イワシ肉中の脂質酸化に及ぼす高圧処理の影響

誌名	日本水産學會誌
ISSN	00215392
著者	田中, 宗彦 卓, 雪儀 長島, 裕二 田口, 武
巻/号	57巻5号
掲載ページ	p. 957-963
発行年月	1991年5月

Nippon Suisan Gakkaishi 57(5), 957-963 (1991)

Effect of High Pressure on the Lipid Oxidation in Sardine Meat

Munehiko Tanaka,* Zhuo Xueyi,* Yuji Nagashima,*
and Takeshi Taguchi*

(Received November 19, 1990)

The model systems consisting of sardine lipids and defatted meat were prepared and pressurized at 1,800 atm. After the pressure was released, the model systems were stored at 5°C. The effect of high pressure treatment on the oxidation of sardine lipids during storage was evaluated by using such oxidation indices as POV, TBA number, oxygen absorption, UV absorption, browning, and fluorescence. It was revealed that the oxidation of sardine lipids was accelerated by the pressure treatment and its extent was relative to intensity and duration of the treatment. In the model system prepared with sardine meat washed with water, the rate of lipid oxidation decreased and was fairly related to the amount of heme iron. When the water content of the model system was altered, the oxidation rates of sardine lipids were 7%>35%>24%>17%. At 17 and 24% water contents, no appreciable oxidation proceeded during the storage of 4 days. On the other hand, at higher water contents the oxidation rate of sardine lipids in the pressurized model system was lower than that in the unpressurized system.

Pressure, which is one of the thermodynamic factors together with heat, has been utilized only to some extent for food processing compared with heat. The main reasons for this are firstly the delayed development of pressure apparatus and secondly the limited processing capacity which is as important factor for food industries. However, because of the recent development of high pressure apparatus, increasing emphasis on pressure treatment as a new alternative food processing technique is focusing attention on the importance of expanding and updating information concerning the effect of high pressure treatment on food quality.

For heat processing it usually takes a fairly long time for heat to penetrate (or conduct), so certain unit operations such as stirring are necessary at the same time. On the other hand, the application of pressure is homogeneous as well as instantaneous irrespective of the shape of food materials, and there is no significant damage to food appearance during processing.

Studies concerning the effect of high pressure during food processing on food quality have been so far conducted mostly on protein denaturation,[1] gel formation,[2,3] enzyme activity,[4] and survival of microorganisms.[5] However, practically no work has been done on lipid which is one of the main constituents of foods.

The aim of this investigation was to determine the effect of high pressure on the oxidation of sardine lipids during the subsequent storage at 5°C.

Materials and Methods

Preparation of the Model System

Fresh sardine *Sardinops melanostictus* (average body weight, 120 g; average body length, 25 cm) were used. After heads, fins, viscera and skins were removed, sardines were dressed and minced by a meat chopper. Minced sardine meat was then freeze-dried. For another preparation of the model system, minced sardine meat was washed once or three times with 5 volumes of ice-cold water (15 min each washing) and freeze-dried. Sardine lipids were extracted four times by chloroform-methanol (2: 1, v/v) from the freeze-dried meat. Each defatted sardine meat thus prepared was mixed thoroughly with sardine lipids obtained from the unwashed sardine meat at the concentration of 20% (w/w). In order to prepare another model system, distilled water was added to the defatted unwashed sardine meat to alter the water content of the model system, to which sardine lipids were mixed in the same manner.

* Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato, Tokyo 108, Japan (田中宗彦，卓　雪儀，長島裕二，田口　武：東京水産大学食品生産学科).

High Pressure Treatment

Each 50 g of the model system thus prepared was vacuum-packed and compressed at 500, 1,000, and 1,800 atm at room temperature for 30 and 60 min, respectively, with a hand-type oil pressure apparatus (Hikari Koatu Co.). After pressure treatment, samples were ground in a mortar with a pestle. Two g portions were placed in an erlenmyer flask (100 m*l*) or 100 m*l* vial with a rubber cap and stored at 5°C in the dark for up to 4 days. In order to determine the effect of pressure on the oxidation of sardine lipids without the presence of sardine meat, sardine lipids in a small collapsible polyethylene bottle (approximately 5 m*l* in volume) were compressed in the same manner. Aliquots (0.4 g) of pressurized sardine lipids were stored under the same condition used for the model system experiments.

Analytical Procedures

After storage at 5°C, sardine lipids in each model system were re-extracted with chloroform-methanol (2: 1, v/v) and subjected to the following analyses.

Peroxide value (POV) of lipids extracted was determined by the iodometric technique[6] and 2-thiobarbituric acid (TBA) number was measured according to the procedure reported by Sinnhuber and Yu.[7] The TBA number was expressed as mg of malonaldehyde per kg of lipids. Oxygen absorption in the head space of a 100 m*l* vial was determined by gas chromatography with a thermo-conductivity detector (Model 373, Gasukuro Kogyo Co.).[8]

Brown color development of sardine lipids was measured by the absorbance at 420 nm of a 2% chloroform-methanol solution. Ultraviolet absorption spectra of a 0.2% chloroform-methanol solution were obtained by a Shimadzu UV-Visible Recording Spectrophotometer type UV-160. Fluorescence intensity of a 0.2% chloroform-methanol solution was measured with a Spectro-fluorometer (Model EP-770, Japan Spectronscopic Co.) with excitation at 380 nm and emission at 440 nm.[9] The fluorescence intensity was compared with that of 1 ppm quinine sulfate in 0.1 N H_2SO_4. Nonheme iron was assayed by the modified Schricker method,[10] and heme iron was calculated as the difference between total iron and nonheme iron. Each analytical component of this study, including preparation of the model systems was repeated 3～5 times. Typical examples of results are reported in this study.

Results and Discussion

Preliminary Experiments

Prior to the experiments with the model systems prepared, high pressure (up to 5,000 atm) was applied to sardine lipids without the presence of sardine meat to elucidate whether pressure influences the oxidation of sardine lipids *per se* during treatment and storage. Sardine lipids obtained from the freeze-dried meat were placed in a small collapsible polyethylene bottle (about 5 m*l* in volume) and pressurized up to 5,000 atm for 60 min at room temperature by a hand-type hydrostatic pressure apparatus (Type KP5B, Hikari Koatu Co.). The POV of sardine lipids to be pressurized was varied between 10 and 40 meq/kg.

Although data not shown, it was found out that POV and TBA numbers of sardine lipids did not change during pressure treatment regardless of pressure intensity and the POV of the starting sardine lipids. However, the oxidation of pressurized sardine lipids proceeded slightly faster than that of unpressurized lipids at the later stage of storge at 30°C. The tendencies observed in the preliminary experiments are agreeable with data reported by Wada *et al.**1 It is concluded that sardine lipids *per se* are not affected by pressure treatment as far as their oxidation is concerned. On the contrary, Ohshima *et al.**2 and Wada *et al.**3 observed that pressure treatment on raw fish meat promoted the oxidation of its lipids during subsequent storage, suggesting that the interaction of lipids with proteins is enhanced by pressure treatment. Considering such observations so far reported, the following studies with the model systems were conducted to clarify the effect of pressure treatment on the oxidation of sardine lipids.

---

*1 S. Wada, S. Ide, T. Mihori, and H. Suzuki: Abstract of papers at the meeting of Japanese Society of Scientific Fisheries (Miyazaki), 1989, p. 205.

*2 T. Ohshima, T. Nakagawa, and C. Koizumi: Abstract of papers at the meeting of Japanese Society Society of Scientific Fisheries (Tokyo), 1989, p. 210.

*3 S. Wada, S. Ide, T. Suzuki, H. Yamanaka, and H. Suzuki: Abstract of papers at the meeting of Japanese Society of Scientific Fisheries (Tokyo), 1990, p. 263.

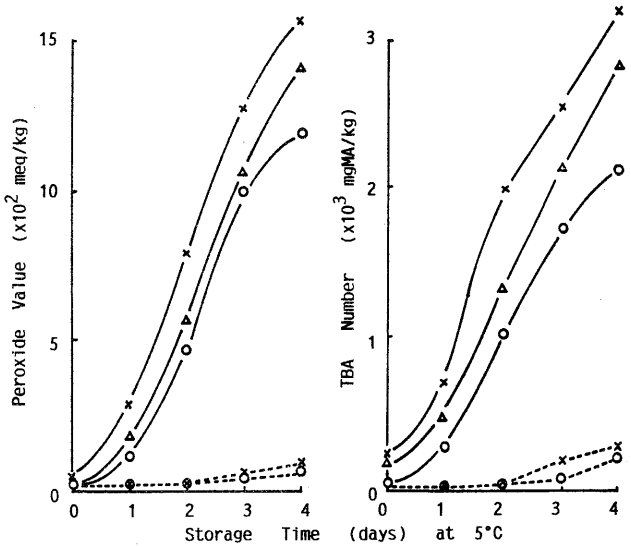

Fig. 1. Changes in peroxide value and TBA number of sardine lipids in the model systems during storage at 5°C in the dark. The model systems were pressurized at 1,800 atm.
——, With meat; ------, without meat.
○, Pressurized for 0 min (control); △, for 30 min; ×, for 50 min.

Effect of Intensity and Duration of Pressure Treatment

The model system consisting of defatted unwashed sardine meat and lipids (20%, w/w) was compressed at 500, 1,000, and 1,800 atm for 30 and 60 min, then stored at 5°C in the dark. Fig. 1 shows changes in POV and TBA number of sardine lipids treated at 1,800 atm during storage. Sardine lipids pressurized without meat were oxidized only to some extent during storage, but the presence of meat significantly accelerated the oxidation even without pressurization. The extent of oxidation further increased by high pressure treatment. Although the data are not shown in this paper, it was found out that the oxidation of sardine lipids with meat was faster with intensity and duration of pressure treatment. It is noteworthy that this tendency was also observed even during pressure treatment, though lipids without meat were not oxidized as was described in the preliminary exeperiments. The accelerated oxidation might be due to denaturation of heme proteins by pressure which facilitates more exposure of the heme to lipids, but we are not sure at this moment if pressurization at 1,800 atm causes the denaturation of heme proteins. However, the interaction between sardine lipids and proteins enhanced by high pressure treatment seemed to play an important role in the acceleration of sardine lipid oxidation, because the oxidation

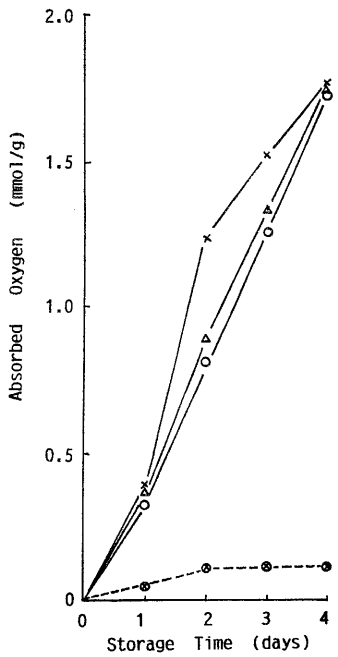

Fig. 2. Changes in oxygen absorption of sardine lipids in the model systems during storage at 5°C in the dark. The model systems were treated at 1,800 atm.
——, With meat; ------, without meat.
○, Pressurized for 0 min; △, for 30 min; ×, for 60 min.

rate of sardine lipids which were mixed with the sardine meat pressurized in advance was nearly equal to that of the control (data not shown).

In this paper, data obtained only at 1,800 atm pressurization are presented, since the effect of this pressure on the oxidation of sardine lipids was most prominent among pressure treatments used in this study. Accelerated oxidation of sardine lipids in the presence of meat by pressure was also affirmed by the oxygen absorption rate of the model systems (Fig. 2). The amounts of oxygen absorbed by lipids during storage also increased with intensity and duration of pressure treatment. However, as is shown in Fig. 2, oxygen absorption by lipids between 0 and 30 min-treatments or at the later storage of 60 min-treatment was not so different as was the development of POV.

Changes in the maximum UV absorbance of sardine lipids (0.2% chloroform-methanol solution) during storage are illustrated in Fig. 3. The maximum absorption was observed at the region between 240 and 250 nm as is given in the inserted figure of Fig. 3, and the maximum absorption tended to shift to longer wavelength during storage. It is obvious from Fig. 3 that the changes of the maximum UV absorbance were fairly similar to those of POV and TBA numbers, though they plateaued out at the later stage of storage. Hence, it is proposed that the maximum absorbance at the wavelength between 240 and 250 nm can be used as an index of the initial sardine lipid oxidation. Ultraviolet absorbance at 233 nm due to conjugated diene in polyunsaturated fatty acids has been employed for the evaluation of the oxidation degree of such fatty acids,[11] but it is not apparent what is responsible for the maximum absorption between 240 and 250 nm of sardine lipids.

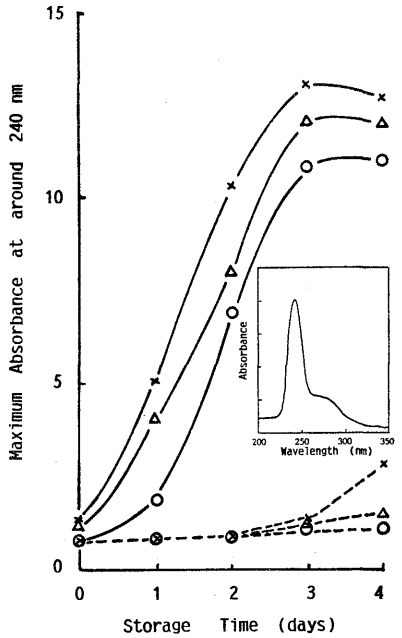

Fig. 3. Changes in UV maximum absorbance of sardine lipids (0.2%) in the model systems at around 240 nm during storage at 5°C in the dark. The model systems were pressurized at 1,800 atm. The insert was the typical UV absorption spectrum of sardine lipids.
——, With meat; ------, without meat.
○, Pressurized for 0 min; △, for 30 min; ×, for 60 min.

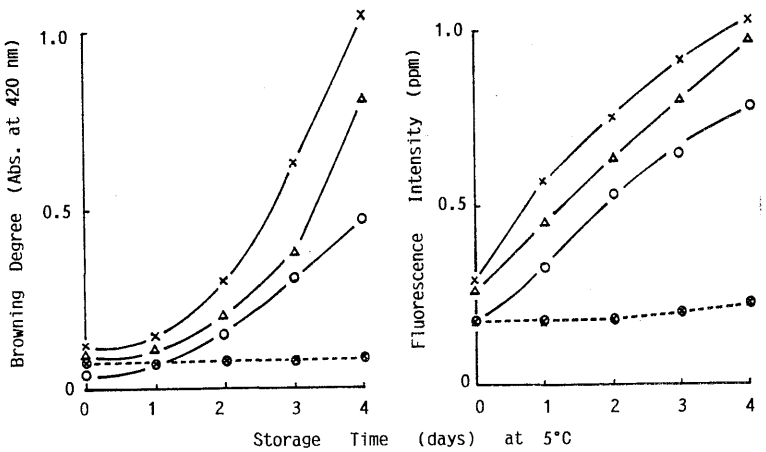

Fig. 4. Developments of brown color and fluorescence in sardine lipids during storage at 5°C in the dark. The model systems were pressurized at 1,800 atm.
——, With meat; ------, without meat.
○, Pressurized for 0 min; △, for 30 min; ×, for 60 min.

Cho *et al.*[9] suggested the determination of relatively stable secondary or termination products of lipid autoxidation such as polymers, fluorescence and browning for the evaluation of the oxidative deterioration in fish products. Figure 4 shows the time courses of browning and fluorescence development during storage of sardine lipids with meat. Development of browning was promoted by pressure and concomitant with intensity and duration of pressure treatment. Since the induction period of browning development was longer than that of POV or TBA number (Fig. 1), it was concluded that brown pigments were formed by the nonenzymatic reaction between sardine meat components and secondary products of lipid oxidation such as aldehydes. However, it was confirmed by the tristimulus color determination that re-defatted sardine meat *per se* did not brown during storage, whereas the model systems became slightly darker during high pressure treatment.

Furthermore, secondary products of lipid autoxidation such as malonaldehyde are known to react with amino acids, proteins, and amino-containing phospholipids to form fluorescent products.[12-14] Therefore the measurement of fluorescent products which are soluble in chloroform-methanol has been successfully used as a quantification method of lipid deterioration.[12,15] Fluorescence of sardine lipids increased almost linearly without an induction period as given in Fig. 4, indicating that the formation of fluorescent products by the browning reaction preceded that of brown pigments. Neither browning nor fluorescence development was observed when sardine lipids were compressed without sardine meat.

In conclusion, the oxidation of sardine lipids in the presence of meat was found to be accelerated during pressure treatment and the subsequent storage at 5°C in the dark. The pressure-induced oxidation was a function of intensity and duration of pressure treatment, so that pressurization in the following experiments was carried out at 1,800 atm for 60 min.

Effect of Washing Meat with Water

In order to figure out which components in sardine meat are responsible for the induced oxidation of sardine lipids by pressurization, minced sardine meat was washed with cold water

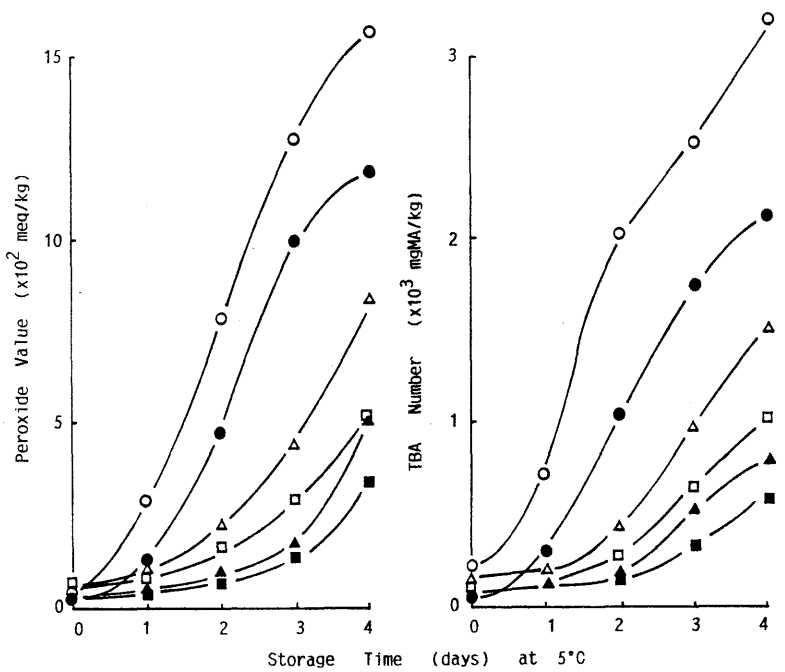

Fig. 5. Effect of washing meat on peroxide value and TBA number developments of sardine lipids during storage at 5°C in the dark. The model systems were pressurized at 1,800 atm for 60 min.

○, Without washing; △, washed once; □, washed three times. ●, ▲, ■; Without pressurization.

Table 1. Iron levels of defatted sardine meat prepared with and without water-washing treatment (μg/g dry wt)

Number of washing	Total Fe	Nonheme Fe	Heme Fe
0	105.3	82.6	22.7
1	95.2 (9.6)	74.0 (10.4)	21.2 (6.6)
3	83.2 (21.0)	65.7 (20.5)	17.5 (22.9)

( ): % decrease by water-washing treatment.
Nonheme Fe was determined by the modified Schricker method (bathophenanthroline disulfonate reagent).

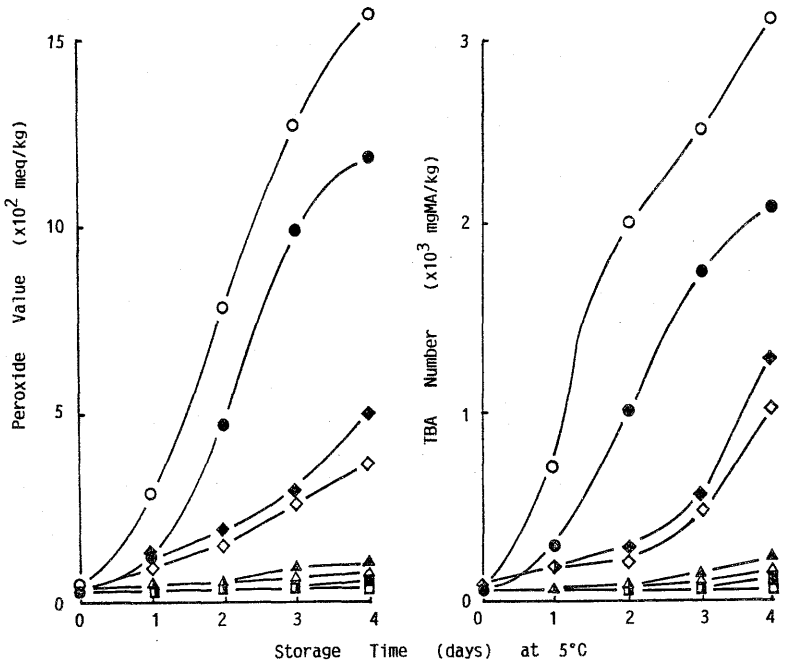

Fig. 6. Effect of water content on peroxide value and TBA number developments of sardine lipids during storage at 5°C in the dark. The model systems were treated at 1,800 atm for 60 min.

○, Water content 7%; □, 17%; △, 24%; ◇, 35%. ●, ■, ▲, ◆; Without pressure treatment.

(known as *mizu-sarashi* treatment in Japan) once or three times. Figure 5 represents changes in POV and TBA number of sardine lipids in the model systems prepared from unwashed and washed meat. Pressure treatment was conducted at 1,800 atm for 60 min. It is evident from the figure that the development of lipid oxidation in the model system became slower with repetition of water-washing treatment. However, the pressure treatment exerted the promotion of sardine lipid oxidation even in the three times-washed model system.

Changes of oxygen absorption and maximum UV absorption as well as developments of browning and fluorescence in sardine lipids during storage are all similar to the tendency presented in Fig. 5 (data not shown). In is suggested that there are some water soluble components present in sardine meat which accelerate the lipid oxidation. Judging from the knowledge accumulated so far, the most possible component responsible would be iron in sardine meat. Table I indicates iron levels of the model systems prepared with and without water-washing treatment. Approximately 10% of total iron was removed by the first washing treatment in which nonheme iron was eliminated faster than heme iron. Repetition of water-washing treatment seemed to remove more heme iron. Nevertheless, water-washing treatment of sardine meat decreased the rate of lipid oxidation as was obvious from Fig. 5, but there was enough iron still left in the meat washed three times to accelerate the lipid oxidation.

Effect of Water Content in the Model System

It is well known that the rate of lipid oxidation is dependent upon the water content (water activity, Aw).[16] Water contents of the model systems in this study were adjusted to 7.1, 16.7, 23.7, and 35.3%, respectively. Their water activities were 0.65, 0.86, 0.92, and 0.97, respectively. Effects of water content on POV and TBA number developments of sardine lipids during storage at 5°C are shown in Fig. 6. The oxidation rates of sardine lipids during storage were quite slow at water contents of 17% (Aw 0.86) and 24% (Aw 0.92). Further increase in water content (35%, Aw 0.97) promoted oxidation, but it was far slower than at water content of 7% (Aw 0.65). It is noteworthy that pressurized sardine lipids in the model systems were less oxidized at the higher water contents, particularly at 35%.

The similar tendency was also observed for the developments of color, fluorescence and maximum UV absorption as well as the amount of oxygen absorbed (data not shown), indicating that pressure treatment at 1,800 atm for 60 min has a reverse effect on sardine lipid oxidation at higher water contents. However, the reason for this peculiar phenomenon is not clear at this time.

According to Labuza *et al.*,[17] a food at Aw=0 oxidizes quite rapidly and the rate decreases with increase in Aw up to about 0.5. They suggested that this was due to decreasing effectiveness of metal catalysts and hydrogen bonding of peroxides because of hydration. Above Aw=0.5, the rate of lipid oxidation is then accelerated proportionally with increase of Aw. The transition point from decreasing to increasing rates of sardine lipid oxidation in the model system used in this study appears approximately Aw 0.7.

Acknowledgment

This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (No. 01560222).

References

1) J. J. Macfarlane: *J. Food Sci.*, **39**, 542–547 (1974).
2) T. Suzuki and J. J. Macfarlane: *Meat Sci.*, **11**, 263–274 (1984).
3) R. Hayashi, Y. Kawamura, T. Nakasa, and O. Okinaka: *Agric. Biol. Chem.*, **53**, 2935–2939 (1989).
4) L. B. Kurth: *J. Food Sci.*, **51**, 663–667 (1986).
5) D. G. Hoover, C. Metrick, A. M. Papineau, D. F. Farkas, and D. Knorr: *Food Technol.*, **43**(3), 99–107 (1989).
6) A. O. A. C.: in "Official Methods of Anaysis of A. O. A. C." (ed. by W. Horwitz), Washington, D. C., 1970, pp. 445–446.
7) R. O. Sinnhuber and T. C. Yu: *Food Technol.*, **12**, 9–12 (1958).
8) H. W. Gardner: *J. Agric. Food Chem.*, **23**, 129–136 (1975).
9) S. Y. Cho, Y. Endo, K. Fujimoto, and T. Kaneda: *Nippon Suisan Gakkaishi*, **55**, 545–552 (1989).
10) K. S. Rhee and Y. A. Ziprim: *J. Food Sci.*, **52**, 1174–1176 (1987).
11) R. Yamauchi, T. Yamada, K. Kato, and Y. Ueno: *Agric. Biol. Chem.*, **47**, 2897–2902 (1983).
12) R. Trombly and A. L. Tappel: *Lipids*, **10**, 441–447 (1975).
13) H. A. Buttkus: *J. Food Sci.*, **32**, 432–434 (1967).
14) C. J. Dillard and A. L. Tappel: *Lipids*, **8**, 183–189 (1973).
15) B. L. Fletcher, C. J. Dillard, and A. L. Tappel: *Anal. Biochem.*, **52**, 1–9 (1973).
16) T. P. Labuza, L. McNally, D. Gallagher, J. Hawkes, and F. Hurtado: *J. Food Sci.*, **37**, 154–159 (1972).
17) T. P. Labuza, H. Tsuyuki, and M. Karel: *J. Am. Oil Chem. Soc.*, **46**, 409–416 (1969).