# Tempeh Oil-Antioxidant(?)

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Previous workers have claimed that tempeh oil contains a potent fat antioxidant. These claims were based solely on measurements of peroxide value during accelerated storage of tempeh oil alone or as an additive to other unsaturated oils. Oxygen absorption rate measurements indicate that tempeh oil actually has weak prooxidant activity. Fermentation of soybeans to produce tempeh generates high levels of free fatty acids. FFA promote rapid decomposition of peroxides so that the levels in the oil never increase substantially during oxidation. Consequently, PV is a poor index of oxidation rate in oils which are high in FFA. Our experience with tempeh oil does not confirm earlier observations of its efficacy as an antioxidant.

Tempeh, a fermented soybean product indigenous to Indonesia, has been a common food item in the Far East for centuries. In 1962, P. Gyorgy reported tempeh to be very stable to rancidity development and suggested that oil extracted from it should find application as an antioxidant (1,2). He described the isolation of 6, 7, 4'-trihydroxyisoflavone, a water-soluble antioxidant from tempeh (3). However, this isoflavone did not show any protective effect when it was added to soybean powder or soybean oil.

More recently, Murakami et al. (4) suggested that the isoflavones diadzein and genistein may be responsible for the antioxidant activity of tempeh. These compounds, identified in fermented soybeans, previously had been reported to possess antioxidant activity (5).

Tempeh and tempeh oil both possess a strong

fermented odor so that it is difficult to assess the development of rancidity by organoleptic methods. For this reason, both Gyorgy and Murakami et al. relied entirely on a chemical test to measure oxidation rates. They used peroxide value measurements during accelerated storage of tempeh oil alone or as an additive to other unsaturated oils. They found tempeh oil to be resistant to peroxide development, whereas unfermented soybean oil generated peroxides quite rapidly under the same conditions. Addition of as little as 10% tempeh oil to soybean oil retarded peroxide development in these tests (6).

Rate of hydroperoxide development is a valid measure of stability only in those instances where peroxides tend to accumulate. If hydroperoxides are not stable intermediates but instead decompose rapidly to form secondary oxidation products, then some other measure of fat stability must be selected. Wagenknecht (7) reported that *Rhizopus oligosporus*, the fungus used to prepare tempeh, possesses strong lipase activity and causes hydrolysis of about one-third of the neutral fat during the fermentation process. Furthermore, Popov and Mizev (8) found that FFA accelerate the decomposition of hydroperoxides to the degree that phenolic antioxidants do not function effectively. Olcott (9) also found FFA to decrease the effectiveness of antioxidants in unsaturated oils.

Since we were unable to confirm the antioxidant activity of tempeh oil by organoleptic evaluation, and we questioned the validity of PV as a stability index, it seemed appropriate to measure the rate of oxygen absorption. This test gives a true measure of resistance to oxidation and is not limited by the degree of stability of intermediate oxidation products.



FIG. 1. Oxygen absorption @ 50 C. \*—\*, 10% tempeh/90% safflower oil; x-x, 10% soybean/90% safflower oil; +—+, safflower oil, and O—O, safflower oil + BHT.

## **EXPERIMENTAL**

Materials and methods. Some samples of tempeh oil and soybean oil were furnished by P. Gyorgy. Tempeh was prepared in our laboratory following the method of Murakami et al. (4). These samples were freeze-dried, and oil was recovered from them by extraction with hexane in a Soxhlet apparatus.

Oil samples were evaluated at a 10% level in refined safflower oil. Rates of oxygen absorption were measured at 50 C following the method of Bishov and Henick (9). Peroxide values were determined by AOCS Official Method Cd 8-53.

Results and discussion. Oxygen absorption rates were measured on oils held at 50 C (Fig. 1). Relative to refined safflower oil as a control, addition of 10% tempeh oil appears to increase the rate of oxygen absorption. Although Gyorgy claimed that fermented crude soybean oil had antioxidant properties, we find that it actually seems to function as a prooxidant. Refined soybean oil (10% level) in safflower oil protects it from oxidation somewhat more effectively than does BHT. These results are quite different from those reported by Gyorgy where fermentation appeared to generate an effective antioxidant. The data shown in Figure 1 was obtained using tempeh oil from Gyorgy. Similar results were found when the oil from our own tempeh was tested in safflower oil.

Oil samples were then held in glass jars at 60 C (Schaal oven test), measuring peroxide values at intervals as shown in Table 1. When only PV is considered, tempeh oil appears to exert some protective effect. We believe this behavior is due solely to the rapid decomposition of hydroperoxides which occurs in the presence of FFA. In systems containing FFA, PV is an unreliable index of stability.

It is unfortunate that the original work on tempeh oil was confined to measurement of peroxide value. In our view, oxygen absorption rate is a much more reliable and

# TABLE 1

Schaal Oven Test (60 C)

| Time (hr)                           | Peroxide values (meq/kg) |     |     |     |     |
|-------------------------------------|--------------------------|-----|-----|-----|-----|
|                                     | 47                       | 114 | 164 | 213 | 336 |
| 10% Tempeh oil + 90% safflower oil  | 4                        | 9   | 14  | 20  | 57  |
| 10% Sovbean oil + 90% safflower oil | 7                        | 16  | 24  | 34  | 93  |
| Safflower oil (0.02% BHT)           | 7                        | 16  | 23  | 30  | 92  |
| Safflower oil                       | 8                        | 17  | 25  | 34  | 102 |

meaningful index of stability to oxidation. We believe our data clearly supports the conclusion that Gyorgy was in error.

### ACKNOWLEDGMENTS

H. Prescott helped prepare samples of fermented soybeans.

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[Received October 28, 1985]