Extraction of oil from *Jatropha curcas* L. seed kernels by enzyme assisted three phase partitioning

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Abstract

Three phase partitioning has been evaluated for extraction of oil from *Jatropha curcas* L. seeds. This process consisted of simultaneous addition of t-butanol (1:1, v/v) and 30% (w/v) ammonium sulphate to the slurry prepared from *Jatropha* seed kernels. Combination of sonication and enzyme treatment with a commercial preparation of fungal proteases at pH 9, led to 97% oil yield within 2 h.

Keywords: Enzyme assisted three phase partitioning; Jatropha seed kernel oil; Three phase partitioning

1. Introduction

Recently, we have described a new approach for extraction of oil from plant materials, in general, and soybean in particular (Sharma et al., 2002a). In this approach, called three phase partitioning (TPP), appropriate amounts of ammonium sulphate and t-butanol were added to an aqueous suspension of soybean meal. This led to the formation of three distinct phases: upper organic phase, lower aqueous phase and interfacial precipitate (consisting mostly of proteins). The oil was found in the organic solvent phase and could be recovered by evaporating the t-butanol. TPP is simple to carry out, has short processing time (about 1 h), and is easy to scale up. It may be also useful to compare this process with solvent extraction. Hexane is the solvent which is generally used in that process. t-Butanol has a higher boiling point (84 °C) than hexane (69 °C), hence addition of volatile organic solvents to the atmosphere during TPP will be much lower even if open systems are used (Rosenthal et al., 1996). Thus, simpler extraction designs will be possible with TPP.

*Jatropha* is a genus comprising 70 species growing in tropical and subtropical countries (Openshaw, 2000). The seed kernel contains 40-60% (w/w) oil (Makkar et al., 1997). The presence of some antinutritional factors renders this oil unsuitable for use in cooking (Makkar et al., 1997; Gubitz et al., 1998). Some efforts are being made for removal of these antinutritional factors (Haas and Mittelbach, 2000). Meanwhile, as such, it has been used for production of biodiesel (Foidl et al., 1996).

The present work demonstrates the application of TPP in extraction of oil from seed kernels of *Jatropha*...
curcas L. An important further step in the process development has been the pretreatment of the plant material with enzymes before TPP.

2. Materials and methods

Jatropha seeds were obtained from Dr. Zope, College of Forestry, Akola, India. ProtizymeM was purchased from Jay sons Agritech Pvt. Ltd., Mysore, India. It is reported to contain mostly acid (pH optimum range 3-4), neutral (pH optimum range 5-7) and alkaline proteases (pH optimum range 7-10) from Aspergillus flavus with a specific activity of 2.1U/mg protein with casein as substrate. All other chemicals and solvents used were of analytical grade.

2.1. Three phase partitioning for extraction of oil from Jatropha curcas L.

Three phase partitioning was carried out as described earlier (Sharma et al., 2002a). Jatropha seeds were cracked, the shells carefully removed and the kernels thus obtained were used for slurry preparation. The slurry was prepared by grinding Jatropha seed kernels (5g/30ml) in distilled water. The pH of the slurry was adjusted to the desired value with 0.1 N NaOH or 0.1 N HCl. The appropriate amount of ammonium sulphate was added and vortexed gently, followed by addition of appropriate amount of t-butanol. The slurry was then incubated at 25 °C for 1 h for the three phase formation. The three phases were then separated by centrifugation at 2000 x g for 10 min. The upper organic layer was collected and evaporated on rotary evaporator (under reduced pressure at 50 °C, for 5 min) to obtain oil. The amounts of oil recovered were calculated as percentages of total oil present in Jatropha seed kernels. The latter was determined by soxhlet extraction using hexane as the solvent as per the standard AOAC procedure (Horowitz, 1984). The FT-IR of oil obtained by TPP was done by taking oil (10 µl) on KBr disc, the spectra was recorded on Nicolet-Protégé-460 spectrometer.

Each extraction by three phase partitioning was run in duplicate and the yields were found to agree between duplicates within 3%.

3. Results and discussion

The solvent extraction of Jatropha seed kernels gave a yield of 44 g oil/100 g Jatropha seed kernels. Jatropha oil is reportedly present in the range of 40-60 g oil/100 g Jatropha seed kernels (Makkar et al., 1997). A value of 44 g oil/100 g Jatropha seed kernels was taken as 100% recovery of oil when calculating the oil recovery by TPP.

The effect of varying ammonium sulphate during TPP is shown in Fig. 1. About 83% (w/w) oil could be obtained by using 30% (w/v) ammonium sulphate. In many other cases as well, where TPP has been used for separation of proteins/enzymes generally ammonium sulphate 30% (w/v) has given best results (Dennison and Lovrein, 1997; Sharma and Gupta, 2001a). In the case of extraction of soybean oil by TPP also, 30% (w/v) ammonium sulphate was found to be optimum (Sharma et al., 2002a). Fig. 2 shows the effect of varying the ratio of volumes of t-butanol to Jatropha slurry. Decreasing the t-butanol volume by half decreases the oil yield whereas increasing t-butanol to twice the volume did not lead to any significant increase. Thus, 1:1 ratio was employed in further studies.

It is well established that oil bodies in plants are trapped in the meshwork of proteins and cellulose/hemicellulose structures. Thus, the treatment with enzymes is an integral part of an approach called aqueous enzymatic oil extraction (AEOE) (Rosenthal et al., 1996). AEOE has been successfully used in extraction of oils from rice bran (Sharma et al., 2001b) and peanut (Sharma et al., 2002b). Thus, it was considered worthwhile to attempt pretreatment of the Jatropha slurry by enzymes before carrying out TPP. In order to distinguish it from other approaches, we would like to call this approach 'enzyme assisted three phase partitioning (EATPP)'.

In an earlier case (Sharma et al., 2002b), a commercial preparation called ProtizymeM had given good results with AEOE. ProtizymeM is a mixture of enzymes, its three main components are proteases with different pH optimum range of 3-4, 5-7 and 7-10. Hence, treatment of Jatropha slurry with ProtizymeM before carrying out TPP was attempted (Fig. 3). Extraction of oil was carried out at three different pH values: 4.0, 7.0 and 9.0, since the enzyme preparation has three proteases with these pH optima.
By enzyme pretreatment at pH 9.0 followed by TPP, 92% oil could be obtained (Fig. 3). It has been recently shown that the particle size of plant material plays an important role in giving optimum oil yield by AEOE. Smaller particle size was found to give higher yields (Rosenthal et al., 2001). Sonication is an efficient way for breaking up bigger particles into smaller ones (Szentmihalyi et al., 2002). It was found that using sonication before enzyme pretreatment increased the oil yield up to 97% (Fig. 4).

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Fig. 3. Effect of enzyme pretreatment in TPP on oil extraction. The pH of the slurry was adjusted to 4.0, 7.0 and 9.0 before adding ProtizymeM (250mg). The slurry was incubated at 50 °C with constant stirring at 100rpm for 1h. Thereafter, ammonium sulphate (30%, w/v) and t-butanol in a ratio of 1:1 (v/v) were added to the slurry. The oil was recovered from upper t-butanol layer as described in the Section 2. A control was also carried out by omitting the addition of enzymes. Control (dotted), enzyme treated TPP (vertically hatched).

Fig. 4. Effect of sonication in TPP on oil extraction. The pH of the slurry was adjusted to 4.0, 7.0 and 9.0 followed by sonication for 5min. After addition of 250mg ProtizymeM, the slurry was incubated at 50 °C with constant stirring at 100rpm for 1h. After this pretreatment, ammonium sulphate (30%, w/v) and t-butanol in the ratio of 1:1 (v/v) (ratio of slurry to t-butanol) were added. The oil was recovered from upper t-butanol layer as described in Section 2. (TPP, three phase partitioning; EA-TPP, TPP in presence of enzyme; sonication + EA-TPP, sonication for 5min followed by TPP in presence of enzymes).

The oil obtained was further analysed by FT-IR spectroscopy to detect the presence of t-butanol in the oil. The characteristics hydroxyl peak at 3368 cm\(^{-1}\) was absent in the IR spectra of Jatropha oil, confirming the absence of t-butanol. The detection limit of FT-IR (as determined with standard solutions of t-butanol) was found to be 1%. Besides, no smell of t-butanol was found in the oil obtained by three phase partitioning (and removal of t-butanol after evaporation at reduced pressure).

4. Conclusion

The novel approach of combining a recently developed technique of TPP with enzyme pretreatment
and sonication constitute an efficient procedure for obtaining oil from Jatropha seed kernels. It should be added that unlike the soxhlet extraction, which is carried out for 24 h, the method developed here takes only about 2 h.

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