

Application of Conjugable Oxidation Products Assay in Assessment of Gamma-Irradiated Palm Olein

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ABSTRAK

Olein sawit didedahkan kepada sinar- γ sehingga dos 12 kGy. Pembentukan peroksida di dalam sampel yang disinari diukur dengan kaedah hasil pengoksidaan terkonjugat (COP assay) dan nilainya dibandingkan dengan nilai-nilai yang diperolehi dari kaedah serapan ultralembayung pada 232 nm. Kedua-dua parameter tidak menunjukkan korelasi yang baik ($r = 0.6321$) dalam julat dos yang digunakan. Kesan utama sinar- γ adalah mengoksidakan asid linoleik yang merupakan asid lemak di tak tepu utama dalam minyak sawit.

ABSTRACT

Samples of palm olein were irradiated with γ -rays up to 12kGy. The extent of peroxidation in irradiated samples was determined by conjugable oxidation products (COP) assay and the result were compared with the UV absorbance at 232 nm. The two parameters were poorly correlated ($r = 0.6321$) within the range of doses used. The effect of γ -irradiation is mainly to oxidise linoleic acid (C18:2) as this component is the major diunsaturated fatty acid in palm oil.

INTRODUCTION

Recently, there has been much concern about the quality of oil palm fruits sent to the mills. Poor quality fruits will produce low quality oil which is not only costly to refine but also fetches low market prices. Factors such as the degree of ripeness, severity of bruising of harvested fruits and delays between harvesting and sterilization determine the quality of oil palm fruits. Bruised or over-ripened fruits will contain a high percentage of free fatty acid (f.f.a.) partly due to the action of a lipase enzyme from microorganisms or fungi. In considering these problems, it was thought that radiation within a preservation dosage of less than 10 kGy (Webb 1985; Diehl 1977) might be effective in reducing the microorganisms or fungi and also deactivating the enzyme.

However, lately there has been increasing concern about the quality and safety of irradiated food. Gamma-irradiation of food, besides improving its quality, might also lead to changes in its physical and chemical properties. It has been known that irradiation of fat-containing food forms lipid peroxides, particularly in the presence of unsaturated fats (Hammer *et al.* 1979; Wolters *et al.* 1987) and lowers the quality of the food. In view of these problems, knowledge about the extent of deterioration is very important if radiation is to be used to improve the quality of palm oil. Therefore, the objective of this investigation was to study the effect of gamma-irradiation on the quality of palm oil using a Conjugable Oxidation Products (COP) assay (Parr *et al.* 1976). The COP assay gives more

information on oxidation products and also differentiates between the oxidation of dienoids and those of more highly unsaturated polyenoic fatty acids.

MATERIALS AND METHODS

Materials

Palm olein was prepared from crude palm oil (CPO) as previously described (Endinkeau *et al.* 1989). Isooctane (HPLC grade), propan-2-ol and absolute ethanol (AR) were obtained from BDH Ltd. Sodium borohydride (NaBH_4) was purchased from Sigma Chem. Co. All other solvents were of reagent grade and were used without further purification.

Preparation of Reagents

A stock solution of reducing reagent was prepared by dissolving NaBH_4 (1 g) in propan-2-ol (100 cm^3). The solution was filtered before being used to prepare an approximately 0.4% (w/v) fresh solution of NaBH_4 in propan-2-ol. A dehydrating reagent was prepared by mixing concentrated sulphuric acid (5.43 cm^3) in absolute ethanol to give a 20% (w/v) solution in 50 cm^3 .

Irradiation

Samples of palm olein (2×20 g) were irradiated with γ -rays in glass tubes (5.5×3.0 cm) at a dose rate 65 Gy/min using a ^{60}Co -gamma source at the University of Salford, England. The dose rate was determined by Fricke Dosimetry (Jayson *et al.* 1975). Samples of oxygenated and aerated solutions were used during irradiation. In oxygenated solutions the oil was bubbled with oxygen 10 min prior to and during the irradiation. For air-saturated solutions, the oil was irradiated as it was, i.e. in its natural form. Samples were irradiated to a full dose ranging from 2 to 12 kGy. At intervals of time, portions of irradiated samples were taken and the COP assay was performed immediately.

Method

The procedure of COP assay was based on that of Parr *et al.* (1976) and Swoboda (1981).

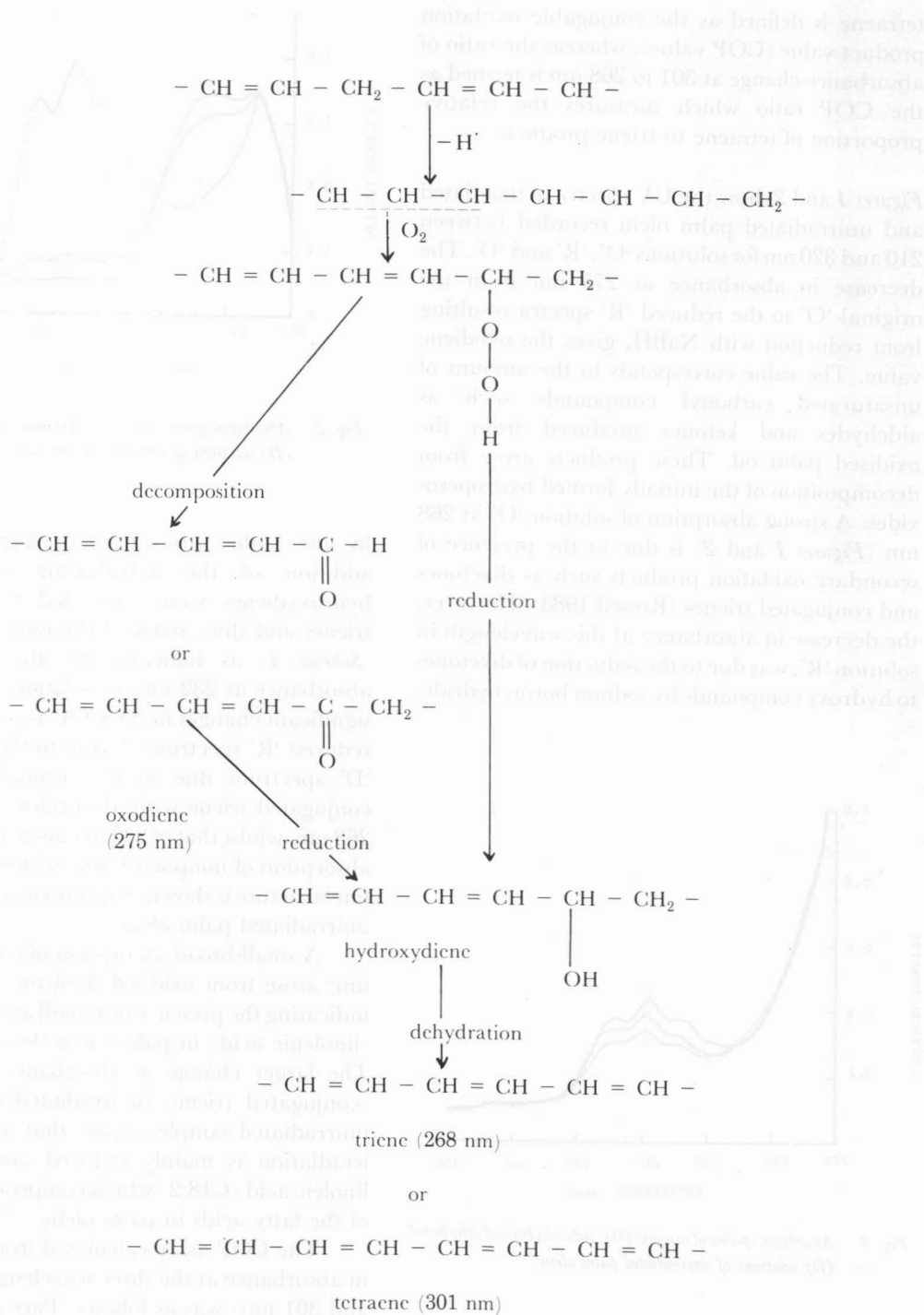
Stock solutions of irradiated and unirradiated oil samples were prepared by dissolving a known weight of each sample (about 0.1 g) in 5.0 cm^3 isooctane-ethanol (1:1 by volume). Aliquots

(1.0 cm^3) of the stock solution were then transferred to each of three 10.0 cm^3 stoppered volumetric flasks which were marked 'O' (original), 'R' (reduced) and 'D' (dehydration). To flask code 'O' was added 10.0 cm^3 isooctane-ethanol (1:1); to flask code 'R' was added 0.4 cm^3 reducing reagent (0.4% w/v) and held at 60°C for 30 min, then cooled and made up to the mark with isooctane-ethanol; flask code 'D' was first treated as 'R' and subsequently 2.0 cm^3 of dehydrating reagent (20% w/v) H_2SO_4 in ethanol) was added with the flask being held again at 60°C for 30 min. After cooling, the solution was made up to 10.0 cm^3 with isooctane-ethanol. The absorbance of each solution, 'O', 'R' and 'D' at the appropriate wavelengths was measured using a SP1800 Spectrophotometer. Solution 'O' was measured at 275 nm (A_{O}^{275}), solution 'R' was measured at 268, 275 and 301 nm (A_{R}^{268} , A_{R}^{275} and A_{R}^{301}) and solution 'D' was measured at 268 and 301 nm (A_{D}^{268} and A_{D}^{301}). A 1.0 cm silica cell containing isooctane-ethanol was used as a blank. The UV absorbance at 232 nm (conjugated diene) was also performed using a SP1800 Spectrophotometer and 0.2% oil solutions in isooctane.

RESULTS AND DISCUSSION

Gamma-irradiation of unsaturated fats (RH) which contain a 1,4-diene structure produces free radical ('R') which reacts with oxygen to form a peroxy radical (ROO'). Subsequent abstraction of a H atom from neighbouring molecule forms a hydroperoxide (ROOH). The compound is unstable and decomposes into various secondary oxidation products namely hydroxydienes, ketodienes and dienals. The ketodienes and dienals are described as oxodienes, (Frankel 1980) in Scheme 1.

In conjugable oxidation product (COP) assay, the hydroperoxide and oxodienes are converted, by reduction and then dehydration, into nonpolar triene or tetraene which are strongly chromophoric and conveniently measured in the ultra violet region. Changes in absorbance resulting from their formation correspond to the amount present. The decrease in absorbance at 275 nm due to reduction of carbonyl compounds is defined as the oxodiene value. The sum changes in absorbance at 268 and 301 nm which measure the amount of triene and



Scheme 1

tetraene is defined as the conjugable oxidation product value (COP value), whereas the ratio of absorbance change at 301 to 268 nm is termed as the COP ratio which measures the relative proportion of tetraene to triene products.

Figures 1 and 2 show the UV spectra of irradiated and unirradiated palm olein recorded between 210 and 320 nm for solutions 'O', 'R' and 'D'. The decrease in absorbance at 275 nm from the original 'O' to the reduced 'R' spectra resulting from reduction with NaBH_4 gives the oxodiene value. The value corresponds to the amount of unsaturated carbonyl compounds such as aldehydes and ketones produced from the oxidised palm oil. These products arose from decomposition of the initially formed hydroperoxides. A strong absorption of solution 'O' at 268 nm (Figures 1 and 2) is due to the presence of secondary oxidation products such as diketones and conjugated trienes (Rossell 1983). However, the decrease in absorbance at this wavelength in solution 'R', was due to the reduction of diketones to hydroxy compounds by sodium boron hydride.

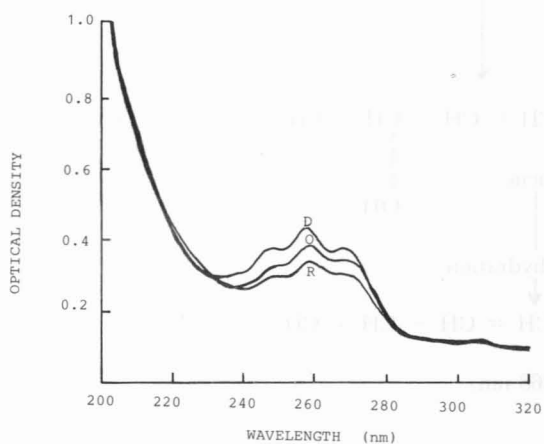


Fig. 1. Absorption spectra at original (O), reduced (R) and dehydrated (D) solutions of unirradiated palm olein.

The increase in absorbance at 232 nm (Figure 2) in solution 'R' compared to 'O' is due to the conversion of carbonyl compounds to conjugated hydroxydienes, and these compounds together with the initially formed hydroxydienes increased the concentration of conjugated dienes as shown

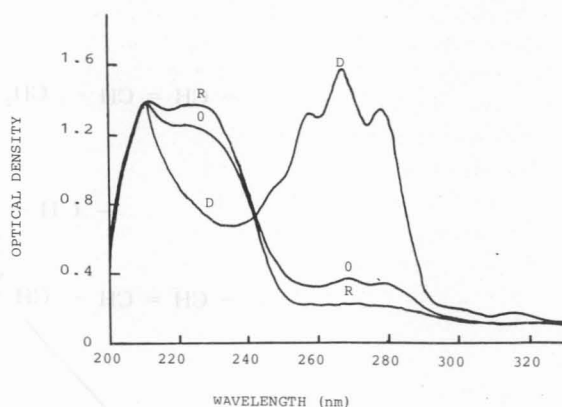


Fig. 2. Absorption spectra of original (O) reduced (R) and dehydrated (D) solutions of irradiated palm olein.

by the higher absorption at 232 nm. Upon addition of the dehydrating reagent, the hydroxydienes were converted to conjugated trienes and thus, reduced the number of dienes (Scheme 1) as indicated by the decrease in absorbance at 232 nm, in solution D. The most significant changes in the COP assay are that the reduced 'R' spectrum change to the dehydrated 'D' spectrum due to the formation of more conjugated triene with absorption maximum at 268 nm, whilst that of 232 nm decreases. A typical absorption of nonpolar triene which has a triplet fine structure is shown in spectra of irradiated and unirradiated palm olein.

A small broad absorption of tetraene at 301 nm, arose from oxidised linolenic acid (C18:3) indicating the presence of a small amount of triene (linolenic acid) in palm olein (less than 0.2%). The larger change in absorbance at 268 nm, (conjugated triene) in irradiated compared to unirradiated samples means that the effect of γ -irradiation is mainly induced autooxidation of linoleic acid (C18:2) which comprises about 10% of the fatty acids in palm olein.

The COP assay calculated from the change in absorbance at the three wavelengths (268, 275 and 301 nm) was as follows (Parr *et al.* 1976):-

$$\text{Oxodiene value} = (A_o^{275} - A_R^{275}) \div 2w$$

$$\text{COP value} = [(A_D^{268} - A_R^{268}) + (A_D^{301} - A_R^{301})] \div 2w$$

$$\text{COP ratio} = (A_D^{301} - A_R^{301}) \div (A_D^{268} - A_R^{268})$$

where w is the weight of the oil sample taken for preparation of the 5.0 cm³ stock solution which gave a strength of 2 × w per 100 cm³ of a final solution in the 10.0 cm³ volumetric flask. Tables 1 and 2 show the results obtained after correction for background (unirradiated sample).

The COP value of irradiated palm olein at a constant dose rate (65 Gy/min) increased steadily with increasing irradiation dose from 2 to 12 kGy (Table 1). It is suggested that as the dose increases

TABLE 1

COP assay of irradiated palm olein in the presence of oxygen

Dose (kGy)	COP Assay*		
	Oxodiene Value	COP Value	COP Ratio
2	0.06	1.05	0.04
4	0.11	1.67	0.04
6	0.19	2.80	0.08
8	0.20	3.36	0.06
10	0.25	3.64	0.08
12	0.32	3.90	0.10
Unirrd.	0.05	0.20	0.13

*COP = (COP_{irrd.} - COP_{unirrd.})

more linoleic acid as well as linolenic acid will be oxidised. However, there is no significant change in oxodiene value, though the values increased slightly with increasing dose. This shows that only small amounts of carbonyl compounds are produced after irradiation. Some of these compounds are volatile, especially those with low molecular weights and may be evaporated during analysis. This factor may also account for the lower yield of carbonyl compounds. These compounds, known as odorous substances, are responsible for the development of an unpleasant smell in deteriorated oils and fats.

Similarly, the COP ratio is very small since the oxidation is mainly of dienoic rather than trienoic in nature. Theoretically, this value varies from zero for a pure triene to 2.8 for a pure tetraene (Fishwick *et al.* 1977). It has been observed that in the aerated solution (Table 2), the COP and oxodiene values were much lower than that of the oxygenated solution. It might be expected that in the presence of low concentrations of oxygen, less hydroperoxides are

TABLE 2

COP assay of irradiated palm olein in aerated solution

Dose (kGy)	COP Assay*		
	Oxodiene Value	COP Value	COP Ratio
2	0.19	0.87	0.05
4	0.19	0.95	0.03
6	0.17	1.13	0.06
8	0.15	1.25	0.05
10	0.10	1.21	0.06
12	0.15	1.33	0.07
Unirrd.	0.09	0.66	0.13

*COP = (COP_{irrd.} - COP_{unirrd.})

formed, accounting for the lower COP and oxodiene values. The relationship between the COP values and the UV absorbance at 232 nm is given in Figure 3. The results show little

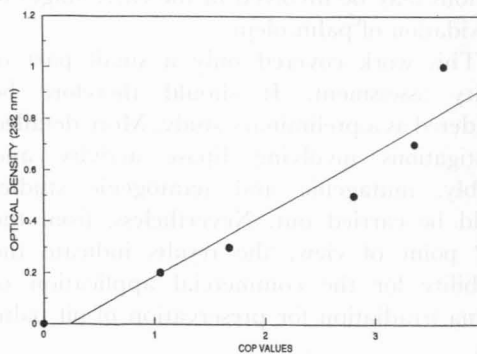


Fig. 3. Correlation between COP values and UV absorbance at 232 nm obtained from irradiated palm olein ($r = 0.6321$, $y = 0.2434x - 0.0578$)

correlation between the two parameters ($r = 0.6321$). This indicated that the yield of conjugated dienes or hydroperoxides by gamma-irradiation is not paralleled with the changes of COP values. Presumably, in the presence of excess oxygen and high irradiation doses, some hydroperoxides undergo other types of reactions, forming non-conjugated products (Gilman 1953; Swern 1961). These reactions, therefore, reduce the number of initially formed hydroperoxides and thus, the conjugated dienes.

Previous work (Parr *et al.* 1976) has shown that some of the commercially available foodstuffs have COP values ranging from 0.5 to 77. A poor quality pork mince and kipper fillets, for example, have COP values of 1.5 and 2 respectively. Based on these values and the values obtained from irradiated palm olein, gamma-irradiation within the range of preservation doses (1-10 kGy) (Webb 1985, Kader 1986) can, therefore, be applied to preserve oil palm fruits. Furthermore, at these doses only small amounts of carbonyl compounds, which impart an unpleasant smell to irradiated samples, are formed.

CONCLUSION

The COP value of irradiated palm olein increased with increasing irradiation dose. Although the COP values obtained (1.05 to 3.9 in oxygenated solution) are acceptable, gamma-irradiation to some extent promotes autoxidation of unsaturated fatty acid, particularly the linoleic acid. The poor correlation between COP values and absorbance at 232 nm indicates that other reactions may be involved in the early stages of autoxidation of palm olein.

This work covered only a small part of quality assessment. It should therefore be considered as a preliminary study. More detailed investigations involving lipase activity and possibly, mutagenic and teratogenic studies should be carried out. Nevertheless, from the COP point of view, the results indicate the possibility for the commercial application of gamma irradiation for preservation of oil palm fruits.

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