EFFECTS OF TRIGLYCERIDE MONOHYDRO-
PEROXIDES ON THE GROWTH OF *E. COLI*Kaneyuki SOHODE¹, Tadashi OGAWA and Setsuro MATSUSHITA²*Research Institute for Food Science, Kyoto University, Kyoto*

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Many experiments (1-4) have been done on the effect of autoxidized products of lipids on living animals, cells, enzymes and biological active substances. However, crude materials such as autoxidized TG or LA have been used without further purification in most such experiments. In a previous paper (5), we reported the effect of pure LAHPO on the growth of *E. coli*. LA can be used as a model substance for TG in studies concerns with the oxidation mechanism. Considerable difference can not be expected between TG and LA oxidation mechanism. However, there are some differences in their molecular structures and sizes. Recently, we prepared TG hydroperoxides (6, 7) from oxidized safflower oil and characterized their properties. In the present investigation we have attempted to examine the toxic effect of TG hydroperoxides on the growth of *E. coli*.

Effect of TGMHPO on the growth of E. coli

TGMHPO was added at various concentrations, and the inhibitory effect of the sample on the growth of *E. coli* was determined (Fig. 1). TGMHPO at 1 μ mole/ml showed almost complete inhibition. These results are very similar to those with LAHPO.

Protection of the growth of E. coli against TGMHPO

Antioxidants were tested as protectants against the inhibitory activity of TGMHPO (Fig. 2). Although complete protection does not seem to be attainable, BHA showed the most remarkable protecting effect. Uric acid and tryptophan which are water soluble antioxidants, showed some effect but less effective than BHA. These results show similarity to those of LAHPO (5), and suggest that the growth inhibitory activity of TGMHPO may be of a radical type, and the protective effect of the antioxidants may be the result of the interaction of TGMHPO radicals with the protectant radicals to bring about the termination reaction.

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Abbreviations: LA, linoleic acid; TG, triglyceride; LAHPO, linoleic acid hydroperoxides; TGMHPO, triglyceride monohydroperoxides; BHA, butylated hydroxy anizole.

Fig. 1. Effect of TGMHPO on the growth of *E. coli*. Each experimental unit consisted of 9.5 ml of culture medium (8) and 0.5 ml of inoculum. 0.02–0.2 ml of TGMHPO in ethanol was added to the tube after 2 hr of preincubation. The mixture was further incubated for 3 hr. The growth rate (O.D. at 600 nm) was measured in order to determine the effect of the test compounds. A, no addition; B, 1 μ mole/ml TG; C, 0.1 μ mole/ml TGMHPO; D, 0.2 ml ethanol/tube; E, 0.5 μ mole/ml TGMHPO; F, 1 μ mole/ml TGMHPO.

Fig. 2. Protective effect of antioxidants on the inhibitory effect of TGMHPO on the growth of *E. coli*. The incubation mixture consisted of 9.1 ml of culture medium and 0.5 ml of inoculum which was preincubated for 2 hr, and 0.2 ml of the test compound (final concentration 1 μ mole/ml) such as BHA, uric acid and tryptophan was added, followed by the addition of 0.2 ml of TGMHPO ethanol solution (final concentration 1 μ mole/ml). The mixture was further incubated for 3 hr. A, no addition; B, BHA+TGMHPO; C, tryptophan+TGMHPO; D, uric acid+TGMHPO; E, TGMHPO.

Effects of TGMHPO on the viability of *E. coli*

The viability was tested to clarify whether the inhibitory effect of TGMHPO on the growth of *E. coli* depends on the inhibition by sterilization or the inhibition by bacteriostatic action. For comparison, LAHPO was used. At 20 hr incubation with 1 μ mole/ml TGMHPO, the inhibition seems to be caused by bacteriostatic action (Table 1). However after 3 hr incubation with 2 μ moles/ml TGMHPO, about 60% of the cells was killed. In the case of LAHPO, about

Table 1. Effect of lipid hydroperoxides on the viability of *E. coli*. Incubated solution (*E. coli* and hydroperoxides) was diluted with physiological saline solution. The solution was cultured on an aga solid medium according to the routine method. The colony was counted, and the viability was expressed as a percentage against the control.

Addition	Incubation time (hr)	Survived cells (%)
Control		100
LAHPO 2 μ moles/ml	3	12.1
LAHPO 4 μ moles/ml	3	0.3
TGMHPO 1 μ mole/ml	20	110
TGMHPO 2 μ moles/ml	3	40.8
TGMHPO 2 μ moles/ml	20	34.4

88% was killed in 3 hr incubation with 2 μ moles/ml and completely with 4 μ moles/ml. Comparing these results, the effect of LAHPO seems to be more drastic than TGMHPO as shown in Table 1. This discrepancy may be due to the differences of molecular structures and sizes, however, the fundamental reaction based on hydroperoxy group seems to be the same. Thus TG hydroperoxides formed primarily in foods are considered to be as toxic as free fatty acid hydroperoxides.

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