A Research Note

Effects of Chlorophyll and β-Carotene on the Oxidation Stability of Olive Oil

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ABSTRACT

Virgin olive oil was purified by silicic acid column chromatography to remove non-triglyceride components. The effects of chlorophyll and β-carotene on the oxidation stability of purified olive oil were studied by a combination of measuring peroxide value and oxygen disappearance in the headspace of sample bottles by gas chromatography. Chlorophyll in the purified oil acted as a photosensitizer for singlet oxygen formation under light. β-Carotene minimized lipid oxidation of purified oil under light storage by its light-filtering effect. Experiments clearly suggested that singlet oxygen was mainly responsible for the photooxidation of the oil containing chlorophyll.

INTRODUCTION

VIRGIN OLIVE OIL, which is obtained by pressing olive fruits, is one of the few oils that are consumed without any further refining process. It gives a characteristic rich flavor and yellow green color due to the presence of chlorophyll, carotenoid and many other minor components. Since chlorophyll and β-carotene have been reported to play important roles in generating or quenching singlet oxygen in model systems (Foote and Denny, 1968; Foote et al., 1970; Endo et al., 1984), the effects of chlorophyll and carotene on lipid oxidation have been a great concern to food scientists. The objective of this investigation was to study the effects of chlorophyll and β-carotene on the oxidation of olive oil in the presence of light during storage.

MATERIALS & METHODS

VIRGIN OLIVE OIL (VO) which was imported from Calamate, Greece was obtained from a local supermarket. The purified olive oil (PO) was obtained by passing 700 mL VO through a chromatographic column (600 mm × 40 mm i.d.) packed with 280g silicic acid (100 mesh, Mallinkrodt) which was activated by the method of Sahasrabudhe and Chapman (1961) and 70g of 2:1 mixture of activated charcoal (J.T. Baker Chemical Co.) and diatomaceous earth (John Manville Products). Peroxides, free fatty acids, phosphorus, chlorophyll and β-carotene in both oils were determined by AOCS (1980) methods. Chlorophyll and β-carotene were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Air-tightly sealed 30 mL serum bottles containing 15g of PO and different levels of chlorophyll or β-carotene were placed on the wire netting which was 20cm above the Sylvania cool-white fluorescent light. The light intensity at the sample level was 4,000 lux and the temperature of samples was 25°C. All samples were analyzed in duplicate.

To study the mechanism of minimizing PO oxidation by β-carotene under light storage, the light-filtering effect of β-carotene was examined. The PO sample bottles were placed on the wire netting and the PO with or without 100 ppm β-carotene was placed between the wire netting and the fluorescent light.

Oxidation stability of sample was determined by a combination of measuring peroxide value of oil and the oxygen disappearance in the headspace of sample bottle by gas chromatography. Peroxide values of samples were determined by AOCS (1980) method. One milliliter of headspace vapor from the sample bottle was removed with a 2 mL gas tight syringe (Hamilton Co., Reno, NV) and injected into a Hewlett Packard 5880A Gas Chromatograph equipped with a thermal conductivity detector and an electronic integrator. A stainless steel column (1.83 m × 0.3 cm i.d.) packed with 80/100 mesh Molecular Sieve 13× (Alltech Associates, Inc., Deerfield, IL) was used and nitrogen gas flow rate was 20 mL/min. The temperatures of injection port, oven and detector were 200°, 35° and 250°C, respectively. The concentration (μmoles) of oxygen in the headspace was calculated by the linear regression analysis of standard curve obtained for the relationship between gas chromatographic peak area and oxygen volume injected.

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RESULTS & DISCUSSION

THE PO did not contain any peroxides, free fatty acids, phosphorus, chlorophyll or β-carotene. The VO contained 0.6% free fatty acids, 6 ppm phosphorus, 8 ppm chlorophyll and 9 ppm β-carotene. Coefficients of variation for PV and headspace oxygen analyses were 2% and 3%, respectively.

Table 1 shows that the addition of chlorophyll resulted in higher peroxides formation and greater oxygen disappearance in the headspace of PO stored under light. Chlorophyll added to the PO thus acted as prooxidant under light storage. The greater was the amount of chlorophyll in the PO, the less was the oxidation stability of oil. Even though results are not presented here, another experiment showed that different levels of chlorophyll added to the PO did not act as prooxidant under dark storage. This agrees with the reports that chlorophyll worked as a sensitizer to generate singlet oxygen in the photooxidation in the model system (Rawls and Van Santen, 1970; Clements et al., 1973; Carlsson et al., 1976).

Table 2 shows that the higher was the concentration of β-carotene in the PO, the lower was the peroxides formation in the PO and the higher was the residual oxygen in the headspace of the bottle under light. This suggested that β-carotene minimized the lipid oxidation of PO which did not contain sensitizers such as chlorophyll under light. The minimization of PO oxidation by added β-carotene under light must not be due to singlet oxygen quenching and/or free radical antioxidant effects because PO does not contain chlorophyll for singlet ox-

Table 1—Effects of chlorophyll on the peroxide formation and headspace oxygen of purified olive oil under light at 25°C

Storage time (hr)	P V (meq/kg Oil) chlorophyll (ppm) 0	2	4	Headspace oxygen (μmoles O_2/mL Headspace) chlorophyll (ppm) 0	2	4
0	0.00	0.00	0.00	9.51	9.51	9.51
3	0.60	3.95	4.72	9.47	9.10	8.93
6	0.70	4.68	6.65	9.44	8.79	8.33
10	1.15	5.65	7.10	9.34	8.48	8.14
16	1.95	7.10	8.90	9.33	7.98	7.58
28	2.10	8.90	11.60	9.03	7.10	6.52
40	3.15	11.30	13.80	8.67	6.43	5.92
52	3.72	13.75	15.60	8.61	5.92	5.34
70	4.50	14.75	17.20	8.35	5.23	4.55

Table 2—Effects of β-carotene on the peroxide formation and headspace oxygen of purified olive oil under light at 25°C

Storage time (hr)	P V (meq/Kg Oil) β-Carotene (ppm) 0	0[a]	5	10	20	Headspace Oxygen (μmoles O_2/mL Headspace) β-Carotene (ppm) 0	0*	5	10	20
0	0.00	0.00	0.00	0.00	0.00	9.43	9.43	9.43	9.43	9.43
12	1.73	0.65	1.09	0.98	0.76	9.13	9.23	9.26	9.33	9.29
24	3.22	0.90	1.70	1.80	1.15	8.41	9.19	8.68	8.83	8.91
36	4.40	1.09	2.70	2.50	2.09	7.99	9.08	8.33	8.37	8.53
48	5.15	—	3.28	2.60	2.42	7.75	—	8.31	8.34	8.49
60	6.50	1.60	4.31	3.46	2.75	7.22	8.92	7.98	8.07	8.39
72	6.56	—	4.52	3.64	3.75	7.00	—	7.76	7.93	7.99
84	7.80	3.60	5.20	4.95	4.02	6.80	7.74	7.62	7.50	7.92

[a] By placing PO with 100 ppm β-carotene between the wire netting and fluorescent light.

ygen production and β-carotene is not a free radical scavenger. The mechanism of minimizing lipid oxidation in the PO by β-carotene under light was further studied by placing the PO with or without 100 ppm β-carotene between the wire netting and fluorescent light. The PO sample bottles on the wire netting above the PO with 100 ppm β-carotene between the wire netting and light showed lower peroxide formation and higher residual oxygen in the headspace than the PO sample bottles on the wire netting above the PO without 100 ppm β-carotene between the wire netting and light as shown in Table 2. Therefore, the oxidation stability of the PO sample on the wire netting above the PO with 100 ppm β-carotene was higher than the PO sample placed on the wire netting above the PO without β-carotene. This suggested that β-carotene in the PO between the wire netting and light filtered out some of the light energy to minimize lipid oxidation of the PO on the wire netting. β-Carotene absorbs light between 400 and 500 nm (Goodwin, 1980) which corresponds to 21% of all energy emitted from light source (Anonymous, 1984). The PO containing β-carotene in the sample bottles will have less energy from light source and thus better oxidation stability than the PO containing no β-carotene.

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t_h	Heating time
T	Temperature
T_c	Cooling temperature
T_h	Heating temperature
T_i	Initial temperature
T_{ref}	Reference temperature
T_S	Heating medium temperature
T_W	Cooling medium temperature
x	Relative retention = $[C/C_o]$
α	Thermal diffusivity
α_c	Thermal diffusivity for the cooling step
α_h	Thermal diffusivity for the heating step
ρ	Density

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