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RESEARCH PAPER

Gas Chromatograph Mass Spectroscopy Method for Analysis of Fatty Acid Composition of Palm Oil Using BPX-70 Capillary Column.

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ABSTRACT

Several oil palm germplasm accessions are available with NRCOP and considerable variations in fatty acid composition (FAC) of palm oil among these accessions are expected. Evaluation of these germplasm for FAC is an important aspect for selection of palms with superior oil quality. The FAC of oil can be estimated by gas chromatography (GC) where known standard fatty acids or their methyl esters are required. Gas Chromatograph Mass Spectroscopy (GCMS) can determine the fatty acids composition more precisely even without the standards and hence unidentified / unknown fatty acids can be detected by GCMS. In the present study, GCMS method was standardized for analyzing the FAC of palm oil samples. Ten random samples of palm oil from African germplasm were used for the present study in a SHIMADZU GC 17A Ver.3, with MS QP5050A. BPX-70 capillary column having 60m length, 0.25mm ID, and 0.25 µm thickness was used for the study. With several experimental trials, the GC parameters were standardized as: 230^{0}C injection port temp., 215 Kpa column inlet pressure, 1.2 ml/ min column flow, 29.7 cm/sec linear velocity, 55 split ratio, 76 ml/ min total flow and 180^{0}C oven temperature. Carrier gas used was 99.999% pure helium. For Mass Spectroscopy, interface temperature was set at 250^{0}C and scanning method of acquisition, ranging from 50 to 500, for mass/charge (m/z) was optimized. Spectrum data was collected at 0.5 sec. interval. Solvent cut time was set at 4 min and 25 min retention time sufficient for separating all the fatty acid of palm oil. Mesocarp from fully ripen fruits were derivatized to methyl ester using 0.5 N Sodium Methoxide with BF_3 as catalyst and extracted with n-Hexane. The average FAC of 10 palm oil samples were recorded as 0.55 % C14:0, 53.02% C16:0, 4.07% C18:0, 38.77% C18:1, 3.59% C18:2. The results are in agreement with the previously reported palm oil composition.

INTRODUCTION

Oil palm is introduced in India as an irrigated crop and the crop is being cultivated successfully in several states. Oil palm germplasm has been collected and a few have been developed indigenously in India. Routinely the oil quality in terms of fatty acids composition (FAC) of the palm oil is being analyzed for several purposes. Usually FAC are determined by Gas Chromatography (GC). In this process, the oil is derivatized to fatty acids methyl esters (FAMEs) and injected in a specific condition to a GC, normally having a Flame Ionization Detector (FID). Individual FAME peaks appear after specific retention time under a set condition of the instrument for the samples and they are compared with the retention time of the standard FAME peaks and thereby the fatty acids composition is determined. Several workers have reported use of GC for analysis of fatty acids and FAMEs. Varian 3700 with a FID, splitter Inlet, glass capillary column (40 m x 0.3 mm ID) coated with XE60 was used isothermally at 220^o C with helium as carrier gas at 1.6 ml/min, split ratio of 1:50 was used for analysis of fatty acids analysis of several edible oils including palm oil (Pieter and Anna, 1985). Similarly after standardizing the GC parametsrs, BPX-70 column (SGE, Australia) was used for the analysis of FAME from different oils in the Gas Chromatography (Naresh Kumar, 2007; Sodeif and Paresh, 2008). Lau *et al*. (2005) reported simultaneous quantification of free fatty acids, free sterols, squalene, and acylglycerol molecular species in palm oil by high-temperature gas chromatography—flame ionization detection.

However when Gas Chromatograph is connected with a specialized detector called Mass Spectorscopy (GC-MS) the instrument becomes extremely sensitive by combining the features of gas-liquid chromatography and mass spectrometry for identification of different substances within a test

sample. The difference in the chemical properties between different molecules in a mixture are separated in the gas chromatograph, and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass to charge ratio (m/z). It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame Ionization Detector) detects multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time) which results in two or more molecules to co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes makes it extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically lends to increased certainty that the analyte of interest is in the sample (Wikipedia, 2009). In recent times GC-MS has been used by several worker for analysis of oil and fats for accurate analysis. A single-laboratory validation of a GC-MS method for the determination of different aromatic hydrocarbons in oils and fats were reported by Adlof and List (2004). Puah et al. (2006) analyzed very long chain fatty acid methyl esters in transesterified palm oil by GC-MS. For the determination of fatty acids composition of oil present in biscuits and margarine, FAMEs were analyzed by Agilent GC–MS and chromatograms obtained were compared with two libraries (NIST & Wily), which provided the best information about the identification of fatty acids present in biscuit as well as margarine samples (Kandhro *et al*., 2008a, Kandhro *et al*., 2008b). Fatty acids composition of the Sterilization and extraction of palm oil from screw pressed palm fruit fiber using supercritical carbon dioxide was analyzed using gas chromatography–mass spectrometry (GC/MS) by Norulaini (2008).

As the oil palm research in the country is new and standardization of methodology is very important for any research laboratory, method of GC-MS to analyze the fatty acid composition of palm oil has been standardized at this centre and is described in this article.

MATREILAS AND METHODS

Instrument and different parameters: SHIMADZU (Japan) make gas Chromatograph model 'GC-17A' coupled with the Mass Spectrometer model GCMS-QP5050A' was used for this study, which has quadrupole mass analyzer. The SGE (Australia) make capillary column BPX-70 (70% Cyanopropyl (Equiv) Polysilphenylene-Siloxane) having length 60m, Inner diameter 0.25mm, Film thickness 0.25 µm was fitted in the GC for separating the fatty acid methyl esters. Helium gas (99.999%) with 700-800 kPa supply pressure was used as carrier gas. Perflurotributylamine (PFTBA) was used as tuning standard for performing resolution adjustment, sensitivity adjustment and mass number calibration. Software used for analysis was GCMS Solution Version 1.1.

The instrument was put on simultaneously with the turbo molecular pump and analysis started under vacuum. Different GC and MS parameters such as lower and higher limit of mass/charge (m/z), injection port temp, oven temperature, Interface temperature, column inlet pressure, column flow, linear velocity, split ratio and total flow were experimented from a lower to higher values to obtain the optimum result. Some reference values from the literature were used while setting up the parameters (Kandhro, 2008a; Anonymous, 2000, Anonymous, 2003a; Anonymous 2003b).

Derivation of oil to Fatty acids methyl ester (FAME) : Oil samples were derivatized to fatty acids methyl ester (FAME) (Morrison and Smith, 1964). Two drops of oil sample was mixed with 5 ml of sodium methoxide in methanol (0.5N) in a screw cap test tube and heated on a boiling water bath at 60–70°C for 15 min. The tube was cooled to room temperature and one drop of BF_3–methanol complex was added to it. It was heated again for 5 min at 60-70°C and allowed to cool to room temperature. n-hexane (2 ml) was added to it, shaken and a few drops of water were added for hexane layer to get separated. The upper (hexane) layer was pipetted out into a microfuge tube and moisture was removed by adding a pinch of anhydrous sodium sulphate.

One ul of FAME sample in hexane was injected to the GC injector port for analysis. Standard FAME samples (Sigma-Aldrich, USA) were also diluted with n-hexane and injected.

Individual FAMEs were identified by comparing the mass spectrum of them with NIST library. They were also compared and matched with the standard FAME for confirmation.

Oil samples: palm oil samples extracted from 10 *dura* oil palm from African accession were used for the study.

RESULTS AND DISCUSSION

The standardization of GCMS method was carried out with a column (BPX-70), which is a recommended column for GCMS. BPX70 is a thermally modified siloxane phase containing a high concentration of cyanopropyl groups. It is ideal for the separation and characterization of saturated and partially unsaturated fatty acids (as the methyl ester derivative) found in edible oils. Baseline separation of consecutive unsaturated fatty acids, (i.e. C18:1, C18:2, C18:3, etc.) even when present in greatly varying concentrations, can be achieved on large inside diameter capillary columns. (Anonymous, 2006). This particular column is being used (by us) at this Centre for analysis of FAC of oil from several plams using GC for past seven years and exhibited very consistent and reproducible results. The same column has been reported to be used for separation of FAMEs from other laboratories (Tan and Che, 2000; Andrikopoulos, 2002; Naresh, 2007, Sodeif and Paresh, 2008). Though all capillary columns used for GC cannot be used for GC MS application, BPX-70 can be used effectively for the GC-MS application.

An essential requirement for GC-MS is high vacuum (at the ion source and analyzer). In the present study, a turbo molecular pump having the capacity of 150L/sec with ceramic bearings in combination with a 50L/min oil rotary pump was connected with the equipment to maintain higher vacuum. The threshold value of back pressure at which the high frequency power (RF power), high voltage detector and filament gets switched off is 40 Pa. In the present study a back pressure of 5-9 Pa was maintained during the operation. During operation, vacuum leak was checked using peak monitor. In this method the peaks of water (m/z 18), air (m/z 28) and PFTBA (m/z 69) were monitored. Peak height of air and PFTBA were greater than that of water ensuring there was no vacuum leak. Optimization of the instrument sensitivity over the mass range was done using PFTBA (Perfluorotributylamine) masses 69, 219, and 502. Relative Abundance of Mass 69 was 100%, Mass 219 was 40-50% and Mass 502 was 2-3%. These tuning parameters were also matched with published literatures (Anonymous, 2000, Anonymous, 2003a; Anonymous 2003b, Lingga, 2004).To maintain a minimum voltage fluctuation, a 5KVA single phase online UPS supplying 220-240V was connected to the instrument to restrict the voltage fluctuation $\pm$5%.

During the study, several experimental trials were conducted and the GC parameters were standardized as: 230^{0}C injection port temp., 215 Kpa column inlet pressure, 1.2 ml/min column flow, 29.7 cm/sec linear velocity, 55 split ratio, 76 ml/ min total flow and 180^{0}C oven temperature.

The mass spectrometer was operated in the electron impact (EI) mode at 70 eV and the interface temperature was set at 250^{0}C. Scanning method of acquisition was used and the mass scan ranged from 50 to 500 m/z with an constant Electron multiplier (Em/ Detector) voltage of approximately 1100V (The detector voltage would be constant during the operation, however it may vary during each tuning. It may be noted that when it exceeds 1500V, ion source and lens need to be cleaned). Spectrum data was collected at 0.5 sec. interval.

The resolution was adjusted so that the peak half-width value obtained with the standard (PFTBA) was near the value entered for spectrum peak half width [Full Width Half Maximum (FWHM)], which was set at 0.5-0.6 u (where "u" denotes one mass number unit). From the earlier study conducted by us with the GC (SHMADZU, Japan Model GC-17A) fitted with a FID detector, it was observed that the solvent (Hexane) get eluted (detected) before 4 minutes. Normally after the sample is injected, a large quantity of solvent is introduced into the analysis system. This results in sharp decrease in the vacuum inside the ion source, which has detrimental effect on the filament and other component. In order to prevent this, the filament is turned off while the solvent is passing through the analysis system (Anonymous 2000-2002). In this experiment with FAME, the set time to turn on the filament once the solvent finishes elution after injection of the samples was 4 min. To complete the analysis 25 min retention time was sufficient for eluting all the FAMEs derivatized from different oil samples.

With the above set parameters, derivatized methyl esters in n-hexane solvent from palm oil (mesocarp oil) was injected and the average FAC of 10 palm oil samples were recorded (Fig. 1). Myristic acid (0.55%), palmitic acid (53.02%), stearic acid (4.07%), oleic acid (38.77%), linoleic acid (3.59%) were the main fatty acids identified from the mass spectrum after comparing with the NIST library. The identified peaks were also confirmed with standard FAMEs. The results were similar to the previously reported palm oil composition (Mandal *et al.*, 2003-04). This method

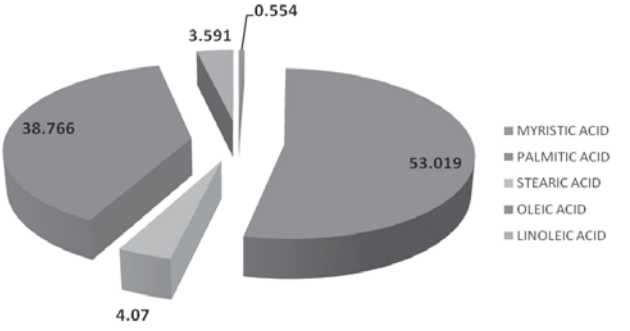

Fig. 1 : Fatty Acid Composition of CPO

can be used for routine analysis of fatty acids composition of palm oil. This method can also be adopted for analysis of other edible oil with some extent of modification.

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