Three phase partitioning for extraction of oil from soybean

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Abstract

Three phase partitioning, a method generally used for protein separation, has been evaluated for extraction of oil from soybean. 82% oil was extracted within 1 h using this process which required simultaneous addition of t-butanol (1:1, v/v) and 30% ammonium sulphate to the soybean slurry.

Keywords: Soybean oil extraction; Three phase partitioning

1. Introduction

Three phase partitioning (TPP) has been used for both upstream and downstream processing of proteins (Dennison and Lovrein, 1997; Sharma and Gupta, 2001; Singh et al., 2001; Roy and Gupta, 2002). The technique consists of adding an organic solvent (usually t-butanol) to a salt solution of protein(s). The salt which has been found to work best is ammonium sulphate and it is usually employed at the concentration below the 'salting out' concentration for proteins. An interfacial layer of protein appears between the upper t-butanol phase and lower aqueous layer. The present work evaluated the possible application of TPP for the extraction of oil from soybean.

Soybean oil is generally extracted from its flour by a multistage process using n-hexane (Kim and Yoon, 1990). The case for 'search for alternative solvents' has been summarized in an excellent report by Lusas et al. (1997). Their final summation is very perceptive, 'Isopropanol will not work the same as hexane if forced to act like it, but its use as a replacement solvent seems feasible'. It appears that what may be desirable is a paradigm shift in the extraction strategy. **TPP**, because of its simplicity and short processing time, may be worth exploring. Thus, this work is not merely focussed at comparing hexane and t-butanol as solvents but is an attempt to explore a novel strategy for oil extraction.

2. Methods

Soybean flour (containing 42% protein and 20% fat) was obtained from Central Institute of Agricultural Engineering (CIAE), Bhopal, India. All other chemicals and solvents used were of analytical grade.

2.1. Extraction of soybean oil by three phase partitioning

The soybean flour (5 g) was dispersed in 30 ml distilled water and gently stirred on a magnetic stirrer to make the slurry. Ammonium sulphate (30%, w/v) was added to this slurry and vortexed gently, followed by the addition of 30 ml organic solvents (t-butanol or n-propanol or isopropanol or ethanol). The mixture was kept at 25 °C for 1 h. Formation of three distinct phases, upper organic phase, lower aqueous phase and interfacial precipitate layer was observed. These were separated by centrifugation at 2000 g for 10 min. The upper organic layer was collected and evaporated (at the particular boiling point) to obtain oil extracted in this phase. The amounts of oil recovered were calculated as percentages of total oil present in soybean meal. Total oil was determined by soxhlet extraction using n-hexane as solvent as the standard AOAC procedure (Horowitz, 1984). The solvent extraction of soybean meal gave a value of 25 g oil/100 g soybean. Soybean oil is reportedly present in the range of 25-27 g oil/100 g soybean (Rosenthal et al., 2001). A value of 25 g oil/100 g soybean was taken as 100% when calculating the oil recovery by **TPP**.

Each extraction was run in duplicate and the yields were found to agree among duplicates within $\pm 6\%$. The reported yields are the lower numbers of the pairs.

3. Results and discussion

TPP was indeed found to extract oil from soybean flour; the oil as expected was found in the organic phase. The ratio (v/v) of organic solvent to the starting aqueous phase has been generally found critical in optimizing the TPP process (Sharma et al., 2000). Fig. 1 shows that the ratio of 1:1 was required to obtain 80% oil; larger amounts of t-butanol did not make a significant difference to the oil yield.

Lusas et al. (1997) have evaluated the different solvents for obtaining soybean oil by the conventional solvent extraction process. Table 1 shows the results obtained with the four alcohols tried for obtaining oil from soybean flour by TPP. t-Butanol gave the best result. It may be added that in various **TPP** based protocols for protein separation, t-butanol has been found to consistently perform better than all other organic solvents (Dennison and Lovrein, 1997; Sharma et al., 2000). The yield of 82% obtained here is lower than 94% reported by Lusas et al. (1997) in a conventional solvent extraction with isopropanol and hexane.

t-Butanol has a higher boiling point (84 °C) than hexane (69 °C). Thus, addition of volatile organic compounds to the atmosphere (Rosenthal et al., 1996) during the process will be much lower even if open systems are used. Simpler extraction designs will be

Fig. 1. Effect of soybean slurry to t-butanol ratio on the oil extraction by TPP. The soybean flour (5 g) was dispersed in 30 ml distilled water and stirred to make the slurry. Ammonium sulphate (30%, w/v) was added to this slurry and vortexed gently followed by addition of varying amount of t-butanol, to achieve different volumetric ratios of slurry to t-butanol. The upper t-butanol layer was collected for oil extraction after keeping these mixtures at 25 °C for 1 h.

Table 1

Extraction of soybean oil by TPP method

Organic solvents	Amount of oil obtained (%, w/w)
t-Butanol	82
n-Propanol	24
Isopropanol	26
Ethanol	6.4

The soybean flour (5 g) was dispersed in 30 ml distilled water and stirred to make the slurry. Ammonium sulphate (30%, w/v) was added to this slurry and vortexed gently followed by addition of 30 ml organic solvent. The upper organic solvent layer was collected for oil extraction after keeping these mixtures at 25 °C for 1 h. In the case of ethanol, the slurry was prepared by dispersing 5 g of flour in 60 ml distilled water.

possible. Lusas et al. (1997) have also suggested that possibility of separation of the organic solvents by chilling. This is considered desirable since chilling uses up much less energy than that required to evaporate the organic solvents by heating. t-butanol with a freezing point of 11 °C compares well with isopropanol (–89.5 °C) and hexane (–95 °C) (Abraham et al., 1988).

It may be added that this TPP based process also simultaneously separates the protein. Estimation and evaluation of this protein meal is in progress. To conclude, TPP can be an efficient alternative approach to oil extraction but more extensive modelling and scale-up studies are admittedly required before one can recommend it for use at the industrial level.

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