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Food Chemistry 82 (2003) 455–463

Food Chemistry

www.elsevier.com/locate/foodchem

Rapid communication

Inhibitory effect of oxidized lipid on the thermal gelation of Alaska pollack (*Theragra chalcogramma*) surimi

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Received 17 December 2002; received in revised form 17 February 2003; accepted 17 February 2003

Abstract

The influence of incorporated oxidized lipid on the thermal gelation of Alaska pollack (*Theragra chalcogramma*) surimi was investigated. Cod liver oil was intentionally oxidized by aeration at 45 °C in the dark for up to 7 days. Cod liver oil oxidized for 2 days (2-CLO) or 5 days (5-CLO) was incorporated at a level of 10% into surimi and suwari gels and kamaboko gels were prepared. The addition of oxidized lipid, especially 5-CLO, inhibited the normal gelation of surimi and it was more profound for the kamaboko gels. SDS–PAGE patterns revealed that the polymerization of myosin heavy chain was inhibited by the addition of 5-CLO. The inhibitory substances for surimi gelation were found to be present mostly in the water-soluble fraction of 5-CLO.

Keywords: Frozen surimi; Oxidized lipid; Cod liver oil; Gelation; Alaska pollack

1. Introduction

Surimi is a refined fish protein product manufactured by washing mechanically deboned fish with cold water to remove blood, lipids, enzymes and sarcoplasmic proteins. The ultimate concentration of myofibrillar proteins resulting from the washing process helps to form an elastic gel when solubilized with NaCl and heated. A variety of formulated seafood analogues, such as crab legs and scallops, have been produced from Alaska pollack surimi. The remarkable gelling properties of surimi have also been utilized for the manufacture of new products by modifying the texture of surimi gels by combination with other gelling and non-gelling ingredients (Lanier, 1986). Lipid is one such ingredient which can be incorporated into surimi products to give rise to modified textural characteristics. In fact, vegetable and animal lipids are often added to surimi products, such as the sausage-type products in Japan, since fish meat can produce a stable emulsion with them (Lee, 1986; Lee, Carroll, & Abdollahi, 1981).

On the other hand, the addition of lipids to surimi products may bring about an adverse effect on the their quality, because the oxidized lipids interact with proteins, causing denaturation, polymerization and changes in functional properties (Funes, Weiss, & Karel, 1982; Smith, 1987). Fish lipids are well known to have a high content of polyunsaturated fatty acids, such as eicosapentanoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) which have health promotion and cardiovascular effects (Leaf & Weber, 1988), but they are fairly susceptible to oxidation, leading to a number of complex chemical changes that eventually give rise to the development of off-flavours in foods, as well as the generation of harmful oxidation products (Cho, Miyashita, Miyazawa, Fujimoto & Kaneda, 1987; Fritsche & Johnston, 1988).

Information on the incorporation of lipid, although not scarce, is of limited value, because it is found mostly in the patent literature and in other publications where experimental detail and quantitative results are not

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0308-8146/03/$ - see front matter
doi:10.1016/S0308-8146(03)00103-1

available. Furthermore, little information has been published concerning the influence of lipid oxidation on the quality of surimi products (Kawasaki, Ooizumi, & Konno, 1992; Takama, Zama, & Igarashi, 1972). In this study, cod liver oil was intentionally oxidized and its effect on the thermal gelation of Alaska pollack surimi was investigated.

2. Materials and methods

2.1. Materials

Blocks (10 kg each) of frozen Alaska pollack (*Theragra chalcogramma*) were purchased from Kanai Gyogyo Company in Kushiro city, Hokkaido, Japan and stored at −35 °C during the study. Cod liver oil, obtained from Toho Yakuhin Co. (Tokyo, Japan), was used without further purification. Other reagents used in this study were obtained from Wako Pure Chemical Ind. Ltd (Tokyo, Japan).

2.2. Methods

2.2.1. Oxidation of cod liver oil

Cod liver oil was intentionally oxidized by air-bubbling at the rate of 100 ml min^{-1} with constant stirring by a magnetic bar at 45 °C in the dark for up to 7 days. The degree of oxidation was monitored using peroxide value (PV; Chapman & Mackay, 1949) and thiobarbituric acid (TBA; Shinnhuber & Yu, 1977) methods. Furthermore, oxidized cod liver oil was fractionated by thin-layer chromatography on Merck Kiesel gel 60 (thickness 0.25 mm) with the developing solvent mixture of petroleum ether, diethylether and acetic acid (80:20:1, v/v/v), and the fatty acid composition was determined by a gas chromatograph (Type GC-14B, Shimadzu Corp., Kyoto, Japan) with a Supelcowax-10 capillary column.

2.2.2. Incorporation of oxidized lipid into surimi product

The frozen surimi was tempered at 4 °C for 16–18 h and then chopped in a Stephan vertical vacuum cutter (model UMC-5, Stephan Machinery Corp., Hameln, Germany) connected to a constant temperature circulating chiller (model CA-1200, Eyela Corp., Tokyo, Japan). NaCl (2.5%) and cod liver oil (10%) were added as percentage by weight of surimi standardized at 80% water and chopped again for 5 min. After chopping, the meat paste was stuffed into stainless steel tubes (diameter 3 cm, height 3 cm). All procedures were conducted in a room maintained at 4 °C. The stuffed samples were heated in a water bath in two ways: heated at 30 °C (suwari or setting gel) for up to 120 min, and heated at 80 °C for 30 min after setting (kamaboko gel).

In another experiment, 10 g of 5-CLO were homogenized with 65 ml of distilled water at 400 rpm for 10 min to extract water-soluble substances. After leaving the mixture at room temperature for 10 min, the water-soluble and water-insoluble (oil) fractions were obtained by using a separatory funnel. The amount of water (65 ml) used to extract water-soluble substances was equivalent to that used to standardize the water content of Alaska pollack surimi (100 g) at 80%. After the water-soluble fraction or water-insoluble fraction was added to surimi instead of water or 5-CLO, the suwari and kamaboko gels were prepared by the same procedure as described earlier.

2.2.3. Evaluation of gel texture

The gels formed in the stainless steel tubes by the heat treatments described earlier were subjected to gel

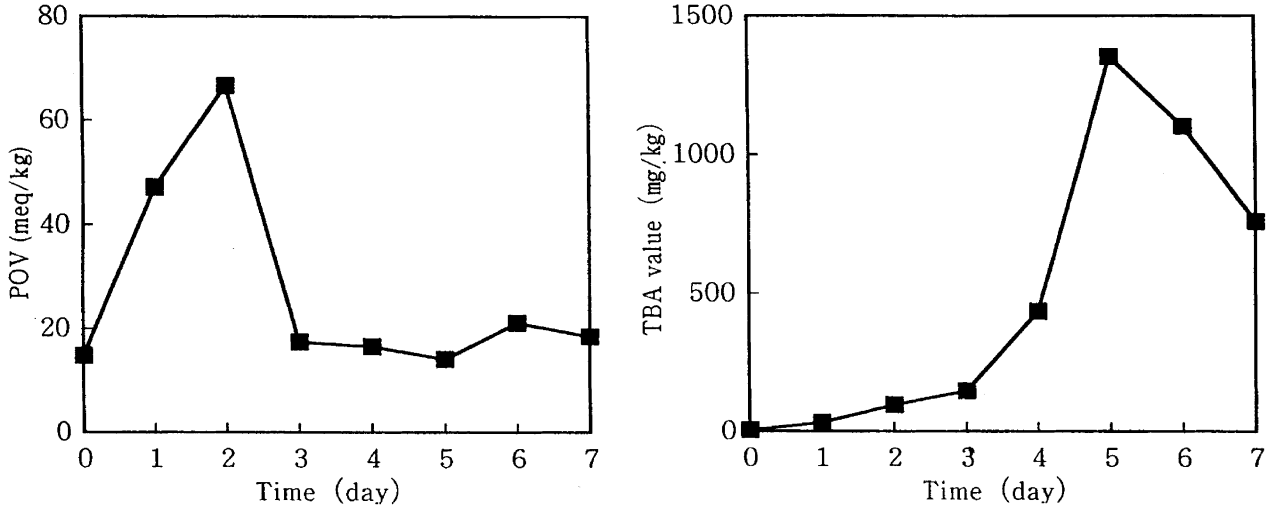

Fig. 1. Changes in POV and TBA value of cod liver oil during the oxidation. Cod liver oil was oxidized by aeration at 45 °C in the dark for 0–7 days.

strength measurement with a rheometer (Type SD-305, Sun Science Corp., Tokyo; spherical plunger, diameter 0.5 cm). Compression on the sample piece was executed with a table speed of 60 mm min^{-1}. The quality of the gels was assessed by measuring their breaking force (g) and breaking strain (cm). The averages with standard errors of eight measurements were calculated.

2.2.4. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

The gels were homogenized with the mixture of chloroform and methanol (1:2, v/v) to remove cod liver oil. Solubilization of the defatted gels for SDS–PAGE analysis was performed by the method of Numakura et al. (1985). SDS–PAGE was conducted in the presence of 0.1% SDS using 5–15% polyacrylamide gradient separating gel and 4.5% acrylamide stacking gel, according to the method of Laemmli (1970). The protein subunit components of the surimi gels were stained with 0.05% Coomassie Brilliant Blue R250 and scanned with a dual wavelength flying spot scanning densitometer (Type CS-9300PC, Shimadzu Corp., Kyoto, Japan).

2.2.5. Statistical analysis

Statistics on a completely randomized design were determined using the GLM procedure in SAS (1988). Duncan's multiple-range test ($P<0.05$) was used to determine significance of differences between means.

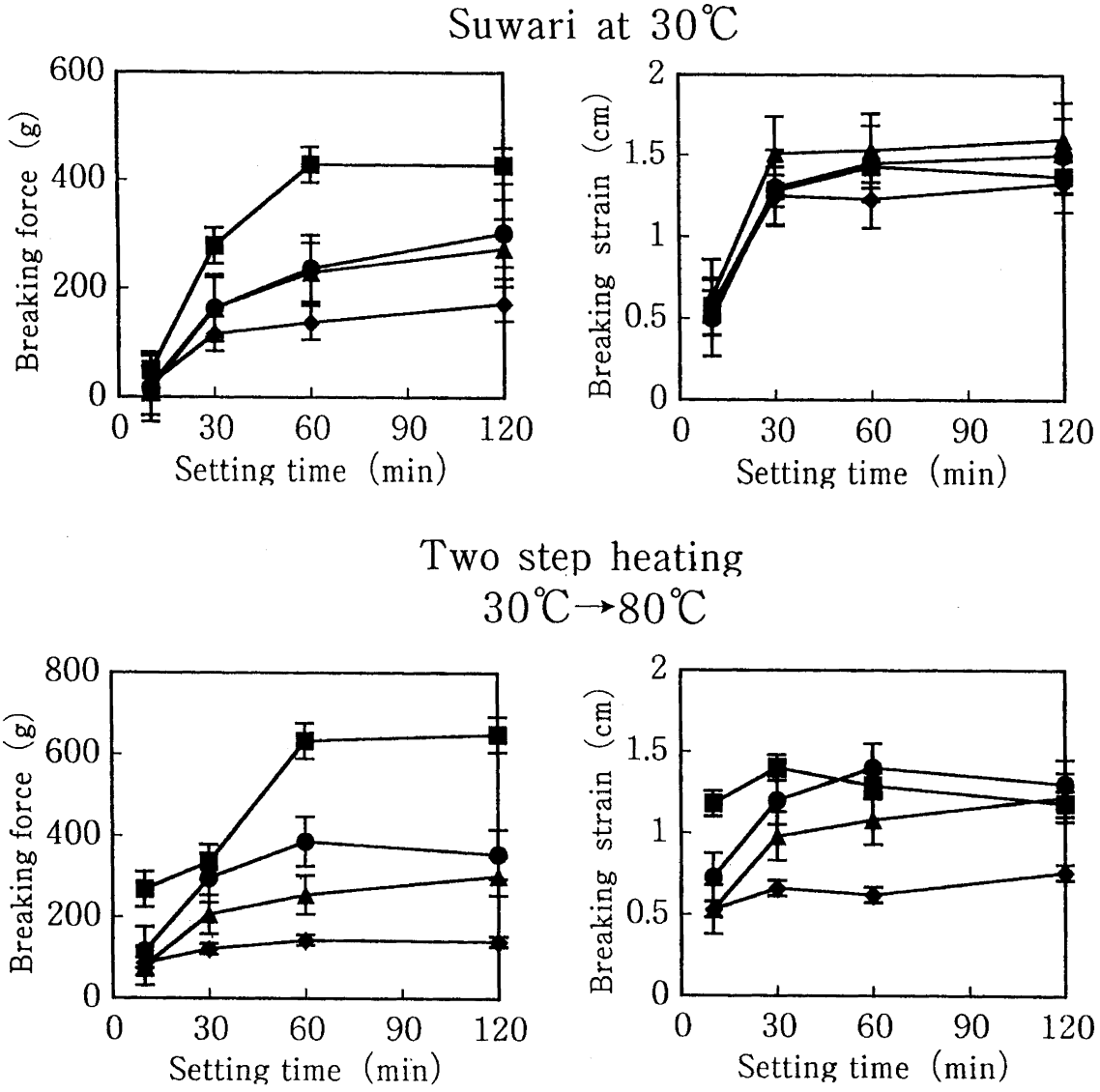

Fig. 2. Effect of oxidized cod liver oil on the breaking force and the breaking strain of surimi gels. Kamaboko gels were prepared by setting at 30 °C for 0–120 min, then heated at 80 °C for 30 min. Cod liver oil was oxidized by aeration at 45 °C. ■: control, ●: 10% cod liver oil, ▲: 10% oxidized cod liver oil (2 days), ◆: 10% oxidized cod liver oil (5 days).

3. Results and discussion

3.1. Oxidation of cod liver oil

Cod liver oil was oxidized by the aeration at 45 °C in the dark for up to 7 days. The extent of oxidation was monitored by PV and TBA methods (Fig. 1). Such abusive treatment is perhaps not representative of conditions likely to be encountered during normal storage of fats and lipids but the oxidized lipids may rapidly induce changes which could normally occur during a long period of storage and shipping. PV was maximized within 2 days of aeration and decreased rapidly thereafter, while the increase of TBA value was initiated after 3 days' storage at 45 °C and reached a maximum level after 5 days. From these results, it is suggested that cod liver oil stored for 2 days accumulates a large amount of hydroperoxides and that, at 5 days, it has a significant amount of degradation products of lipid oxidation.

Furthermore, the polar fraction and the free fatty acid spots appeared on the thin-layer chromatograms after the storage of 3 days and their size increased with a concomitant decrease of the triglyceride spot (data not shown). From gas chromatography, it was revealed that the fatty acid composition of cod liver oil did not change during 3 days of storage at 45 °C but that the content of EPA and DHA decreased significantly after 4 days of storage (data not shown). These findings seem to correspond to the changes of PV and TBA values (Fig. 1). Therefore, cod liver oils stored for 2 days (maximum PV, 2-CLO) and 5 days (maximum TBA, 5-CLO) were used as a source of oxidized lipid for the experiments on the thermal gelation of surimi.

3.2. Effect of oxidized cod liver oil on the thermal gelation of surimi

Fig. 2 shows the effect of cod liver oil incorporation on the thermal gelation of surimi. The addition of 10% unoxidized cod liver oil (0-CLO) caused a remarkable decrease of the breaking force and a slight decrease of the breaking strain of the suwari and the kamaboko gels, which is in agreement with the report of Ishikawa, Nakaya, and Ito (1991). This could be due to the reduced concentration of myofibrillar proteins in the surimi as the result of adding 0-CLO. The texture of suwari gels with 10% 2-CLO was the same as that with 10% 0-CLO, while the addition of 10% 5-CLO in surimi led to the further decrease of the breaking force. These findings seem to indicate that the addition of oxidized cod liver oil prevents the normal gelation of Alaska pollack surimi, which became more significant when the suwari gels were heated at 80 °C for 30 min to prepare the kamaboko gels (Fig. 2). These phenomena could be due to further oxidation of cod liver oil during the two-step heating, which was elucidated by the determination of TBA values of kamaboko gels (TBA values of kamaboko gels were much larger than those of suwari gels, data not shown).

On the other hand, Kawasaki, Ooizumi, and Konno (1991, 1992) reported that the addition of the triglyceride oxidation products of sardine oil to carp myofibrils changed their ATPase activity and induced the polymerization of myosin heavy chain (MHC). Therefore, the protein subunit compositions of the suwari gels were determined by SDS–PAGE in this study to clarify the inhibitory effect of oxidized cod liver oil on the thermal

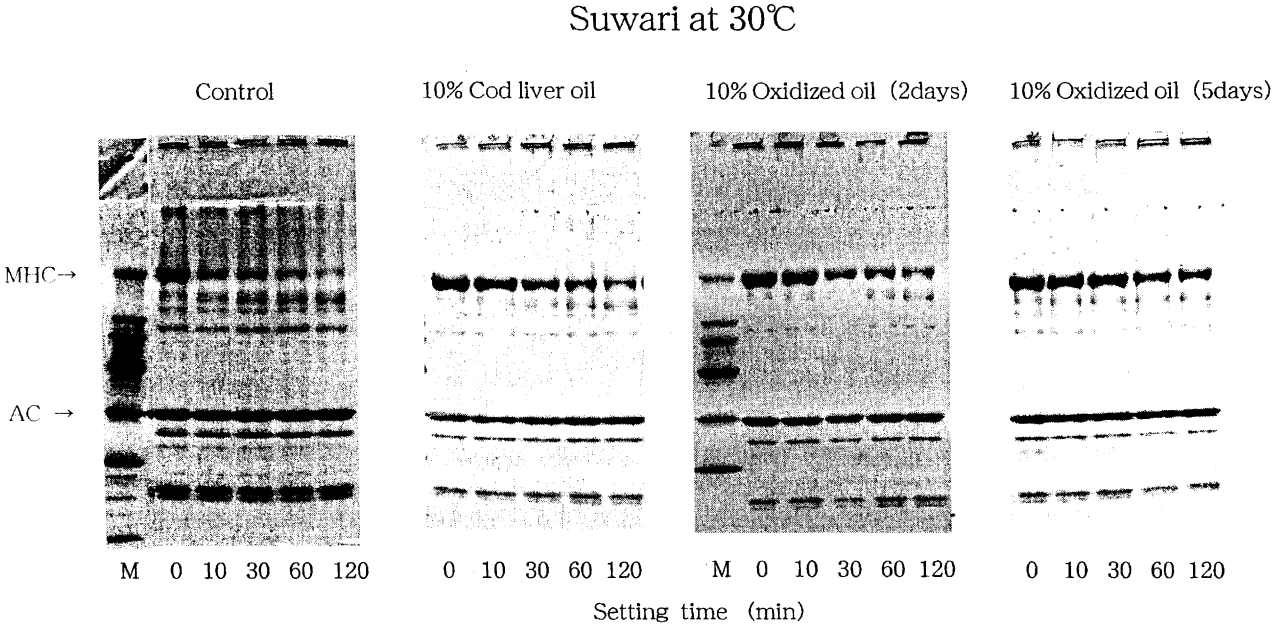

Fig. 3. Changes in SDS–PAGE patterns of SDS-urea solubilized proteins of suwari gels by the addition of oxidized cod liver oil. Cod liver oil was oxidized by aeration at 45 °C for 2 or 5 days. MHC: myosin heavy chain, AC: actin, M: molecular weight marker.

gelation of Alaska pollack surimi. Fig. 3 presents the change in SDS–PAGE patterns of suwari gels. In the case of the control suwari gel (without cod liver oil), the MHC content decreased due to its polymerization during setting at 30 °C with a concomitant increase of the components heavier than MHC, as reported by many researchers (Niwa, Yamada, Kanoh, & Nakayama, 1989; Numakura et al., 1985). On the other hand, the polymerization of MHC during setting was slowed down by the addition of 10% 0-CLO and 2-CLO. This tendency was more obvious in the suwari gel with 10% 5-CLO, confirming the inhibitory effect of oxidized cod liver oil on the breaking force and the breaking strain of suwari gels (Fig. 2). SDS–PAGE patterns of kamaboko gels with 0-CLO, 2-CLO, and 5-CLO were similar to those of suwari gels (data not shown).

3.3. Effect of 5-CLO on the thermal gelation of surimi

From the results given in Figs. 2 and 3, it can be concluded that hydroperoxides of cod liver oil do not influence the gelation of surimi. This is in contrast to the findings of Kawaski and Ooizumi (1996) who reported that hydroperoxides produced during the autoxidation

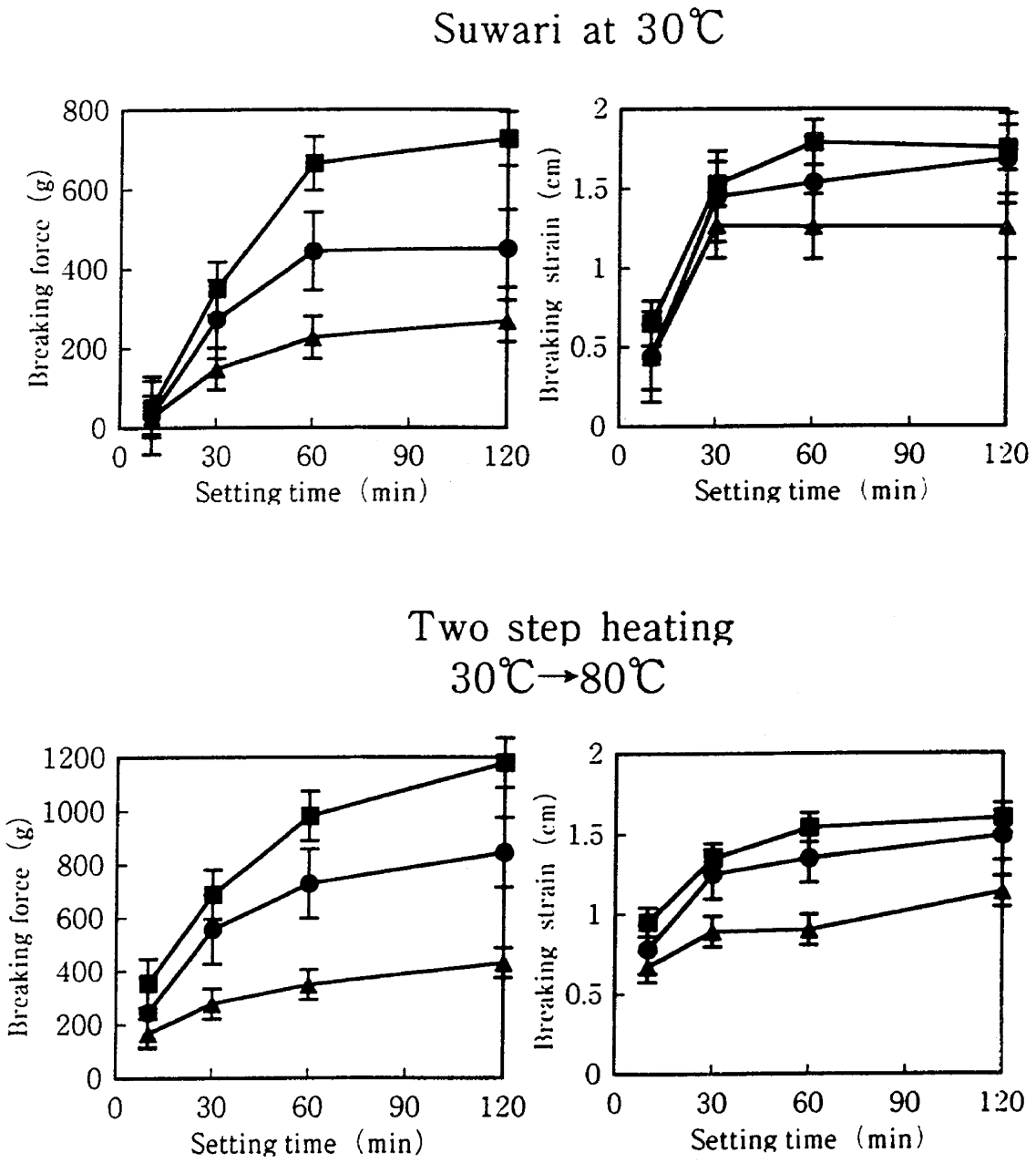

Fig. 4. Effect of the water-soluble fraction from oxidized cod liver oil (5 days) on the breaking force and the breaking strain of suwari and kamaboko gels. ■: control, •: from cod liver oil, ▲: from oxidized cod liver oil.

of lipids caused the cross-linking of MHC. On the other hand, the oxidation products in 5-CLO were responsible for the significant inhibition of surimi gelation. Therefore, in the next experiment, 5-CLO was separated into water-soluble and water-insoluble fractions, prior to adding to surimi.

Fig. 4 depicts the effect of the water-soluble fraction on the breaking force and the breaking strain of suwari and kamaboko gels. The water-soluble fraction from 0-CLO was also used. The addition of the water-soluble fraction of 0-CLO reduced the breaking force of the suwari and kamaboko gels to some extent. On the other hand, it is quite obvious that the water-soluble fraction of 5-CLO caused a drastic decrease of both the breaking force and strain, suggesting that there are some water-soluble substances formed by the oxidation in 5-CLO which retard the gelation of surimi during setting. This was confirmed by the stepwise addition of this fraction (0–25%) to surimi (Fig. 5). The textural properties of the suwari and kamaboko gels decreased with the increased addition of the water-soluble fraction from 5-CLO.

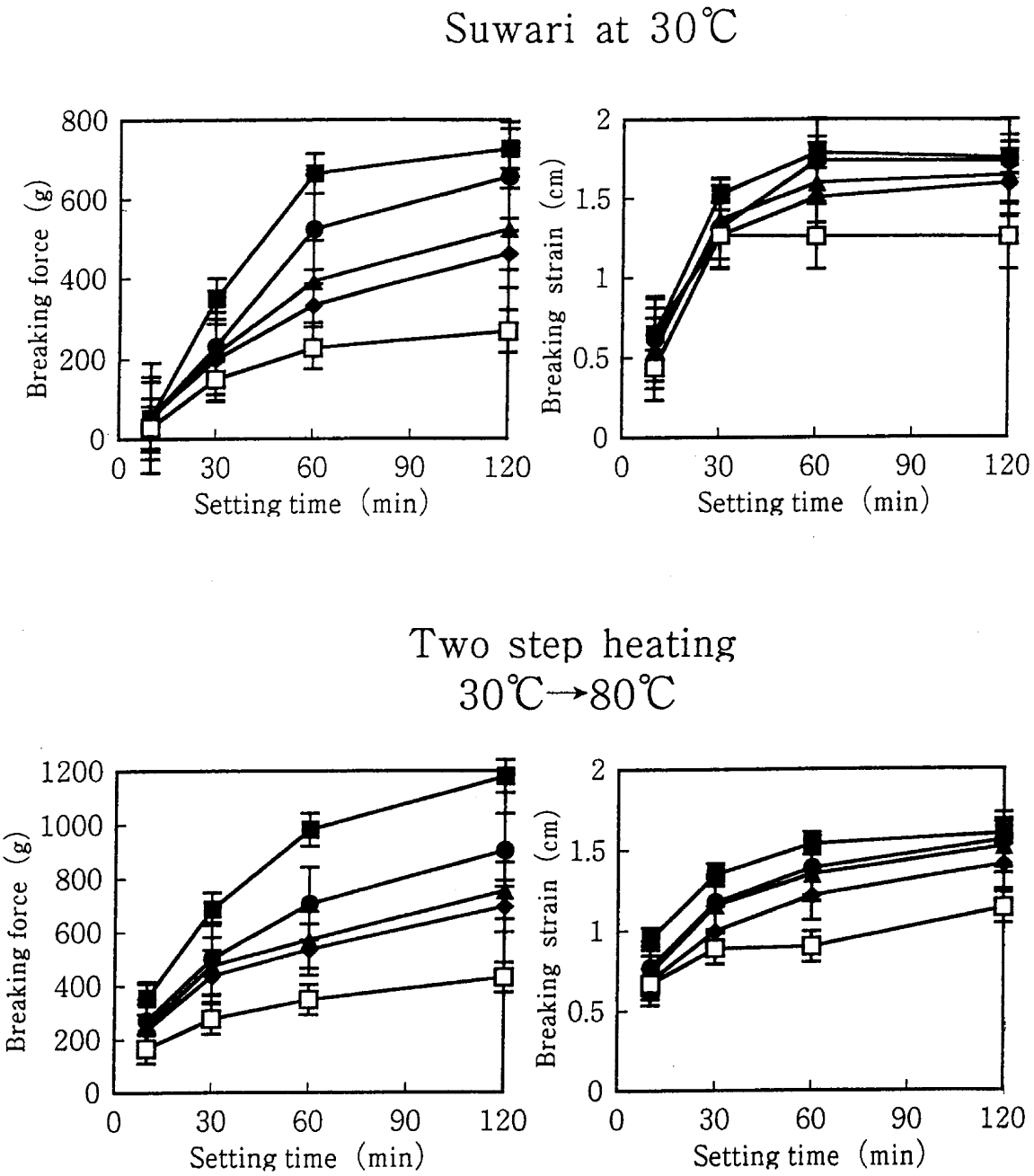

Fig. 5. Effect of the amount of the water-soluble fraction incorporated on the breaking force and the breaking strain of suwari and kamaboko gels. ■: control, ●: 5%, ▲: 10%, ◆: 15%, □: 25%.

Fig. 6 illustrates the changes of breaking force and strain of the suwari and kamaboko gels, into which the water-insoluble fraction of 0-CLO or 5-OCL was incorporated at the level of 10%. It is of relevance to note that the textural properties of gels with 0-CLO or 5-CLO were almost the same as those with the water-insoluble (oil) fraction from 0-CLO or 5-CLO, respectively, indicating that irrespective of the degree of oxidation, the oil fraction of oxidized cod liver oil does not contain substances which inhibit or promote the gelation of surimi during setting. It is well known that such compounds as peroxides, aldehydes, acids, and epoxides as well as free radicals and polymers are formed as the result of the oxidation of unsaturated lipids (Aubourg, 1993). Interaction of the above compounds with surimi proteins, especially myosin, may lead to the inhibition or promotion of surimi gelation. Among those compounds, malonaldehyde could be one of the most reactive substances which can easily cross-link protein molecules (Li & King, 1996; Nair, Cooper, Vietti, & Turner, 1986; Tironi, Tomas, & Anon, 2002).

In order to further confirm the inhibitory effect of the water-soluble fraction of 5-CLO on the gelation of Alaska pollack surimi, SDS–PAGE was performed (Fig. 7). In the case of surimi gels with the water-soluble fraction from unoxidized cod liver oil, the amount of MHC decreased with the setting time at 30 °C, suggesting the occurrence of the polymerization of MHC, as observed in normal surimi gels (Fig. 3). On the other hand, the amount of MHC did not change during set-

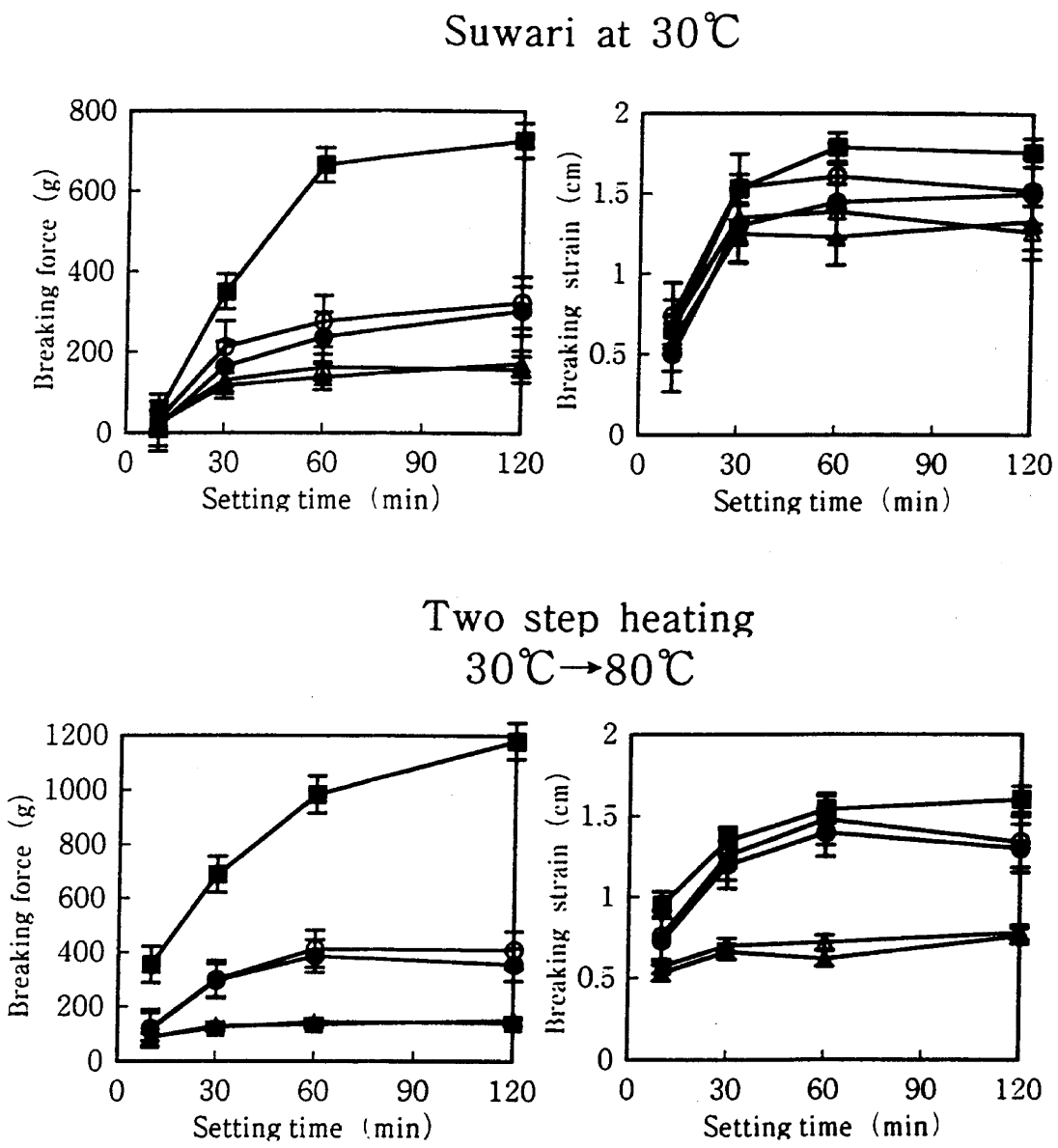

Fig. 6. Effect of oil fraction from oxidized cod liver oil (5 days) on the breaking force and the breaking strain of suwari and kamaboko gels. ■: control, ●: cod liver oil (0-CLO), ▲: oxidized cod liver oil (5-CLO), Oil fraction; ○: from cod liver oil, △: from oxidized cod liver oil (5 days).

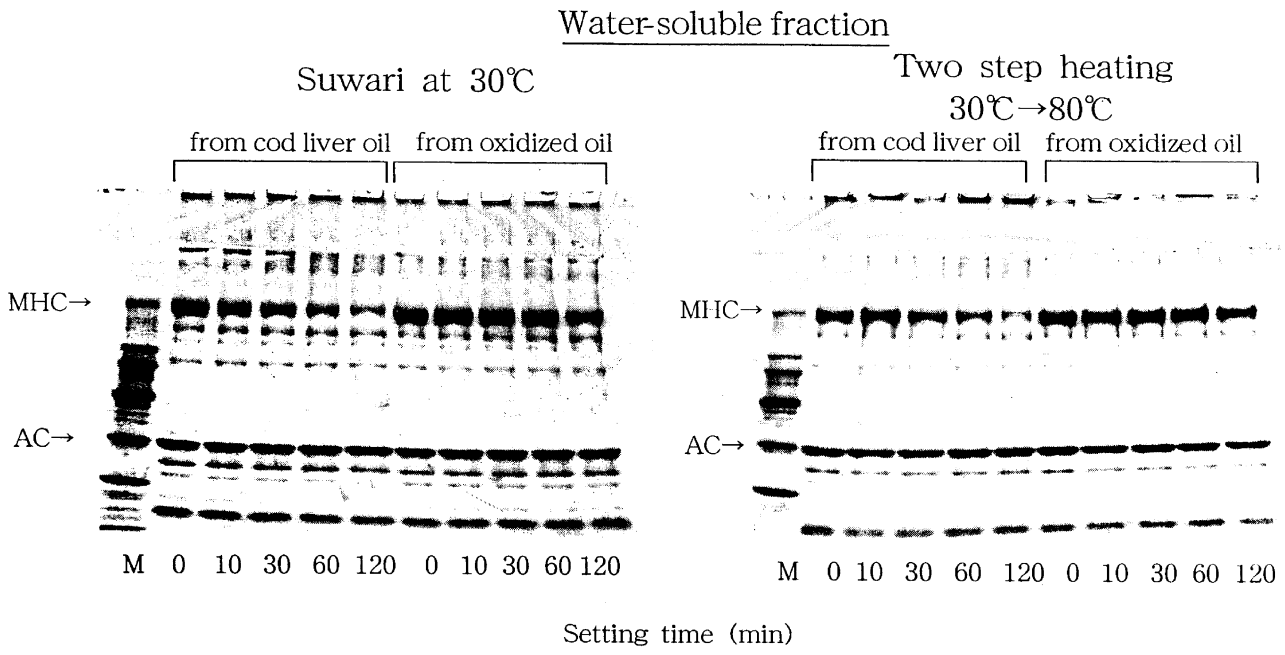

Fig. 7. Changes in SDS–PAGE patterns of surimi gels containing the water-soluble fraction of oxidized cod liver oil (5 days). MHC: myosin heavy chain, AC: actin, M: molecular weight marker.

ting when the water-soluble fraction of 5-CLO was mixed in surimi, indicating that some inhibitory substances for the polymerization of MHC are present in this fraction.

4. Conclusion

The results of this study showed that the thermal gelation property of Alaska pollack surimi was markedly lost by the incorporation of oxidized cod liver oil. The drastic loss of textural properties of surimi gels by the addition of 5-CLO was due to the presence of water-soluble substances which were formed during the later stage of lipid oxidation. Further study on the identification of water-soluble substances responsible for the deterioration of suwari and kamaboko gels and the elucidation of their inhibitory mechanism are now in progress.

Acknowledgements

This study was financed in part by the Exchange Program in the Field of Fisheries Sciences under the Core University System, supported by the Japanese Society for the Promotion of Science (JSPS).

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