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Extraction of soybean (*Glycine max.*) with hexane–acetic acid: Effect on oil quality

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Soybean flakes were extracted in a Soxhlet extractor with hexane and hexane containing different concentrations of acetic acid for different periods. A concentration of 3% acetic acid in hexane and extraction for 3 h were found to be optimum for maximum extraction of total lipids and phospholipids. Compared to hexane, acidic hexane extracted 5·8% and 191·3% more total lipids and phospholipids respectively. Quality characteristics of acidic hexane-extracted oil showed that the Lovibond colour intensity and free fatty acid (FFA) content were higher compared to hexane-extracted oil. However, the fatty acid composition did not show any changes.

INTRODUCTION

India produces nearly 1·8 million tonnes of soybean annually and most of it is solvent-extracted using hexane to obtain oil and the meal. Although hexane is the primary choice of solvent adopted by the oilseed processors, use of various alternative solvents such as alcohols, methylene chloride and mixtures of azeotropic solvent systems based on hexane and alcohols have been reported (Johnson & Lusas, 1983). Hensarling *et al.* (1974) have shown that extraction of cottonseed with hexane containing 2–25% acetic acid resulted in a higher extractability of total lipids, phospholipids and gossypol. Recently, Hensarling and Jacks (1983) have shown that extraction of lipids from soybean using hexane, containing 5% acetic acid at room temperature, yielded higher amounts of total lipids and phosphorus as compared to that of pure hexane extraction. However, systematic studies on the extraction of total lipids from soybean as a function of concentration of acetic acid in hexane and duration of extraction have not been carried out. Also, no information is available on the quality characteristics of acidic hexane-extracted oil. This paper deals with these aspects in relation to the extraction of total lipids and phospholipids from soybeans and also some of the quality characteristics of the extracted oil as compared to that of hexane-extracted oil.

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MATERIALS AND METHODS

Soybean seeds of yellow 'Clark-63' variety were obtained from Shakti Soya Ltd (Coimbatore, India). The seeds were cleaned and dehulled according to the method of Shamanthaka Sastry *et al.* (1969). The dehulled soybean was equilibrated to 12% moisture and passed through flaking rolls (Kranmaskiner Malmo Type J. No. 6725) to obtain flakes of 0·3 mm thickness. The flakes were dried in a through-flow drier for 6 h at 50°C. The moisture content of soy flakes was 5%.

Extraction of lipids

A known quantity (35 g) of soybean flakes was extracted with hexane or hexane containing different concentrations of acetic acid (1–5% v/v) for different periods (1–4 h) in a Soxhlet extractor. The solvent to flakes ratio in the extractor was 1·5:1 and the time of siphoning of the solvent was maintained constant in order to have a uniform contact time of the different solvents with the flakes. The temperature in the extractor was around 60°C. After the extraction, the solvents were removed *in vacuo* in a rotary flash evaporator at

50°C. The oil, which is free from solvent, is stored at 4°C until use.

Determination of phospholipids

A known quantity of soybean oil (50–100 mg) was digested (Marinetti, 1962) and the phosphorus content was determined according to the method of Taussky and Shorr (1953). A factor of 25 was used to convert the phosphorus values to phospholipids (Chapman, 1980).

Free fatty acid determination

The free fatty acid (FFA) content in the oil was determined according to the AOCS (1978) procedure and the values are expressed as percent oleic acid.

Lovibond colour measurement

The colour of the oil was measured in the Lovibond Schofield Tintometer Type 1A using ¼ in cell.

Fatty acids composition

Fatty acid methyl esters were prepared by base-catalysed transmethylation reaction of the lipids using 0·5 M sodium methoxide (Christie, 1982) and analysed in a gas chromatograph (Shimadzu GC–9A) equipped with flame ionisation detector and a stainless steel column (152·4 cm × 3·17 mm i.d.) packed with 20% diethylene glycol succinate on 80–100 mesh Chromosorb W support at a column temperature of 180°C, and the injection port and FID temperature at 210°C. Nitrogen was used as carrier gas at a flow rate of 40 ml/min. The area and the relative percentage of fatty acid methyl esters were obtained with a Shimadzu integrator (Shimadzu C-R3A Chromatopac). The composition of each peak was identified with those of standards run on the same column under similar conditions.

RESULTS AND DISCUSSION

The total lipid content of soy flakes extracted with hexane and hexane containing varying concentrations of acetic acid for different periods are presented in Table 1. The total lipid content of hexane-extracted soy flakes was 19·0% at 2 h. Further increase in extraction beyond 2 h did not improve the extraction of lipids. Acidic hexane extraction showed that, as the concentration of acetic acid is increased, the total lipid extraction is also increased. A concentration of 3% acetic acid in hexane and extraction for 3 h was found to be optimum for the removal of lipids (20·0%). Further increase in the concentration of acetic acid did not result in any significant improvement in the extraction of lipids. Thus, the results of Table 1 showed that extraction of soy flakes, with hexane containing 3% acetic acid for 3 h, resulted in 5·8% higher amounts of total lipids compared to that of hexane alone. Using 2 g soy meat in 6 ml solvent, and extraction period of 5 min each for three successive extractions, Hensarling and Jacks (1983) have reported approximately 11% higher yields of total lipids with hexane containing 5% acetic acid compared to that of hexane alone. However, with the present experimental conditions, only 5·8% higher yield of total lipids could be achieved. Increased extractibility of total lipids could be due to greater accessibility of acidic hexane solvent than hexane to membranes which surround the spherosomes, the storage site of oils of oilseeds (Jacks *et al.*, 1974).

Table 1. Effect of acidic hexane extraction on the total lipids (%) of soybean flakes

Percent acetic acid concentration in hexane (v/v)	Extraction (h)			
	1	2	3	4
0	18·6 ± 0·2	19·0 ± 0·3	18·9 ± 0·1	19·0 ± 0·1
1	19·0 ± 0·2	19·3 ± 0·1	19·5 ± 0·2	19·8 ± 0·2
2	19·2 ± 0·1	19·4 ± 0·1	19·8 ± 0·2	19·9 ± 0·3
3	19·2 ± 0·1	19·5 ± 0·2	20·0 ± 0·1	20·1 ± 0·2
5	19·3 ± 0·2	19·5 ± 0·1	20·1 ± 0·1	20·1 ± 0·1

The values are means of triplicate determinations ± standard deviation.

Since the acidic hexane solvent is polar in character, the effect of extraction of soy flakes as a function of acetic acid concentration in hexane and duration of extraction on the phospholipid content of the oil, is given in Table 2. The phospholipid content of hexane-extracted oil ranged from 1·13 to 1·27 g/100 g oil, the maximum being at 3 h extraction. The values are higher (2·05–3·75%) for acidic hexane-extracted oil. As the concentration of acetic acid in hexane is increased, the phospholipid content is also increased. However, a maximum value of 3·7% was obtained when soy flakes were extracted with hexane containing 3% acetic acid for 3 h as against 1·27% for hexane-extracted oil. This

Table 2. Effect of acidic hexane extraction on the phospholipid content g/100 g oil of soybean oil

Acetic acid concentration in hexane (%)	Extraction (h)			
	1	2	3	4
0	1·13±0·01	1·18±0·01	1·27±0·03	1·27±0·01
1	2·05±0·05	2·10±0·01	2·23±0·01	2·21±0·01
2	2·56±0·02	2·89±0·01	3·00±0·02	3·01±0·02
3	3·21±0·01	3·43±0·03	3·70±0·02	3·72±0·03
5	3·26±0·01	3·68±0·03	3·73±0·04	3·75±0·04

See Table 1 footnote.

represents an increase of 191·3% or 2·9 times that of hexane-extracted oil. Further increase in acetic acid concentration or duration of extraction, did not show increase in the phospholipid content. Hensarling and Jacks (1983) have reported that, from soy meals, the amount of phosphorus extracted by hexane containing 5% acetic acid at room temperature or 60°C was 16 to 35-fold greater than the amounts extracted by hexane.

The FFA content, Lovibond colour and fatty acids composition of hexane and hexane containing 3% acetic acid-extracted oil are given in Table 3. For comparison, data on hexane containing 5% acetic acid-extracted oil are also included. The FFA content of hexane-extracted oil is 0·5%. Hexane containing 3% acetic acid-extracted oil showed an FFA of 0·75%. The value further increased to 1·08% when the concentration of acetic acid was 5%. The percent increase in FFA would be 50–116% depending on the concentration of acetic acid in hexane. Thus some degree of hydrolysis of triglycerides occurs during extraction with acidic hexane. To check whether any traces of acetic acid left in the oil would effect the apparent FFA values, FFA analysis was carried out in oil stored at 4°C for 3 months. There were no significant variations in FFA contents compared to initial values.

The colour of acidic hexane-extracted oil was more of reddish yellow as compared to that of hexane-extracted oil. The Lovibond colour value of hexane-extracted oil showed a value of 8Y 0·9R, while that of hexane containing 3% acetic acid was 20Y 3·5R and the value further increased to 30Y 4·5R in hexane containing 5% acetic acid-extracted oil. Soybean contains the carotenoid pigment, lutein (Proctor & Palaniappan, 1989). The high colour intensity of the acidic hexane-extracted oil could be due to the extraction of these pigments.

Although FFA content of the acidic hexane-extracted oil is higher, the fatty acid composition of the extracted oil did not show any variations compared to that of hexane-extracted oil.

In conclusion, the results that are reported here show that 3% acetic acid concentration, in hexane, is the optimum for the extraction of total lipids and phospholipids. From the solvent recovery point of view, lower concentration of acetic acid in hexane is advantageous as the solvent forms an azeotropic mixture and hence is easily recoverable (Hensarling & Jacks, 1983). Hexane partially extracts phospholipids and the residual lipoidal materials, especially phospholipids, have been claimed to cause bitter and beany flavours in soybean meal (Johnson & Lusas, 1983). Acidic hexane extracts more phospholipids into the oil and therefore the quality of the meal is upgraded. However, the presence of higher amounts of phospholipids and FFA level could possibly lead to some losses of oil during refining.

Therefore, it would be better to use the acidic hexane solvent as a secondary extraction solvent rather than as a primary one. Currently work is in progress in the quality evaluation of soybean meal treated with acidic hexane as secondary solvent.

Table 3. Quality characteristics of hexane and acidic hexane-extracted soybean oil

	Hexane	Hexane containing 3% acetic acid	Hexane containing 5% acetic acid
FFA (%)	0.5	0.75	1.08
Fatty acids (%)			
$C_{16:0}$	13·6	12·7	13·7
$C_{18:0}$	1·8	1·7	1·7
$C_{18:1}$	28·0	25·6	26·3
$C_{18:2}$	48·2	50·9	50·0
$C_{18:3}$	8·2	9·1	8·2
Lovibond colour	8Y 0·9R	20Y 2·5R	30Y 4·5R

Values are averages of two independent determinations.

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