Evaluating the supercritical extraction effects on the chemical compositions of the extracted oil using GC/TOF MS

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Abstract. Gas chromatography time of flight mass spectrometry (GCTOFMS) is becoming an attractive and alternative full-spectrum technique against others traditional instruments. In this study, the GCTOFMS was used to evaluate the effect of supercritical temperature and pressure on the chemical composition of the extraction yields. A total of 12 samples obtained from 3x4 general factorial design were analyzed for its chemical components using GCTOFMS. A list of compounds was identified and matched to the NIST library with a hit over 80% on the basis of the specific mass determination of their molecular ions and their major fragments. All the overlaps peaks were separated. All the extracted oils possess similar chemical components but varied in each compound concentration. Moreover, the aromatic ethers group was identified as the main group in the supercritical extracts with the presence of Myristicin as the highest peak, whereas, in the soxhlet extraction this group concentration was found lower. This result reveals the great effect of the extraction condition and the used method on the chemical compositions of the extraction yield and the concentration of each compound. This finding indicates and confirms the potential use of supercritical extraction in producing richer extract and fractions.

Key words: Gas Chromatography: Nutmeg: Supercritical: Time of flight.

1. Introduction
These Gas chromatography time of flight mass spectrometry (TOFMS) is becoming an attractive and alternative full-spectrum technique against others traditional instruments. The novelty and the potent of this technique led to the detection and quantification of low levels of several compounds [1]. ToF-MS has many characteristics including the high-speed full spectrum acquisition without mass spectral skewing which led to preserve high resolution and maximize sensitivity. This has made the TOF MS very attractive for the screening of target and non-target compounds and for their reliable accurate mass confirmation. Thus, it has been applied in many different field such as environmental research [2], biological samples [3], in the presence of pesticides in food [4] and in food analysis [5]. Fragrance and essential oils contains monoterpenoids, sesquiterpenoids, saturated and unsaturated hydrocarbons, alcohols, carbonyl derivatives, and esters. The common analysis tool for identifying these compounds is GC. These compound classes possess similar chromatographic behavior, and may have common isobaric molecular ions or MS in GC [6]. TOF-MS has significantly more reliable interpretation for compound identification. Its ability in generate broader peak that enables Character-impact odorants to be identified more than traditional GC [6]. TOF MS very attractive for the rapid screening of compounds and for their ability to produce an accurate mass confirmation [7]. Moreover, it is a rapid sample screening and definitive identifications in the presence of low amounts of matrix [5]. Fay [8] selected seafood extract as a representative example for complex food flavors. Several
unidentified flavor components molecules of the seafood extract using GC-quadrupole MS were successfully identified using GC-TOFMS.

Supercritical extraction techniques have been used widely in extraction and fractionation [9][10][11]. Thus, many researchers have used it in the extracting of nutmeg oil [12]. Nutmeg is a native South East Asian plant that has many significant properties [13]. Up to now, few papers have been published regarding GC analysis of nutmeg oil, with less information about GC-TOFMS has been conducted or published yet. Therefore, in this study, the GC-TOFMS will be applied for the determination of chemical components of the supercritical and soxhlet extract of nutmeg seed and to study the effect of the supercritical extraction temperature and pressure on the chemical composition of the extracted oil.

2. Material &Methods

2.1. Chemicals & Material
Commercial liquid carbon dioxide gas with purity of 99 g kg\(^{-1}\) was purchased locally from Malaysian Oxygen, Penang, Malaysia in a gas cylinder at temperature below -5°C. Nutmeg samples were purchased from a farm in Balik Pulau, Pinang, Malaysia. n-Hexane from AR grad obtained from R&M marketing UK., Ethyl acetate HPLC grade was purchased from Merck.

2.2. Supercritical carbon dioxide (SC-CO\(_2\)) extraction
The SC-CO\(_2\) extraction was conducted using a supercritical fluid extractor SFX™ 220 (ISCO, Inc., Lincoln, NE. USA; model SFX 220) with 2.5 ml extractor vessel capacity. General factorial design of two variable multi-levels was applied with thirty six runs to investigate the operating parameters that influence the supercritical extraction condition of nutmeg oil that give the highest yield. The first independent variable studied was extraction pressure (MPa) with 4 levels as follow (20.7, 27.6, 34.5 and 41.4 MPa). The second independent variable was temperature with three levels (40, 50 and 60 °C). Response is the oil yield at each design point was recorded. Extraction was carried out at all the design points and each extraction was run in three replicates, and the average was calculated. About 1.5 g of ground nutmeg seed was placed into the extractor vessel. The extraction was then performed under various experimental conditions in accordance with the full factorial design.

2.3. Soxhlet extraction
About 15 g of nutmeg grinded seeds were extracted using n-Hexane in a soxhlet apparatus for the duration of 8 h as described by AOCS Standard Method (30.036) [14]. The result of extraction yield was expressed as the percentage of crude extract.

2.4. GC-TOFMS
The GC-TOFMS instrument was used in this experiment, which consist of a gas chromatogram 7890 (Agilent Technology) and a 7890 series auto sampler injector with controller, coupled with a LECO Pegasus© III GC-TOFMS with electron impact ionization equipment. All data were processed and analyzed with LECO ChromaTOF® software. Gas chromatography was carried out using MS capillary column-HP-5 (30 m x 0.32 mm x film thickness 0.25 µm) with a constant flow rate of helium at 1 mL/min as a carrier gas. The GC oven temperature programmed for 70 °C, hold for 2min, then the temperature gradually increased to 280 °C at the rate of 20 °C/min and was hold for 20min. Sample injection volume was 3 µL in the splitless injections mode. Injection port temperature was set to 280 °C for the entire run with total run time was 1950 seconds. Electron impact ionization was performed at electro energy -70 eV was used. Ion source temperature was set to 250 °C. The detector voltage is 1550 volt. Data acquisition was at the mass range 30 to 700 amu, at a rate of 20spectra/sec. As for the processing data method the ratio of signal to noise (S/N) was set to 10.0 (to enable the scan of all compounds peaks). Minimum/maximum molecular weight was set to 30/750 respectively. All the detected peaks on the chromatogram were determined by comparing their mass ionization spectrum with NIST library.
3. Result and Discussion

3.1. Chemical constituent identification using GC-TOF/MS

In this experiment, non-target screening of nutmeg seed oil extracted by using SFE was carried out using LECO Pegasus© III GCTOF-MS. The LECO ChromaTOF® software automatically detected peaks according to a defined parameters, showing their mass spectra to be searched in the library, and produced a hit list with positive matches (library match >70 %). A large list of compounds was identified and was matched to the NIST library. It has been well established that the TOFMS capability in identifying non target compounds was due to its capacity in recording the full spectrum for all the detected compounds at the same time. The amount of each identified compound is represented as qualitatively (% area) in an automated search was applied to determine the chemical constituent of oil at each supercritical extraction condition. The data processing method gives the (TIC) total ion chromatograph profile of extracted oil and (AIC) analytical ion chromatograph. The AIC spectrum offers information similar to the (TIC) but with higher signal-to-noise ratio. The AIC is the sum of the extracted-ion chromatograms for the ions in the found peaks.

3.2. Chemical constituent of the supercritical extracted oil of nutmeg seed M. fragrans.

A total of 12 samples which represent all the extracted oils that were obtained from the supercritical extraction were tested and analyzed for it chemical components using GCTOFMS. The results showed that all the extracted oils were very much similar in the chemical components, but varied in each component concentration. The major chemicals compounds that were present in the extracted nutmeg were listed in Table 1.

| No | Compound Name         | Formula    |
|----|-----------------------|------------|
| 1  | Eugenol               | C7H8O2     |
| 2  | Isoeugenol            | C10H12O2   |
| 3  | Methoxyeugenol        | C11H14O3   |
| 4  | Methylleugenol        | C11H14O2   |
| 5  | Myristicin            | C11H12O3   |
| 6  | Asarone               | C12H16O3   |
| 7  | Safrole               | C10H10O2   |
| 8  | Decanoic acid, methyl ester | C11H22O2 |
| 9  | α-Phellandrene        | C10H16     |
| 10 | Terpinolen            | C10H16     |
| 11 | Sabinen               | C10H16     |
| 12 | 4-Carene,             | C10H16     |
| 13 | Anisole,              | C19H24O3   |
| 14 | a-Thujene             | C10H16     |
| 15 | α-Pinen              | C10H16     |
| 16 | κ-Terpinen            | C10H16     |
| 17 | Copaene               | C10H16     |

However, the result of the GCTOF analysis showed that more than 80 % of the detected compounds were identified with a high similarity of more than 70 % matching with NIST library and during acquisition time of 1200 sec. After the 1200 sec, only few compounds were detected, and these compounds showed low similarity with the NIST library except of few compounds with significant similarity such as methoxyeugenol. This is due to the high resolution capability of the GCTOFMS, and the advantages of using multichannel ion detector. According to Veriotti & Sacks [16] Time-of-flight (TOF) MS can be used to obtain reliable extracted ion chromatograms with high spectral
acquisition rates, as this will allow for the characterization of many narrower GC peaks. Interestingly, the area percentage of each compound varied between the extracted oils. Some components were present in all the supercritical extraction oil and increased with increasing temperature. Figure 1a, shows the analytical-ion chromatogram (AIC) for nutmeg oil extracted using supercritical extraction at extraction temperature of 40 °C with pressure of 20.7, 27.6, 34.5, and 41.4MPa. Myristicin was found as a true peak with exact mass of 192.1, it is also the largest peak in the chromatogram that appeared at 727.2 sec, with a hit similarity of 86.6 % and above with the NIST library. Figure 1b shows the matching of the Myristicin in the extracted sample with the NIST library. Moreover, the chromatogram has become very complex and overfilled with peaks with increasing the extraction pressure of 41.3MPa. The chromatogram also showed very obvious differences between the extractions at different pressure, as the peaks become sharper, higher and more compounds peaks appeared. However, the Myristicin peak has a significant interference from 1-Octanamine by increasing the pressure. Despite with this extensive overlap, components identification by the provided software of LECO ChromaTOF was successful, and few other peak overlaps were also successfully detected.

**Figure 1a.** shows the AIC of the supercritical extraction of nutmeg seed at extraction temperature of 40 ° C using pressure

**Figure 1b.** Myristicin peak compound in the supercritical extraction vs NIST library.
The AIC for the supercritical extraction at extraction temperature of 50 °C using the extraction pressures set of 20.7, 27.6, 34.5, and 41.4MPa is shown in figure 2. Myristicin which appears again as the largest peak in the chromatogram, while (1-Octanamine) overlaps significantly with it, yet, spectral overlap was detected successfully and both two compounds peaks were successfully identified. Myristicin was detected as a true peak (with exact mass of 192.1) at 727.2 sec, which hit the NIST library significant with similarity of 86 % and above. Note that the supercritical nutmeg oil samples at a fixed temperature of 50 °C exhibited similar AIC for the extracted samples at different pressures. Á-Phellandrene was identified as well as Sabinene which scored library matching with 90. However, the extraction pressure affect it presence as well. The peak area percentage of sabinen increased gradually with increasing the pressure to 41.3MPa. Á-pinene appears and overlaps with sabinen at the retention time of 495.4s and hit the library with 87.6 %. This result confirmed that at constant temperature increasing the pressure increases the extraction yield and improved the presence of some compounds. It can be seen from the chromatogram at figure 3 that more number of peaks appeared at the highest extraction pressure of 41.3MPa. This is due to the solvating power of the SC-CO₂ fluid, which improved the isolation and extraction of some compounds from the matrix. Also, terpinenol-4 hit the library matching with a similarity of 86.7 % at the retention time of 594.9s with an exact mass of 154.13. Whereas, (-)-limonene matched the NIST library significantly with 91%. Moreover, the result of the AIC showed that the peaks in the region between 400 and 600s were mostly terpen compounds that belong to the monoterpens family. While, á-phellandrene, 1-terpinen-cis appeared at with similarity above 80 %. It was found from the AIC, that the presence of the aromatic ether group followed the monoterpen group, which starts to appear at the region from 600s to 800s with higher similarity above 80 % such as isoeugenol and asaron. Interestingly Methyleugenol hit significant similarity of 92.5 % which formed a peak area about 7.6 % as well as methoxyeugenol aromatic ether and a phenylpropane compound.

Figure 2. Shows the AIC of the supercritical extraction of nutmeg seed at extraction temperature of 50°C using different pressures.

Figure 3 shows the AIC for the supercritical extraction oil of nutmeg at extraction temperature of 60 °C using the extraction pressures set of 20.7, 27.6, 34.5, and 41.4MPa, also giving the highest peak area for the myristicin. It can be seen that the chromatogram has more peak than the extracted sample at temperature of 50 °C, which confirmed our previous result for the extraction yield. In addition, it is perceived from AIC chromatogram, that the extraction oil at the highest pressure of 41.3MPa has more peaks with higher peak area from other extraction oil at lower pressure which indicates that increasing the pressure increased the extraction yield. This fact theoretically and practically acceptable, as increasing the pressure of a supercritical gas, will easily separate the extracted organic compounds from the gaseous phase [16].
However, few components started to appear at the highest extraction pressure of 41.3MPa, which is nearly absent in the extracted oil at all lower pressure such as 3-carene, α-linalool and 4-terpineol, which were overlapped and successfully separated and identified by the supplied software. Figure 4 shows the AIC of the supercritical extraction oils of nutmeg at the lowest extraction pressure of 20.7MPa and the highest 41.3MPa respectively using the extraction temperature of 40, 50 and 60 °C. It can be seen from the AIC that at a fixed operating pressure, many chemical components were extracted by increasing the extraction temperature from 40 to 60 °C. At higher pressure of 41.3MPa, the chromatogram was overfilled with many peaks. It was perceived that at a fixed operating pressure increasing temperature has positive effect on the presence of some chemical components while it reduces the presence of others. The result also shows that using SFE extraction at temperature of 50 °C and pressure of 20MPa, high concentration of myristicin can be fractioned and obtained from nutmeg seed. Spricigo et al [18] extracted and identified the chemical composition of the nutmeg essential oil obtained by and found that the myristicin compound is behind the characteristic aroma of nutmeg. However, many other researchers also identified the chemical composition of the nutmeg oil with the presence of myristicin higher in SF than traditional method [19].
Sabinene, Alfa Phellendren, safarol, copaene was also existing at all different temperatures and increased by increasing the temperature as shown in figure 5. Sabinene increased with temperature increment at a fixed extraction pressure with a similarity of 90.9 % matching with the NIST library as shown in figure 6a. This is due to the increase in solubility or solvent density which increases the solute presence in the extracted oil. This result was in agreement with previous study from [20]. Figure 6b, shows the molecular structure of Sabinen.

Moreover, few other compounds concentration increased by increasing the extraction temperature from 40 to 60 such as elemicin, eugenol and farnesene.
3.3. Chemical Constituent of the extracted oil using soxhlet and compared to the supercritical extraction.

Figure 7 shows the AIC of the soxhlet extraction compared to the supercritical extraction oils of nutmeg. The result of the chromatogram of the comparison between the soxhlet extraction and the supercritical extraction at the highest pressure of 41.3MPa at temperature of 40, 50 and 60 °C shows quite full spectrum. It was found that at the region from 400s -600s the soxhlet extraction has many peaks with higher peak area, these peaks is representing the terpenes, monoterpen and Alcohol group particularly, Sabinen gives the highest peak area as well as Terpinenol-4, and á-phellandren. Whereas, at the region of 600 and above the supercritical peaks were more and with higher peak area than the soxhlet. Nevertheless, the supercritical extraction was richer in the aromatic ether group than the soxhlet extraction. Particularly, myristicin at temperature of 50°C gives the highest peak in the chromatogram. This result explained the difference in the extraction yield as the supercritical extraction gives higher yield than the soxhlet. Moreover, supercritical extraction was richer in the aromatic ether group which is behind its strong smell and fragrances.

![Figure 7. Shows the AIC of the supercritical extraction of nutmeg seed at extraction pressure of 41.3MPa using set range of different temperature compared with soxhlet.](image)

4. Conclusion

It can be concluded that GCTOFMS effectively identified and detected the chemical constituent of the nutmeg seed extract using simple method that uses little amount of sample. Moreover, using the GCTOFMS the effect of supercritical extraction temperature and pressure was confirmed represented by the various presences of chemical compounds. In addition, TOF-MS detected the overlaps spectral compounds significantly that matches well with the reference spectrum in the NIST library and identify each compound. In addition, our result confirms that the SFE can be used to extracted the desired compound or for fractionation purposes by optimizing its extraction parameters.

5. References

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