Short Communication

Antioxidative Stability of Tempeh

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Antioxidant activities of 6,7,4′-trihydroxyisoflavone and the compound 0.58 substance, (Rf value 0.58 on Silicagel TLC with cyclohexane 9 : ethylether 1) isolated from tempeh were measured by oxygen absorption. Both are active in preventing consumption of dissolved oxygen in an emulsified solution of safflower oil.

In response to the short communication on Tempeh Oil-Antioxidant (?) by Stahl and Sims (1), it is necessary to report our further experimental work on 6,7,4′-trihydroxyisoflavone [a new isoflavone isolated from tempeh (2), and chemically synthesized for the study on its antioxidative activities (3)] and the other "0.58 substance" (Rf value, 0.58 on Silicagel TLC with developing solvent, cyclohexane 9 : ethylether 1) (4). The antioxidative and antihemolytic effects of 6,7,4′-trihydroxyisoflavone on vitamin A and sodium linoleate in aqueous solution at pH 7 have been reported (3). Later studies using the rapid method for evaluation of antioxidation by Yagi (5) not previously published are presented here.

EXPERIMENTAL PROCEDURES

According to the method described by Yagi (5), changes in oxygen content in a reaction mixture were measured with the Beckman Oxygen Analyzer (Model 777) attached to a recorder (Hitachi OPD 33) and connected to a sensor. The reaction mixture, which contained 10 g safflower oil, 180 ml water and one ml Tween 20, was blended for emulsification. Measurements were made immediately after addition of one ml of a test solution and one ml of a catalyst: 1% Fe SO_4. 7 H_2O or 0.02% hemin.

RESULTS

Antioxidant activities of 6,7,4′-trihydroxyisoflavone measured by oxygen absorption method. As shown in Figure 1, 100 μg or 50 μg of 6,7,4′-trihydroxyisoflavone/40 ml emulsified solution of safflower oil (2.2 g) in the presence of the metal catalyst gave almost complete protection against oxygen consumption for the 10-min test period. In the control, almost 100% of the oxygen was consumed in this time.

Antioxidant activities of unknown compound (0.58 substance) from Tempeh by AOM. Results of the experiment on the 0.58 substance isolated from tempeh oil are shown in Figure 2. As seen there, 160 μg of the 0.58 substance/40 ml emulsified solution of safflower oil (2.2 g) protected against about 85% of dissolved oxygen consumption within several min, compared with 50% in the control in the presence of metal catalyst. However, 40 μg of the 0.58 substance using 0.02% hemin as catalyst gave little protection and was no more effective than 10 μg of BHT.

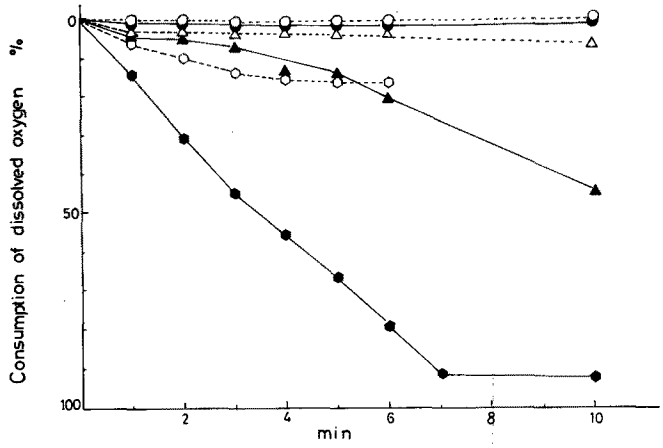

FIG. 1. Antioxidant activities of 6,7,4′-trihydroxyisoflavone(F2) measured by the oxygen absorption method. ○ - - - - ○, added F2, 100 μg/40 ml (catalyst, 1% $FeSO_4 \cdot 7H_2O$); ●——●, added F2, 50 μg/40 ml (catalyst, 1% $FeSO_4 \cdot 7H_2O$); △ - - - - △, added F2, 25 μg/40 ml (catalyst, 1% $FeSO_4 \cdot 7H_2O$); ▲——▲, added F2, 12 μg/40 ml (catalyst, 1% $FeSO_4 \cdot 7H_2O$); ○- - -○, added F2, 100 μg/40 ml (catalyst: 0.02% Hemin), and ●——● , control (catalyst, 1% $H_2SO_4 \cdot 7H_2O$).

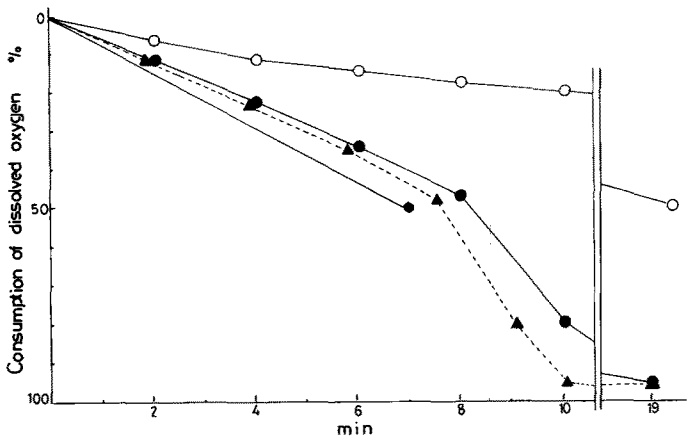

FIG. 2. Antioxidant activities of 0.58 substance measured by the oxygen absorption method. ○——○, added 160 μg/40 ml 0.58 substance (catalyst, 1% $FeSO_4 \cdot 7H_2O$); ●——●, added 40 μg/40 ml 0.58 substance (catalyst, 0.02% Hemin); ▲ - - - ▲, added 10 μg/40 ml BHT (catalyst 0.02% Hemin), and ●——● , control (catalyst, 1% $FeSO_4 \cdot 7H_2O$).

DISCUSSION

Stahl and Sims (1) emphasized that fatty acids liberated from soybeans during the fermentation of tempeh promote rapid decomposition of peroxide. Therefore, peroxide values determined during storage are a poor index of oxidation rate in oils high in FFA. From the

results of oxygen absorption rates measured on oil at 50 C following the method of Bishov and Henick (6), Stahl and Sims (1) pointed out that their data clearly support the conclusion that the report by György was in error.

Stahl and Sims (1) did not mention our work of 1968 (3), in which the results of antioxidant activities in vitro and in vivo of 6,7,4′-trihydroxyisoflavone were reported together with discussion. The reference on the isolation of 6,7,4′-trihydroxyisoflavone is György et al. (2) and for the test of protection of peroxide development on the addition of as little as 10% tempeh oil to soybean oil is György et al. (7) and not György et al. (2) mentioned by them (1).

As mentioned by Yagi (5), antioxidants are classified in three types, according to the mechanism of action: chain breakers (or free radical inhibitors); peroxide decomposers, and metal inactivators. Flavonoids also were reported to act as chain breakers as well as metal inactivators. Chelated quercetin with cupric ion showed considerable loss of antioxidant activity when measured by the AOM test.

Our results using the AOM test clearly indicated that 6,7,4′-trihydroxyisoflavone and the 0.58 substance protected well against oxygen consumption for about a 10-min test period. Other reasons for stability of tempeh and mechanisms for its stability should be studied further.

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