CHAPTER III

Studies on Local Soyabean Oil

(A) Determination of Phospholipid Content from Oil

(B) Adsorption of Heated Oil Components on Magnesium Silicate

(C) Determination of Lipid Peroxidation Products from Oil
Soyabean (*Glycine max*) - also termed as Soybean (U.S) is a legume (Leguminosae) indigenous to orient. It is an annual plant with a height of 60-125 cm. The cotyledons are the parts of the seed that emerge from the soil as the seedling develops. The pods generally contain one to four seeds. Varieties of Soyabean native to Asia have yellow, green, black or brown seed coats. The growing season for Soyabean is controlled by response to photoperiod and temperature. Soyabean is grown as a soil building crop. The valued portion of the plant is seed which is rich in protein and fatty oil. Many vegetable products are derived from soya due to high oil content, low price and universal availability. Recent studies have demonstrated intake of Soya food and Soyabean oil are associated with reduced cardiovascular risk. Enhancement of antioxidant contents from Soyabean oil has been reported. These have mostly been found to be isoflavones. In a different study, for higher recovery of olive oil, enzymatic pretreatment has been suggested.

Preparation for extraction consists of conditioning the beans, cracking these into small particles giving flakes. The flakes are extracted with hexane. The oil-laden solvent is separated (from the flakes) and distilled for recovery of crude Soyabean oil. The oil has substantial proportion of essential fatty acids and is used for edible purposes. Since the oil is produced from local crop
and said to be economical and popular with the lower/middle class folk in the surrounding areas, it was thought worthwhile to have in depth study of the oil, extracted from the locally available source. The material for the present study was collected from a local farm and authenticated at the Botany Department of the University. The beans (in the form of flakes) were extracted with hexane in Soxhlet.
(A) DETERMINATION OF PHOSPHOLIPID CONTENT FROM OIL

To be suitable for edible products, crude oil from the extraction would require processing. Phospholipids present in the crude oil will precipitate during storage and contribute to deterioration during use of the oil. Residual phosphorus in processed oil is a measure of the quality of oil. Removal of phospholipid content improve colour and enhance oxidative stability. Residual phosphorus is determined by colorimetric determination which is tedious. The objective of the present work was to have an alternative method. In the present work FTIR has been used to identify phospholipid content. The method saves time and large volumes of solvents.

Experimental:

The oil was obtained from seeds in yield of 18%. For sample preparation, water (2%v/v) was added to crude oil, followed by heating at 90°C for half an hour with stirring. The oil was centrifuged at 5,000 rpm for twenty minutes and decanted. The phospholipid rich layer was washed with acetone and solvent later removed. The sample was purified by passing through a column of silicic acid (30 x 2.5 cm) and eluting with methanol. Later the solvent was evaporated. Finally the sample was taken in hexane, concentrated and subjected to FTIR.
Fig. III/A: FTIR Spectrum – Phospholipid Content of Soyabean oil
Results:

Fig. III/A shows the FTIR spectrum of the sample. Band at 1730 cm\(^{-1}\) corresponds to C = O due to ester group present in phospholipid. Phosphate stretches are seen at 1169, 1084 and 1063 cm\(^{-1}\). P = O vibration at 1250 cm\(^{-1}\) and quaternary amine head group (PC and PE) stretching at 970 cm\(^{-1}\).

Total phospholipid content from some seed oils have been quantitated by TLC/FID and HPLC earlier in our Laboratory,\(^9\) the present method is rapid, efficient, reproducible and ecofriendly.
(B) ADSORPTION OF HEATED OIL COMPONENTS ON MAGNISIUM SILICATE

The Soyabean oil, as any edible oil, is often put to high
temperature heating (frying temperature, 180°C) during cooking
and is used several times particularly in the fast-food joints,
which have come up in large numbers in the past few years. The
quality (nutritive and chemical) of the oil gets affected – everytime
it is put to heat treatment, as has been studied in this Laboratory
in an earlier study. In the present study, the heated oil has been
passed through the adsorbent (magnesium silicate) and the
adsorbed constituents have been analyzed.

Experimental:

Magnesium silicate (Sardary Chemical Company, Ontario,
Canada) have been used for filtration since it is known to have
high adsorptive capacity. The present set of experiments were
carried to investigate if any of the components of the oil content
are adsorped on its surface, FTIR has been used to observe the
adsorption behavior.

1% by weight of magnesium silicate was added to the used
oil (10g) at 150°C. The hot mixture was stirred for 5 minutes, then
filtered through Whatman 41 filter paper. The filter cake was
washed with hexane to remove adsorbed oil. Hexane was removed
and the dried adsorbent was analyzed by FTIR. (Model : Shimadzu
8201 PC).
Fig. III/B: FTIR Spectrum – Heated Soybean oil adsorbed on Magnesium silicate
Results and Discussion:

Fig. III/B shows the FTIR spectrum. Stretching and deformation bands at 3008 cm\(^{-1}\) (C-H stretch – alpha to double bond \([R-C = C (H)R']\) of unsaturated fatty acid) and at 2926 and 2680 cm\(^{-1}\) (a symmetric and symmetric vibrations of methylene (CH\(_2\)) groups. Carbonyl (C = O) stretch H bonded to adsorbent is shown by band at 1725 cm\(^{-1}\)and methylene 'wag' is the band at 1460 cm\(^{-1}\). Band at 1550 cm\(^{-1}\) (between C = O stretch and methylene 'wag' bands) corresponds to carboxylate (RCOO') group, which is formed by loss of H ion and ionic adsorption to metal oxide on surface of magnesium silicate, thus in addition to oil adsorbed by H bonding, some is chemisorbed to magnesium silicate.

Magnesium silicate has adsorbed carboxylic acid content from used heated oil under conditions of temperature and contact time of filtration.

The used adsorbent (magnesium silicate) shows adsorptive capacity for part of the heat treated oil constituents.
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(C) DETERMINATION OF LIPID PEROXIDATION PRODUCTS FROM OIL

Fatty acid profile of the oil shows it to be rich in unsaturated acids. On high temperature heating of the oil, lipophilic per oxidation products (mainly aldehydes) are formed. No single method is available to identify these products. In the present work, HPLC has been applied and using the method various non polar and polar peroxidation products have been identified.

On high temperature heating (in presence of air) due to peroxidation of the fatty acids present in the oil, different lipophilic aldehydes are formed$^{13-15}$, some of these can inhibit enzyme activity$^{16,17}$.

There are several methods$^{18-20}$ to determine the peroxidised products but no single method can identify all the formed products. The method used in the present study (monitoring by HPLC) achieves separation and identification of a wide range of nonpolar and polar peroxidation products.

Experimental:

Standard carbonyl compounds and derivatives were obtained from Sigma Chemical Company. Solvents used were of HPLC grade. HPLC instrument (with UV/vis detector) was from Waters with a computing integrator (Hewlett-Packard). For separations ODS C$_{18}$ reversed phase column and disposable syringes with 0.2 $\mu$m filter (Chrom Tech) were used. Isocratic elution (methanol : water) for 10 minutes followed by linear gradient (methanol) for 15
minutes, flow rate 0.8 ml/min (detection 378 nm, injection volume 100 ml). For preparation of dinitrophenyl hydrazine reagent: 10 mg of it was treated with 20 ml hydrochloric acid. The contents were mixed and heated to 50°C for ½ hr., cooled and extracted with hexane. For derivatization of standards; 50 ml of standard was treated with 5 ml of the above reagent in presence of 5 ml methanol and 0.1 ml hydrochloric acid. Soyabean oil was heated (in presence of air) at 180°C for 6 hours. After cooling, 3 ml of oil was treated with 5 ml reagent and mixed thoroughly.

For separation of nonpolar and polar carbonyl compounds\textsuperscript{21,22} (peroxidised products), the above derivatised oil was extracted with 10 ml methylene chloride and 500 µl from this applied to TLC plate. The non polar and carbonyl compounds were separated by their $R_f$ values. The nonpolar and polar regions were individually removed from TLC plates. The collected portions were washed with methanol and centrifuged (1360xg) for 10 minutes to remove the silica. The supernatants were collected. 100 µl from non polar fraction was injected to HPLC column, using isocratic elution (methanol : water 75:25 v/v) for 20 minutes followed by (methanol alone) for 20 minutes. Similarly 100 µl from polar fraction was injected using methanol : water (50:50 v/v) for 20 minutes followed by methanol alone for 20 minutes. Identification was done by comparing the retention times of standards with those of the oil sample.
Fig. III/C1: GLC Profile - FAME - Soyabean oil
Results:

The GLC fatty acid profile (Fig. III/C1) of Soyabean oil shows presence of 5 peaks –

Peak 1 : Palmitic acid (5%)
Peak 2 : Stearic acid (1%)
Peak 3 : Oleic acid (51%)
Peak 4 : Linoleic acid (22%)
Peak 5 : Linolenic acid (14%)

Oleic acid and linoleic acid are the major unsaturated fatty acids followed by linolenic acid.
Fig. III/C2: HPLC Profile - Nonpolar peroxidised fraction - Soyabean oil
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The non polar peroxidised content were found to have $R_f$ 0.55 fraction and the polar fraction $R_f$ 0.23 on TLC.

HPLC profile of nonpolar fraction (Fig. III/C2) shows presence of 17 peaks –

Peak 1 : Butanal
Peak 2 : Butanone
Peak 3 : Pentanal
Peak 4 : Pentanone
Peak 5 : Unidentified
Peak 6 : Hexenal
Peak 7 : Hexanal
Peak 8 : Heptadienal
Peak 9 : Heptenal
Peak 10 : Octanal
Peak 11 : Unidentified
Peak 12 : Unidentified
Peak 13 : Nonenal
Peak 14 : Decadienal
Peak 15 : Decanal
Peak 16 : Unidentified
Peak 17 : Unidentified
Fig. III/C3: HPLC Profile – Polar peroxidised fraction - Soyabean oil
HPLC profile of polar fraction (Fig. III/C3) shows presence of 13 peaks –

Peak 1 : Unidentified
Peak 2 : Hydroxyhexenal
Peak 3 : Unidentified
Peak 4 : Unidentified
Peak 5 : Hydroxyoctenal
Peak 6 : Unidentified
Peak 7 : Unidentified
Peak 8 : Hydroxynonenal
Peaks 9-13: Unidentified

Hexanal and Heptenal are formed from linoleic acid, whereas Heptadienal and Octanal are formed from linolenic and oleic acids.23
REFERENCES


