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ORIGINAL PAPER

Determination of Coenzyme Q_9 and Q_{10} in Developing Palm Fruits

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Abstract A series of biochemical changes took place as oil palm fruits developed into maturity. Among these are the active synthesis of glycerides as well as unsaponifiable compounds such as carotenes, vitamin E, sterols and squalene. Coenzyme Q_9 and Q_{10} were found to be present at different stages of the oil palm fruits development. The presence of coenzyme Q_9 was detected as early as 4 weeks after anthesis (WAA) and its concentration diminished as the oil palm fruits ripen. Coenzyme Q_{10} on the other hand, can only be detected from 12WAA onwards and its concentration remained at an elevated level throughout the remaining development period of the oil palm fruits. Their occurrence pattern suggested that there is a strong relationship between the concentration of coenzyme Q_9 and coenzyme Q_{10} with the age of the oil palm fruits.

Keywords Coenzyme Q · Palm fruits · Glycerides

Introduction

As oilseeds develop into maturity, their chemical constituents change with different stages of development. Biosynthetic changes of oil constituents such as triglycerides, diglycerides and monoglycerides in plants or oilseeds are well documented [1–5]. In most plants, the formation and accumulation of oil normally begin after the husk, shell and endosperm are already formed [6, 7]. The fruiting behavior of oil palm fruits also shows a similar pattern [8–10].

It has been reported that the oil palm fruits start to develop at approximately 2 weeks after anthesis (WAA) and it takes about 20 weeks to ripen thereafter [8, 10]. The fruits' maturation process proceed with noticeable biochemical and morphological changes [8–12]. Among the biochemical changes in the developing palm fruits include the composition of unsaponifiable matter such as carotenes, chlorophyll, tocopherols and tocotrienols [8–12]. These changes are in agreement with the changes observed in other oilseeds such as sunflower seeds and olive oil [1, 13, 14].

This study reports on the occurrence of coenzyme Q_9 and Q_{10} at different stages of the developing palm fruits. The coenzyme Q_9 and Q_{10} were reported to be present at about 5 and 10–80 ppm respectively,in crude palm oil [15–19]. Molecular structures of coenzyme Q_9 and Q_{10} are depicted in Fig. 1. The presence of these coenzymes Q are of particular interest as coenzyme Q_{10} has been shown to exhibit beneficial health properties such as being antioxidant, giving relief from angina, lowering the risk of heart attack as well as boosting the immune system [20–24].

Materials and Methods

Oil Palm Fruits

Palm fruits aged 4, 8, 12, 14, 16, 18, 20 and 22 weeks after anthesis (WAA) were obtained from the Malaysian Palm

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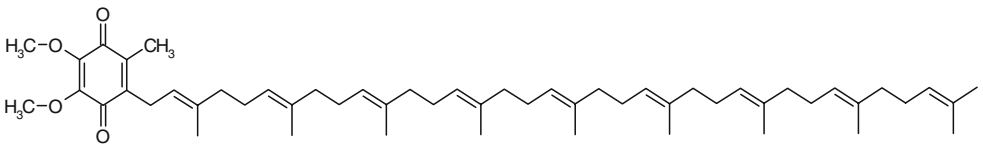
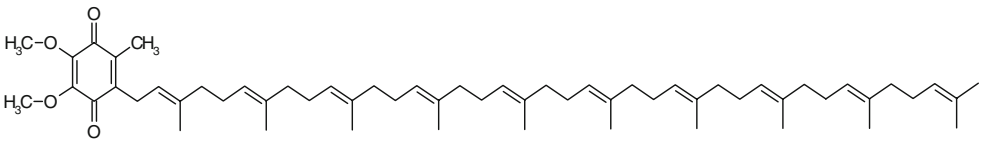
Fig. 1 Coenzyme Q_9 and Q_{10}



Coenzyme Q_9



Coenzyme Q_{10}

Oil Board research station in Bangi, Selangor, Malaysia. The fruits were tagged and ages varied ±1 week.

All solvents used were of chromatography grade purchased from Merck (Darmstadt, Germany), J.T. Baker, GmbH or Mallinkroft. Carbon dioxide of 99.995% purity was purchased from Malaysian Oxygen (MOX), Malaysia. Nitrogen used for blow drying or nitrogen blanketing was purchased from the same source.

Sample Pretreatment of Palm Fruits

Palm fruits aged 4WAA were surface washed with water and dried at room temperature. The palm fruits were separated from the bunch and peeled to separate the kernel from the mesocarp. Thereafter, the peeled palm fruits were dried in an oven at 60 °C until there was no change in mass.

The dried peeled fruits were blended with 1 L methanol:hexane (2:1) and left to soak overnight. The solvent extracts were then filtered and set aside. The peeled fruits were soaked for another night in 1 L of the similar solvent mixture. Both the filtered solvents were subjected to rotary evaporation to remove the solvents leaving behind the fruit extract. The fruit extract was then further dried under a vacuum.

These were then repeated for the palm fruits of 8, 12, 14, 16, 18, 20 and 22WAA.

Analyses of Coenzyme Q

We took 4 g of the extract obtained by the method above and this was dissolved in hexane and subjected to flash chromatography. The column used was silica 4.0 × 7.5 cm length. The mobile phase was 100% hexane at 0.5 bar. Fractions were collected until the eluant turned pale yellow in color. Thereafter the mobile phase was changed to 100% ethanol. Fractions were collected until the eluant turned colorless.

The second (ethanol) fraction was subjected to rotary evaporation to remove excess solvent. The residue was then transferred to a 1-mL volumetric flask. The flask was topped to the line with hexane. Thereafter the sample was injected into a JASCO SFC system coupled with a UV variable wavelength detector. The column used was Metaphase RP C18, 4.6 mm I.D. × 250 mm length. The mobile phase used was supercritical fluid carbon dioxide ($SC\text{-}CO_2$) and ethanol in the ratio of 3.0:0.1 mL/min. SFC conditions were set to be 50 °C and 180 bar.

The coenzyme Q_9 and Q_{10} was calibrated using standard prepared in a similar manner.

Analyses of Glycerides and Free Fatty Acids

A weighed amount (0.2 g) of the extract obtained by the method described above was transferred to a 2-mL volumetric flask. A volume of 0.2 mL triacontane and 0.3 mL BSTFA were then added into the volumetric flask. The flask was then topped up to the line with dichloromethane.

The mixture was then heated at 60 °C for 2 h to allow the silylation to take place. Thereafter the mixture was subjected to GC analysis.

A Hewlett Packard 590 series II plus gas chromatograph was used for the analysis. The column used was an SGE 1.5 m × 0.32 mm I.D. BPX5 0.25 μm capillary column. The initial oven temperature was set at 100 °C for 1 min and increased to 400 °C at a rate of 10 °C/min. The injector and detector temperatures were set at 370 °C. Oven equilibrium time was 3 min under a pressure of 6.60 psi. The carrier gas, helium was set at flow velocity range from 1.99–2.0 mL/min/cm/s. The split ratio between compressed air and hydrogen gas was 0.0–1.

Regression and Statistical Analyses

Analyses of components in developing palm fruits were carried out on five batches of fruits collected. Concentration

Table 1 Concentration of coenzymes Q_9 and Q_{10}, glycerides and free fatty acids in developing palm fruits

Weeks after anthesis (WAA)	Concentration (ppm)					
	Coenzyme Q_9	Coenzyme Q_{10}	Monoglycerides	Diglycerides	Triglycerides	Free fatty acids
4	23 ± 2	ND	1,520 ± 20	3,300 ± 30	30,00 ± 25	5,500 ± 150
8	46 ± 5	ND	1,860 ± 25	2,340 ± 35	500 ± 40	2,270 ± 100
12	57 ± 3	ND	1,750 ± 20	3,510 ± 25	1,020 ± 60	2,230 ± 100
14	82 ± 6	ND	1,400 ± 15	6,570 ± 15	8,790 ± 80	2,000 ± 80
16	30 ± 2	36 ± 4	1,250 ± 15	20,500 ± 80	24,100 ± 100	1,450 ± 70
18	11 ± 2	53 ± 4	420 ± 10	21,560 ± 90	35,4690 ± 200	2,00 ± 40
20	ND	76 ± 5	100 ± 15	22,000 ± 90	9,60,200 ± 400	20,000 ± 150
22	ND	80 ± 6	80 ± 5	23,500 ± 110	8,63,420 ± 350	84,230 ± 250

of each component of the oil palm fruits are presented as the average and standard deviations of the five batches of fruits studied.

The regression model for coenzyme Q_9 was performed as a function of the age (WAA) and other components present in the developing oil palm fruits. Regression analyses for coenzyme Q_{10}, mono-, di- and triglycerides as well as free fatty acids were performed in a similar manner.

Results and Discussion

The extract obtained from different stages of oil palm fruits development showed obvious color changes. As the oil palm fruits developed to maturity, physical changes that were observed include the color changes from green to orange due to the diminishing chlorophyll and the elevated carotenes. Up to 16WAA, the oil palm fruits were greenish yellow in color. By 18WAA onwards, the extracts were orange in color. The oil palm fruits started to feel oily at 12WAA onwards and by 18WAA, the fruits extract was a semi-liquid. Table 1 shows the concentration of the glycerides (mono-, di- and tri-) as well as free fatty acids throughout the development of the oil palm fruits. The negative coefficients of the glycerides and free fatty acids (Table 2) showed that there is a weak relationship between these components and the age of the oil palm fruits.

Chromatograms of Coenzymes Q_9 and Q_{10} analyzed by method developed using SFC are depicted in Fig. 2. Table 2 shows the concentration of coenzyme Q_9 and Q_{10} in the oil palm fruit extracts. Coenzyme Q_9 was detected in the developing palm fruits as early as 4WAA (Table 1). Its concentration increased until 14WAA and declined thereafter. At 20WAA onwards, the occurrence of coenzyme Q_9 can no longer be detected. Coenzyme Q_{10} on the other hand, can only be detected in the developing palm fruits from 16WAA onwards. Its concentration displayed a steady increase until 20WAA.

Besides conversion of Coenzyme Q_9 to Q_{10}, the active oil deposition in the oil palm fruits starting from 12WAA also contributed to the sudden increase in concentration of coenzyme Q_{10}. Deposition of oil in palm fruits started at 12WAA and is almost complete by 18WAA. By 18WAA, the fruits extract exist as an orange oil. This is in agreement with prior studies carried out by Arrifin (1990) and Choo et al. (2004) [8, 10].

18WAA seems to be an important stage in the maturation of oil palm fruits. Most of the unsaponifiable compounds in palm oil have been formed and their concentrations are at a maximum at this period of time [10].

Table 2 Regression model for presence of palm oil components at different stages of developing palm fruits

No.	Regression model	R^2
1.	$Co\ Q_9 = -22.18772 + 4.28919WAA - 3.17655Co\ Q_{10} + 0.01033MG + 0.00406DG + 0.00009TG + 0.00098FFA$	0.9904
2.	$Co\ Q_{10} = -7.85737 + 1.07874WAA - 0.27011CoQ_9 + 0.00351MG + 0.00146DG + 0.00003TG + 0.00035FFA$	0.9995
3.	$MG = 1913.1749 - 81.8407WAA + 22.2954CoQ_9 + 89.1528Co\ Q_{10} - 0.1618DG - 0.0036TG - 0.0029FFA$	0.9675
4.	$DG = 5811.7374 - 513.9744WAA + 144.5161Co\ Q_9 + 609.8170Co\ Q_{10} - 2.6685MG - 0.0202TG - 0.0240FFA$	0.9969
5.	$TG = 293783.6058 - 27722.1884WAA + 7390.6159CoQ_9 + 29828.7277Co\ Q_{10} - 137.1373MG - 46.9700DG - 1.0167FFA$	0.9960
6.	$FFA = 83356.3 - 9221.5WAA + 2646.8Co\ Q_9 + 11235.5Co\ Q_{10} - 36.0MG + 18.1DG - 0.3TG$	0.7364

Co Q_9 coenzyme Q_9, *Co Q_{10}* coenzyme Q_{10}, *MG* monoglycerides, *DG* diglycerides, *TG* triglycerides, *FFA* free fatty acids, *WAA* weeks after anthesis

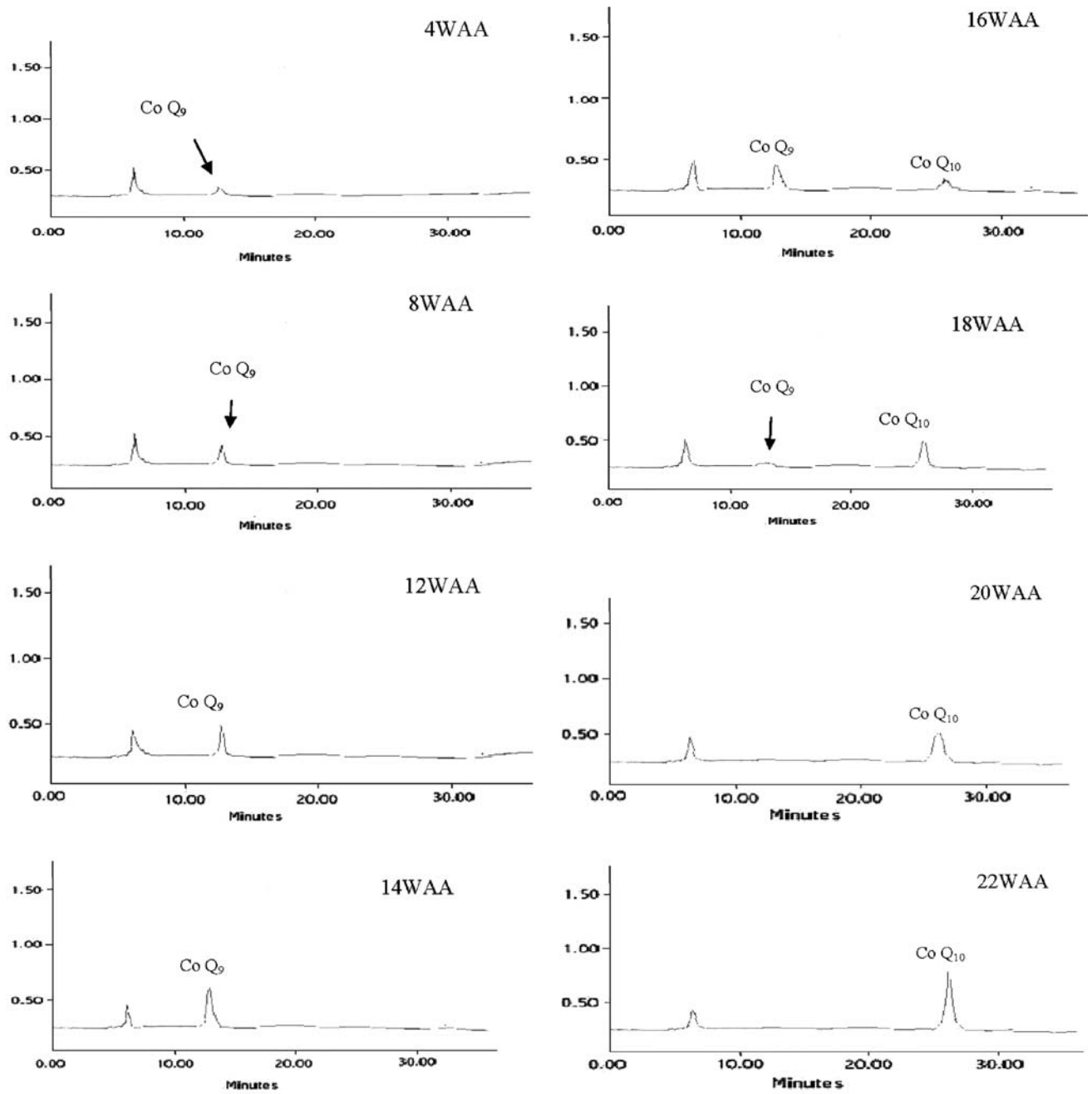


Fig. 2 Chromatogram of coenzyme Q_9 and Q_{10} in developing palm fruits

The changes in the lipid constituents in the oil palm fruits also helped in the active biosynthesis of lipid soluble unsaponifiable matter such as the coenzyme Q. By the time the oil palm fruits are ripe enough to be plucked for commercial oil extraction at 20WAA, the composition of Coenzyme Q_{10} was at about 76 ppm, which is in agreement with the concentration reported in CPO [10, 25].

The regression equations showed there is a strong direct relationship between the concentration of coenzyme Q_9, coenzyme Q_{10} and the age of the oil palm fruits as shown in Table 2.

Equation 1 (Table 2) denotes a strong inverse relationship between the concentration of coenzyme Q_9 and the concentration of coenzyme Q_{10}. This supports the findings where the presence of coenzyme Q_{10} was detected only when the coenzyme Q_9 diminished. Both Eqs. 1 and 2 showed that the presence of coenzyme Q_9 and Q_{10} is linked with the age of the oil palm fruits. The negative correlation

for coenzyme Q_9 and coenzyme Q_{10} however, showed that the presence of each of these coenzymes is the reverse of the other. From the regression model, it can be seen that the age of palm fruits has a more profound effect on the presence of coenzyme Q_9 than coenzyme Q_{10}. On the other hand, the presence of other components in palm fruits, i.e. monoglycerides, diglycerides, triglycerides and free fatty acids do not have a more significant effect on the concentration of the coenzyme Qs than the age of the palm fruits.

Although the concentration of Coenzyme Q_{10} is still increasing when the oil palm fruits are left to ripen till 22WAA, nevertheless, it was found that 20WAA is deemed to be the prime for commercial oil extraction when other factors such as oil content, fatty acids content and oil extraction rate are taken into consideration. The concentration of triglycerides is quite independent of other factors with the exception of the age of the oil palm fruits as denoted in Eq. 5 in Table 2. The same pattern is observed in the concentration of free fatty acids where it is quite independent of other factors. At 22WAA, the concentration of free fatty acids had increased by more than 300% compared to its concentration at 20WAA. The concentration of the oil component, triglycerides, on the other hand had decreased by 10% during the period of 20 to 22WAA. The increase in the concentration of free fatty acids lowered the quality of the oil which would make it difficult to meet the standards specification for processed palm oil.

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