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Full Length Research Article

An Efficient Alternative for Oil Extraction from Tissue Cultured *Arachis hypogaea* and its Optimization

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Abstract

Peanut oil is one of the healthiest oils. It is a vegetable oil that is naturally trans fat-free, cholesterol free, and low in saturated fats. Peanut consumption may improve lipid profiles without promoting weight gain. Peanut oil is used to lower cholesterol and prevent heart disease, to extract the maximum amount of oil we have experimented in varying concentrations of Ammonium sulphate in different dilutions of tertiary-butanol. Varying parameters (like temperature, pH) were analyzed using peanut purchased from the market and the peanut grown through tissue culture. The main purpose of this work was to compare the plants and to provide a method for maximum extraction of peanut oil. Peanut oil is high in unsaturated fats, especially monounsaturated fat, like olive oil. It is also a source of antioxidant, vitamin E and phytosterols, which benefit heart health. Maximum oil production was seen at room temperature having pH 7 and also found maximum in 1:1 concentration (slurry: tertiary butanol). Maximum oil was obtained at 30% ammonium sulphate concentration.

***Key words*:** Ground nut, TPP, Oil Extraction, Tissue culture, *Arachis hypogaea*

Groundnuts (*Arachis hypogaea*) are legumes but are generally considered as nuts. Arachis oil also called as groundnut oil is extracted from groundnuts i.e., the seeds of *Arachis hypogaea* Linn). Epidemiologic studies indicate an inverse association between the frequency of nut consumption and body mass index [1]. Groundnut consumption has been associated with improved overall diet quality and nutrient profile [2-3]. Peanut seeds contain 24-28% (w/w) protein and 45-52% (w/w) oil [4]. (Table 1) shows nutritional values per 100gms of groundnuts [5].

Table 1 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

Many cuisines of India extensively use Groundnut oil for its nutty flavour and taste. Ground nut oil also is considered a healthy option when compared to other oil varieties due to its high nutritional values such as potassium, magnesium, copper, niacin, arginine, fibre, α-tocopherol, folates, phytosterols, flavonoids, diacylglycerols, phosphatides, pigments, flavour compounds, sterols and also rich in vitamins, minerals and bioactive materials and high MUFA and PUFA content [5]. In traditional medicine, groundnut oil is applied tropically for treating arthritis, joint pains, dry skin, eczema, scalp crusting and scaling, and other skin disorders. Groundnuts (*Arachis hypogaea* L.) are identified as one of the chief oil crops and about 50% of the groundnuts grown are used for oil extraction [6]. Groundnut oil is also a perfect choice for healthier frying as it can be heated to a higher temperature when compared to other oils, and this results in lower oil pick-up in the food. Due to its increasing demand, there has been a hike in the prices of good quality groundnut oil in Indian markets [7].

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Keeping the high demand for good quality groundnut oil it has now become an important concern to look forward to newer breeding techniques and also better extraction procedures so that the yield of the oil can be increased. The objectives of the present study are to use plant tissue culture techniques for the in vitro growth of groundnuts in a controlled environment and then shift the resulting plant onto the ground for its maturity [8]. These groundnuts can then be subjected to various oil extraction procedures and a comparison can be made concerning to the amount of the oil extracted from the lab-grown seeds and commercially bought seeds.

MATERIALS AND METHODS

Tissue culture

Groundnut was purchased from a local shop of Maharashtra. Seeds were then washed with Dextron-20 and pretreated sodium hypochloride and ethanol to remove the contamination, they are then inoculated in plant tissue culture basal media. The pH of the medium is adjusted to 5.8 and then the prepared media is autoclaved for periods of 120 min at a temperature of 120 °C. Two hormones Cytokinin (Kinetin) and Auxin (IBA) are used in different concentrations for the growth of the peanut plant. Proper care has been taken, all the in vitro cultures are maintained at a temperature of ±25 °C and a photoperiod of 12 hours is provided daily. After a period of 10 days, the cotyledons are carefully removed from the seeds and then are subjected to mild surface disinfection and then again carefully replaced in a fresh medium so that these cotyledons may further develop into explants, the embryonic leaflets are cautiously dissected from their embryos and then cultured in 10cm diameter cultured bottles. Once these explants grew to a certain length, they are then removed and planted in the garden.

Table 2 Growth details viz. plant height, root length and number of leaves with respect to BA+NAA (mg/l in 20 replicates)

BA+NAA (mg/l in 20 replicates)	Plant height (cm)	No. of leaves	Root length (cm)
0.5+00	7.5	5.5±0.86	6.5±0.16
1.0+00	7.9	5.0±0.29	7.1±0.31
2.0+00	7.9	5.2±0.28	7.5±0.12
00+0.5	8.2	6.55±0.19	6.7±0.14
00+1.0	8.5	6.65±0.17	3.5±0.11
0.5+2.0	8.7	5.10±0.31	3.8±0.15
0.5+0.5	7.7	5.05±0.25	6.2±0.17
1.0+1.0	8.6	6.10±0.30	6.7±0.17
2.0+2.0	9.0	6.15±0.24	5.5±0.15

Fig 1 Day-1

Fig 2 Day-5

Fig 3 Day-15

Fig 4 Day-25

Extraction method of oil

Typically, the most popular method for separation used is the steam distillation and aqueous enzymatic extraction [6] but three-phase partitioning technique stands to be more promising with respect to the yield of the final product. Three-phase partitioning (TPP) is an advanced technique which is widely used in bio-separations for separating and purifying bio compounds in recent years. Also, it proves to be more effective and economical due to the absence of steam and energy requirements. The partitioning of the liquid into three different phases depends mainly on the concentration of alcohol and salt used. In this study, we used TPP technique for the separation of ground nut oil from crude mixture. Ammonium sulphate salt is used in the current study which precipitates the enzymes and lipids from crude oil samples and Tertiary butanol is used as an alcoholic base which assists in the extraction of lipids/oils from the crude mixture.

Fully grown ground nuts are collected and washed thoroughly in fresh running water once. These cleaned groundnuts are then properly washed with distilled water and then placed inside Oven for 4 hours. The temperature of the oven is maintained at 30 °C. Once all the groundnuts are fully dried with no visible moisture content on them, the nuts from the shells are removed. Also, the skin of the groundnuts is removed and then powdered. 1 gram of groundnut powder is weighed accurately. 5ml distilled water is added to the powder and the pH of the solution is adjusted to 7.0. The entire mixture is then kept for 1 hour on a magnetic stirrer so that a uniform slurry is formed. This also provides good incubation time for the slurry. Now add 30% Ammonium sulphate to this mixture

so that phase separation takes place. Leave the mixture without disturbing aside for a few minutes till the layers get separated. Now add 1:1 Tertiary-butanol and vortex gently. Centrifuge it at low speed for phase formation. The centrifuge tubes are then left for 1 hour at 25-30 °C for Three-phase partitioning. Using a separating funnel t-Butanol layer and oil layer are separated. This mixture is then heated till t-Butanol evaporates. This leaves only extracted oil from the earlier mixture. Weigh an empty Eppendorf tube and transfer the oil into the Eppendorf tube, the difference in weight gives the amount of oil extracted. This oil is then distilled using the Soxhlet method so as to remove any traces of the solvent. The same procedure was repeated with varying concentrations of Ammonium Sulphate and Tertiary-butanol. Also, the pH and temperature of the solution were varied each time.

Fig 6 Day-45

Fig 7 Day-60

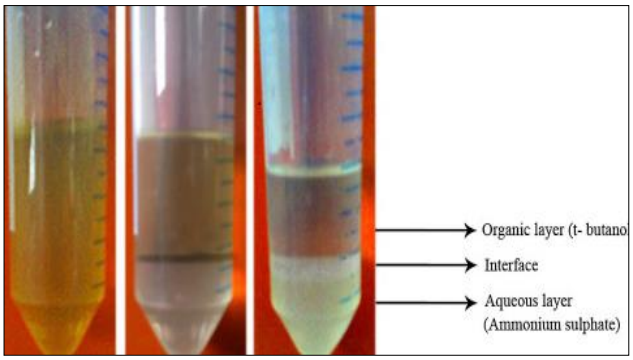


Fig 8 Three phase partition

RESULTS AND DISCUSSION

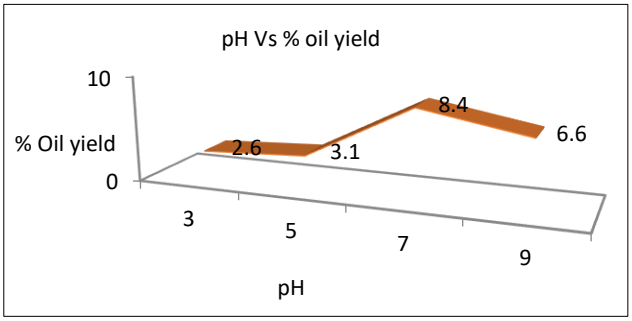
Ground nut oil is subjected to a TPP (three-phase partitioning) method of separation. During the TPP procedure, parameters like temperature, pH and concentration of ammonium sulphate and t- Butanol are varied to figure out the optimum conditions for the highest oil yield. Under standard temperature and pressure conditions, Tertiary-butanol is completely miscible with water, but upon the addition of specific salts such as Ammonium sulphate, the miscible solution separates into three phases, a lower aqueous phase, an upper t-butanol phase and an intermediate phase which usually forms in between these two phases consisting of the debris of the substrate added. The oil gets extracted into the upper layer in combination with t- butanol [9].

Effect of pH on % oil yield

(Table 3) shows the effect of pH on % oil yield. It is observed that when the pH of the medium is increased from 3 to 7, there is a gradual increase in the weight of oil extracted in grams and when the pH is further increased to 9 no significant increase is seen. Thus, it can be concluded that maximum oil extraction is seen at a neutral pH value. Also, it can be observed from the data that oil extraction is more favourable at basic pH rather than at acidic pH [10].

Table 3 Effect of varying pH on % oil yield

pH	Weight of oil (in gms)	% Oil yield
3	0.060	2.6
5	0.070	3.1
7	0.189	8.4
9	0.150	6.6



Graph 1 Graph showing pH during extraction process vs % yield of oil

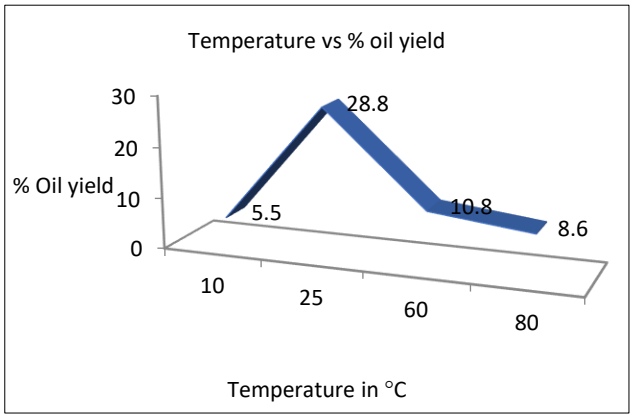
Effect of varying temperature on % oil yield

Table 4 Effect of varying temperature on % oil yield

Temperature	Weight of oil (in gms)	% oil yield
10^0C	0.124	5.5
25^0C	0.649	28.8
60^0C	0.245	10.8
80^0C	0.143	8.6

Graph 2 shows temperature effects on the oil extraction. It is observed that lower temperatures did not favour much oil extraction. The highest yield is obtained at room temperature

and upon the further increase in temperature, no significant increase in yield is observed. The authors opine that this might be due to the formation of emulsion at higher temperatures, which is preventing the oil collection [11].



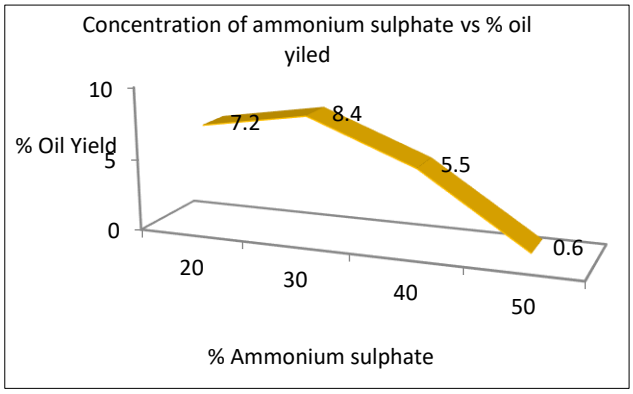
Graph 2 Graph showing temperature of extraction vs % yield of oil

Effect of varying concentration of ammonium sulphate on % oil yield

The amount of yield is studied by the addition of various concentrations of Ammonium sulphate salt to the slurry and the results obtained are as shown in (Table 3). (Table 5) shows that the highest amount of oil i.e., 0.19 gms is obtained when the concentration of ammonium sulphate used is 30%. This shows that a good amount of oil can be easily extracted when using low salt concentrations for extractions and as the salt concentration is increased further the weight of oil extracted also declines [12-13]. This may be because the overall solution becomes supersaturated concerning to salt and the extra amount of salt simply precipitates out. Also, from (Graph 3) it can be concluded even that by using very low salt concentrations good amounts of oil can be extracted. Further adding more amount of the same salt simply reduces the oil extraction capacity. Thus, it can be concluded that the optimum concentration of ammonium sulphate which can be safely used should be between 20%-30% and not above this [14].

Table 5 Effect of varying concentration of ammonium sulphate on % oil yield

Concentration	Weight of oil (in gms)	% Oil yield
20%	0.13	7.2
30%	0.19	8.4
40%	0.121	5.5
50%	0.013	0.6



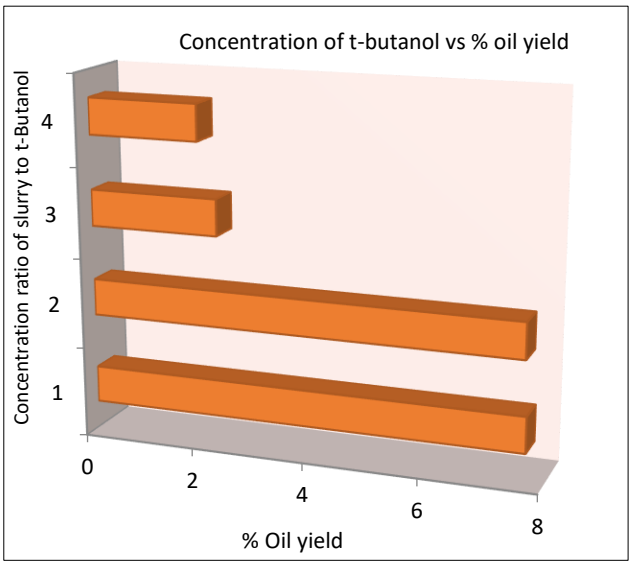
Graph 3 Graph showing concentration of ammonium sulphate vs % yield of oil

Effect of varying concentration of t- butanol concentration on % oil yield

In the current study, the authors also focused on the concentration ratio or dilution ratio of oil slurry to t-Butanol during extraction. From (Table 6) it can be observed that when a dilution ratio of 1:1 is used 1:1 (slurry: tertiary butanol), the weight of oil extracted is more. When the concentration of t-Butanol is increased further the results are not so promising and also have shown a decreasing trend. With this, it can be concluded that at low dilution ratios maximum amounts of oil can be extracted [15-17].

Table 6 Effect of varying concentration of t- butanol concentration on % oil yield

Dilution	Weight of oil (in gms)	% Oil yield
1:1	0.173	7.68
1:2	0.172	7.64
1:3	0.052	2.31
1:4	0.043	1.98



Graph 4 Graph showing concentration of t-butanol vs % yield of oil

CONCLUSION

From the current work done on oil extraction, it can be concluded that maximum oil extraction is seen at a neutral pH value when using TPP (three phase partitioning) method of separation. It is observed that lower temperatures did not favour much oil extraction. The highest yield is obtained at room temperature and upon the further increase in temperature, no significant increase in yield is observed. The authors opine that this might be due to the formation of emulsion at higher temperatures, which is preventing the oil collection. The optimum concentration of ammonium sulphate which can be safely used should be between 20%-30% and not above this. TPP technique stands to be more promising concerning for the yield of the final product.

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Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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