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Methods for Refining of Brebra *(Millettia ferruginea)* Oil for the Production of Biodiesel

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Abstract: Oil from brebra (*Millettia ferruginea)* is the potential raw material for biodiesel production. The refining of crude oil requires a series of purification steps to produce quality biodiesel. The objective of the study was to produce refined oil from brebra seed for biodiesel production. Crude oil extraction was carried out using mechanical press and solvent extraction methods. Impurities of the oil were determined by standard assay methods. Solubility characteristics of precipitates were determined. The data generated from nature of solubility were used to design refining technique. The appropriate method was found to be hexane-ethanol refining techniques. From crude oil extract using solvents, 84.66% and 15.34% were pure oil and residue, respectively. From crude oil extract by mechanical press, 86.83% and 13.17% were refined oil and residue, respectively. The quantity of refined oil extract by mechanical press was significantly ($p < 0.05$) increased than extracted by hexane. Crude oil contained 4.4% protein and 7.2% phospholipid. These impurities were responsible for the inferior quality of the biodiesel from crude oil. It is possible to recover the used solvents from their respective components by using Rotavapour and reuse for another oil refinery batch. Brebra oil can have important application for production of biodiesel.

Key words: Hexane-ethanol · Impurities · Oil extraction · Phospholipid · Protein · Refinery · Solvent

INTRODUCTION

Our modern technological society relies very heavily on fossil fuels as an important source of energy but use of fossil fuel as energy is currently encountered with a number of problems, such as the limiting supply of fossil fuels, global warming and the need to reduce dependence on fuel imports [1-2]. One of the most promising renewable sources used to reduce dependence on petroleum is biomass [3]. Among the biomass sources, vegetable oils and animal fats have attracted much attention as a potential resource for production of an alternative fuel such as biodiesel for the replacement of the petroleum-based diesel fuel. However, the main challenges in the production of biodiesel are its cost and availability of fats and oils resources [2]. Therefore, we have to search biodiesel from cheep and non-edible oil sources. Algae mainly microalgae [4] and vegetable oil [5] from non-edible source are the best raw material for biodiesel production. Among oil producing plants brebra (*Millettia ferruginea*) seed oil can serve as oil source for biofuel. The oil from brebra may not only be used for biodiesel production but it can serve as raw material for soap production and even as oil for human consumption if the method is developed to remove rotenone (mild toxic to human) from the oil.

Brebra, *Millettia ferruginea* (Hochst.) Baker, Leguminacae is a large tree growing up to length of 25 meter high. It is endemic to Ethiopia and widely distributed in the country within the agro climatic zones of 1000-2600 meter above sea level [6]. The tree is used for fish poisoning where mature pods and seeds are ground to fine powder and is spread over the surface of water [7]. Moreover, it serves as shade tree for coffee (*Coffea arabica*) in Harargae region, Easter Ethiopia [8] and South part of Ethiopia [9]. *Millettia ferruginea* is also a N_2 fixing leguminous tree species that is known to have significant positive effect for intercropping crops in the southern parts of Ethiopia [6, 10]. It is also used as agro forestry and for erosion control [6]. Despite these and other significant benefits of brebra the plant seed oil and protein are not investigated for production of

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economically important products like oil for soap, biodiesel and as well as proteins for different purposes (microbial media, human food and animal feed).

It is well known that oil seeds and their products are the most valuable agricultural crops in the world trade. Oil crops are processed into products by utilizing their seeds or the products derived by a partial removal of one of the major seed components. Extraction of oil is one of the major aspects involved in oilseed processing [11]. There are two things when considering the methods by which oil is extracted from a plant: the refinement level and the physical process used to extract the oil. Many technological processes are carried out in order to obtain refined vegetable oils from crude oils. Therefore, refining is a process of removing gross impurities from crude oil by means of chemical and physical treatment techniques [12]. The refining process probably has more impact on a vegetable oil's quality and economic performance than any of the other processes during the conversion to a finished product. Inadequately refined oils will affect the operation of all succeeding process [13]. With regard to later method, oils are extracted by squeezing the seed, grain or fruit at high pressure. In chemical or solvent extraction, the oil is separated from its components with hexane or other petroleum solvents. The oil is next refined, depending on the nature and the use of the oil [11].

Naturally occurring oils are complex mixtures of triglycerides and other materials, such as coloring materials, sugars, waxes, partial glycerids, free fatty acids, lipid soluble proteins and phospholipids [12]. Some, depending on the proposed use of the oil, components should be removed from the oil. This refining of the oil is an expensive procedure which is composed of a number of stages. Because of quality and economic importance of refining, a large amount of work has been done both to improve and to simplify refining processes [14].

Most of the impurities have unfavorable effects on intended use and shelf life of the oil and therefore have to be removed from the vegetable oils by chemical or physical refining processes [15]. The common oilseeds as soybean, cottonseed, sunflower and rapeseed are rich sources of phospholipids [16-17]. Phospholipids pose many problems for the storage and processing of the crude oil and are removed from oil during refining by a process known as degumming [18-19]. Normally, there are two types of phospholipids: hydratable (HPL) and non-hydratable (NHPL) and they are removed from oil by the degumming process. Traditional degumming processes, including water degumming, superdegumming, top degumming, acid treatment and other ones, cannot guarantee the achievement of low phosphorus contents required for physical refining and they are not always optimally suited for all oil qualities because of the high content of non-hydratable phospholipid [20].

Additionally, innovative extraction techniques, such as co-solvent amended extraction, were studied as potential replacement processing steps that may offer reduced production costs, improved extraction efficiency (phospholipids and protein capture) and a much more environmentally friendly process [14]. Depending on the nature of the oil type or source, the refinery process should be investigated and optimized. Therefore, operational parameters should be optimized during preliminary processing and isolation of oil. In this study, pretreatment, dehulling, oil extraction and oil purification procedures are optimized. Pretreatment is important to maintain the quality of the products. Dehulling is significant to avoid seed cots of the seeds in order to improve the quality of oil and protein of brebra oil cake. The well known oil purification (refining) process involves degumming, neutralization, drying, bleaching and deoderization [21].

According to this investigation, this level of brebra oil is recommended for industrial usage such as biodiesel production, soap production, lubricants for metal surfaces and textile chemicals as well as for human consumption if the rotenone is properly removed. Before this investigation, there is no any scientific report about the extraction and refinery processes of oil produced from brebra seed. Therefore, the main objective of this study is to develop appropriate refinery method of the oil for the production of biodiesel and other bioproducts. Data generated from this study is very important to produce quality biodiesel and soap from this non-edible oil and even for human consumption after developing of methods for removing of rotenone.

MATERIALS AND METHODS

Oil Purification (Refining Process): Crude oil was extracted from brebra seed using two methods: mechanical press and solvent extraction. When brebra seed oil obtained through the two extraction methods were tested for the preparation of biodiesel, the resulting product was brown to dark brown in colour containing which indicates that the crude oil contains impurities. Therefore, a purification step using standard acid degumming [21] or other methods is essential. In this work, 2% hot water containing 0.1% sulphuric acid and phosphoric were added separately to the separated crude oil samples and

then they were cooled to 45°C and allowed to stand for 60 min. The resulting precipitate was separated by centrifugation. Any trace of water left in the oil was removed using anhydrous sodium sulfate.

Determination of Hydrophobic Protein and Phospholipids from Brebra Seed Oil: The presence and quantity of hydrophobic proteins in the crude oil and different precipitates was measured following the Biuret method [22] with bovine serum albumin (BSA) serving as standard. The standard curve was prepared by making five dilutions (0 - 0.431μg/ml) from a 1 mg/ml protein stock solution of bovine serum albumin (BSA). The absorbance of samples (including the standards) was read at 540 nm.

The amount of phospholipid found in the different samples was determined by phosphorus assay [23]. The standard curve was prepared by preparing five dilutions (0-0.75 μg/ml) from a 100 μg P/ml KH_2PO_4 stock solution. The absorbance of cool samples (including the standards) was read at 800 nm. Distilled water was used as blank.

Determination of the Solubility Characteristics of Precipitated Samples: In addition to the analysis of contents of phospholipids and proteins of samples, further analysis was carried out on the solubility characteristics of precipitated samples, since these precipitated substances were affecting the quality of biodiesel. Precipitates were collected from crude oil, biodiesel and acid degumming refined oil and their solubility properties were tested. Solvents used were water, methanol, ethanol, acetone, chloroform and concentrated inorganic acids. Based on the solubility characteristics of these precipitates, the following refinery method was designed.

Oil Refining Process: Crude oil obtained by mechanical press and solvent extraction was mixed with hexane (1:1 ratio) and the precipitate formed was removed through filtration. To two volumes of hexane oil mixture, one volume of 96% ethanol was added, mixed and allowed to stand for 1 h in a separatory funnel for phase separation. The bottom layer containing hexane and oil was separated from the upper layer containing ethanol, phospholipids and the solvent from both phases recovered using Rotavapor [24].

Statistical Analysis: Data were analyzed with SPSS 17.0 for windows. The results are given as means of triplicate samples. Appropriate statistical analysis of variance (ANOVA) was done to determine the significance differences among means followed by Duncan's multiple range tests. The statistically significant difference was defined as $p < 0.05$.

RESULT AND DISCUSSION

Oil Impurities: After standing overnight brebra crude oil obtained using both mechanical press and solvent extraction formed a thick white precipitate that gradually settled down to the bottom of the container. In addition, biodiesel prepared from this crude oil turned dark brown up on addition of the catalyst (KOH) and formed white precipitate as well. This indicates that the oil contained substantial quantities of impurities. Therefore, in order to prepare pure oil suitable for biodiesel preparation it was important to develop a refinery method.

Oil Purification (Refining Process) Through Acid and Water Degumming: To check if the impurity could be removed following standard acid-water degumming process [14], the crude oil was treated with dilute solution (1%) of sulphuric and phosphoric acids followed by three times washing using hot water. Although a white mass of precipitate was removed following acid-hot water treatment, the biodiesel prepared from it formed a precipitate after standing overnight. Its colour was also changed to dark brown indicating, qualities not acceptable for vegetable oil derived biodiesel [25-26]. This indicates that the acid-hot water degumming process was not effective in removing impurities that interfere in the process of biodiesel production. To develop an appropriate method it was important to know the chemical composition of the impurities.

Protein and Phospholipids Content of the Crude Oil: To determine the chemical nature of the impurities, precipitates formed under different treatment conditions and the crude oil were tested for the presence of phospholipids and hydrophobic proteins. As shown in Fig. 1 the crude oil contained appreciable quantity of phospholipid and protein. Nearly half of the phospholipid was precipitated up on addition of hexane while the other half remained suspended in the oil (Fig. 1). Addition of acid was not effective in precipitating the protein fraction (Fig. 1). Substances precipitated by acid treatments could be waxes and other impurities.

In addition to phospholipids (7.2%), the crude oil contained 44 mg protein per gram oil as determined by the Biuret method (Fig. 1). The protein was not detected in any of the precipitates. This indicated that other refining processes need to be employed to remove all the impurities.

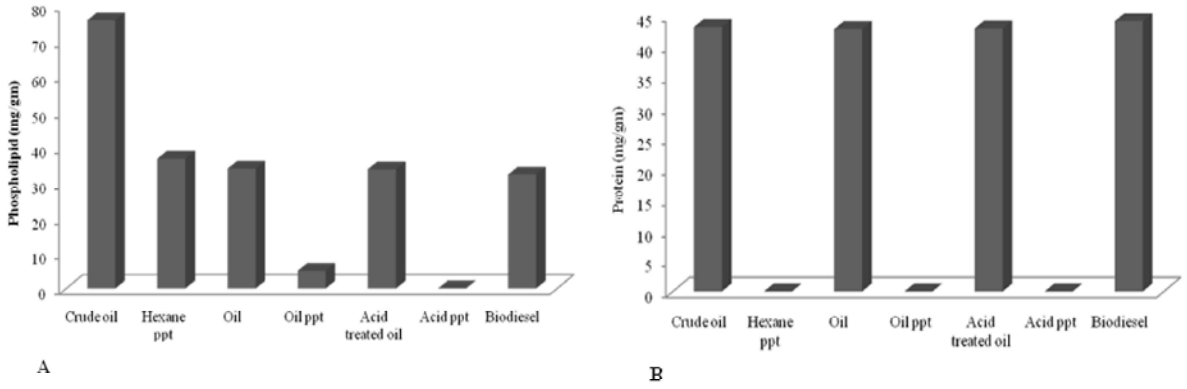

Fig. 1: Determination of phospholipid (A) and protein (B) from brebra crude oil, hexane treated oil, oil treated by acid water degumming method, biodiesel and its different components

Fig. 2: Separation of impurities by co-solvent method (top part ethanol and ethanol soluble polar substances and lower phase contains hexane-oil mixture)

To develop a solvent extraction method, the solubility of the precipitate formed under different treatment condition in different solvents was tested. As shown in table 1, all the precipitates were soluble in ethanol suggesting that this solvent could be used in the refinery process. It is well known that different substances are soluble in different solvents depend on degree of polarity. According to Hansen [27], polar portic, polar aprotic and non-polar substances are dissolved by polar portic, polar aprotic and non-polar solvents, respectively. In this study, hexane and chloroform were used as non-polar, acetone as polar aprotic, while water, acid and methanol were used as polar portic solvents. Their solubility potential was evaluated using precipitated substances from crude oil of brebra seed.

A white precipitate was formed up on mixing of hexane and crude oil indicating that the impurity is poorly soluble in hexane. When equal volumes of crude oil, hexane and ethanol were mixed and allowed to stand, two phases were formed (Fig. 2). The above phase contained ethanol, phospholipids, proteins and other impurities. The bottom phase consisted essentially of hexane and oil. The bottom phase was separated from the upper phase and the solvent (hexane) removed using Rota evaporator leaving the refined oil. According to this finding, non-polar substance (oil) was moved along with hexane and polar portic substances moved together with ethanol.

Table 1: Solubility characteristics of different substances obtained from Brebra oil that treated with different solvents

	Solubility in:						
Precipitate from:	Water	Boiling water	Inorganic Acid (HCl)	Methanol	Ethanol	Acetone	Chloroform
Acid treated[1]	Insoluble	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble
Acid water treated oil[2]	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble
Biodiesel[3]	Soluble	Soluble	Soluble	Soluble	Soluble	Insoluble	Insoluble
Solvent extracted oil	Insoluble	Soluble	Insoluble	Insoluble	Soluble	Soluble	Insoluble
Mechanically extracted oil	Insoluble	Insoluble	Insoluble	Insoluble	Soluble	Soluble	Soluble

[1]= Oil precipitates formed by only acid treatment

[2]= Precipitate from acid and water treated oil after over night

[3]= Biodiesel precipitates (precipitates obtained from biodiesel)

(A) (B)

Fig. 3: Biodiesel produced from the oil that was refined by acid-water degumming process (A) and hexane-ethanol solvents (B) of brebra seed oil. Brown black colour at the bottom of two separatory funnels is glycerol while the top parts are biodiesels with different colour

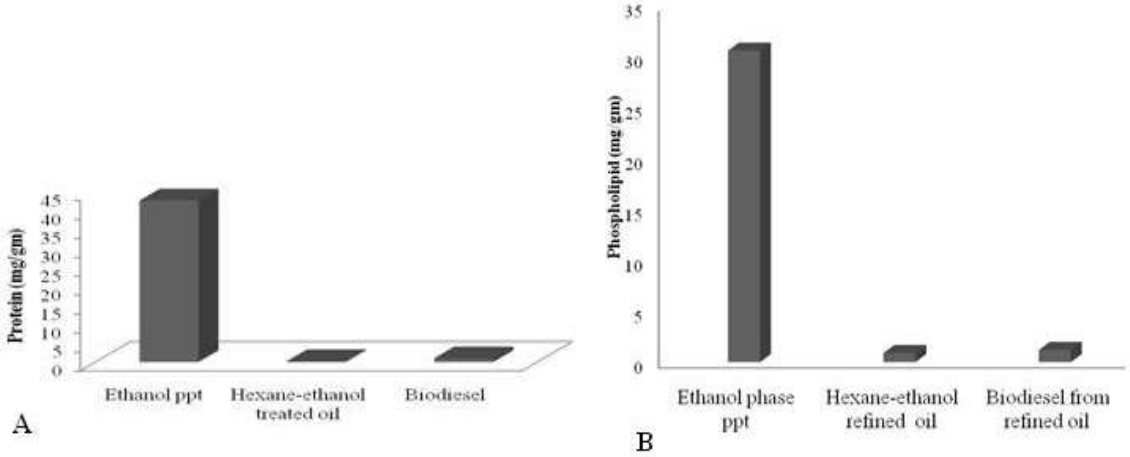

Fig. 4: Determination of protein and phospholipid from the oil refined by hexane-ethanol solvents and acid-water treated oil and their different components

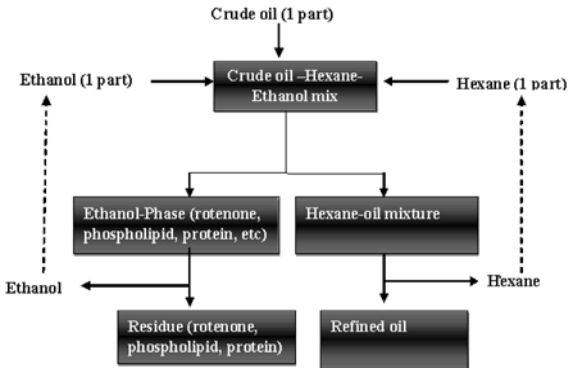

Fig. 5: (Summary) flow chart for refinery of brebra oil through the use of co-solvent refinery techniques (hexane and ethanol)

Oil refined following the above process appeared light yellow in colour and no precipitate formed even after standing for several days. When this oil was used for biodiesel production, the resulting product appeared clear light yellow in colour (Fig. 3). This shows that the separation was efficient in removing all the impurities. No protein and phospholipid was detected from the refined oil and biodiesel prepared from it. On the other hand the upper ethanol phase contained high concentration of protein and phospholipid (Fig 4). The overall refining process is shown in Fig. 5.

Out of 77.91 g of crude oil extracted using solvent extraction 65.96 g (84.7%) was pure (refined) oil and 11.95 g (15.3%) was impurities (consisting of protein, phospholipid and other impurities). Similarly, 86.8 % of the crude oil obtained through mechanical press was refined oil and the remaining 13.2% was accounted for impurities (Table 2). In this study, the amount of refined oil from the crude oil extract by mechanical press was significantly ($p < 0.05$) increased than refined oil extract by hexane.

Table 2: Efficiency of refinery of crude oils from brebra (means of two solvent systems hexane-ethanol)

Type of crude oil	Amount in gram
1) Oil extracted by mechanical press	
Crude oil	881 (100%)
Refined oil	765 (86.8%)[d]
Total residue	116 (13.2%)[a]
2) Oil extracted by hexane	
Crude oil	77.92 (100%)
Refined oil	65.96 (84.7%)[c]
Total residue	11.96 (15.4%)[b]

Values (n=3) at column with different alphabetic superscripts are significantly different at P<0.05; Means were tested by ANOVA and ranked by Duncan's multiple range test

On the other hand, the quantity of residue produced after refinery of crude oil from mechanical press was significantly ($p < 0.05$) reduced than the residue from crude oil extracted by hexane.

Based on the above methods a refinery of brebra oil was developed (Fig. 5). In oil refinery process, the recovered hexane and ethanol can be reused for the next batch of oil refinery process. The residue left as byproduct can serve as raw material for production of phospholipid and protein. Generally, refinery of crude oil using short-chain alcohols as solvent, especially ethanol [28] has many advantages. Ethanol has low toxicity, easy recovery in the process, good values of selectivity and distribution coefficient for free fatty acids [28-29] and low losses of nutraceutical compounds [30].

Although triglycerides are the major components of vegetable oils, other components, like phospholipids, proteins, colouring materials, sugars, waxes and other impurities are also present [14]. For some industrial applications these impurities could interfere in the production processes. Crude oil from brebra contained about 4.4% protein and 7.2% phospholipid. Some of these impurities might be responsible for the inferior quality of the biodiesel prepared from crude brebra seed oil.

The refinery processes developed in this study could allow fractionation of at least three important products from brebra crude oil: refined (pure oil), protein and phospholipid. The oil can have important application for the production of biodiesel. The presence of potentially marketable co-products is expected to lower the production cost of biodiesel. Moreover, because of its low rotenone content [31], the refined oil, subject to toxicological evaluation, could also find important application as animal feed supplement or for human consumption.

Phospholipids, proteins and other impurities recovered from the upper ethanol phase could find important applications as well. For example, phospholipids could find important application in pharmaceutical, cosmetic and food industries. It can also find various industrial uses such as paints, textiles, lubricants and waxes.

CONCLUSIONS

It has been discovered that the phospholipids, proteins, colored materials and other minor components of brebra oil can be removed by co-solvent (Hexane-ethanol) refining techniques. This method is not as such expensive procedure that consists of limited stages. Another advantage of this procedure is that it is possible to recover the used solvents and reuse for another oil refinery batch. The oil produced in this refinery process may not need further treatment, such as bleaching and deodorization, since the main objective is to get appropriate oil for biodiesel production. Brebra oil can find important application for the production of biodiesel. Co-products, such as phospholipid and protein which should be removed during refinery process, are expected to lower the production cost of biodiesel. Biodiesel can provide the quality of multiple energy services: cooking fuel, heat, electricity and transportation fuels. Thus, brebra biodiesel may help to reduce the amount of currency expenditure for the imported petroleum and address environmental problems.

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