Project No. : F0271/20001A

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Isolation of Palm Vitamin E Using Supercritical Fluid Chromatography

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ABSTRACT Crude palm oil contains 600 – 1000 ppm of tocols in the form of tocopherols and tocotrienols. These palm tocols were successfully isolated using the Supercritical Fluid Chromatography (SFC) and characterized using various spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR as well as Mass Spectrometry (MS). The palm vitamin E showed unique spectroscopic characteristics of their isomers. The tocotrienols gave rise to peaks that are absent in the ^{1}H NMR and MS of tocopherols thus distinguished them from the tocopherols. The individual isomers of both the tocopherols and tocotrienols were characterized based on the chemical shifts of both the protons and carbons in the ^{1}H and ^{13}C NMR. Their identities were further confirmed using Mass Spectrometry (MS). Based on their spectroscopic characteristics, the vitamin E in palm oil were identified to be ?-tocopherol (?-T), ?-tocotrienol (?-T3), ?-tocopherol (?-T), ?-tocotrienol (?-T3) and ?-tocotrienol (?-T3).

INTRODUCTION

Palm oil consists of mainly glycerides with about 1 % minor components such as carotenes, tocols or better known as vitamin E, sterols as well as hydrocarbons such as squalene (1).

The palm vitamin E consists of ?-tocopherol (?-T), ?-tocotrienol (?-T_3), ?-tocopherol (?-T), ?-tocotrienol (?-T_3) and ?-tocotrienol (?-T_3). The palm tocotrienols and ?-tocopherol have been successfully analysed in the past using chromatographic method such as HPLC (2,3). Detectors such as UV-Variable wavelength as well as fluorescence detector were used for the detection of palm vitamin E analysed by HPLC. Recent studies have revealed the coupling of HPLC-MS and HPLC-NMR for the separation and identification of tocopherols and tocotrienols (4,5).

Analyses carried out using HPLC uses large amount of organic solvents in general. A more advanced method, supercritical fluid chromatography (SFC) has been developed for the isolation of palm vitamin E. SFC is a powerful tool as it combines the advantages of

both HPLC and GC in terms of efficiency, sensitivity and capability to analyse thermally labile compounds.

The supercritical fluid used in this study is the supercritical carbon dioxide (SC-CO_2) (6,7). Being non-toxic, SC-CO_2 is the eluant of choice for the chromatography of food products such as the palm vitamin E (8,9).

The presence of these palm vitamin E isomers were identified using various spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR as well as mass spectra (MS).

MATERIALS AND METHOD

Materials. Crude palm oil (CPO) was obtained from Keck Seng (Malaysia) Berhad. Solvents used for SFC were of chromatographic grade purchased from Merck and degassed with nitrogen.

Methods. CPO was saponified with absolute ethanol, KOH and pyrogallol. The unsaponifiable matter was extracted using hexane and washed with distilled water and ethanol These are then rotavap to distill off the solvents and later pumped to dryness. The unsaponifiable matter was dissolved in dichloromethane and injected into SFC.

RESULTS AND DISCUSSIONS

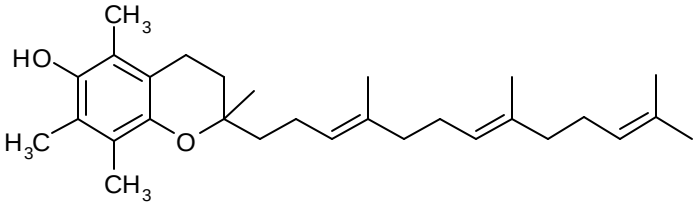
There are about 600 – 1000 ppm of vitamin E in the form of tocopherols and tocotrienols in crude palm oil. The NMR shows the protons and carbons coupling of the molecule and their chemical shifts reveal the exact locations of the side chain and methyl groups that differentiate between the ?, ? and ? isomers of vitamin E. The MS later confirm the identities of the isomers by way of the fragmentations of the molecule that gave rise to the peaks in the spectra.

Vitamin E

^{1}H NMR

The tri-methylated ?- isomers can be distinguished by the absence of C-5 proton signal at ? 6.2 – 6.6 ppm. Further identification of these isomers can be made by the detection of C-7 aromatic proton at ? 6.8 ppm present only in the ?- isomer.

The absence of olefinic proton signals denote a saturated molecule which is the case for tocopherols. The tocotrienols, on the other hand, is three times unsaturated at its side chain. Thus, the tocopherols and tocotrienols can be distinguished from each other based on the ^{1}H NMR.



Molecular Structure of ? -Tocotrienol

^{13}C NMR

The ? -isomers can be distinguished from the others with the C - 4a carbon resonates at higher field (? 122ppm). When C - 5 is protonated as in the case for ?- and ? - isomers, the chemical shift of C-4a carbon is increased to ? 124ppm. The protonation of C-7 has the effect of lowering the signal of C-8b methyl group downfield to ? 16.0 ppm showing the difference from the ? - and ?- isomers in which their signals for C-8b is at around ? 11ppm.

The difference between the tocopherols and tocotrienols lies in the unsaturation of the tocotrienols side chain. These two types of palm vitamin E can be easily distinguished by looking at the chemical shifts of C-4', C-8' and C-12'.

Mass Spectra

The MS is a very useful tool for the identification of certain compounds based on their principal and fragments m/z. The principal m/z of the respective vitamin E are 430 (? - T), 424 (? - T_3), 416 (? - T), 410 (? - T_3) and 396 (? - T_3). The vitamin E tend to loss its side chain at C3 - C4 bond and rearrange itself to form the stable tropylium ions.

Besides the principal fragmentation, cleavages through the non-aromatic rings of the vitamin E without hydrogen transfer is also possible to give weaker ion peaks. The isoprenoid side chain of the tocotrienols tend to fragmentate into smaller ions (m/z 69). The intense peak at m/z 69 is a characteristic feature to distinguished the tocotrienols from the tocopherols.

CONCLUSION

SFC is a good tool to identify palm vitamin E. The individual palm vitamin E, the ? - T, ? - T_3, ? - T, ? - T_3 and ? - T_3 can be distinguished through spectroscopic technique such as ^{1}H and ^{13}C NMR as well as through their mass spectra.

Acknowledgements The authors wish to thank Datuk Director General of MPOB, Datuk Dr. Yusof Basiron for his permisson to present this paper. Thanks are also due to MPOB and MU for the research assistantship and research grant (Vot F) respectively for Ng M.H. to conduct her Ph.D study in University of Malaya.

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