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Separation of Coenzyme Q_{10} in Palm Oil by Supercritical Fluid Chromatography

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Abstract: Palm oil is known to host a variety of phytonutrients; some having antioxidant property such as the carotenes and vitamin E. These antioxidants are also present in the oil recovered from the palm-pressed fibre. Study was carried out to investigate the presence of coenzyme Q_{10}, yet another non-glyceride compound which possesses antioxidant property in crude palm oil (CPO) and palm fibre oil. Separation of coenzyme Q_{10} in CPO and palm fibre oil was carried out using supercritical fluid chromatography with ultra violet detection. ^{1}H and ^{13}C NMR were used for its characterisation. Calibration with authentic standard shows that there are 10-80 ppm coenzyme Q_{10} in CPO while its concentration in palm fibre oil is 1000-1500 ppm.

Key words: Coenzyme Q_{10}, crude palm oil, NMR, palm pressed fibre, supercritical fluid chromatography

INTRODUCTION

The presence of carotenes and vitamin E in palm oil has been well documented since 1980s[1-5]. These minor components of palm oil had gained attention worldwide due to their beneficial health properties. Studies have shown that the carotenes and vitamin E exhibit antioxidant property whereby they are able to scavenge free radicals that lead to diseases as well as the ageing process[6-10]. The presence of yet another powerful antioxidant in palm oil has recently been investigated. This compound, known as coenzyme Q_{10} or ubiquinone is ten times more powerful as antioxidant than the vitamin E[11].

Coenzyme Q_{10} (Fig. 1) has been detected in commercial red palm olein in concentration ranging from 18-25 ppm[5]. Hamid and co-workers[12] reported the presence of 10-80 ppm coenzyme Q_{10} in crude palm oil with analyses carried out by preparative thin layer chromatography (pTLC) and high performance liquid chromatography (HPLC) simultaneously.

The importance of coenzyme Q_{10} is established with the fact that it shows promising results when administered to patients with cardiac or heart diseases[13-15]. In addition, it has also been shown to be effective in the prevention of lipid peroxidation and oxidative damage in haemoglobin[13, 16- 18].

In view of its beneficial properties, the presence of coenzyme Q_{10} in palm oil need to be further studied.
The presence of coenzyme Q_{10} in palm pressed fibre was also investigated in this study. Palm pressed fibre is the fibrous material left behind after the oil palm fruits have been pressed for its oil yielding CPO. Previous study has shown that the palm pressed fibre contain vast amount of phytonutrients such as carotenes and vitamin E[3]. Thus, it is worth looking into the content of coenzyme Q_{10} in the palm pressed fibre.

As the carotenes and vitamin E are present in a much higher amount in palm oil (500 -700ppm and 600-1000ppm respectively) than the coenzyme Q_{10}, their presence tend to mask the occurrence of coenzyme Q_{10}[1,5,19]. Thus, palm oil samples for coenzyme Q_{10} analyses need to undergo pre-treatment before they can be analysed using SFC.

MATERIALS AND METHODS

Crude palm oil and palm pressed fibre were obtained from MPOB Experimental Mill in Labu, Negri Sembilan, Malaysia. All solvents used were either of analytical or chromatography grades purchased from Merck (Darmstadt, Germany), J.T. Baker and HmBG. 99.995% carbon dioxide used for supercritical fluid chromatography (SFC) was obtained from Malaysian Oxygen, Malaysia. Coenzyme Q_{10} standard was purchased from Sigma Aldrich.

Extraction of palm fibre oil: Palm pressed fibre obtained fresh from the mill was soaked overnight in 95% ethanol. Thereafter, the solvent was filtered. Palm fibre oil was obtained after distillation of excess solvent.

Saponification: Approximately 5g CPO was refluxed in the dark for 1 hour under steam bath and nitrogen atmosphere with 30 mL absolute ethanol, 5 mL

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potassium hydroxide (50%w/v) and 1g pyrogallol. Thereafter, the refluxed mixture was cooled to room temperature.

The unsaponifiable compounds were extracted using hexane until the upper layer of the mixture turned colorless. Thereafter, the sample was washed with distilled water until the drained washing water is neutral when tested with phenolftalein. Excess solvents were then distilled off and the unsaponifiable matter obtained was pumped to dryness.

The same saponification procedure was repeated with 3 g of palm fibre oil.

Extraction of coenzyme Q: The unsaponifiable matter from CPO and palm fibre oil was subjected to open column chromatography to extract coenzyme Q_{10}. An open column with 2cm internal diameter was wet-packed with hexane to a height of 7cm. The unsaponifiable sample of CPO or palm fibre oil was dissolved in hexane and loaded to the top of the silica. Thereafter, hexane was used to elute the compounds present until the eluting solvent was pale yellow in color. After that, ethanol was used to elute the remaining compounds until the eluting ethanol was colorless.

Both the hexane and ethanol fractions were rotary evaporated to dryness and weighed prior to injections into the SFC.

An SFC JASCO Model SUPER-200 SFC system with a UV-970 variable wavelength UV/VIS detector equipped with high pressure flow cells was used. Column used was Metaphase RP C18 4.6mm I.D. x 250mm length. Temperature and pressure were set at 50^0C and 180 kg/cm^2. Mobile phase was CO_2 and methanol with the flowrate of 3.0/0.2 ml/min (CO_2/MeOH).

Both the hexane and ethanol fractions from open column chromatography were dissolved in dichloromethane prior to injections. Separation of coenzyme Q_{10} was compared using authentic standard.

Spectroscopic characterisation: Coenzyme Q_{10} isolated from SFC was dried under vacuum for 24 hours. Thereafter, it was dissolved in d-choloroform ($CDCl_3$) for ^{1}H and ^{13}C NMR analyses.

RESULTS AND DISCUSSION

Separation of coenzyme Q_{10} in the ethanol fractions of CPO is depicted in Fig. 2. Chromatograms were shown at 275nm, which is the λ_{max} of coenzyme Q_{10}.

No coenzyme Q_{10} is detected in the hexane fractions of both samples. This is much anticipated as the coenzyme Q_{10} is more polar in nature and is not able to be carried by non-polar mobile phase such as hexane. On the other hand, carotenes, being non-polar are found in the hexane fraction.

Fractionation of unsaponifiable matter by open column has successfully separated the coenzyme Q_{10} from the carotenes and the remaining obstacle is the separation of coenzyme Q_{10} from vitamin E in the ethanol fractions which can easily overcome by using a reversed-phase C18 column for SFC whereby all the palm oil vitamin E isomers eluted much earlier than the coenzyme Q_{10} when a reversed stationary phase was used.

Detection of coenzyme Q_{10} was carried out by comparing the peak and retention time with an authentic standard. In addition, coenzyme Q_{10} is easily recognisable through UV spectra where its λ_{max} is at 275nm while the λ_{max} for vitamin E isomers are 290 - 300nm. Further characterisation using ^{1}H and ^{13}C NMR confirmed the presence of coenzyme Q_{10} in palm oil. Fig. 3 and 4 show the ^{1}H and ^{13}C NMR spectrum of coenzyme Q_{10} while their chemical shifts are depicted in Tables 1 and 2.

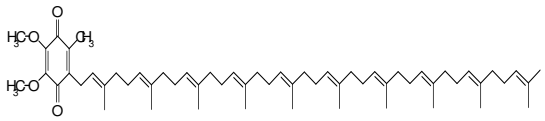

Fig.1: Molecular structure of coenzyme Q_{10}

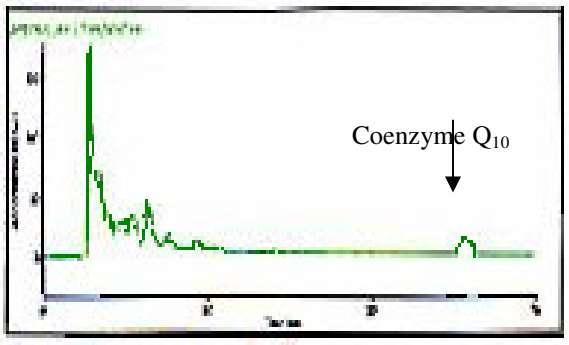

Fig. 2: Separation of coenzyme Q_{10} by SFC

Most of the protons and carbons in coenzyme Q_{10} resonance at the same chemical shifts as its structure is fairly simple; a basic quinone structure attached to 10 repetitive terpenic side chain (Fig. 1). In ^{1}H NMR, the allylic protons of the side chain resonance at 5.2 ppm while its α- protons resonance at higher field of 2.05ppm.

Table 1: ^{1}H NMR chemical shifts of coenzyme Q_{10}

Protons	Chemical Shifts (δ)/ppm*
39', 40' -CH_3	1.6-1.7
1', 4', 5', 8', 9', 12', 13', 16', 17', 20', 21', 24', 28', 29', 32', 33', 36', 37'-CH_2	2.05
4, 5 -OCH_3	3.2
2', 6', 10', 14', 18', 22', 26', 30', 34', 38' -CH_2	5.2

in $CDCl_3$

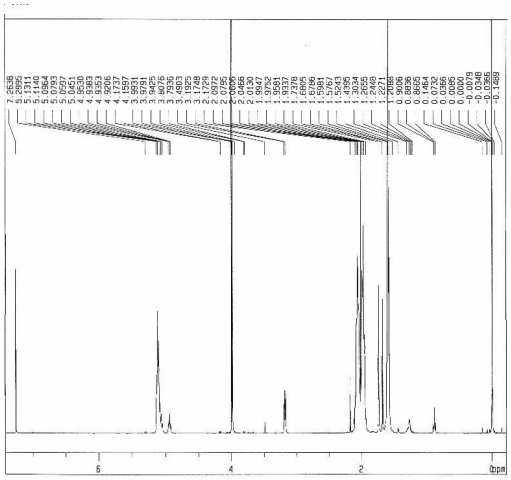

Fig. 3: ^{1}H NMR of coenzyme Q_{10}

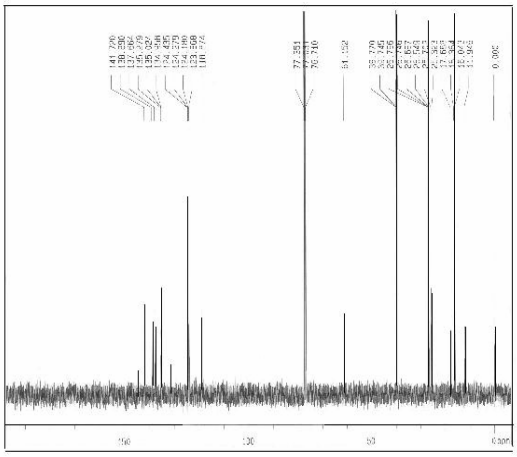

Fig. 4: ^{13}C NMR of coenzyme Q_{10}

Table 2: ^{13}C NMR Spectral Data of Coenzyme Q_{10}

Carbons	Chemical Shift (δ)/ppm*
3, 6	142
3', 7', 11', 15', 19', 23', 27', 31', 35'	135
2', 6', 10', 14', 18', 22', 26', 30', 34', 38'	125
4', 8', 12', 16', 20', 24', 28', 32', 36'	40
1', 5', 9', 13', 17', 21', 25', 29', 33', 37'	27
3'a, 7'a, 11'a, 15'a, 19'a, 23'a, 27'a, 31'a, 35'a, 39'a, 40'	17

* in $CDCl_3$

Calibration with authentic standard showed that coenzyme Q_{10} is present in 10-80 ppm in CPO and 1000-1500 ppm in palm fibre oil. Separation of coenzyme Q_{10} using SFC has shown good linearity and repeatability.

CONCLUSION

Coenzyme Q_{10} is present in CPO and PFO in concentration of 10-80 ppm and 1000-1500 ppm respectively. While this is not a substantial amount in both type of palm oil, the method developed using supercritical fluid chromatography is able to isolate and detect this compound with good linearity and repeatability. Characterisation using ^{1}H and ^{13}C NMR confirmed the identity of coenzyme Q_{10} separated by the SFC.

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