

# Studies on Antioxidant Activity of Tempeh Oil

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## ABSTRACT

Various oils, such as soy, cottonseed, corn, safflower, and lard may be protected from autoxidation by the addition, often in small amount, of tempeh oil. Tempeh is obtained from cooked, dehulled soybeans by fermentation with the fungus *Rhizopus oligosporus*. The oil is extracted from dried tempeh with hexane/alcohol 2:1 and recovered by evaporation of the solvents. This crude oil was used in most of the experiments quoted.

## INTRODUCTION

Tempeh, a fermented soybean preparation, has been used widely for centuries in the Far East, especially Indonesia. Whole soybeans are soaked in water and dehulled; they then are boiled to soften the beans. The cooked beans are inoculated with a culture of the fungus *Rhizopus oligosporus* (or by a technique like that used in cheese manufacture with previously prepared tempeh) and spread out in layers 1-2 in. deep. In equatorial Indonesia the fungus grows rapidly at ambient temperature, and overnight the layers of soybeans are densely invaded with the fungus, and the surface covered with a white layer of fungus, giving an appearance similar to that of Camembert cheese. The tempeh is fried or incorporated in other cooked dishes. It may be dried and reconstituted for later use. In studies with dried tempeh, it has been found that it has remarkable antioxidant activity compared with the original soybeans (1).

Fishing is one of the most important home industries in Indonesia. The fisherman (with family) starts fishing around 6 a.m. and returns home in the heat of the day around 11 a.m. The freshly caught, cleaned fish already is showing signs of beginning rancidity. This can be prevented if the cleaned, opened fish very soon after being caught is covered with dry tempeh powder. The fish remains fresh in

contrast with untreated controls (2).

In studies carried out on rats kept on a vitamin E low diet, a greater resistance of red blood cells to in vitro dialuric acid-induced hemolysis was demonstrated in rats fed dried tempeh than in rats fed plain boiled, dried soybeans (1). Since dialuric acid hemolysis is due to lack of antioxidants, the positive results with tempeh indicate the presence of active antioxidants.

A powerful antioxidant has been isolated from tempeh and recognized as 6,7,4'-trihydroxyisoflavone (3). In in vitro tests, this compound has been proven to be one of the most active antioxidants among the natural flavonoids. This trihydroxyisoflavone is water-soluble and active in aqueous milieu, but it will not exert its antioxidant effect when mixed with soybean powder or soybean oil. It is also inactive as an oral supplement, probably on account of its water solubility (4).

This conclusion led to the study of oil components of tempeh, free from water soluble constituents.

## METHODS

The procedure for the preparation and separation of the active factors in tempeh was as follows: powdered tempeh was extracted with 10 volumes of 2 parts of hexane and 1 part alcohol. After removal of the solvent the oily residue, referred to hereafter as crude tempeh oil, was used for study of its antioxidant activity in various concentrations and at several temperatures (up to 60 C). The amount of oil recovered is essentially the same as that recovered from untreated soybeans. The samples were stored with exposure to air for periods of time up to several months. In some experiments, the sample was exposed in a thin layer in open scintillation vials.

For measurement of autoxidation, the peroxide value (PV) of the samples was determined by the method of Wheeler (5). A 0.5 g sample of oil was weighed into a 300 ml glass-stoppered flask. Chloroform (10 ml) was added to dissolve the sample followed by 15 ml glacial acetic acid and  $1 \pm 0.1$  g powdered potassium iodide (KI). The preparation was refluxed over the water bath and then boiled for 3 min. The flask was cooled in ice water. Distilled water (75 ml) was added and the flask shaken. A

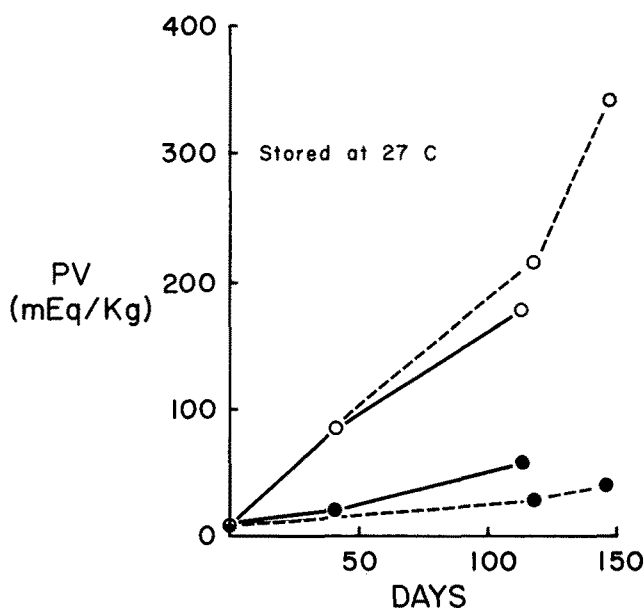


FIG. 1. Peroxide value (PV) of cottonseed oil-tempeh oil mixtures, 150 days, 27 C. ○-○ = Cottonseed oil, ●-● = cottonseed oil + 10% tempeh oil, ○-○ = cottonseed oil, and ●-● = cottonseed oil + 20% tempeh oil.

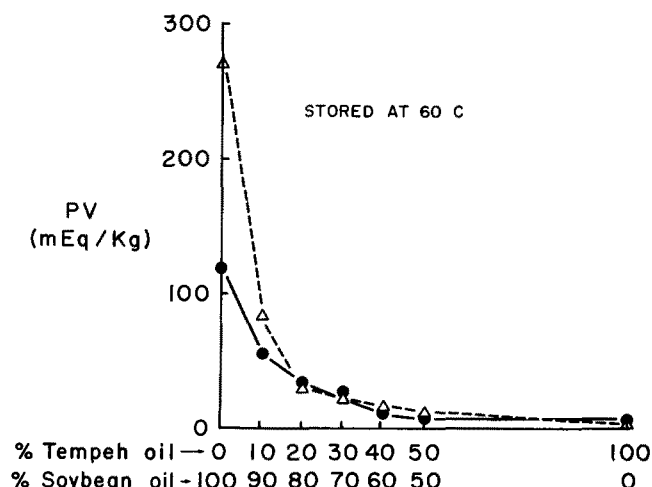


FIG. 2. Peroxide value (PV) of soybean oil-tempeh oil mixtures, 35 and 43 days, 60 C. △-△ = PV after 43 days, and ●-● = PV after 35 days.

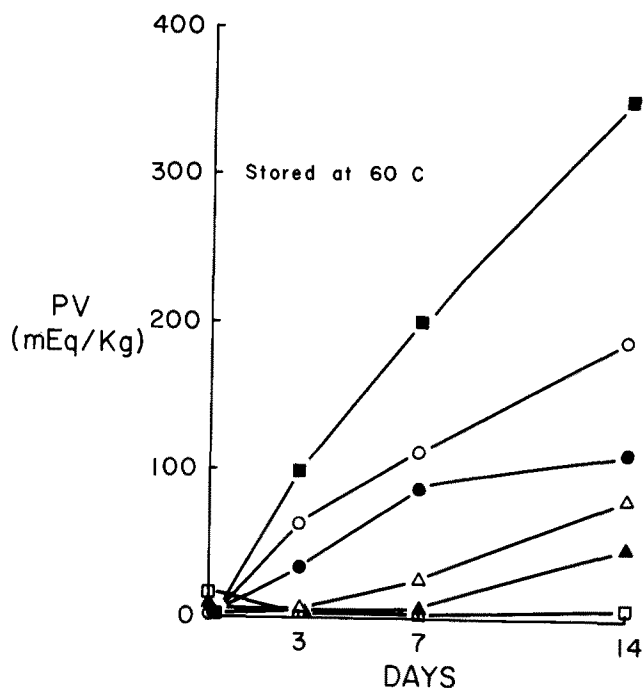


FIG. 3. Peroxide value (PV) of cottonseed oil-tempeh oil mixture, 14 days, 60 C. ■-■ = cottonseed oil, ○-○ = cottonseed oil + 10% tempeh oil, ●-● = cottonseed oil + 20% tempeh oil, △-△ = cottonseed oil + 30% tempeh oil, ▲-▲ = cottonseed oil + 50% tempeh oil, and □-□ = tempeh oil.

drop of 1% starch solution was added and the preparation titrated with N/1000 Na thiosulfate until the iodine color disappeared. PV is reported as milliequivalents of iodine/kg oil sample.

### RESULTS

Observations on the antioxidant activity of the crude tempeh oil are shown in Figures 1-7.

Figure 1 shows the protection given cottonseed oil by addition of only 20% tempeh oil after storage at room

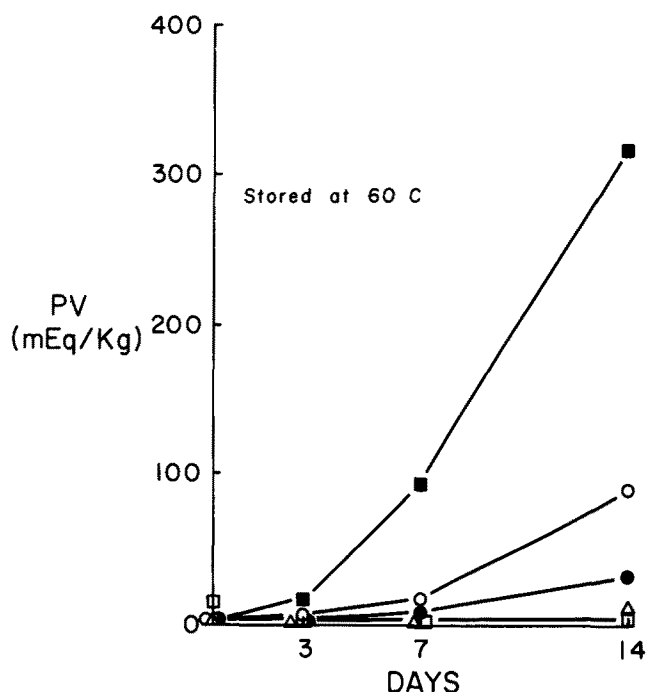


FIG. 4. Peroxide value (PV) of corn oil-tempeh oil mixture, 14 days, 60 C. ■-■ = corn oil, ○-○ = corn oil + 10% tempeh oil, ●-● = corn oil + 20% tempeh oil, △-△ = corn oil + 30% tempeh oil, and □-□ = corn oil + tempeh oil.

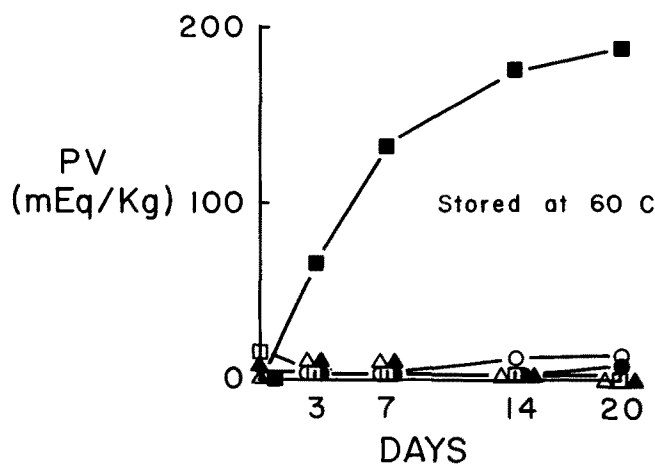


FIG. 5. Peroxide value (PV) of lard-tempeh oil mixtures, 14 days, 60 C. ■-■ = lard, ○-○ = lard + 10% tempeh oil, ●-● = lard + 20% tempeh oil, △-△ = lard + 30% tempeh oil, ▲-▲ = lard + 50% tempeh oil, and □-□ = tempeh oil.

temperature for 5 months.

Results obtained when oils were exposed to a temperature of 60 C for 2 or more weeks may be seen in Figures 2-6. Definite retardation of peroxidation was observed with all five oils tested: soybean, cottonseed, corn, lard, and safflower. Only limited inhibition was observed with safflower oil (Fig. 6) with its high content of unsaturated fatty acids. The most pronounced antioxidant activity was found with lard, where the lowest level of tempeh oil used gave complete protection.

Samples of soya oil without and with added tempeh oil were incubated for 18 days at 37 C and were compared with undiluted Crisco Oil, which is soya oil with commercial antioxidants (polysorbate 80, polyglycerides, butylated hydroxytoluene, butylated hydroxyanisole, and methyl silicone). The antioxidants in the Crisco Oil afforded ca. the same degree of protection as 25% of tempeh oil in the tempeh-soy mixtures (Fig. 7).

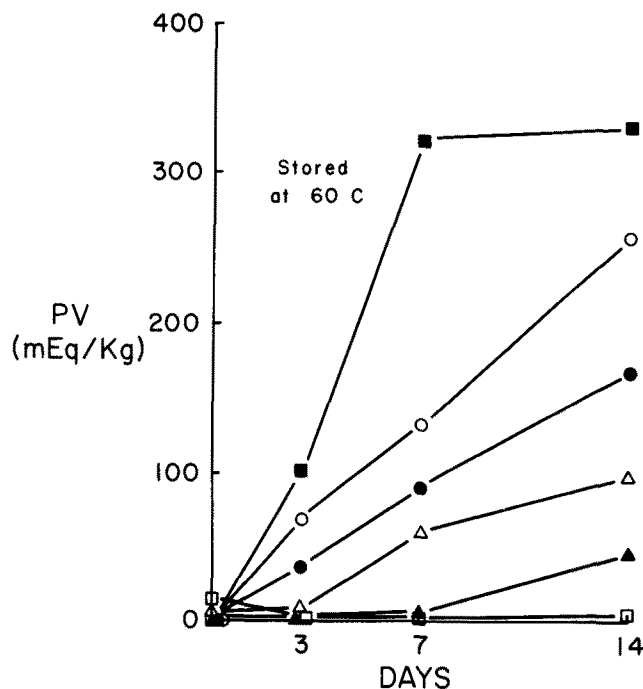


FIG. 6. Peroxide value (PV) of safflower oil-tempeh oil mixtures, 14 days, 60 C. ■-■ = safflower oil, ○-○ = safflower oil + 10% tempeh oil, ●-● = safflower oil + 20% tempeh oil, △-△ = safflower oil + 30% tempeh oil, ▲-▲ = safflower oil + 50% tempeh oil, and □-□ = tempeh oil.

## DISCUSSION

The experimental results reported indicated that the tempeh oil, extracted from the fermented soybeans, even in low concentration, exerts an effective antioxidant effect upon almost all oils tested, even after exposure to air and at temperatures up to 60 C for many weeks. This mixture appears to be, at present, the best natural way of preserving various kinds of oil in fresh condition for long periods of time.

Soy oil, which, under ordinary commercial conditions (in bottles without complete elimination of air), develops undesirable flavor within a few months, retains, for an almost indefinite period of time when mixed with a small amount of tempeh oil, its normal physical, chemical, and gustatory qualities. This conclusion applies to other oils and fats, including lard.

The active antioxidant principle in tempeh oil is fat-soluble but different from vitamin E, the content of which does not increase after *Rhizopus* fermentation. The chemical character of the active principles in tempeh oil has not been established yet.

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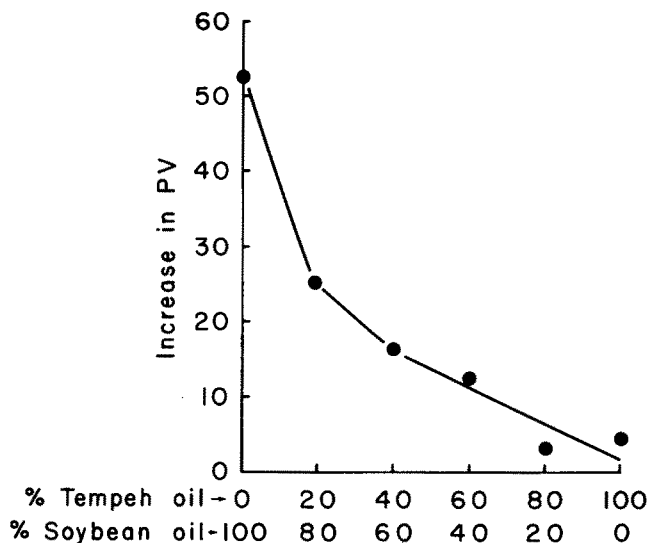


FIG. 7. Peroxide value (PV) of soybean oil-tempeh oil mixtures as compared with soybean oil protected with commercial samples incubated for 18 days at 37 C 1 g in open scintillation vials.

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