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Preventive Action of Vegetables on Degradation of Methyl 13-Hydroperoxyoctadecadienoate

Md Khorshed Alam[a], Rumiko Shimizu, Eiko Yamada,
Naomichi Baba and Shuhei Nakajima
(Department of Bioresources Chemistry)

The preventive actions of some vegetables on the degradation of 13-hydroperoxyoctadecadienoate were investigated. Among these, kidney bean extract prevented the degradation of the hydroperoxide at about 60% followed by green pepper, cucumber, spinach, pumpkin, mushroom, carrot and shungiku to different degrees.

Key words : Methyl 13-hydroperoxy octadecadienoate; Degradation of hydroperoxide

Introduction

Interaction of lipid hydroperoxides and their degradation products with proteins and other biological components can affect vital cell functions by causing damage to membranes and enzymes[1]. Membrane deterioration caused by free radical and active oxygen mediated reactions may contribute to the aging process[2]. In complex food systems, the interactions of lipid hydroperoxides and their oxidation products with proteins and other components have important impacts on flavor stability and texture during processing, cooking and storage. Polyunsaturated fatty acids undergo autoxidation to give hydroperoxy forms[2,3] which are easily degraded to various oxidation products[1,4] that are called secondary products of lipid peroxidation. Secondary products, including polymers[5,6], aldehydes[7,8], hydroperoxyalkenals[9] and hydroperoxyepoxides[10] are known to be toxic in a number of biological systems. Thus, self-evident is the importance of development of new strategy for the prevention of autoxidation of unsaturated lipids whether their hydroperoxides themselves are toxic or not. It is also known that the use of antioxidants and the treatment or storage of foods containing unsaturated lipids at low temperature as well as exclusion of air have been major ways to minimize undesirable hydroperoxidation. On the other hand, there are very few studies on the prevention of degradation of lipid hydroperoxides to the toxic products described above. Recent studies suggest that hydroperoxy unsaturated fatty acids themselves seem to be not so toxic and can be metabolized in the digestive organs[5]. Viewed from the above situation, one important aspect of food and lipid peroxidation chemistry is to consider how such degradation can be prevented. There is a possibility that some vegetables might prevent the degradation of lipid hydroperoxides since the main source of food-related antioxidant is various kind of plants[11]. Interestingly, in recent years, there is strong evidence for an inverse relation between vegetable and fruit intake for cancers of the lung, oral cavity, larynx, pancreas, bladder and cervix[12,13].

The objective of this study is to evaluate the effect of vegetable extract on the prevention of degradation of one model compound for hydroperoxidized lipid : methyl 13-hydroperoxy octadecadienoate (ML-OOH).

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a) *Graduate School of Natural Science and Technology*

Materials and Methods

Linoleic acid was purchased from Tokyo Kasei Chemical Co. (Tokyo, Japan), soybean lipoxygenase from Sigma Chemical Co. (St. Louis, Mo. USA). ML-OOH was prepared by enzymatic oxidation of linoleic acid using soybean lipoxygenase, and the carboxyl group was methylated with diazomethane in 70% yield after column purification. Fresh vegetables purchased from local market were washed 3 times thoroughly in tap and deionized water. The washed vegetables were homogenized and shaken vigorously in a separatory funnel with $CHCl_3$: MeOH (3:1). Lower organic phase was taken and the solvent was evaporated in vacuo. To observe the preventive action of vegetables on degradation of ML-OOH, the hydroperoxide (100 mg) was mixed with vegetable extract (660 mg) in $CHCl_3$ (5 ml) and then stirred at 60℃ in the dark. A control (experiment) was carried out taking ML-OOH (100 mg) in $CHCl_3$ (5 ml) and stirred under the same conditions. Aliquots were taken at 0, 10, 24, 48, 72 and 96 h and the remaining hydroperoxide was isolated by silica gel column chromatography using hexane : ethyl acetate (100 : 0 → 90 : 10 → 80 : 20). The weight of the isolated ML-OOH was measured and the percentage of the weight to the ML-OOH added was regarded as % preventive activity (Fig. 1). The purity of the hydroperoxide was confirmed by ^{1}H NMR and peroxide value. The reliability of this quantitative determination was confirmed by ^{1}H NMR method using 1-naphthylethyl alcohol as an internal standard. The same procedure was followed for the study of the preventive action of kidney bean at different concentrations (Fig. 2). To observe the effect of heated kidney bean extract, the extract was heated without solvent for 20 minutes at 100℃ in water bath and the experiment was carried out as described above (Fig. 3). In another experiment, we separated the inner bean and outer shell of kidney beans and extracted them separately using the same solvent system used for whole vegetable extraction. These extracts were used to observe the effect of the beans and the shell on prevention of ML-OOH degradation where the experimental procedure was the same as for whole vegetables (Fig. 4).

Results and Discussions

Most foods contain quite complicated biomolecules, including oils and fats, and we sometimes consume them together with vegetables and fruits. It is known that these oils and fats

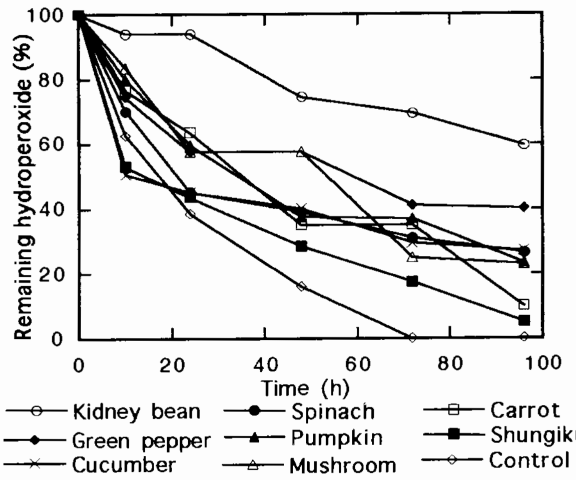

Fig. 1 Preventive action of vegetables on the degradation of methyl 13-hydroperoxy octadecadienoate.

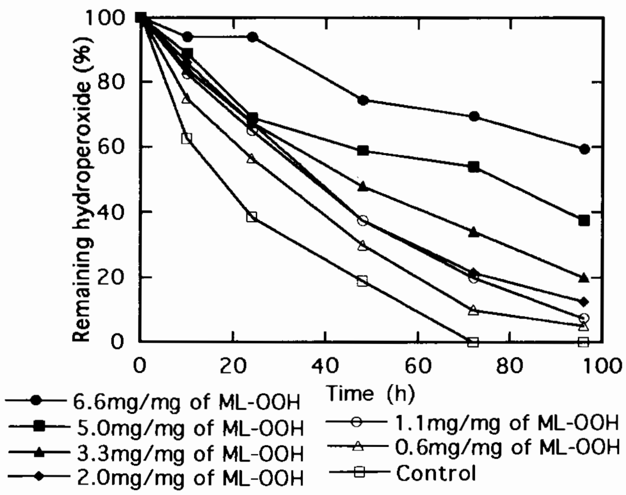

Fig. 2 Prevention of the degradation of methyl 13-hydroperoxy octadecadienoate by kidney bean at different concentrations.

are very often contaminated with lipid hydroperoxides, particularly in the case of stale foods. Therefore, it seems rational to investigate the interaction of vegetables with hydroperoxides. For the first time, however, we examined rather the lipophilic components of vegetables, since whole vegetables or plants have various biochemical functions, most of which are due to enzymes and may exert various chemical changes on external substances as substrates contained in other foods. For example, lipoxygenases from soybean, tomato, wheat etc. produce hydroperoxides of unsaturated fatty acids, and hydroperoxide lyases[14] in various plants can decompose those hydroperoxides to afford different aldehydes and other compounds. On the other hand, lipophilic components of vegetables may contain vegetable oils including triglycerides as well as glycerophospholipids, chlorophylls and other small lipophilic compounds like naturally occurring antioxidants. However, these lipophilic fractions from vegetables do not have any enzymatic activities. Thus, chloroform-methanol soluble fractions of vegetables were examined in the present study.

The vegetable extracts, after evaporation of the solvent were mixed with pure ML-OOH prepared from linoleic acid using soybean lipoxygenase and the mixture was stirred under conditions described in the experimental section. The remaining hydroperoxide fraction was isolated by silica gel column chromatography eluted with hexane-ethyl acetate and the amount was determined directly by weighing. This remaining hydroperoxide fraction was confirmed by its brown color on TLC with potassium iodide solution. Fig. 1 shows the preventive action of vegetables, namely spinach (*Spinacia oleracea* L.), *Chrysanthemum coronarium* L. var. spatiosum Bailey (Japanese name shungiku), pumpkin (*Cucurbita moschata* Duch. var. Toonas Makino), green pepper (*Capsicum annuum* L. var. angulosum Mill), kidney bean (*Phaseolus vulgaris* L.), mushroom (*Flammulina velutipes* sing), carrot (*Panax schinseng* Nees) and cucumber (*Cucumis sativus* L.) on the degradation of ML-OOH. Preventive assessment was done separating the remaining ML-OOH and calculating the % of the remaining ML-OOH in the reaction mixture at a particular time which varied from vegetable to vegetable. Among the eight vegetables, the highest preventive activity was found in kidney bean followed by green pepper, cucumber, spinach, pumpkin, mushroom, carrot and shungiku. In case of kidney bean, which showed the highest preventive activity, about 60% of the added hydroperoxide

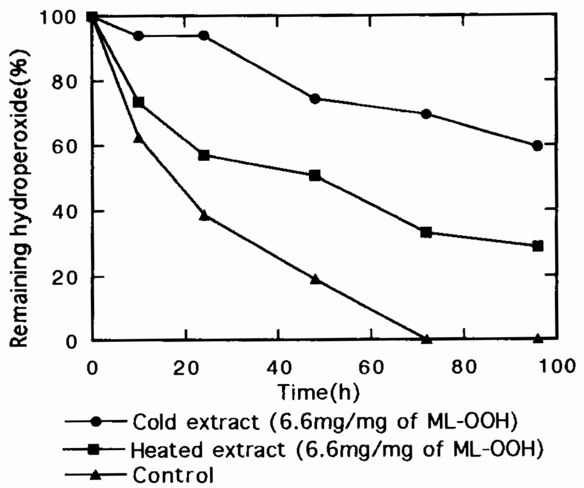

Fig. 3 Preventive action of kidney bean with and without heat treatment on the degradation of methyl 13-hydroperoxy octadecadienoate.

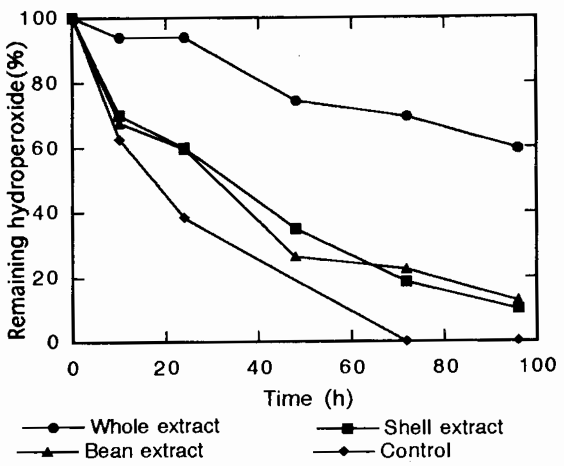

Fig. 4 Preventive action of kidney bean shell and bean on the degradation of methyl 13-hydroperoxy octadecadienoate.

remained undegraded even at 96 h and 60℃. However, in the control run case, the added hydroperoxide was completely depleted within 72 h. As evident from Fig. 1, the average degradation rate of ML-OOH in control run was 0.28 mg/h whereas in presence of kidney bean, the degradation rate reduced to 0.08 mg/h, i.e., about one third of the control value. Shungiku showed least activity and the average degradation rate is very near to the control.

Fig. 2 shows that the preventive action of kidney bean on degradation of ML-OOH is related to vegetable extract concentrations and preventive activity decreases as the concentration is decreased. In the case of kidney bean, 5% of the added ML-OOH still remains undegraded at 96h even when the concentration is reduced to one tenth of its original concentration. It is thus evident that kidney bean extract can indeed prevent the degradation of ML-OOH. To simulate the cooking conditions of vegetables, kidney bean extract was heated for 20 min. at 100℃ after removal of the solvent in order to evaluate whether the preventive activity of kidney bean on degradation of linoleic acid hydroperoxide is affected by heat. Fig. 3 reveals that kidney bean extract retains the preventive activity against degradation even after the heat treatment although the activity was almost halved in comparison to unheated extract. Pinsky et al[15] reported that some vegetables and fruits have lipoxygenase inhibitory activity that might be due to the presence of antioxidants in those vegetables and fruits. This enzyme promotes the oxidation of lipids containing unsaturated fatty acids providing hydroperoxides[16]. Thus the above results suggest that vegetables prevent degradation of ML-OOH, and kidney bean was found to have the highest activity among the tested vegetables. A further experiment was conducted in which beans and shells of the kidney bean were examined separately for preventive activity. As shown in Fig. 4, both activities were reduced drastically. This indicates that the activity might be responsible for some complex system involving chemical and/or physicochemical factors rather than a simple chemical principle like antioxidant. Although the mechanism of the preventive action is unknown, the results suggest the importance of the interactions between foods containing lipid hydroperoxides and fresh vegetables when we intake both at the same time, as well as during the process of cooking them together.

References

1) Frankel, E. N. : Secondary products of lipid oxidation. *Chem. & Phys. lipids,* **44**, 73-85 (1987).

2) Pryor, W. A. : The role of free radical reactions in biological systems. in *Free Radicals in Biology,* (Pryor, W. A. ed.), pp. 1-49, Academic Press, New York, (1976).

3) Mead, J. F. : Free radical mechanisms of lipid damage and consequences for cellular membranes. in *Free Radicals in Biology*, (Pryor, W. A. ed.), pp. 51-68, Academic Press, New York, 1976.

4) Esterbauer, H. : Aldehydic products of lipid peroxidation. in *Free Radicals, Lipid Peroxidation and Cancer*, (McBrien, D. C. H. and T. F. Slater eds.), pp. 101-128, Academic Press, London, 1982.

5) Ohfuji, T. and T. Kaneda : Characterization of toxic compound in thermally oxidized oil. *Lipids*, **8**, 353-359 (1973).

6) Morita, M. and M. Tokita : Methyl linoleate dimer and methyl 8- phenyl octanoate in the copper catalyzed decomposition products of methyl linoleate hydroperoxides. *Agric. Biol. Chem.*, **48**, 2567-2568 (1984).

7) Dianzani, M. U. : Biochemical effects of saturated and unsaturated aldehydes. in *Free Radicals, Lipid Peroxidation and Cancer*, (McBrien, D. C. H. and T. F. Slater eds.), pp. 129-158, Academic Press, London, 1982,.

8) Summerfield, F. W. and A. L. Tappel : Determination by fluorescence quenching of the environment of DNA crosslinks made by malondialdehyde. Biochim. *Biophys. Acta*, **740**, 185-189 (1983).

9) Yoshioka, M. and T. Kaneda : Studies on the toxicity of the autoxidized oils : the toxicity of hydroperoxyalkenals. *Yukagaku*, **23**, 321-326 (1974).

10) Imagawa, T., S. Kasai, K. Matsui and T. Nakamura : Detrimental effects of methyl hydroperxy-epoxy-octadedecenoate on mitochondrial respiration. *J. Biochem.*, **94**, 87-96 (1983).
11) Larson, R. A. : The antioxidants of higher plants. *Phytochemistry*, **27**, 969-978 (1988).
12) Peto, R., R. Doll, J. D. Buckley and M. B. Sporn : Can dietary beta-carotene materially reduce human cancer rates? *Nature*, **290**, 201-208 (1981).
13) Willett, W. C. : Diet and health : What should we eat? *Science*, **264**, 532-537 (1994).
14) Gardner, H. W. : Recent investigations into the lipoxygenase pathway of plants. *Biochim. Biophys. Acta*, **1084**, 221-239 (1991).
15) Pinsky, A., S. Grossman and M. Trop : Lipoxygenase content and antioxidant activity of some fruits and vegetables. *J. Food Sci.*, **36**, 571-572 (1971).
16) Kaneko, T., K. Kaji and M. Matsuo : Cytotoxicities of a linoleic acid hydroperoxide and its related aliphatic aldehydes toward cultured human umbilical vein endothelial cells. *Chem. Biol. Interactions*, **67**, 295-304 (1988).

Methyl 13-hydroperoxyoctadecadienoate の分解に対する蔬菜類の抑制効果

モハムド　コルシェド　アラム・清水るみ子・山田　英子
馬場　直道・中島　修平
（生物資源開発学講座）

不飽和脂質の酸化防止に関する研究は今日なお活発に行われており，その食品学的重要性と問題の難しさには変わり無い。自動酸化によって生成した過酸化物は極めて容易に分解し，その分解生成物は食品の重大な劣化を招き，健康を害することは良く知られている。本研究ではMethyl 13-hydroperoxyoctadecadienoateの分解の抑制について検討したところ，ある種の蔬菜のクロロホルム－メタノール抽出物に分解阻止効果があることが明らかになった。例えばいんげん豆抽出物は過酸化物に対して，60℃，96時間の加熱においても約60％の分解抑制作用を示すことが判明した。