# Enzyme-Assisted Aqueous Extraction of Peanut Oil

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**ABSTRACT:** Enzyme-assisted aqueous extraction of oil from oilseeds is a relatively recent technique. In the present study, peanut oil was extracted under optimized aqueous extraction conditions using Protizyme™, which is predominantly a mixture of acid, neutral, and alkaline proteases. The optimal conditions were: enzyme concentration of 2.5% (w/w) in 10 g of peanut seeds, pH 4.0, 40°C, and 18 h incubation with constant shaking at 80 rpm. Centrifuging the mixture at 18,000 × *g* for 20 min separated the oil with a recovery of 86–92%. The merits of this process over existing solvent extraction and/or mechanical pressing methods are discussed.

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**KEY WORDS**: Enzyme-assisted aqueous extraction of oil, peanut oil.

Peanuts are an important food source of lipid and protein in developing as well as developed countries, and peanut oil is one of the major oils in the human diet (1). Peanut seeds contain 27–29% (w/w) protein and 40–50% (w/w) oil (2). Oil from peanuts is conventionally extracted by either mechanical pressing or solvent extraction. Mechanical pressing is a less efficient process, leading to low oil recovery (40–60%). Solvent extraction, although its recovery is in the 90–98% range, has inherent disadvantages of poor quality of protein in oil cake (meal), and high investment, and energy requirements (3). 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) (4). Hence, there is a need to explore alternative safe and efficient oil extraction processes that may also result in edible protein.

Aqueous enzymatic oil extraction is one such alternative eco-friendly process based on simultaneous isolation of oil and protein from oilseed by dispersing finely ground seed in water and separating the dispersion by centrifugation into oil, solid, and aqueous phases. The presence of certain enzymes during extraction enhances oil recovery by breaking cell walls and oil bodies (5). It is a very effective approach for oil extraction in case of coconut (6), soybean (7), and corn germ (8), giving oil recovery in the range of 90–98% and good quality protein meal.

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Attempts to extract peanut oil by aqueous extraction have also been made. Rhee *et al.* (9) described a multistep process (including an aqueous extraction step) for extracting peanut oil (9), obtaining 98% oil recovery. However, Lanzani *et al*. (10) reported only 72% oil recovery by using single-step aqueous extraction. In their work, use of an enzyme preparation containing cellulase, protease, and α-1,4-galacturonide glucanohydrolase increased oil recovery by only 6% (10). The present work with peanuts focuses on evaluating the effect of using a commercial mixture of three proteases in aqueous enzymatic oil extraction on the overall oil yield.

## MATERIALS AND METHODS

*Materials*. Deshelled peanut seeds available in the local market were used throughout the investigation. Protizyme™ was obtained from Jaysons Agritech Pvt. Ltd. (Mysore, India). It is reported to contain mostly acid, neutral, and alkaline proteases from *Aspergillus flavus.* Its specific activity (with casein as substrate) (11) was 2.1 U/mg protein. Papain, trypsin, and chymotrypsin (containing specific activities of 11.3, 3.8, and 6.3 U/mg protein, respectively, toward casein as substrate; determined as in the case of Protizyme™) were procured from Sisco Research Laboratory (Bombay, India). All other reagents used were of analytical grade unless otherwise specified.

*Enzyme-assisted aqueous oil extraction of peanut oil. (i) Use of a commercial enzyme preparation containing different proteases*. Peanut seeds (10 g) were soaked in water for 2 h and then dehusked. The dehusked seeds were ground (without addition of any water to the soaked seeds) to a thick paste by using a mixing blender at high speed. This paste was then dispersed in distilled water at 1:2 (wt/vol) paste-to-water ratio followed by gentle stirring with a magnetic stirrer. Protizyme™ (250 mg) was added before the pH of the slurry was adjusted to the desired pH (by adding an appropriate amount of 0.5 N HCl or NaOH). The enzyme mixture was then incubated overnight at 40°C followed by centrifugation at 18,000 × *g* for 20 min. The upper oil phase was carefully collected by using a Pasteur pipette. A control (aqueous oil extraction) was also carried out for the above extraction during which no enzyme was added.

*(ii) Use of purified papain, chymotrypsin, and trypsin*. Enzyme-assisted aqueous oil extraction was performed according to the procedure described above except that papain, trypsin, and chymotrypsin (with 21 U of caseinolytic activity

that was the same level as present in 250 mg of Protizyme™) were added instead of Protizyme™.

In both cases, oil recovery was calculated as percentages of total oil present in peanut seeds. Total oil was determined by solvent extraction using hexane in a Soxhlet apparatus following the standard AOAC procedure (12). The solvent extraction of peanut samples gave a value of 42 g oil/100 g peanut. During a second round of extraction, no more oil could be extracted from the residual peanut meal. Peanut oil is reportedly present in the range of 40–50 g oil/100 g peanut (2). A value of 42 g oil/100 g peanut was taken as 100% recovery of oil when calculating the oil recovery under various conditions in aqueous extraction.

## RESULTS AND DISCUSSION

In most of the aqueous enzymatic oil extraction procedures reported in the literature, commercial enzyme preparations containing varieties of enzyme activities, such as lipase, cellulase, pectinase and amylases, were used (10,13). For example, Lanzani *et al.* (10), while working with peanut seeds, used a mixture of protease, cellulase, and α-1,4-galacturonide glucanohydrolase for maximal oil recovery. The enzyme Protizyme™ used in this study contained a mixture of three proteases having pH optima in the range of 3–4, 5–7, and 7–10. In addition, small amounts of amylase, lipase, cellulase, phospholipase, and deaminase were present as per the supplier's specifications. Initially, pH 4.0 was chosen for extraction, because it is reported that this pH facilitates peanut oil recovery in aqueous oil extraction (pH 4.0 being the isoelectric point of many peanut proteins) (9).

The level of enzyme required for optimal recovery was determined by using enzyme levels of 50, 100, 250, and 500 mg (w/w). Slurry was made of 10 g peanut seeds in 20 mL distilled water, pH 4.0, overnight incubation at 40°C and 80 rpm. Control was run under similar conditions except the addition of enzyme. All the experiments were run in duplicate, and the difference in the individual values was less than 5%; 250 mg enzyme was found to give optimal oil recovery of 91% (w/w). Unlike the work presented by Lanzani *et al.* (10), the effect of using enzyme was quite drastic and significantly enhanced oil recovery. In all subsequent experiments, 250 mg enzyme in 20 mL slurry was used. The control of shaking speed during extraction is fairly critical. Table 1 shows that the decrease in shaking speed led to a decrease in oil recovery. Increasing the speed led to emulsification (both with or without enzyme) and reduced the amount of clear oil obtained at the top. The temperature of 40°C was chosen in the above experiment because this is the reported optimal temperature of the enzyme. The process temperature seems to be a critical parameter because decreasing the temperature merely by 3°C to 37°C significantly reduced the amount of oil extracted. Increasing the temperature led to reduced oil recovery presumably because the enzyme becomes thermo-inactivated (14) (Fig. 1). The time of the extraction process in the experiments above was about 18 h. This seemed to be an optimal time required for extraction (Fig. 2).

**TABLE 1**
**Effect of Shaking Speed on Oil Recovery by Aqueous Enzymatic Extraction[a]**

| Shaking speed (rpm)* | Oil yield (%, w/w) without enzyme | Oil yield (%, w/w) with enzyme |
|---|---|---|
| 50 | 23 | 50 |
| 80 | 44 | 92 |
| 100 | 38 | 83 |
| 200 | 37 | 78 |

[a]Slurry made of 10 g peanut seeds in 20 mL distilled water, pH 4.0, overnight incubation at 40°C, with 250 mg of enzyme. The mixtures were stirred at different rpm with constant shaking. All the experiments were run in duplicate, and the difference in the individual values was less than 5%.

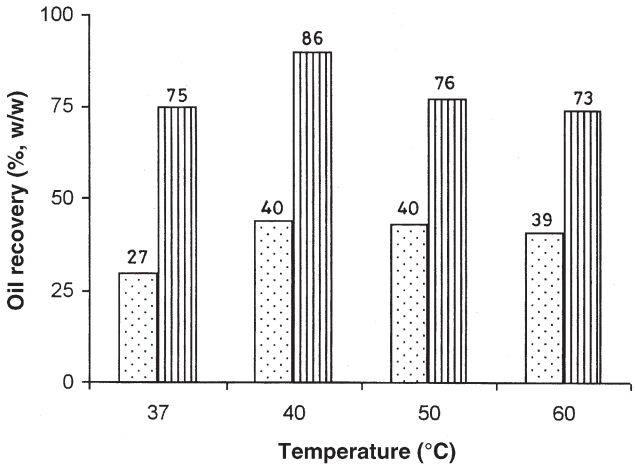


**FIG. 1.** Effect of varying temperature on oil recovery by aqueous enzymatic oil extraction. Slurry made of 10 g peanut seeds in 20 mL distilled water, pH 4.0, with 250 mg enzyme, incubated overnight at different temperatures with 80 rpm constant shaking. A control was run at different temperatures without adding enzyme. Stippled bars, without enzyme; lined bars, aqueous enzymatic extraction.

Although the foregoing experiments were carried out at pH 4.0, it was fortuitous that one of the proteases present in the enzyme preparation has a reported pH optimum around 4.0. As the enzyme preparation also has two other proteases with pH optima around 7.0 and 10.0, aqueous enzymatic oil extraction was also attempted at these pH values. However, pH 4.0 gave the best results (Fig. 3).

The Protizyme™-assisted aqueous oil extraction was compared with aqueous enzymatic oil extraction, in which some well-characterized enzymes (*viz.,* papain, trypsin, and chymotrypsin) were used (Table 2). Papain gave better results as compared to trypsin or chymotrypsin. This work showed that aqueous enzymatic oil extraction gave higher yield than simple aqueous oil extraction. The enzyme preparation used here (*viz.,* Protizyme™) also contained small amounts of lipase, cellulase, and amylase. Their presence may facilitate the recovery of oil. Parameters such as pH, temperature, time, shaking speed, and, of course, amount of enzyme need to be optimized during aqueous enzymatic oil extraction. Working with a mixture of proteases having pH optima over a wide range

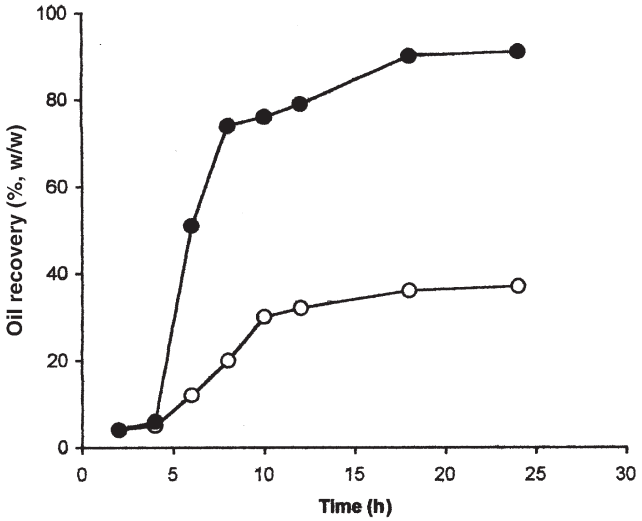


**FIG. 2.** Effect of varying time on oil recovery from peanut seeds by aqueous enzymatic extraction. Slurry made of 10 g peanut seeds in 20 mL distilled water, pH 4.0, with 250 mg enzyme, incubated at 40°C with constant shaking at 80 rpm for different time intervals. A control was run for different time intervals without adding enzyme; (○), without enzyme; (●), aqueous enzymatic extraction.

means that one can choose any pH for the extraction in a specific case to suit the isoelectric point of the major protein fraction. Reasonable amounts of oil can also be extracted by using well-characterized proteases. In this case, a nonspecific protease papain gave better results as compared to specific proteases trypsin and chymotrypsin.

Good recovery in this environmentally friendly process shows that it is possible to avoid solvents, which are harmful

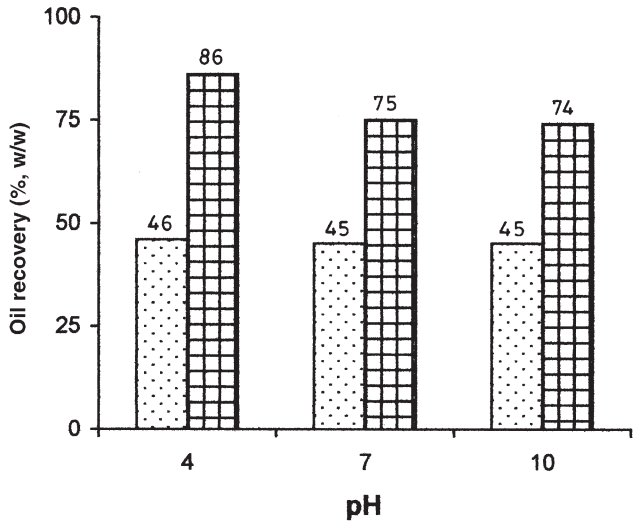


**FIG. 3.** Effect of pH on aqueous enzymatic oil extraction from peanut seeds. Three different sets of peanut slurry (10 g in 20 mL) were taken. Enzyme (250 mg) was added to each set before adjusting the pH of the slurry to 4.0, 7.0, and 10.0. The enzyme mixture was then incubated overnight at 40°C with constant shaking at 80 rpm. A control was run for each set without adding enzyme. Stippled bars, without enzyme; cross-hatched bars, aqueous enzymatic extraction.

**TABLE 2**
**Enzymatic Aqueous Oil Extraction by Using Different Proteases**

| Enzyme | Amount of enzyme added (mg) | Oil recovered (%, w/w) |
|---|---|---|
| Papain | 488 | 76 |
| Chymotrypsin | 500 | 61 |
| Trypsin | 162 | 67 |

[a]Slurry made of 10 g peanut seeds in 20 mL distilled water, pH 7.0, incubated overnight with different proteases containing units of activity similar to that of Protizyme™ (Jaysons Agritech Pvt. Ltd., Mysore, India) at 40°C with constant shaking at 80 rpm. All the experiments were run in duplicate and the difference in the individual values was less than 5%.

to the environment as well as to the remaining protein cake. At the moment, the cost of the enzyme is a major factor that will prevent adoption of this technology. However, increasing environmental concerns coupled with developing of more efficient downstream processing technology for enzymes (15,16) is likely to make this a viable technology for oil extraction in the future.

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## REFERENCES

1. Gunstone, F.D., *The Lipid Handbook*, edited by F.D. Gunstone, J.L. Harwood, and F.B. Padley, Chapman and Hall, London, 1986.
2. Mattil, K.F., F.A. Norris, and D. Swern, Extraction of Fats and Oils, in *Bailey's Industrial Oil and Fat Products,* 3rd edn., edited by Daniel Swern, John Wiley & Sons, London, 1964, pp. 637–717.
3. Gandhi, A.P., K. Jha, S.K. Khare, and N. Ali, Status of Soymeal Production and Its Utilization for Human Food in India, Souvenir SOPA convention, Indore, India, 1992, pp. 24–34.
4. Marlowe, I.T., T.J. Giddings, S.J. Richardson, and A. Stentiford, *U.K. Industry and Ozone Pollution from Volatile Organic Compound Emissions*, Warres Spring Laboratory–The Environmental Technology Executive Agency of the Department of Trade and Industry, London, 1991, Report 878(PA).
5. Rosenthal, A., D.L. Pyle, and K. Niranjan, Aqueous Enzymatic Processes for Edible Oil Extraction, *Enzyme Microb. Technol. 19*:402–420 (1996).
6. McGlone, O.C., C.M. Octavio, A.L.-M. Canales, and J.V. Carter, Coconut Oil Extraction by a New Enzymatic Process, *J. Food Sci. 51*:696–698 (1986).
7. Kim, I.K., and S.H. Yoon, Effect of Extraction Solvents on Oxidative Stability of Crude Soybean Oil, *J. Am. Oil Chem. Soc. 67*:165–167 (1990).
8. Bocevska, M., D. Karlović, J. Turkulov, and D. Pericin, Quality of Corn Germ Oil Obtained by Aqueous Enzymatic Extraction, *Ibid. 70*:1273–1277 (1993).
9. Rhee, K.C., C.M. Cater, and K.F. Mattil, Simultaneous Recovery of Protein and Oil from Raw Peanuts in an Aqueous System, *J. Food Sci. 37*:90–93 (1972).
10. Lanzani, A., N.C. Petrini, O. Cozzoli, C. Gallavresi, C. Carola, and G. Jacini, On Use of Enzymes for Vegetable Oil Extraction, A Preliminary Report, *Riv. Ital. Sostanze Grasse 21*:226–229 (1975).

11. Fujimura, M., T. Mori, and T. Tosa, Preparation and Properties of Soluble–Insoluble Immobilized Proteases, *Biotechnol. Bioeng. 29*:747–752 (1987).
12. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 14th edn., edited by W. Horowitz, Association of Official Analytical Chemists, Washington, DC, 1984.
13. Singh, R.K., B.C. Sarker, B.K. Kumbhar, Y.C. Agrawal, and M.K. Kulshreshtha, Response Surface Analysis of Enzyme-Assisted Oil Extraction Factors for Sesame, Groundnut, and Sunflower Seeds, *J. Food Sci. Technol. 36*:511–514 (1999).
14. Gupta, S., and M.N. Gupta, Mechanisms of Irreversible Thermoinactivation and Medium Engineering, in *Thermostability of Enzymes*, edited by M.N. Gupta, Narosa, India/Springer Verlag, Germany, 1993, pp.114–142.
15. Gupta, M.N., and B. Mattiasson, Novel Technologies in Downstream Processing, *Chem. Ind.*:673–675 (1994).
16. Roy, I., and M.N. Gupta, Current Trends in Affinity-Based Separations of Proteins/Enzymes, *Curr. Sci. 78*:587–591 (2000).

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