Impact of Vinegar Acetic Acid on Hydrolysis and Oxidation of Lipids in Tissues of the Oyster, *Crassostrea gigas*, at 37 °C

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Diarrhetic shellfish poisoning symptoms following the ingestion of vinegar-pickled oysters could reflect free polyunsaturated fatty acids (PUFA) or their oxidation products. Oyster digestive glands (DGLs) were treated with 4% acetic acid (vinegar), and the degradation of lipids at 37 °C was investigated. A significant decrease in total fatty acid in phospholipid (PL) and an increase in free fatty acid were observed after the treatment of DGLs with acetic acid. Saturated fatty acids were released from triglyceride (TG) and PL at the same level. On the other hand, the amount of PUFA released from TG was 1.5 times higher than that from PL. Lipid oxidation products increased in the acid-treated oyster DGLs. PUFA and lipid oxidation products in DGLs treated with the acid were higher than that in PBS-treated ones at 37 °C. Acetic acid was considered to have an impact on the release of PUFA formation in the raw oysters.

Keywords: *Diarrhetic shellfish poisoning; fatty acids; oyster, Crassostrea gigas; acetic acid; lipid peroxidation*

INTRODUCTION

The cause of gastroenteritis induced by raw oysters is usually attributed to bacteria or viruses, and processors therefore ensure careful handling of oysters during transporting and marketing. In spite of this, several outbreaks per year of gastroenteritis induced by consumption raw vinegar pickled oysters, which is a popular style of eating raw oysters in Japan, have been reported (Sekine et al., 1989; Yamashita et al., 1993). It is considered, then, that gastroenteritis might be caused not only by bacteria or viruses but also by substances formed during the pickling of oysters. Formation of free polyunsaturated fatty acid (PUFA) in raw oysters treated with 4% acetic acid, the same as vinegar acetate concentration, was reportedly highest at 37 °C (Sajiki, 1994). Furthermore, mice toxicity was stronger in the fraction containing free fatty acids, their oxidative metabolites, and monoglyceride than in other lipid fraction such as triglyceride (TG). It has been reported that free eicosapentaenoic acid (EPA), one of the PUFAs, abundantly found in marine products, and its autoxidized substances caused mouse lethal toxicity (Sajiki and Takahashi, 1992) and diarrhea in rabbits (Sajiki et al., 1993). Since free PUFAs had stronger toxicity to mice than saturated fatty acid (SFA), monounsaturated fatty acids (MFA), and esterified PUFA (Takagi et al., 1984), it is considered that the formation of PUFA in acetic acid treated oyster at 37 °C, equivalent to the body temperature, might be related to gastroenteritis induced by the ingestion of oysters. However, the mechanism of PUFA release from acetic acid treated oyster at 37 °C has not been clarified yet. For the purpose of clarifying the mechanism of PUFA formation and reconfirmation of the synthesis of lipid oxidative metabolites in acetic acid treated oysters, changes of fatty acid compositions in lipid classes and concentrations of conjugated dienes, thiobarbituric acid reacting substances (TBARS), and Schiff bases were investi-

gated. Since digestive glands (DGLs) are likely to be a strong site for lipids in bivalves (Napolitano and Ackman, 1992), we selected DGLs of oysters as raw materials in this study.

MATERIALS AND INSTRUMENTS

Live oysters collected in January 1993 from the Pacific coast of Tohoku district, Japan, were used.

Pentadecanoic acid, 1,2-dipentadecanoylphosphatidylcholine, and tripentadecanoylglycerol used for internal standard were purchased from NuCheck Prep, Elysian, MN, and 1,1,3,3-tetraethoxypropane purchased from Tokyo Kasei Kogyo Co. Ltd., Tokyo, was used as malonaldehyde.

Silica gel plates (20 cm × 20 cm, 0.25 mm thickness, E. Merck, Darmstadt, Germany) were heated for 2 h at 120 °C for activation and kept in a desiccator until use.

A gas chromatograph (GC-14A, Shimadzu, Kyoto) equipped with an SP-2330 capillary column [0.32 mm (i.d.) × 30 m, Supelco, Bellefonte, PA], an HPLC Model 880-PU with Finepak SIL C$_{18}$ column, a UV-970 detector (Japan Spectroscopic Co., Ltd., Tokyo), a spectrophotometer (Ubest-30, Japan Spectroscopic Co., Ltd., Tokyo), and a fluorescence spectrofluorometer (MPF-4, Hitachi, Ltd., Tokyo) were used as analytical instruments.

EXPERIMENTAL METHODS

Preparation of Experimental Samples from Oyster DGLs. For analysis of fatty acids composition, DGLs were separated from 10 live oysters and each cut into halves. Immediately after killing, lipid was extracted from a half part of each DGL, as nontreated control. Two volumes of 4% acetic acid (pH 2.45) was added to the other half, and the halves were incubated for 3 h at 37 °C. After incubation, lipids were extracted from the samples. For the determination of PUFA and lipid oxidative metabolites in DGLs, eight DGLs of oysters, each divided into three pieces, were treated as follows: (i) nontreated control; (ii) 4% acetic acid was added and the DGLs were allowed to stand for 3 h at 37 °C (as acid-treated DGLs); (iii) added phosphate-buffered saline (PBS, pH 7.4, as PBS-treated DGLs) was added and the DGLs were allowed to stand at 37 °C for 3 h. For lipoperoxide determination, 10 whole DGLs were combined and homogenized.

Analysis of Fatty Acid Composition of Lipid Classes in DGLs. Lipids were extracted with chloroform/methanol (2:1) (Folch et al., 1957) containing 0.001% BHT after the

Table 1. Fatty Acid Concentrations[a] in Lipid Classes of Oysters with and without Acid Treatment

class	nontreated			acid-treated[c]		
	PL[b]	TG[b]	FFA[b]	PL[b]	TG[b]	FFA[b]
saturated	1.85 ± 0.25	2.33 ± 0.70	0.21 ± 0.05	1.02 ± 0.23**	1.94 ± 0.49	2.65 ± 0.49**
mono	0.95 ± 0.13	1.24 ± 0.35	0.06 ± 0.04	0.57 ± 0.13**	1.10 ± 0.28	1.42 ± 0.35**
poly	3.69 ± 0.53	2.83 ± 1.17	0.04 ± 0.05	3.07 ± 0.66**	2.20 ± 0.85	1.51 ± 0.48**
others	1.52 ± 0.17	0.52 ± 0.24	0.03 ± 0.03	1.38 ± 0.23	0.65 ± 0.32	0.35 ± 0.18**
total	8.00 ± 1.00	6.92 ± 2.33	0.32 ± 0.14	5.99 ± 1.07**	5.88 ± 1.72	5.93 ± 1.27**

[a] Micromoles per gram. Mean ± SD of 10 replicates. Differences between with and without treatment are tested using two-way analysis of variance. ** $p < 0.01$. [b] PL, phospholipid; TG, triglyceride; FFA, free fatty acid. [c] Incubated at 37 °C for 3 h with 4% acetic acid.

Table 2. Fatty Acid Composition[a] in Lipid of Oyster DGLs with and without Acid Treatment

fatty acid	nontreated			acid-treated[c]		
	PL[b]	TG[b]	FFA[b]	PL[b]	TG[b]	FFA[b]
14:0	3.65 (0.67)	4.09 (0.83)	4.71 (6.45)	4.76 (1.40)	3.73 (1.25)	3.71 (0.47)
15:0	0.53 (0.14)	0.75 (0.07)	ND	0.38 (0.30)	0.73 (0.12)	1.05 (0.26)
15:1(n−5)	ND[d]	ND	ND	ND	ND	ND
16:0	14.39 (0.76)	24.48 (2.41)	54.94 (20.98)	8.88 (1.57)	23.78 (2.83)	31.62 (2.80)
16:1(n−7)	0.78 (0.15)	2.25 (0.74)	ND	0.52 (0.20)	2.22 (0.75)	2.13 (0.39)
18:0	4.53 (0.53)	5.10 (1.60)	9.24 (8.89)	2.82 (0.71)	5.66 (1.74)	8.92 (1.58)
18:1(n−7)	2.78 (0.36)	5.07 (0.83)	0.73 (2.31)	1.93 (0.40)	5.31 (0.97)	5.43 (0.72)
18:1(n−9)	2.11 (0.21)	5.80 (0.82)	1.76 (3.71)	1.82 (0.43)	5.91 (0.98)	5.21 (0.42)
18:2(n−6)	0.64 (0.24)	2.06 (0.10)	ND	0.72 (0.28)	1.94 (0.11)	1.41 (0.17)
18:3(n−3)	0.75 (0.29)	2.25 (0.40)	ND	0.86 (0.19)	1.88 (0.26)	1.65 (0.29)
18:4(n−3)	1.86 (0.55)	5.34 (0.99)	ND	2.34 (0.41)	4.41 (0.53)	2.59 (0.40)
20:1(n−7)	3.75 (0.38)	2.57 (0.46)	13.92 (6.97)	2.21 (0.45)	2.75 (0.63)	7.80 (1.74)
20:1(n−9)	1.00 (0.17)	0.53 (0.22)	ND	0.68 (0.31)	0.60 (0.12)	1.67 (0.32)
20:1(n−11)	1.41 (0.15)	1.97 (0.65)	ND	2.22 (0.38)	2.16 (0.38)	1.48 (0.23)
20:4(n−6)	3.23 (0.49)	1.63 (0.12)	ND	3.88 (0.51)	1.60 (0.21)	1.54 (0.17)
22:1(n−9)	0.05 (0.11)	0.15 (0.17)	ND	ND	0.14 (0.16)	0.18 (0.24)
22:2(n−7)	4.22 (0.68)	1.46 (0.49)	ND	5.55 (1.01)	1.68 (0.23)	1.64 (0.25)
20:5(n−3)	16.94 (1.25)	15.46 (1.73)	8.45 (9.91)	18.24 (1.89)	13.70 (1.98)	10.14 (2.12)
22:4(n−6)	0.84 (0.42)	0.66 (0.36)	ND	0.83 (0.45)	0.57 (0.32)	0.21 (0.27)
22:5(n−6)	0.16 (0.20)	0.07 (0.11)	ND	0.05 (0.16)	0.07 (0.12)	ND
22:5(n−3)	1.22 (0.44)	0.35 (0.31)	ND	1.17 (0.63)	0.27 (0.29)	0.16 (0.25)
22:6(n−3)	16.13 (0.92)	10.64 (2.09)	ND	17.18 (1.65)	10.09 (2.35)	5.61 (0.98)
saturated	23.11 (1.14)	34.42 (4.09)	68.89 (16.66)	16.83 (2.33)	33.86 (4.89)	45.20 (4.10)
monoene	11.88 (0.45)	18.34 (2.26)	16.41 (7.52)	9.39 (1.94)	19.09 (2.70)	23.89 (2.29)
polyene	45.98 (1.95)	39.92 (3.83)	8.45 (9.91)	50.82 (3.68)	36.20 (4.97)	24.94 (3.45)
others	19.02 (1.58)	7.30 (2.51)	6.29 (7.30)	22.96 (1.31)	10.85 (5.35)	5.98 (3.09)

[a] Weight percent. Mean ± (SD) of 10 replicates. [b] PL, phospholipid; TG, triglyceride; FFA, free fatty acid. [c] Incubated at 37 °C for 3 h with 4% acetic acid. [d] ND, not detected.

addition of 1,2-diheptadecanoylphosphatidylcholine, triheptadecanoylglycerol, and free heptadecanoic acid as internal standards. The extracts were separated by TLC on silica gel plates with petroleum ether/diethyl ether/acetic acid (74:15:1 by vol) as a solvent system. After visualization, the bands using iodine vapors, phospholipids (PL), triglyceride (TG), and free fatty acids (FFA), were scraped and fatty acids were transmethylated with 6% H_2SO_4 in methanol at 70 °C for 45 min. Fatty acid methyl esters were analyzed by GC. The injection port was at 250 °C, and the column temperature was kept at 160 °C for 10 min and increased to 200 °C at 4 °C/min. Flame ionization detector (FID) was used. Helium was used as a carrier gas (LFV, 1.41 mL/min). Identification of fatty acids was done by comparing retention times with those of authentic standards and GC−MS analysis.

Determination of Free PUFA in DGLs. PUFA were extracted with ethanol and further reextracted with Sep-Pak according to the method previously reported (Sajiki, 1994). PUFA were analyzed by HPLC at 195 nm. Conditions of HPLC were as follows: solvent, acetonitrile/methanol/water/ phosphoric acid (200:100:125:0.4 by vol); flow rate, 1.0 mL/ min; column temperature, 40 °C. Identification of peaks was made by comparing authentic standards.

Measurement of Lipid Oxidative Metabolites in DGLs. Conjugated dienes, Schiff bases, and thiobarbituric acid reacting substances (TBARS) were measured to assess lipid oxidative metabolism. For the determination of conjugated dienes and Schiff bases, samples were prepared by extraction of DGLs with 99% ethanol at 3 h after the treatments with and without PBS or acetic acid. Conjugated dienes were assayed by spectrophotometry at 234 nm (Sevanian et al., 1988). An absorption coefficient of 2.54×10^4 (at 234 nm) was used to estimate the amount of conjugated dienes. Schiff bases were expressed as relative fluorescence intensity (percent) using fluorescence emission at 420 nm and excitation at 360 nm (Tappel, 1975). TBARS in DGLs were determined according to the method of Ohkawa et al. (1979) using 15% homogenized DGL samples with PBS−saline (pH 7.4) using tetraethoxy-propane as standard. As for the determination of lipid- and water-soluble Schiff base in the treated DGLs as a function of time, 20 volumes of chloroform/methanol (2:1) (Folch et al., 1957) was added to samples at each reaction time and they were mixed vigorously. After the same volume of water was added, they were further mixed. Water- and lipid- soluble fractions were separated by centrifugation at 3000g for 2 min.

Statistical Analysis. Data were analyzed by one-way or two-way analysis of variance, and the difference among treatments was tested by least significant difference (Snedecor and Cochran, 1969).

RESULTS AND DISCUSSION

The total fatty acids and individual mass percentage of total fatty acids in PL, TG, and FFA of the nontreated and the acid-treated DGLs are shown in Tables 1 and 2. In the acid-treated oysters, total fatty acids concentrations in PL decreased significantly ($p < 0.01$). The rates of decrease of SFA, MFA, and PUFA in PL were 0.63, 0.38, and 0.62 μmol g^{-1} (3 h)$^{-1}$, respectively. The

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decrease rates of SFA, MFA, and PUFA in TG were 0.39, 0.14, and 0.63 μmol g^{-1} (3 h)$^{-1}$, respectively. Fatty acids in FFA of oyster DGLs increased ($p < 0.01$) very quickly with the acid treatment. SFA, MFA, and PUFA in FFA class in the acid-treated DGLs were 12.6, 23.7, and 37.8 times those of nontreated ones, respectively. The rates of increase of SFA, MFA, and PUFA were 2.44, 1.36, and 1.47 μmol g^{-1} (3 h)$^{-1}$, respectively. These data indicated that the treatment of DGLs at 37 °C enhanced the release of fatty acids in esterified lipids of PL and TG which may be caused by the activation of lipolytic enzyme in the samples. This agreed with the findings of Jeong et al. (1990) on lipid deterioration during frozen storage in oysters and with the findings of Dyer and Fraser (1959) and Tsukuda (1976) in fish. Although the amounts of PUFA released in PL and TG were the same, the actual amount of released fatty acids in TG (1.89 μmol/g as fatty acid) was higher than in PL (1.24 μmol/g as fatty acid) considering their characteristics, TG as triesters, PL as diesters. This result suggests that the free PUFA formation could be caused more by PUFA release from TG than from PL in the acid-treated DGLs.

Jeong et al. (1990) reported that the percentage of SFA, mainly 16:0 in PL and TG, increased in oyster while PUFA, mainly 20:5 and 22:6, decreased during frozen storage. The percentage of SFA, mainly 16:0 and 18:0 in PL, decreased while PUFA, mainly 20:5, 22:2 and 22:6, increased in acetate-treated oyster DGLs (Table 2). It seemed that the effect of frozen storage on the release of SFA and PUFA was different from that of the acid treatment.

The sum total of SFA decrease in TG (1.17 μmol/g) and that of PL (1.26 μmol/g) was equal to the SFA increase in FFA (2.44 μmol/g). The same tendency was observed in the MFA, the sum total of decrease in TG (0.42 μmol/g) and PL (0.76 μmol/g) was almost the same as the increase in FFA (1.36 μmol/g), whereas the sum of PUFA decrease in TG (1.89 μmol/g) and PL (1.24 μmol/g) was twice higher than the PUFA increase in FFA (1.47 μmol/g). These data suggest that a part of the PUFA released by the acid treatment at 37 °C might have been further changed to other metabolites in DGLs. Moreover, fatty acids 20:5 > 22:6 > 16:0 and 16:0 > 20:5 > 22:6 were predominant in PL and TG of live oyster DGLs (Table 2), which agreed with the data reported previously (Jeong et al., 1990) using the same species of oyster collected along a different coast of Japan. The percentage of PUFA possessing more than two double bonds was high (Table 2). Above all, the sum percentage of 20:5 and 22:6 in total PUFA was very high, 71.9 for PL and 65.4 for TG. Since PUFA are easily oxidized and abundantly contained in DGLs, it is easy to consider that many oxidative metabolic substances derived from these PUFA are synthesized in the acid-treated DGLs. This finding agrees well with the data in the previous paper (Sajiki, 1994), in which conjugated dienes derived from EPA such as hydroxy- and hydroperoxyeicosapentaenoic acids increased and other unknown substances appeared in the acid-treated DGLs.

Lipid oxidative metabolites in DGLs 3 h after the treatment are listed in Table 3. A significant increase ($p < 0.01$) in the concentration of conjugated dienes was observed in the acid-treated DGLs. Ethanol-soluble Schiff base was also significantly higher ($p < 0.01$) in the acid-treated DGLs than in nontreated and PBS-treated DGLs, where the PBS value was significantly

Table 3. Conjugated Diene, Schiff Base, and TBARS Values in Oyster DGLs Treated with Acid and PBS[a]

treatment	conjugated diene (mg/g of wet wt)	Schiff base[b] (%)	TBARS value (μmol/g of wet wt)
none	5.21 ± 0.12[a]	17.6 ± 0.36[a]	1.05 ± 0.06[a]
PBS[c]	5.53 ± 0.26[a]	22.2 ± 0.25[b]	1.14 ± 0.08[a]
acid[c]	7.61 ± 0.07[b]	25.4 ± 0.75[c]	0.56 ± 0.03[b]

[a] Mean ± SD of three measurements; pool of five oyster DGLs for each treatment. Different superscript letters in the same column are significantly different at $p < 0.01$. The same superscript in the same column is not significantly different at $p < 0.01$. [b] Represented as relative fluorescence intensity. [c] Incubated at 37 °C for 3 h.

Table 4. Concentrations[a] of Free Polyunsaturated Fatty Acids in Oyster DGLs with Various Treatments

fatty acids	nontreated	acid-treated[b]	PBS-treated[b]
18:3(n−3)	0.003 ± 0.007[a]	0.091 ± 0.048[b]	0.001 ± 0.015[a]
18:4(n−3)	0.001 ± 0.004[a]	0.115 ± 0.035[b]	0.006 ± 0.008[a]
20:4(n−6)	ND[a]	0.053 ± 0.021[b]	0.001 ± 0.004[a]
20:5(n−3)	0.009 ± 0.011[a]	0.725 ± 0.113[b]	0.037 ± 0.043[a]
22:6(n−3)	0.003 ± 0.005[a]	0.441 ± 0.061[b]	0.013 ± 0.010[c]
total	0.018 ± 0.018[a]	1.426 ± 0.240[b]	0.064 ± 0.067[a]

[a] Mean (mg/g of wet wt) ± SD of eight replicates. Different superscript letters in the same fatty acid are significantly different based on lsd ($p < 0.01$). The same superscripts in the same fatty acid are not significantly different. Significance of differences are tested according to the method described under Experimental Methods. [b] Incubated at 37 °C for 3 h.

higher ($p < 0.01$) than that in nontreated ones. On the other hand, the TBARS value in the acid-treated DGLs decreased remarkably ($p < 0.01$). Furthermore, their total concentrations increased 79-fold after the acid treatment (Table 4), although free PUFA concentrations of 18:3, 18:4, 20:4, 20:5, and 22:6 were very low in live oyster DGLs.

Significant increases of conjugated dienes and Schiff bases in the acid- and PBS treated DGLs, which depended on the free PUFA concentrations, supported the assumption that oxidative metabolism would be induced in the acid-treated oyster at 37 °C.

To clarify the correlation between Schiff base and TBARS in the acid-treated DGLs, changes of TBARS value and water- or lipid-soluble Schiff bases in DGLs treated with PBS at 5 or 37 °C and with acetic acid at 37 °C were analyzed. As shown in Figure 1, the TBARS value decreased in all treatments. Both treatments of PBS and acetic acid at 37 °C enhanced dramatically the decrease of TBARS within 30 min after the incubation. In contrast, water-soluble Schiff bases increased within 30 min in the acid-treated DGLs but not in the PBS treated DGLs. Lipid-soluble Schiff bases were barely detected in the acid-treated DGLs. It has been considered that the aqueous Schiff base product, RN=CH—CH=CH—NH—R, is malonaldehyde cross-linked with soluble amino compounds such as amino acids, proteins, nucleic acids, and their bases (Tappel, 1975). These reactions might be considered as one of the reasons for increasing aqueous Schiff base concentrations in the acid-treated DGLs, although TBA is known not necessarily to react only to malonaldehyde. Also, the fact that the protein concentration in the supernatant of DGLs homogenate decreased (data not shown) agrees with the hypothesis mentioned above. Oysters possess a xenobiotic metabolism system, such as flavin-containing monooxygenase and NADPH-independent benzo[a]pyrene hydroxylase (BPH), which is considered to be a co-oxidation pathway involving a one-electron transfer of oxygen from a lipid hydroper-
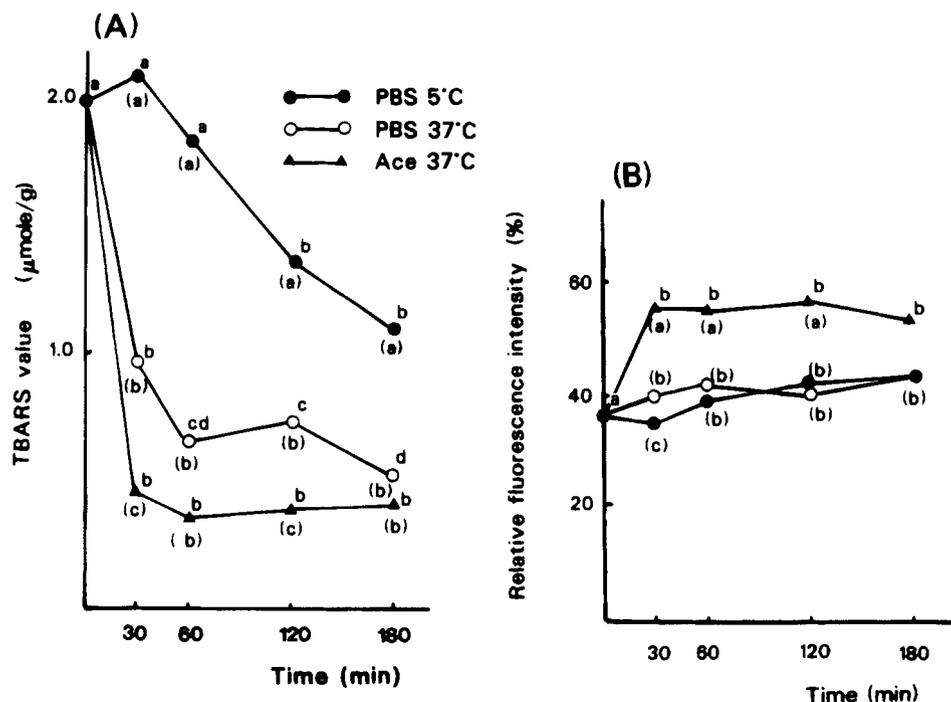
(A)

(B)

Figure 1. Changes of TBARS value (A) and Shiff base in water soluble fraction (B) as a function of time after the various treatments in oyster DGLs at different conditions. Values are represented as means (n = 3). Values with different letters are statistically different (p < 0.01) among assay times in the same treatment. No statistical difference in values of water soluble Schiff base in DGLs treated with PBS at both 5 and 37 °C was observed among assay times. Values with different letters in parentheses are statistically different (p < 0.01) among the treatments in the same time.

oxide (Schlenk and Buhler, 1989). Furthermore, it is reported that *in vitro* transformation of xenobiotics is influenced by a fluctuation of pH and may be involved in prostaglandin synthase in oyster visceral mass (Schlenck and Buhler, 1990). It is interesting to explore whether EPA metabolism along this pathway causes gastroenteritis induced by raw oysters.

In PBS-treated DGLs, the concentration of free PUFA was much lower than that in the acid-treated DGLs, while it was higher than in nontreated DGLs (Table 4). Considering the facts that fatty acids in TG and PL were reportedly reduced due to the actions of an endogenous lipolytic enzyme system in fresh muscles of fish (Tsukuda, 1976; Hardy et al., 1979) or oyster (Jeong et al., 1990) and that the temperature around 37 °C is optimum condition for activation of endogenous enzymes, the temperature would be important in the release of PUFA. It appears that acetic acid had more of an impact on the release of PUFA in the acid-treated DGLs than temperature.

It has been reported that PUFA are abundantly contained in sn-2 position in alkyl and alkenyl type PL of oyster (Koizumi et al., 1990), although no data are available in acyl type PL. Judging from the data that the decreases in concentration of SFA and PUFA were similar (Table 1), both phospholipase A_1 and A_2 might be involved in the acceleration of fatty acids release. The pH optima of both phospholipase A_1 and phospholipase A_2 in oyster DGLs are known to be, respectively, around pH 3 and 7 (Sajiki, unpublished results).

It is considered that PUFA are easily oxidized because of the possession of many double bonds, and their oxidative metabolites were toxic to various organisms (Oarada et al., 1989; Kanazawa and Ashida, 1991). In our previous works, autoxidative products of EPA reacted more strongly to hemoglobin (Sajiki et al., 1992) and resulted in a higher possibility of diarrhea in rabbits than free EPA (Sajiki et al., 1993). Recently, it has been

reported (Grataroli et al., 1992) that fish oil induced the decrease in *ex vivo* production of prostaglandin E_2 (PGE_2), an enzymically oxidized metabolite of arachidonic acid (AA) in gastric mucosa, because of the decrease in the membrane AA level, and a high dose of fish oil enhanced catabolism of PGE_2. From the facts that PGE_2 possesses cytoprotective properties and it is also effective in the treatment of peptic ulcer, Grataroli et al. (1992) suggested that excessive consumption of fish oil concentration is not recommended. Thus, $\omega 3$-PUFA may have a negative influence on gastric function. Further study to clarify the role of released fatty acids and their oxidative metabolites produced in the acid-treated oyster DGLs at 37 °C on gastric disorders such as vomiting and diarrhea is necessary.

It is well-known that one cause of gastroenteritis induced by bivalves is ascribed to toxins from dinoflagellate (Hallegraeff, 1993). Especially, dinophysistoxin 3, a compound that bound with PUFA (22:6), had been reported as a highly toxic substance (Yanagi et al., 1989). These compounds derived from algae may be also considered as causative toxins, although the question remains whether these toxins are produced in oyster by the acid treatment at 37 °C.

In conclusion, SFA were liberated from both classes of PL and TG at almost the same concentration in the acid-treated DGLs at 37 °C, while PUFA in TG seemed to be liberated more easily than those in PL. Released PUFA from TG and PL were further oxidized to lipoperoxides. PUFA release and lipid oxidative metabolite formation in raw oyster were accelerated by incubation at 37 °C. However, addition of acetic acid further accelerated the PUFA release and lipid peroxidation in raw oyster incubated at 37 °C. It is considered that malonaldehyde produced by PUFA oxidation may be changed to Schiff base in the early stage of the reaction with acetic acid at 37 °C. The mechanism of PUFA

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release and the production of Schiff base in oyster treated with acetic acid should be further investigated.

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