at a rate of 3°C/min, maintaining the temperature at 300°C for a further 5 minutes. The mass spectrometer was operated in electron impact mode at 70eV within the scan range of 40-550 m/z. GC-MS chromatograms were compared with the NIST library, which yielded a significant amount of information^{41,44,45}.

Statistical analysis: The factorial design with a complete randomization design (CRD) was chosen. The studies were conducted in triplicates. All values were presented as mean ± standard error. The experimental data analysis of variance (ANOVA) was conducted using SAS software⁴⁶. Least significant difference (LSD) was determined using Fisher's LSD test at a significance level of P < 0.05. Values of probability below 0.05 were considered significant.

Results and Discussion

Characteristics of the castor seed samples: In this investigation, two novel castor seed varieties were derived from the Melkasa Agricultural Research Center (MARC). The two castor seed varieties identified are ABARO and HIRUY (Figure-1). The characteristics of these varieties were investigated by studying their seed weights, seed moisture content, and seed color. The 'HIRUY' variety exhibited a statistically higher seed weight (52.97g) compared to the 'ABARO' variety (48.89g). The colour difference between the genotypes indicated that 'HIRUY' has a brown color and 'ABARO' has a purple color. Most of the varietal difference in seed weight and color is due to the genetic differences between the two varieties. ANOVA showed a statistically significant difference between the castor genotypes in terms of seed weight (Table1), with P<0.001, whereas there was no significant difference between the castor genotypes in seed moisture content (Table-1), with P > 0.05 (Table-1).



Figure-1: The seeds of HIRUY (a) and ABARO castor seeds (b).

The data obtained are consistent with reports in the literature, which indicated that a weight of 100 castor seeds falls within a range of 10.1 to 73.3g⁴⁷. The current study's result, showing the weight of 100 HIRUY variety seeds to be 52.97 g (Table-1), is comparable to values reported in the literature, which averaged 52.5 g per 100 seeds²⁸. Larger seeds generally have a relatively higher oil content compared to smaller seeds. Studies have demonstrated that in castor oil, the endosperm is the site where oil is stored, and it is typically larger in seeds with a greater size. Consequently, the individual seed weight may directly impact the amount of oil extracted per seed, and choosing seeds with heavier weight should be taken into account when making recommendations for production.

Table-1: The weight (g) of 100 seeds and the seed characteristics.

	Seed weight (g)	Seed color
ABARO	48.89 ^b	Purple
HIRUY	52.97 ^a	Brown
CV	0.952	
LSD	1.099	
Significance level	***	

Where, Ns – Non-significant; * significant (5%); ** significant (1%); *** significant (0.1%); LSD = least significant difference and CV (%) = coefficient of variation.

Effect of seed variety and oil extraction method on oil yield:

Several factors that make castor seed oil a suitable feedstock for biodiesel production include lower heat energy requirements for conversion to fuel, high oxygen content for complete combustion, and the need for minimum production costs^{42,48}. This fuel has properties similar to those of conventional fuels and enhanced lubrication capabilities⁴⁸. Oils were extracted from the two castor seed varieties (ABARO and HIRUY) using

two distinct oil extraction methods to evaluate the impact of seed variety and extraction method on oil yield. Table-2 and Figure-4 indicate that HIRUY variety yielded more oil than ABARO variety. The analysis of variance showed that the oil extraction method had a significant main effect ($P < 0.001$) on oil yield from castor beans, as did the variety ($P < 0.001$), and there was also a significant ($P < 0.01$) interaction effect between the extraction method and variety on oil yield (Table-2).

Table-2: The main factor effect of variety and oil extraction method on oil yield.

Variety	Oil yield (%)
ABARO	46.212 ^b
HIRUY	50.698 ^a
LSD (5%)	1.001
Significance level	***
Oil extraction method	
Soxhlet	53.528 ^a
Mechanical Screw Press	43.382 ^b
LSD	1.001
Significance level	***
CV	1.332

Where: ns – Non-significant; * significant (5%); ** significant (1%); *** significant (0.1%); LSD = least significant difference and CV (%) = coefficient of variation.

Main factor effect of variety and oil extraction method on oil yield: A comparison of the mean oil yield percentages revealed a significant difference among various castor bean varieties, as demonstrated in Table-2. The HIRUY variety showed a higher oil yield percentage (50.698%) than the ABARO variety, which had the lowest oil yield (46.212%) (Table-2). Observed differences in oil yield amongst castor bean varieties are likely due to genotypic variations, with HIRUY showing a higher oil yield percentage compared to other tested varieties in Ethiopia's Central Rift Valley. This discovery aligns with studies indicating that the larger seed size of the particular variety contributes to increased oil production²⁸. The Soxhlet oil extraction method demonstrated the most pronounced effect, resulting in an average oil yield of 53.528%. The mechanical oil extraction method showed a lower average oil yield of 43.382% from both varieties as indicated in Table-2. The variations in oil yield among the oil extraction methods may be caused by the difference in oil extraction efficiency. The pressure, solvent, and heat employed in the extraction process could directly impact the quantity of oil obtained⁴⁸⁻⁵⁰.

Interaction effect of variety and oil extractor machine on oil yield: The interaction between variety and oil extraction method has significantly impacted the oil yield of castor bean ($p < 0.01$). The results suggest that different castor bean varieties have varying responses to the oil extraction methods. A higher oil yield of 56.693% was obtained when the HIRUY variety was subjected to the Soxhlet extraction method compared to the ABARO variety, which achieved a yield of 50.363% (Figure-2). The lower oil yield, at 42.06%, seen in the ABARO variety was obtained using a mechanical oil extraction method, which was lower than the 44.703% yield observed in the HIRUY variety (Figure-2). The current outcome aligns with research that involved evaluating 48 castor accessions using the Soxhlet oil extraction method, which found that there is sufficient variability in castor genotypes' oil yield⁵¹. The range of observed oil content in this study was found to be between 42.4% and 53.53%, with a mean value of 42.53%. Various researchers have documented the results of their studies on oil yield evaluation, which involved testing multiple castor bean varieties; they noted significant variation in percent oil yield across the assessed varieties. The results of this study are consistent with earlier studies, which found that the oil yield fell between 42.5% and 54.86% for the castor varieties examined⁵²⁻⁵⁵.

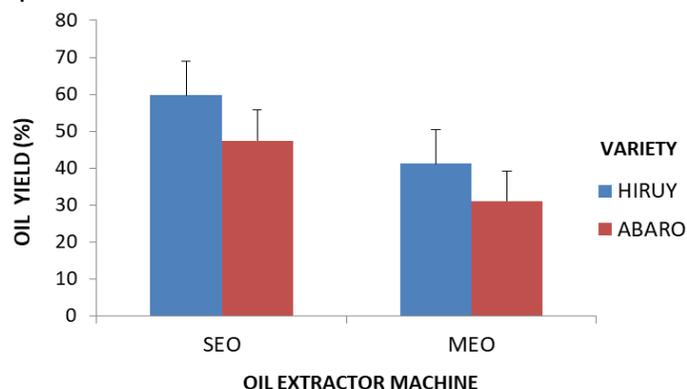


Figure-2: Interaction factor effect of variety and oil extractor machine on oil yield. Where SEO is oil extracted by Soxhlet and MEO is oil extracted by mechanical screw press.

Physical and chemical properties: Whichever extraction method is used, crude and refined oil must be assessed for their physico-chemical properties, which include specific gravity, refractive index, pH value, kinematic viscosity, acid value, free fatty acid, saponification value, moisture content, iodine value, and peroxide value²⁹. Conversion of vegetable oils through trans-esterification with methanol and the use of acid/base catalyst yields fatty acid alkyl esters (biodiesel) and glycerol^{49,57-59}. It is worth noting that while the oil obtained through the Soxhlet oil extraction method was higher in quantity (Figure-2), it may have undergone chemical alterations due to the solvent and heat employed during extraction and the extended extraction time. On the other hand, the oil extracted by mechanical methods may retain its natural chemical properties as it is extracted by pressure alone.

It is anticipated that this will produce a biodiesel of good quality. To evaluate the impact of extraction methods on oil characteristics, key physicochemical properties including specific gravities, kinematic viscosities, pH levels, free fatty acid values, acid values, peroxide values, saponification values, and iodine values of the extracted oils²⁹ and the biodiesel produced were measured in accordance with established protocols.

Specific gravity: The specific gravity value is a measure of the relative density of the oil, typically denoted as SG⁴⁰. A comparison of the mean SG values for the crude oil revealed that 'ABARO' exhibited a statistically higher SG value (0.979) compared to 'HIRUY' (0.969). The SG values of the refined oils indicated that 'ABARO' had a statistically higher value (0.967) compared to 'HIRUY' (0.958) (Table-3). The data indicated that the SG of crude oil was greater than that of the refined oil. The elevated SG of the crude oil can be attributed to the presence of some impurities. Removal of impurities is expected to enhance the quality of refined oil to be used for biodiesel. Consistent with earlier studies, this study's results revealed that the SG of crude oil (0.9628) was greater than that of refined oil (0.9618)^{33,35}. The data also aligns well with the ASTM standard range of SG (0.957-0.968) for oil quality suitable for biodiesel production.

Kinematic viscosity: The kinematic viscosity (KV) is the resistance to flow with vibration in the castor oil³³. The KV is a significant property of oil utilised for producing biodiesel, and it seems to decrease when the oil's purity is elevated. The presence of free fatty acids, gums, phospholipids, and other oil impurities is thought to be responsible for this, and they must be removed via degumming before biodiesel production can take place. The results indicated that the KV value for crude oil derived from ABARO variety was greater (271.667 mm²/s) than that from HIRUY variety (259 mm²/s) (Table-3 and Figure-4). The refined oil's KV data also demonstrated that ABARO had a

higher value (214.333mm²/s) than HIRUY's (202.667 mm²/s) (Table-3 and Figure-4). The data showed that oil from HIRUY variety had superior quality than ABARO for biodiesel production, which requires smooth ignition in both cold and hot temperatures, potentially due to a genotype difference. The kinetic viscosity value of this study was lower than the reported values for crude oil (234.07mm²/s) and refined oil (224.16mm²/s)^{33,35}.

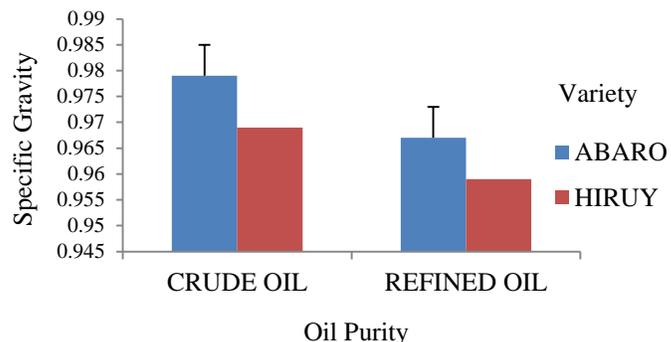


Figure-3: The SG of crude and refined oils of castor bean varieties 'HIRUY' and 'ABARO'.

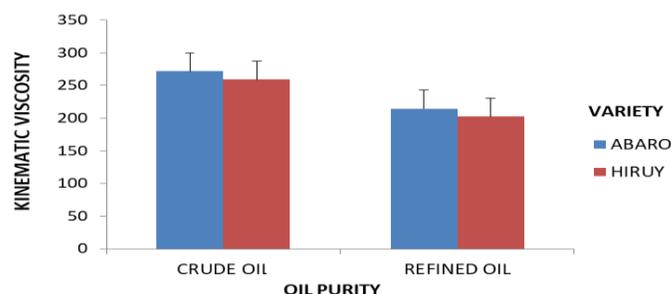


Figure-4: The kinematic viscosity for crude and refined oil of castor bean varieties 'HIRUY' and 'ABARO'.

Table-3: The physical properties of the crude and refined castor oil from ABARO and HIRUY varieties.

Variety	Crude oil				Refined oil			
	SG	KV	RI	pHV	SG	KV	RI	pHV
ABARO	0.979 ^a	271.67 ^a	1.469 ^a	5.683 ^b	0.967 ^a	214.333 ^a	1.475 ^a	6.61 ^b
HIRUY	0.969 ^b	259 ^b	1.47 ^a	6.03 ^a	0.959 ^b	202.667 ^b	1.477 ^a	6.743 ^a
CV	0.209	1.202	0.136	0.929	0.116	1.504	0.11	0.773
LSD	0.0046	7.228	0.0045	0.121	0.0025	7.108	0.0037	0.117
Significance level	**	**	Ns	**	**	**	Ns	*

Note: SG- specific gravity, KV- kinematic viscosity, RI- refractive index, PH- pH. Where, ns – Non-significant; * significant (5%); ** significant (1%); *** significant (0.1%); LSD = least significant difference and CV (%) = coefficient of variation.

Refractive Index: The refractive index (RI) is a physical property which determines the degree to which light travels bent or refracted when it enters a material (e.g. oil). Thus, the higher the RI value, the higher the degree of unsaturation of the oil, which is related to the iodine content. It is therefore preferable to use unsaturated oil in order to obtain a biodiesel that is liquid at room temperature and flows smoothly through the engine³⁹. The data on the RI showed that the crude and refined oil samples from the HIRUY variety (1.47 and 1.477) were slightly higher than the corresponding values for the ABARO variety (1.469 and 1.475) (Table-3). The average comparison of the RIs showed that there was no significant difference between the castor varieties in crude and refined oil (Table-3, Figure-5). However, the average comparison showed that there was a slight difference between the crude and refined oil RI values (Figure-5). The current study's result was also consistent with ASTM guidelines for the range of 1.476 to 1.479 and a report by Akpan et al., which indicated refractive index values 1.4686 for crude castor oil and 1.4674 for refined castor oil³¹.

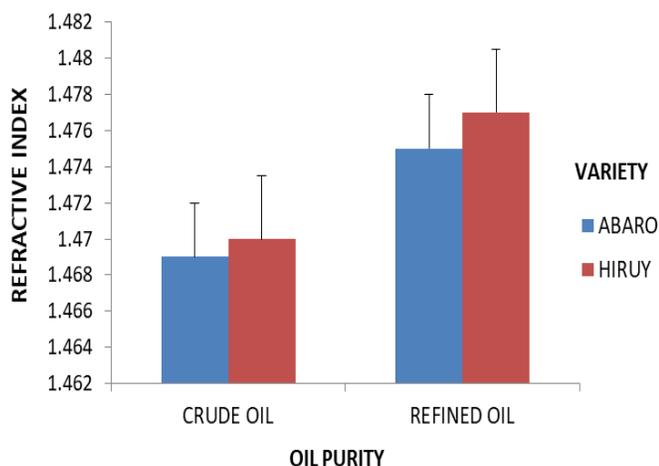


Figure-5: Effect of castor bean varieties 'HIRUY' and 'ABARO' on refractive index for crude and refined oil.

pH value: The pH value is used for determining the acidity or alkalinity of oil samples³⁸. The average of the pH values of the oils in this study showed that ABARO oil is more acidic (5.683) than HIRUY oil (6.03) (Table-3, Figure-6). On the other hand, the refined oil of the ABARO variety had a higher acidity (6.41) than the refined oil of the HIRUY variety (6.52) (Table-3, Figure-6). In addition, data showed that crude oil is more acidic than refined oil (Figure-6). This can be attributed to the presence of free fatty acids and other impurities in the oil. The refined oil is, therefore, of higher quality for the preparation of castor-based biodiesel. The data also showed that the quality of the HIRUY oil for biodiesel production is better than that of the ABARO oil.

This may be caused by a genotype difference. The findings of this study are in line with the ASTM standard range of the pH value of refined oil (6.54) for the production of biodiesel.

Similarly, Akpan and colleagues reported the pH of crude oil and refined oil to be 6.11 and 6.34, respectively.

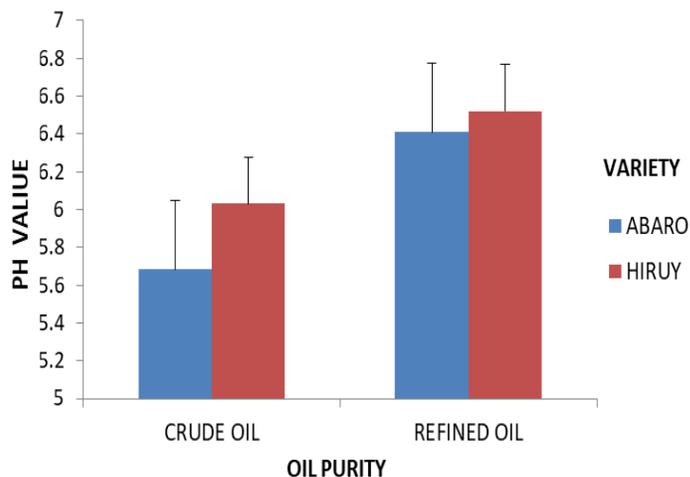


Figure-6: The pH value of crude and refined oil of two castor bean varieties 'HIRUY' and 'ABARO'.

Chemical properties: Free fatty acid: The acidity of crude oil is often expressed as the percentage of free fatty acids (FFA) in the sample⁵⁶. The average comparison of the FFA values of oils in this study showed that the FFA values of ABARO oil were higher (1.696%) than those of HIRUY oil (1.485%). Similarly, the FFA value of refined oil from ABARO was also shown to be relatively higher (0.9%) than that of HIRUY (0.826%) (Table-4, Figure-7). The relatively lower FFA value for HIRUY oil indicated that the oil of this variety is of higher quality for biodiesel production. The data obtained in this study are comparable to those reported by Imasuen et al. which reported that the FFA of castor oil seed oil ranged from 0.90 to 1.06 %³.

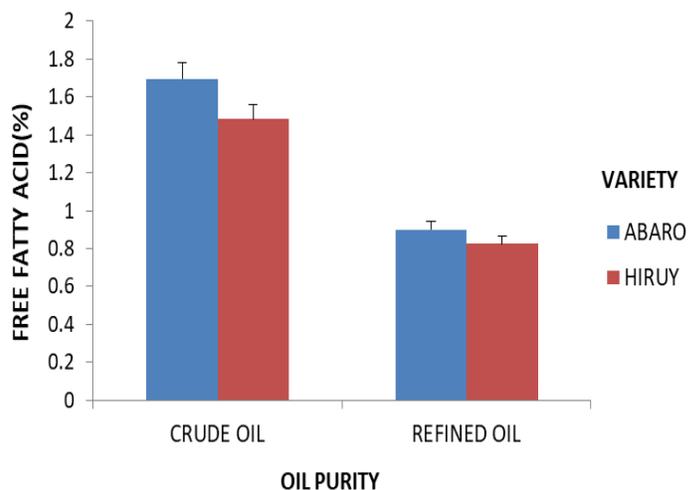


Figure 7: The effect of castor bean varieties 'HIRUY' and 'ABARO' on FFA content of crude and refined oil.

Acid value: The acidity value (AV) is defined as the number of milligrams of sodium or potassium hydroxide needed to

neutralize free fatty acids in one gram of oil. This is a relative measure of acidity, since free fatty acids are normally formed during the degradation of glycerides from oil⁵⁶. The average comparison showed that the AV of ABARO crude oil was higher (3.367 mg per g) than that of HIRUY crude oil (3.003 mg per g). The average content of refined oil from ABARO was also relatively higher (1.799 mg per g) than that of HIRUY (1.66 mg per g) (Table- 4, Figure-8). Of the oils from these two castor seed varieties, HIRUY oil was found to have a relatively lower AV, which suggests that it can be considered as a better raw material for the production of biodiesel in good quantity and quality. The results of this study are in line with the ASTM standard which stated that the AV of refined oil for biodiesel production to be in the range (0.4-4 mg per g) for the extraction of crude oil for use in the production of biodiesel. The data obtained in our study were comparable to the reported AV values (1.84 - 2.12 mg per g) of castor oil in the literature⁵⁵, indicating that the Ethiopian castor oil oil is within acceptable levels for biofuel production.

Saponification value: The saponification value (SV) is the number of mg potassium hydroxide required for the saponification of 1 gram of oil. Higher SV leads to a decrease in biodiesel yield. Therefore, it has to be reduced prior to biodiesel production by neutralising the oil⁴¹. Therefore, a lower SV is recommended in order to achieve a higher yield of biodiesel. The average comparison of the data from our study showed that the SV of ABARO crude oil is slightly higher (183.566 mg KOH per g) than that of HIRUY crude oil (180.019 mg KOH per g) (Table-4, Figure-9). A similar trend was observed for refined oil samples from the ABARO variety, which was relatively higher than the HIRUY SV (175.42 mg KOH per g) (Table-4, Figure-9). The SV of crude oil was higher than that of refined oil. Of these two varieties, HIRUY showed a relatively lower SV than ABARO, which is better for high biodiesel yield as well as for quality (Figure 9). The data obtained in this study are in line with literature reports which indicate that the VSP of refined oil for the extracted oil used for biodiesel production is in the range (175-187 mg KOH per g)^{35,55}.

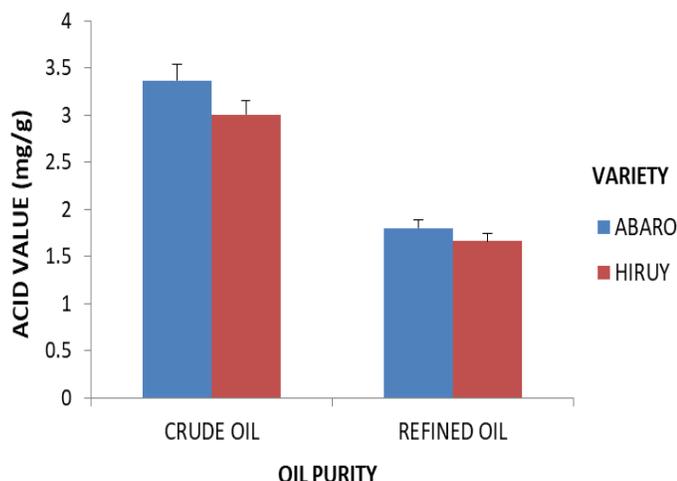


Figure-8: The acid value of crude and refined oil of castor bean varieties 'HIRUY' and 'ABARO'.

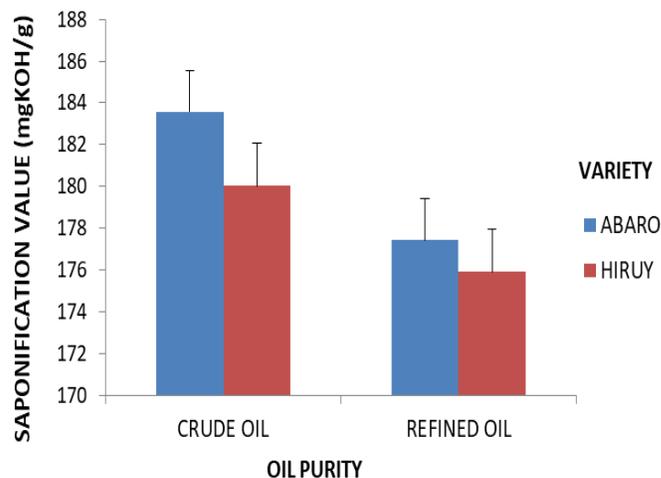


Figure-9: Saponification value of crude and refined oil 'HIRUY' and 'ABARO' castor bean varieties.

Table-4: The chemical properties of the crude and refined oil samples obtained from ABARO and HIRUY castor seed varieties.

Variety	Crude oil						Refined oil					
	MC	FFA	AV	SV	PV	IV	MC	FFA	AV	SV	PV	IV
ABARO	0.393 _a	1.696 _a	3.367 _a	183.566 _a	8.086 _a	85.023 _b	0.290 _a	0.9 _a	1.799 _a	177.42 _a	6.06 _a	82.803 _b
HIRUY	0.368 _a	1.485 _b	3.003 _b	180.019 _b	7.316 _b	89.883 _a	0.274 _a	0.826 _b	1.66 _b	175.896 _b	5.42 _b	84.516 _a
CV	6.87	3.915	2.15	0.278	1.905	0.798	5.841	1.891	1.524	0.154	4.567	0.332
LSD	0.059	0.141	0.155	1.148	0.332	1.583	0.037	0.037	0.145	1.118	0.594	0.63
Significance level	Ns	**	**	**	**	**	Ns	**	**	**	*	**

Note: MC- moisture content, FFA- free fatty acid, AV- acid value, SV- saponification value, PV-peroxide value, IV- iodine value. Where, ns – Non-significant; * significant (5%); ** significant (1%); *** significant (0.1%); LSD = least significant difference and CV (%) = coefficient of variation.

Peroxide value: The peroxide value (PV) of the oil is a measure of the extent of spoilage, when oxygen is not activated by the presence of a strong antioxidant. Therefore, lower PV oil is preferred to avoid spoilage of biodiesel by oxygen during storage⁴⁰. The mean PV values for this study were found to be 8.086 meq/g and 7.316 meq/g for ABARO and HIRUY crude oil, respectively (Table-4, Figure-10). Refined oil of the ABARO variety showed a relatively higher PV (6.06 meq/g) than that of the HIRUY variety (5.42 meq/g) (Table-4, Figure-10). The result showed a significant difference between the two varieties in terms of spoilage rate, both crude and refined. It is expected that the HIRUY oil will show a relatively lower level of spoilage than the ABARO oil. This makes the HIRUY variety a potential source for the production of high-quality biodiesel. In addition, the crude and refined oil data in this study were comparable with literature reports which show that the PV values for crude and refined oils are 6.93 and 5.90 meq/g, respectively.

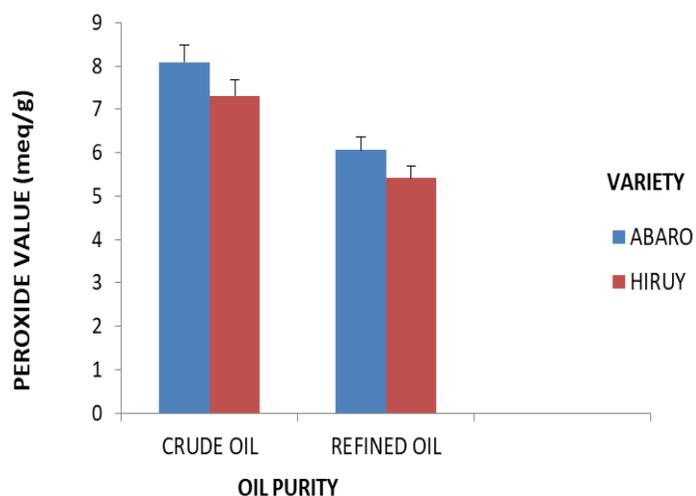


Figure-10: The effect of castor bean varieties 'HIRUY' and 'ABARO' on peroxide value of crude and refined oil.

Iodine value: Iodine value (IV) is a parameter used to determine the amount of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. The higher the IV, the more unsaturated fatty acid bonds are present in the oil, appear to be more fluid, and are liquid at room temperature and winter conditions⁴⁰. The IV values of the crude oils were 85.023 and 89.883 from ABARO variety and HIRUY variety, respectively. These data also showed IV value of crude oil from HIRUY variety to be higher than that of ABARO variety. Similar trend was observed for IV values of refined oils extracted from the two castor varieties used in the study (Table-4, Figure-11).

The findings of this study indicate that crude oil IV values are slightly higher than refined oil IV values and are also in line with reported literature IV values for crude and refined oil of 86.98 and 84.23 respectively³⁵. It was also found that the data were comparable to the Akpan et al report who reported that the

IV of crude castor oil was 87.72 and that the IV of refined castor oil was 84.8³¹ were not available for use in the survey. Data indicate that the HIRUY variety could be a potential source of improved biodiesel oil which could exist at room temperature in liquid or liquid form, as well as in low-temperature areas.

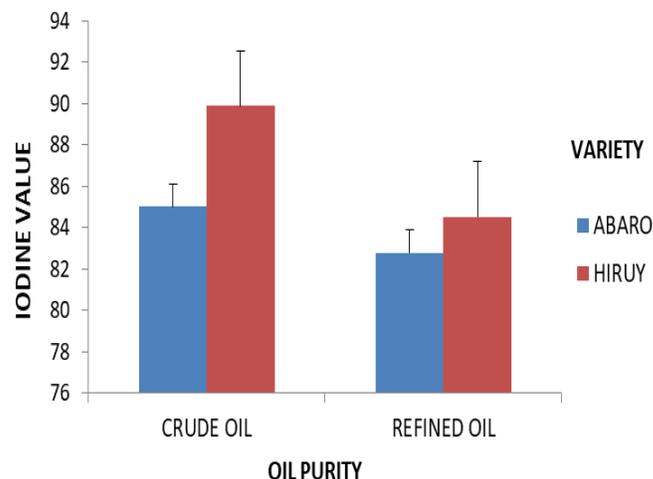


Figure-11: Effect of variety on iodine value of crude and refined oil.

Biodiesel production and GC-MS analysis: Biodiesel production: The biodiesel was prepared from refined oils from the castor seed varieties studied and from experimental procedures discussed in the Experimental part of the present article. The yield of biodiesel from ABARO and HIRUY crude oil was 73.87% and 74.76%, respectively. Similarly, 87% and 89% of the biodiesel was obtained from ABARO and HIRUY refined oils, respectively. This study was found to be in line with the study report by Oluwole et al. (2014) which reported a significant difference in biodiesel yields between four castor oil varieties⁵⁷. The biodiesel yield calculated in this study was also very similar to the Lemma et al study, which reported biodiesel yields between 48.68% and 93.34%⁵⁸.

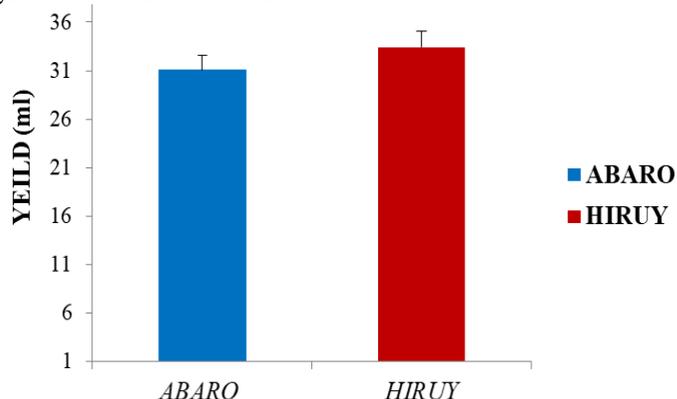


Figure-12: The biodiesel yield of 'HIRUY' and 'ABARO' variety.

GC-MS analyses of biodiesel: The chemical composition of biodiesel has an effect on its quality. It is therefore mandatory to analyse its chemical composition to determine its quality before

using chromatographic techniques such as gas chromatography-mass spectrometry (GC-MS). The methyl ester composition of biodiesel produced from castor oil varieties (HIRUY and ABARO) was found to be similar in terms of type and percentage of the esters with minor variations (Table-5). An analysis of the GC-MS data showed that biodiesel from ABARO oil has a higher content of methyl ricinoleate (90.915%) and a smaller content of other esters such as methyl esters (2.607), methyl esters (1.473), methyl esters (1.909), methyl esters (1.112) and methyl esters (0.98). Similarly, the highest methyl ricinoleate content (91.582) is followed by the low methyl linoleate content (2.394), methyl linolenate (2.102), methyl linolenate (2.035), Methyl palmitate (1.034) and methyl stearate (0.851) were found in the biodiesel formulated from HIRUYO oil (Table-5).

The findings of our study are in line with previous reports which have shown that methyl ricinoleate is the highest methyl ester (91.068) in seed oils^{34,41}. The maximum content of methyl ricinoleate (84.2%) was reported in 10 accession batches of castor beans⁵⁹. The existence of methyl ricinoleate is therefore one of the main reasons for the production of biodiesel from castor bean raw materials. Data for biodiesel produced from HIRUYA and ABARO showed higher unsaturated methyl ester

content (98.114) and a lower unsaturated methyl ester content (97.906) respectively. The study data showed that the unsaturated methyl ester content was higher than that of the Tanzanian castor oil biodiesel (88.3 to 96.2%)⁶⁰.

Conclusion

In this study, screw-press and Soxhlet extraction methods, integrated with factorial analysis, were used to compare the oil yield of two indigenous castor varieties in Ethiopia (ABARO and HIRUY). In addition, the biodiesel yield of ABARO and HIRUY varieties and the fatty acid methyl ester mass spectra of the biodiesel from these varieties were used for the comparison. The oil and biodiesel production was significantly influenced by the castor varieties released. Mass spectra by gas chromatography allowed a clear distinction to be made between the compositions of methyl ricinoleate and methyl stearate among the seven identified fatty acid methyl esters. The biodiesel produced from both varieties of castor contained a high content of unsaturated fatty acid methyl ester which makes the biodiesel a liquid at room temperature. These will make the two indigenous varieties a good alternative source of energy for the production of biodiesel.

Table-5: The GC-MS analysis of castor varieties *ABARO* and *HIRUY* based biodiesel.

Fatty acid methyl ester	ABARO area	ABARO area (%)	HIRUY area	HIRUY area (%)
Methyl Palmitate; C16:0	37484.88	1.112174	40243.57	1.034049
Methyl stearate; C18:0	33090.56	0.981795	33119.67	0.851002
Methyl Oleate; C18:1 n9	83374.77	2.473724	81827.09	2.102527
Methyl Linoleate; C18:2 n9,12	87872.48	2.60717	93204.76	2.394873
Methyl Linolenate; C18:3	-	-	79226.25	2.035699
Methyl Ricinoleate; C18:1 n9, 12-OH	3064223.8	90.91531	3564223.83	91.58185
Methyl Ecosenoate; C20:1 n11	64369.2	1.90983	-	-
Saturated	70575.44	2.093968	73363.24	1.88505
Unsaturated	3299840.3	97.9060	3818481.93	98.11495
Total	3370415.72	100	3891845.17	100

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