Extraction of Edible Oil From Groundnut By using Solvents and Enzymes

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Abstract - The methods of oil aqueous extraction process (AEP) assisted by enzymes are, over the last 50 years, an alternative designed to replace traditional methods of extraction using organic solvents. To extract the oil using an AEP, the use of specific enzymes, able to hydrolyze some or all components of seeds can significantly increase the yields of extraction. Hydrolyzing the different constituents of cell walls (cellulose, hemicellulose, pectin, proteins, etc.), enzymes is able to enhance the liberation of the oil. A number of physico-chemical parameters must also be considered for the better expression of the enzymatic mixture, while maintaining the quality of oils and meals. An experimental study was performed in order to investigate the effect of an enzymatic pre-treatment process for extraction of oil from groundnuts. In the present study celluclast 1.5L was used for the pre-treatment. The effect of enzyme concentration (5-10%), temperature (50-60 ℃), pH (5.0-6.0), reaction time (1-7 h) on free oil liberated was studied. Residual oil was collected by subjecting the treated meal to soxhlet extraction for 4 h. The optimal conditions were: enzyme concentration of 7.5% (w/w) in 10 g of peanut seeds, pH 5.0, 50°C, and 5 h with constant shaking at 450 rpm. Centrifuging the mixture at 8500 for 20 min separated the oil with a recovery of 71-73.1%.

Keywords: Extraction; ediableoil; solvents; enzymes.

I. INTRODUCTION

'Lipids' are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. These are insoluble in water but soluble in organic solvents such as chloroform, hydrocarbons, alcohols, esters. Lipid is a chemical term being used for different types of compounds like triacylglycerol. They are relatively simple molecules, for example the fatty acid they are more complex and contain phosphor or sulpho groups, amino acid, peptides and their derivatives. Lipids can be classified as derived, simple, or complex. The derived lipids include fatty acids and alcohols, which are building blocks for simple and complex lipids. Simple lipids composed of fatty acids and alcohols include acylglycerols, ether acylglycerols, sterols and their esters and wax esters. Complex lipids include glycerophospholipids, glyceroglycolipids and sphingolipids. These structures yield three or more different compounds on hydrolysis.

Particularly common and practically useful system is the division in to neutral lipids and polar or amphiphilic lipids. Thus neutral lipids include simple hydrocarbons, carotenes, triacylglycerol's, wax, esters, and sterol esters.Other lipids such as fatty acids, polyprenols, sterols in which hydrophilic function has relatively little impact on the overall molecular characterization. However, it is evident that oils and fats are one of the major components of lipids. Oils and fats are predominantly tri esters of fatty acids and glycerol, commonly called 'triglycerides or triacylglycerol's'. Substances which are solid or semisolid at room temperature are called 'fats' and those which are liquids are called 'oils'.

Fatty Acids

Fatty acids are the building blocks for triacylglycerol. Aliphatic carboxylic acids with 4 or more carbon atoms are called fatty acids. In nature, they occur with an even number of carbon atoms, with very few exceptions. The natural oils and fats contain saturated and unsaturated fatty acid. Saturated means that all the carbon valences (except in the carboxylic acid group) are satisfied. Fatty acids having hydrogen deficient carbon atoms bonded by double or triple valences are called unsaturated. According to the number of double bonds present they are called as mono unsaturated or di-, tri-, tetra-, penta, hexa-enoic acids etc. If the fatty acids contain more than one double bond, those are called as poly unsaturated fatty acids (PUFA).

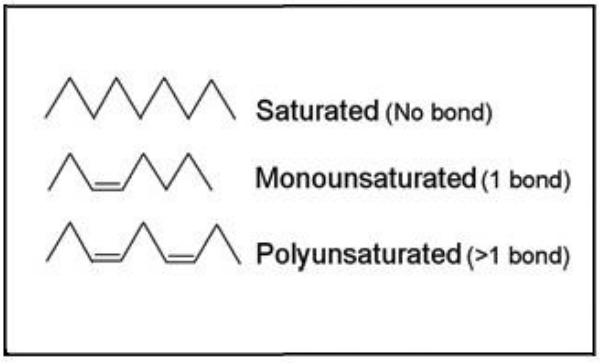

Fig 1: Types of Fatty Acids

(According to the number of double bonds)

The most common fatty acids present in the edible oils are lauric, myristic, palmitic (saturated), oleic (mono unsaturated), linoleic and linolenic (poly unsaturated) acids and the classification is shown in Fig1.

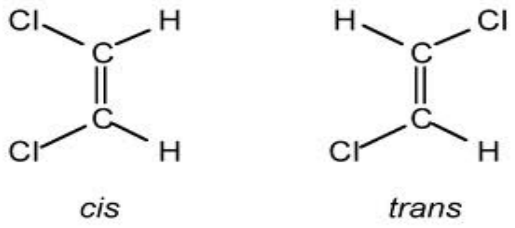

Fig 2: Structure of Cisand Trans-Isomers

However, some oils contain unusual fatty acids like hydroxy fatty acids (ricinoleic acid in castor oil) also. Other fatty acids are arachidonic (AA), eicosapentaenoic (EPA) and docosahexanoic (DHA) acids present in fish oil. Examples of few common unsaturated and saturated fatty acids are tabulated below in Table 1 and 2.

Most of the hydrogen in double bonds of natural fatty acids is found on the same side of the bond, indicating a '*Cis* position' and the respective structures are shown in Fig 2. But, some of the hydrogen atoms may move to the other side of the bond during dehydrogenation process, to produce '*Trans*-isomers'.

The properties of the fatty acids which depend up on their degree of saturation or unsaturation with respect to hydrogen are retained by them when they are in combination with glycerin as triglycerides.

Table 1: Saturated fatty acids

Common name	Chemical structure	C:D
Caprylic acid	CH3(CH2)6COOH	8:0
Capric acid	CH3(CH2)8COOH	10:0
Lauric acid	CH3(CH2)10COOH	12:0
Myristic acid	CH3(CH2)12COOH	14:0
Palmitic acid	CH3(CH2)14COOH	16:0
Stearic acid	CH3(CH2)16COOH	18:0
Arachidic acid	CH3(CH2)18COOH	20:0
Behenic acid	CH3(CH2)20COOH	22:0
Lignoceric acid	CH3(CH2)22COOH	24:0
Cerotic acid	CH3(CH2)24COOH	26:0

Table 2: Unsaturated fatty acids

Common name	Chemical structure	Δ*x*	*C*:*D*	*n*−*x*
Myristoleic acid	CH3(CH2)3**CH=CH**(CH2)7COOH	*cis*-Δ9	14:1	*n*−5
Palmitoleic acid	CH3(CH2)5**CH=CH**(CH2)7COOH	*cis*-Δ9	16:1	*n*−7
Sapienic acid	CH3(CH2)8**CH=CH**(CH2)4COOH	*cis*-Δ6	16:1	*n*−10
Oleic acid	CH3(CH2)7**CH=CH**(CH2)7COOH	*cis*-Δ9	18:1	*n*−9
Elaidic acid	CH3(CH2)7**CH=CH**(CH2)7COOH	*trans*-Δ9	18:1	*n*−9
Vaccenic acid	CH3(CH2)5**CH=CH**(CH2)9COOH	*trans*-Δ11	18:1	*n*−7
Linoleic acid	CH3(CH2)4**CH=CH**CH2**CH=CH**(CH2)7COOH	*cis*,*cis*-Δ9,Δ12	18:2	*n*−6
Arachidonic acid	CH3(CH2)4**CH=CH**CH2**CH=CH**CH2**CH=CH**CH2 **CH=CH**(CH2)3COOHNIST	*cis*,*cis*,*cis*,*cis*-Δ5Δ8,Δ11,Δ14	20:4	*n*−6
Erucic acid	CH3(CH2)7**CH=CH**(CH2)11COOH	*cis*-Δ13	22:1	*n*−9

Triglycerides

As above stated, fats may be split in to glycerin and fatty acids, the resulting mixture containing three molecules of fatty acid for each molecule of glycerin as shown in Fig 3.

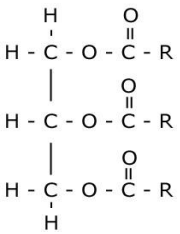

Fig 3: Structure of Triglyceride

Since there are a number of different fatty acids that occur in natural fats, a great many different triglycerides are encountered in nature. They include mono and diglycerides, free fatty acids, phosphatides, sterols, fatty alcohols, fat soluble vitamins and other substances.

Groundnuts

The groundnut belongs to the pea and bean family and is a leguma but is considered as nut because of its high nutrition value. The groundnut is the native of South America, where it has been cultivated for several countries. In 16th century the Portuguese took it from Brazil to other countries and it spread in India

also. Groundnut is the only nut that grows beneath the earth. They become mature in about two months, when the leaves of the plant turn yellow. The groundnut is particularly valued for its protein contents, which is of high biological value. Groundnut contains more protein then meat, two and half more then eggs and more then any other vegetable food. The proteins in groundnut are well balanced.

Groundnuts (*Arachishypogaea*), famous by its Indian name mongphaliis a species in the legume or "bean" family (Fabaceae). The peanut was probably first domesticated and cultivated in the valleys of Paraguay.] It is an annual herbaceous plant growing 30 to 50 cm (1.0 to 1.6 ft) tall. The leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet); each leaflet is 1 to 7 cm (⅜ to 2¾ in) long and 1 to 3 cm (⅜ to 1 inch) across. The flowers are a typical peaflower in shape, 2 to 4 cm (0.8 to 1.6 in) (¾ to 1½ in) across, yellow with reddish veining. The specific name, *hypogaea* means "under the earth"; after pollination, the flower stalk elongates, causing it to bend until the ovary touches the ground. Continued stalk growth then pushes the ovary underground where the mature fruit develops into alegume pod, the peanut – a classical example of geocarpy. Pods are 3 to 7 cm (1.2 to 2.8 in) long, containing 1 to 4 seeds.

Fig 4: Groundnut Crop

Fig 5: Shelled groundnuts with skin

Groundnuts have all the benefits of a perfect fruit, in fact they can be considered in the same league as fruits when it comes to contributing to our diet and health.

Health Benefit:

- Groundnuts contain five important nutrients namely food energy, protein, phosphorous, thiamin and niacin. It maintains and repairs body tissues.
- Eating fresh roasted groundnuts with jaggery and goat's milk is very nutritious for growing children, pregnant women and nursing mothers. It builds a resistance against all infections, such as Hepatitis and tuberculosis.
- Groundnuts contain 13 different vitamins (including Vitamin A, B, C and E) along with 26 essential trace minerals, including calcium and iron.
- Groundnuts also contain zinc, good for protecting brain function, and boron, which helps to maintain strong bones.
- Ground nuts or groundnut products are useful in the treatment of hemophilia, and inherited blood diseases, which cause hemorrhage. It is also useful in nose bleeding and in cases of excessive bleeding during menstruation in women.
- Groundnuts have good dietary fiber content so they are very good for digestion.
- Groundnuts are valuable in diabetes. It is also useful in diarrhea, especially chronic diarrhea, which is more frequent immediately after a meal. The patient can use it by drinking goat's milk in which lemon is squeezed with a handful of fresh roasted groundnuts.

Table 3: Nutritional Value of Groundnuts (per 100 grams)

Water	6.50gm
Energy	567kcal
Energy	2374Kj
Protein	25.80g
Fat	49.24g
Carbohydrate	16.13
Fibre	8.5 gm
Sugar, total	3.97gm
Calcium	93 mg
Iron	4.58mg
Magnesium	168mg
Phosphorus	376mg
Potassium	705mg
Sodium	18mg
Zinc	3.27mg
Copper	11.44mg
Manganese	1.934mg

Selenium	7.2 mcg

Groundnut Oil

Groundnut oil also known as peanut oil or arachisoil, is a mild tasting vegetable oil derived from groundnuts. The oil is available in refined, unrefined, cold pressed, and roasted varieties, the latter with a strong peanut flavor and aroma, analogous to sesame oil.

It is often used both for general cooking, and in the case of roasted oil, for added flavor. Groundnut oil has a high smoke point relative to many other cooking oils, so is commonly used for frying foods. Its major component fatty acids are oleic acid (46.8% as olein), linoleic acid (33.4% as linolein), and palmitic acid (10.0% as palmitin). The oil also contains some stearic acid, arachidic acid, arachidonic acid, behenic acid, lignoceric acid and other fatty acids.

Applications of Groundnut Oil:

- Peanut oil is high in monounsaturated “good” fat, and low in saturated “bad” fat, which is believed to help prevent heart disease and lower cholesterol. It is also used to decrease appetite as an aid to weight loss. Some people use it to help prevent cancer.
- Peanut oil is sometimes applied directly to the skin for arthritis and joint pain, dry skin, eczema, scalp crusting and scaling without hair loss, and other skin disorders that cause scaling.
- Rectally, peanut oil is used in ointments and medicinal oils for treating constipation.
- Pharmaceutical companies use peanut oil in various products they prepare for internal and external use.
- In manufacturing, peanut oil is used in skin care products and baby care products.

Oil Extraction

The production process of vegetable oil involves the removal of oil from plant components, typically seeds. This can be done via mechanical extraction using an oil mill or chemical extraction using a solvent. The extracted oil can then be purified and, if required, refined or chemically altered.

Mechanical Extraction

Oils can also be removed via mechanical extraction, termed "crushing" or "pressing." This method is typically used to produce the more traditional oils (e.g., olive, coconut etc.), and it is preferred by most "health-food" customers in the United States and in Europe. There are several different types of mechanical extraction. Expeller-pressing extraction is common, though the screw press, ram press, and Ghani (powered mortar and pestle) are also used..

Solvent Extraction

The processing vegetable oil in commercial applications is commonly done by chemical extraction, using solvent extracts, which produces higher yields and is quicker and less expensive. The most common solvent is petroleum-derived hexane. This technique is used for most of the "newer" industrial oils such as soybean and corn oils. Groundnuts, Coconut, Palm, Grape seed and Rice Bran are typically solvent extracted. Even the most perfect expellers leave at least six percent of oil in the expeller cake. It is possible to recover these losses using a solvent extraction plant. Supercritical carbon dioxide can be used as a non-toxic alternative to other solvents.

Enzymatic Pretreatment

The idea to develop an aqueous extraction process (AEP) to produce oil from oilseeds was born in the 1950s. In those days, such a process seemed cheaper and less than the processes of extraction by solvent which supplied then the best yields. Groundnut oil which contains 40~50% oil and 27-29% protein, is often used in cooking, because it has a mild flavor and a relatively high smoke point. Due to its high monounsaturated content, it is considered healthier than saturated oils, and is resistant to rancidity.The traditional approach for extracting oil from groundnut is solvent extraction by using hexane. In the last few decades, aqueous (enzymatic) extraction has been attempted to extract oil/protein from many oil-bearing materials, such as coconut, sun flower, rice bran, soybeans.The commercial hexane used as the most common solvent for oil extraction is listed among hazardous air pollutants associated with neurological and respiratory disorders on prolonged exposure (the International Standard Organization permits only 50 ppm residual hexane in oilseed meal).Hence, there is a need to explore alternative safe and efficient oil extraction processes that may also result in edible protein.

II. MATERIALS AND METHODS

Groundnuts used in the experiments were purchased from the local market. Celluclast 1.5L was supplied by Novozymes (Denmark). All the chemicals Hexane, citric acid and sodium citrate were of analytical grade and were procured from M/s. Sd Fine chem. Pvt. Ltd., Mumbai.

2.1 Enzyme

Enzyme is a protein molecule acting as catalyst in enzyme reaction. Enzyme inhibition is a Science of enzyme-substrate reaction influenced by the presence of any organic chemical or Inorganic metal or biosynthetic compound due to their covalent or non-covalent interactions with enzyme active site.

The Enzyme used in the lab experiment is Celluclast1.5L. Celluclast is a lipase used for breaking down cellulose into glucose, cellobiose and longer glucose polymers.

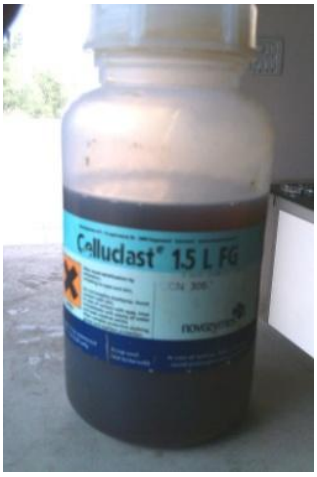

Fig 6: Purchased Celluclast 1.5L

2.1.1 Source:

Celluclast 1.5 L was manufactured by Novozymes was purchased.Cellulase that hydrolyzes (1,4)-beta-D-glucosidic linkages in cellulose and other beta-D-glucans

2.1.2 Uses:

Celluclast can be used whenever the aim is to break down cellulosic materials into fermentable sugars, the reduction of viscosity of soluble cellulosic substrates, or the increase in yield of valuable products of plant origin.

2.1.3 Activity:

Celluclast catalyzes the breakdown of the glucose polymers that comprise cellulose to glucose, cellobiose (i.e., pairs of glucose units) and longer chains of glucose units. For practical purposes, the optimum conditions for activity of this enzyme preparation are in the range pH 4.5-6.0, and about 50-60 °C.

2.1.4 Storage:

Generally at a storage temperature of 25 °C, you can expect the enzyme to maintain its declared activity for at least 3 months. At lower temperatures (5-10 °C), the shelf life is considerably increased.

2.2 Hexane:

Hexane is significant constituents of gasoline. They are all colorless liquids at room temperature, odorless when pure, with boiling points between 50 and 70 °C. They are widely used as they are easily evaporating non-polar solvents. Hexane is used as solvent for the extraction of oil from oil seeds.

The "hex" prefix refers to its six carbons, while the "ane" ending indicates that its carbons are connected by single bonds. Cooking oils are daily necessities used in all over the world and different types of oilseeds are grown at everywhere. Besides food purpose, vegetable oil is also the source of bio-diesel, the new environmental friendly fuel. The cake from oil press section could also be used as raw material for solvent extraction plant, the chemical way to extract oil. This kind of extraction uses chemical solvent (Like Hexane) to dissolve oil content contained in cake or oil seeds. Oil is collected by vaporizing solvent out which is later recycled. In configuring the solvent extraction plant, pre-pressing may be involved in which case seeds are lightly pressed leaving about 14% to 18% oil in pressed cake. Solvent extraction will further process these cakes and leave only 2% oil in the final cake (meal). This method results in higher capacity; Lower power consumption, lower wear & tear / maintenance and high extract efficiency.

III. PHYSICAL CHEMICAL PROPERTIES OF HEXANE

Physical state and appearance: Liquid.

Odor: Gasoline-like or petroleum-like (Slight.)

Taste: Not available.

Molecular Weight: 86.18g/mole

Colour: Clear Colourless.

pH (1% soln/water): Not applicable.

Boiling Point: 68°C (154.4°F)

Melting Point: -95°C (-139°F)

Specific Gravity: 0.66 (Water = 1)

Vapour Pressure: 17.3 kPa (@ 20°C)

Vapour Density: 2.97 (Air = 1)

Odor Threshold: 130 ppm

Water/Oil Distribution Coefficient: The product is more soluble in oil; log(oil/water) = 3.9

Iconicity (in Water): Not available.

Dispersion Properties: See solubility in water, diethyl ether, and acetone.

Solubility: Soluble in diethylether, acetone. Insoluble in cold water, hot water

2.3 Buffer Solution:

A buffer is an aqueous solution consisting of a mixture of a weak acid and its conjugate, or vice versa. Its pH changes very little when a small amount of strong acid or base is added to it and thus it is used to prevent changes in the pH of a solution. Buffer solutions are used as a means of keeping pH at a nearly constant value in a wide variety of chemical applications.To prepare 0.1 M Sodium citrate 29.41 g was taken in 1liter Standard flask

and made up to the mark with distilled water and to prepare 0.1M citric acid 18.48 g was taken in another 1 liter Standard flask and make up to the mark with distilled water.

From the above Standardized solutions, take 295 ml of 0.1 M sodium citrate and 20.5 ml of 0.1 M citric acid wastaken in a1 liter standard flask and 1000 ml of distilled water was added up to the mark. The pH of the buffer was checked using Thermo Scientific pH meter as shown below in Fig 7 and the pH was adjusted to 5 using 1N NaOH. If it is below the desired pH add NaOH to raise it to the correct pH. If it is above the desired pH add phosphoric acid to lower it to the desired pH [5.0]. Similar method was applied to prepare buffers with pH 5.5, 6.0.

Fig 7: pH meter

2.4 Experimental Setup:

The experimental set up consists of a hotplate showing a digital display of temperature and rpm. The maximum stirring speed is 700rpm. The temperature can be set upto 180^{o}C.

The ground nut powder is weighed and enzyme, pH buffer solution is added to a round bottom flask. A magnetic bead is placed into the round bottom flask which helps in stirring. The temperature and RPM can be set as per our interest.

Fig 8: Experimental setup for study

2.5 Centrifuge:

Fig-9: Floor model Centrifuge

A centrifuge is a device for separating particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed, generally driven by an electric motor (or, in some older models, by hand), that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis.The centrifuge works using the sedimentation principle, where the centripetal acceleration causes denser substances to separate out along the radial direction (the bottom of the tube). By the same token lighter objects will tend to move to the top (of the tube; in the rotating picture, move to the Center).

In a solution, particles whose density is higher than that of the solvent sink (sediment), and particles that are lighter than it float to the top. The greater the difference in density, the faster they move. If there is no difference in density, the particles stay steady. The maximum speed is 15,200 rpm and can hold up to 3L of samples.

2.6 Soxhlet extractor:

Fig 10: Soxhlet Extractor

A soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von soxhlet. It was originally designed for the extraction of a lipid from a solid material. However, a soxhlet extractor is not limited to the extraction of lipids. Typically, a soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The sample is placed in the thimble. Normally a solid

material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. The extraction solvent to be used is taken into a distillation flask and the soxhlet extractor is now placed onto this flask. The soxhlet is then equipped with a condenser. The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material is slowly filled with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hs or days.During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded

4.7 Rotary Evaporator

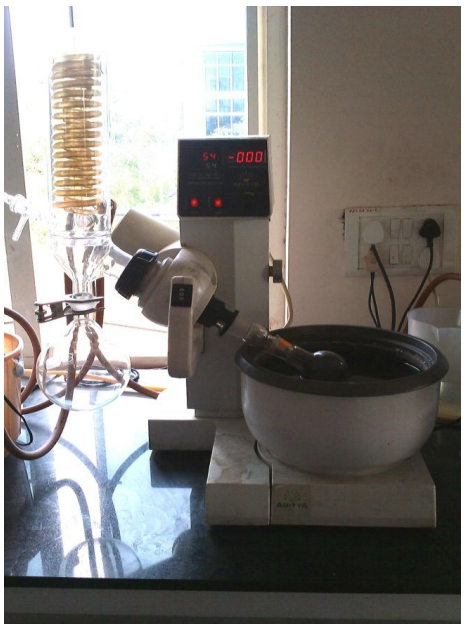

Fig 11: Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation.

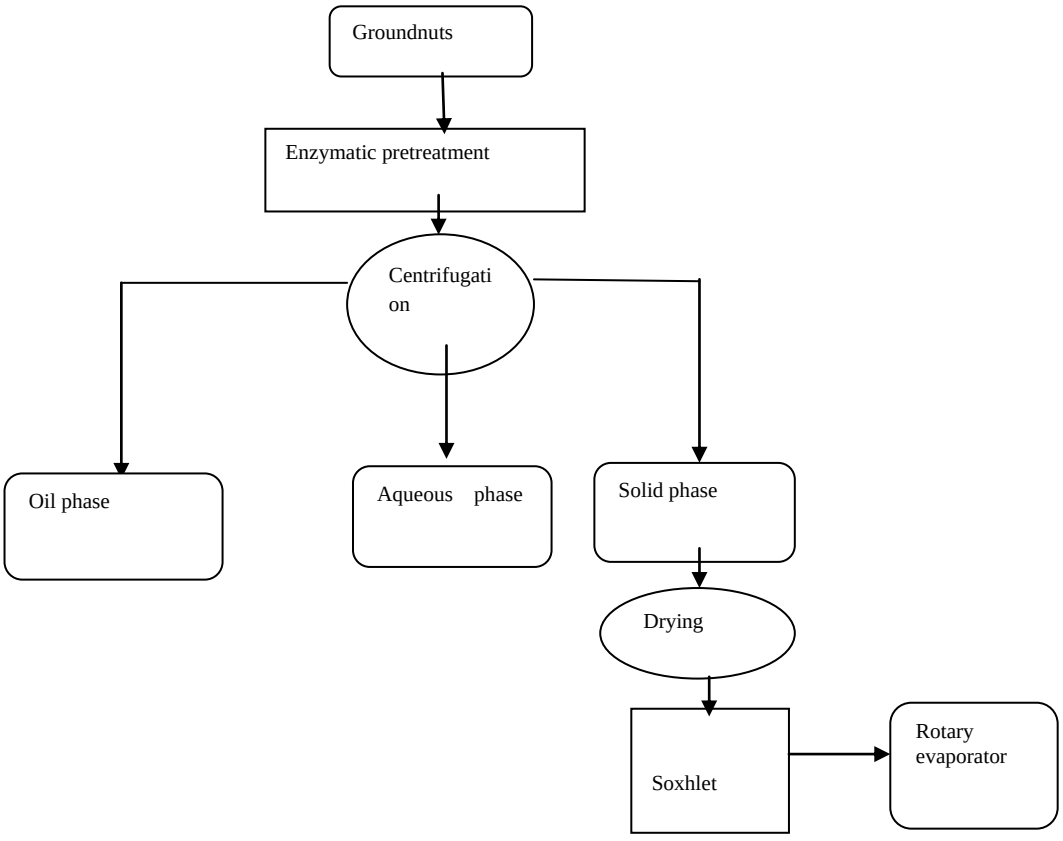

Fig 12: Flowchart of the method.

Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts.

A simple rotary evaporator system was invented by Lyman C. Craig.

1. The main components of a rotary evaporator are:
2. A motor unit that rotates the evaporation flask or vial containing the user's sample.
3. A vapor duct that is the axis for sample rotation, and is a vacuum-tight conduit for the vapor being drawn off of the sample.
4. A vacuum system, to substantially reduce the pressure within the evaporator system.
5. A heated fluid bath (generally water) to heat the sample.
6. A condensate-collecting flask at the bottom of the condenser, to catch the distilling solvent after it re-condenses.
7. A mechanical or motorized mechanism to quickly lift the evaporation flask from the heating bath.

The vacuum system used with rotary evaporators can be as simple as a water aspirator with a trap immersed in a cold bath (for non-toxic solvents), or as complex as a regulated mechanical vacuum pump with refrigerated trap. Glassware used in the vapor stream and condenser can be simple or complex, depending upon the goals of the evaporation, and any propensities the dissolved compounds might give to the mixture (e.g., to foam or "bump"). Commercial instruments are available that include the basic features, and various traps are manufactured to insert between the evaporation flask and the vapor duct. Modern equipment often adds features such as digital control of vacuum, digital display of temperature and rotational speed, and vapor temperature sensing.

3.1 Enzymatic pre-treatment:

- The groundnuts were ground using a blender into fine granules.
- 10 grams of blended groundnuts were weighed in each 250 ml round bottom flasks.
- To the above sample 7.5% of enzyme (i.e., 0.75g of enzyme) was dissolved in 50 ml of (Oil to buffer ratio of 1:5) pH 5.0 buffer solution was added.
- The seeds in parallel synthesizer was kept at 50^{o}C for 5 hs at 450 rpm.
- The stirred round bottom flasks were then added 1.5ml of solvent (Commercial Hexane).
- And again were kept for continuous stirring for 15 minutes.
- Then the mixture in the round bottom flasks was transferred to centrifuge bottles.
- The round bottom flasks were given a hexane wash to avoid wastage of oil.
- Then the centrifuge bottles were kept in the centrifuge and speed was set to 8500rpm for 20 minutes.
- The centrifuge bottles were taken out after 20 minutes carefully without disturbing the layers.
- The oil and the cake were separated with the help of funnel and cotton.
- The Hexane layer was collected into the conical flasks.
- The cake was collected in petri dishes and was placed in the hot air oven bearing temperature around 110^{o}C.
- The cake was dried until it had no moisture in it.
- The Hexane layer and water were separated using separating funnels.
- The hexane which was evolved was kept rotor to evaporate the solvent and to extract oil.
- Then the cake after drying completely was scraped crushed into powder and was placed in thimbles.
- 3-4 boiling chips were put into the round bottom flasks which were fitted with the soxhlet.
- The thimbles were then placed into the soxhlet and hexane was passed through the thimbles into the round bottom flasks.
- Then the soxhlet apparatus was set and the temperature was set around 35^{o}C.
- The soxhlet was run for about 4 h.
- Continuous water supply was given to condensers to avoid evaporation of solvent.
- After 8 h, soxhlet apparatus was turned off and was let to cool.
- Once it was cooled down, the solvent was taken and kept rotor to extract oil.

  The received oil percentage was added to the percentage received in the hexane layer.

IV. RESULTS AND DISCUSSIONS

The effect of process parameters like concentration, temperature, pH, and reaction time on enzymatic pre-treatment was studied. These parameters play a major role in the yield.

4.1 Effect of time

Reaction time plays a major role in the extraction of oil. The effect of time on enzymatic pre-treatment of

groundnuts were studied on varying time from 1-7 h and by keeping other parameters constant i.e., 7.5% of enzyme, temperature at 50°C, 450 rpm of continuous stirring, oil to buffer ratio of 1:5 is shown Fig The free oil yield increased with the increase in reaction time. After 1h of reaction 25.5% oil was extracted, by increasing time to 3h increased oil yield to 55.2%, increasing to 5h gave 73.1% oil yield and further increasing to 7 h 77.1% oil was extracted. As we can see in the table that increase in reaction time was actually an increase in the oil

Table 4: Enzymatic Aqueous Oil Extraction by different reaction time

Reaction Time (h)	***Oil yield (%)***
1	25.5
3	55.2
5	73.1
7	77.1

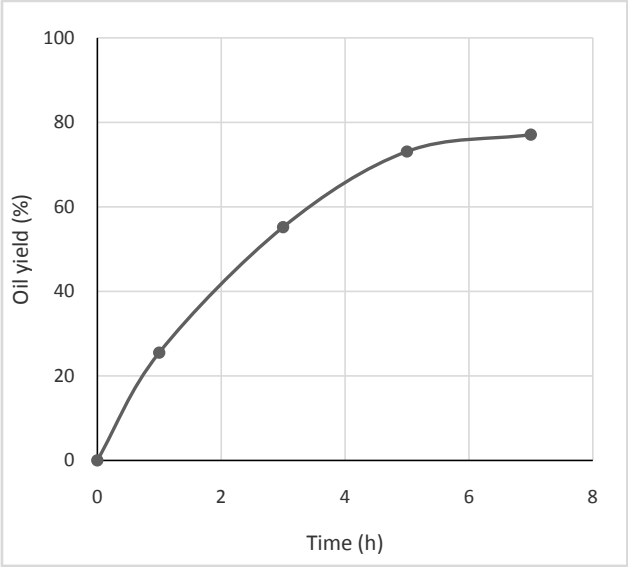

Fig 13: The reaction time was varied by keeping other parameters constant. The oil yield increased with the increase in the reaction time.

4.2 Effect of enzyme

The effect of enzyme on enzymatic pre-treatment of groundnuts were studied on varying enzyme concentration from 1-10% and by keeping other parameters at constant i.e., reaction time for 5hs, temperature at 50°C, 450 rpm of continuous stirring, oil and buffer in the ratio of 1:5. The oil yield increased with the increase in enzyme concentration.

The concentration of enzyme was varied to check the oil yield respectively. The experimental results showed that the oil yield increased with the increase in concentration of enzyme. The enzyme enhances the oil recovery from the groundnuts.

Table 5:Enzymatic Aqueous Oil Extraction by using different concentration of enzyme

Concentration of enzyme (%)	**Oil yield (%)**
1	38.3
2.5	55.6
5	66.8
7.5	73.1
10	77.3

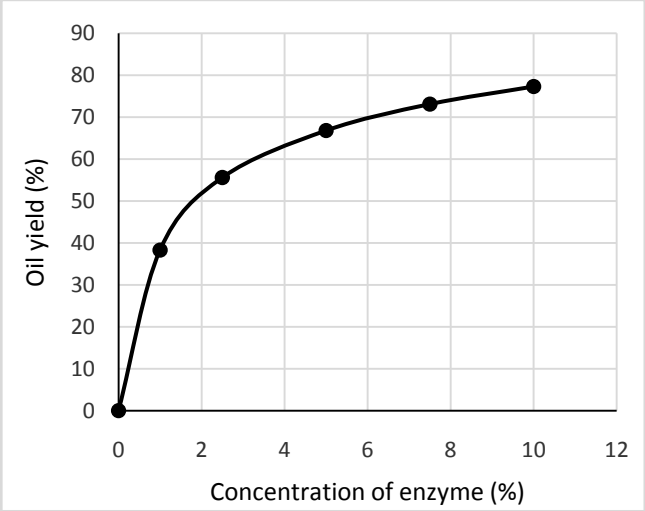

Fig 14: The concentration of enzyme was varied by keep other parameters constants. The oil yield % was more when the enzyme concentration was more.

4.3 Effect of pH:

The effect of pH on enzymatic pre-treatment of groundnuts were studied on varying pH from 4.5-6.0 and by keeping other parameters at constant i.e., enzyme concentration of 7.5%, reaction time for 5h, temperature at 50°C, 450 rpm of continuous stirring, oil and buffer in the ratio of 1:5. The activity range of pH for Celluclast is 4.5-6.0. pH range was varied and the results were showed that oil yield was more at 5.0 pH.The below table shows the obtained oil% based on the pH range.

Table 6:Enzymatic Aqueous Oil Extraction by varying pH buffer solutions.

pH	***Oil yield (%)***
4.5	61.9
5.0	73.1
6.0	53

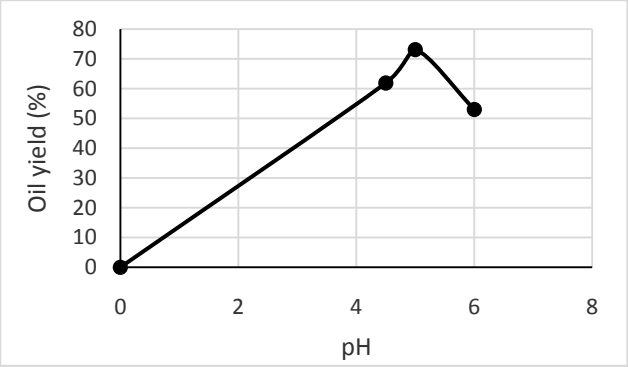

Fig 15: The aqueous oil extraction was done using 3 pH ranges i.e., 4.5, 5.0, 6.0. The results show that the enzyme activity is more at pH 5.0 therefore resulting in higher oil liberation.

4.4 Effect of temperature:

The effect of temperature on enzymatic pre-treatment of groundnuts were studied on varying temperature from 50-60^{o}C and by keeping other parameters at constant i.e., pH 5.0,enzyme concentration of 7.5%, reaction time for 5hs, 450 rpm of continuous stirring, oil and buffer in the ratio of 1:5.The activity range of temperature for Celluclast is 50-60^{o} C. The experiment was performed for 3 different temperatures and the results were showing that the oil recovery decreased with the increase in temperature. The below table shows the obtained oil% based on the temperature.

Table 7:Enzymatic Aqueous Oil Extraction by performing the experiment under different temperatures

Temperature(^{o}C)	***Oil yield (%)***
50	73.1
55	69.28
60	63.5

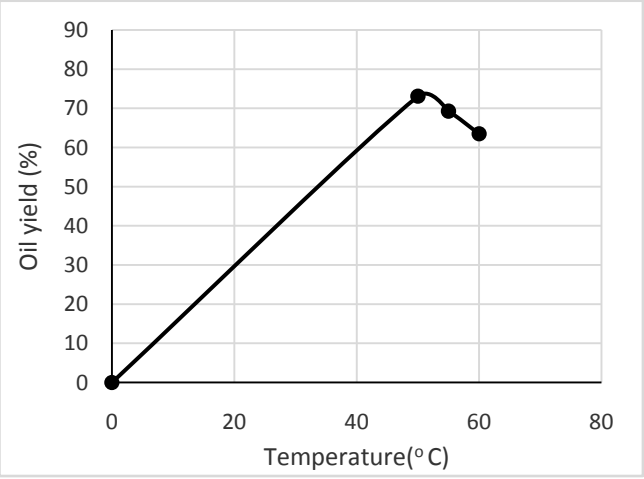

Fig 16: Effect of varying temperature on oil recovery by aqueous enzymatic oil extraction. The oil % obtained from the three different temperatures i.e., 50 ^{o}C, 55 ^{o}C, 60 ^{o}C were recorded. The results signified that the oil recovery is more 50 ^{o}C as 73.1% oil was liberated.

V. CONCLUSION

Using Celluclast enzyme can increase the oil yield greatly compared to without enzyme. The optimal conditions for enzyme should be favorable so as to enhance the pre-treatment. The time, concentration of enzyme play a major role in pre-treatment as more the reaction time, more the oil yield will be and in the same way more the concentration of enzyme, more the oil recovery will be from the groundnuts. The study confirmed that at pH 5.0 the oil yield is more when compared to pH 4.5 and 6.0.The obtained results signified that the temperature which is more suitable for celluclast enzyme is 50^{o}C.

Enzymatic pre-treatment is one of the safest methods for oil extraction in both large scale and small scale. There are no losses of nutrition and proteins in the oil extracted using enzymatic pre-treatment.

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