Effect of Heating on Mutagenicity of Fruit Juices in the Ames Test

Mendel Friedman,* Robert E. Wilson, and I. Irving Ziderman†

Western Regional Research Center, U.S. Department of Agriculture—Agricultural Research Service, 800 Buchanan Street, Albany, California 94710

Mutagenic responses of freshly squeezed orange juice, fresh orange juice heated for 0.5–30 min at 100 °C, and several commercial orange juices were investigated in four bacterial strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA2637) with and without microsomal activation. The response without activation was similar in all cases, ranging 2–3 times the background values. The experiments with microsomal activation produced barely visible pinpoint colonies that do not appear to be normal revertants. Our results do not duplicate previously reported high mutagenic activity of fresh orange juice after heating, or of commercial varieties. In preliminary studies, no increase in the number of revertants was apparent after freshly prepared apple, grape, and pear juices were heated. The possible chemical basis for the mutagenic and antimutagenic activities of fruit juices is discussed.

Nonenzymatic and enzymatic browning is ubiquitous in fruit juices, including apples (Toribio and Lozano, 1984), grapefruit (Lee and Nagy, 1988), lemon (Robertson and Samaniego, 1986), orange (Lee and Nagy, 1988), and pear (Beveridge and Harrison, 1984). Such browning damages the appearance, quality, and possibly the safety of the juices. With respect to safety, Ekasari et al. (1986, 1989) reported that fresh orange juice was mutagen-free but that heating for 2 min at 93 °C (usual pasteurization conditions) produced large amounts of mutagens, as measured by the Ames test. Mutagen formation was related to dose and to heating time. Commercial juice, but not fresh juice, was also reported to contain significant mutagen levels.

In related studies, Stich et al. (1982) found that although freshly prepared apple juice had only a minor effect on chromosomes of Chinese hamster ovary cells, stored and canned apple juice induced severe damage. They ascribe this effect to the formation of browning products during storage and canning. Although mutagen formation could indeed be due to browning (Powrie et al., 1986; MacGregor et al., 1989), it could also be induced by aldehydes and ketones produced by carbohydrates or vitamin C (Bjeldanes and Chew, 1979; Yamaguchi and Nakagawa, 1983), by flavonoid aglycons derived from naturally occurring flavone glycosides (MacGregor, 1984, 1986; Friedman and Smith, 1984; van der Hoeven et al., 1983), or by mycotoxins (Friedman et al., 1982). It is also noteworthy that extracts from fruits and vegetables contain antimutagenic compounds (Shinohara et al., 1988; Stich and Rosin, 1984) and that SH-containing amino acids and peptides can suppress mutagenic activities of naturally occurring and processing-induced food ingredients (De Flora et al., 1989; Molnar-Perl and Friedman, 1989; Friedman, 1973, 1984; Friedman et al., 1982).

Our objective was to investigate the formation of mutagens in freshly prepared, heated, and commercial orange juices using the Ames *Salmonella his* reversion assay with strains TA98, TA100, TA102, and TA2637. For comparison, we also assayed the mutagenic activities of unheated and heated freshly prepared apple, grape, and pear juices. Possible sources of mutagenic activity of fruit juices are discussed.

† Visiting scientist at WRRC. Permanent address: Israel Fiber Institute, Ministry of Industry and Trade, P.O. Box 8001, 91080 Jerusalem, Israel.

Table I. Quantitative Plate Test of Fresh Orange Juice Heated for Various Time Periods in *Salmonella typhimurium* TA100 Strain (Duplicate Values Are Revertants per Plate (1 mL of Juice))

test compound	TA100
orange juice, unheated	185, 213
orange juice, heated, 0.5 min	249
orange juice, heated, 2 min	237, 276
orange juice, heated, 3 min	272, 273
orange juice, heated, 5 min	258, 283
orange juice, heated, 30 min	272, 304
H_2O control	118, 132
positive control (NQNO, 0.1 μg/plate)	574, 596

Table II. Quantitative Plate Tests of Fresh Unheated and Heated Orange Juices in *Salmonella typhimurium* Strain TA100 and Histidine and Lysine Content of the Juices[a] (Duplicate Values Are Revertants per Plate)

test compound	TA100	His, μg/mL	Lys, μg/mL
orange juice, unheated	227, 253	6.6	26.8
orange juice, heated, 0.5 min	239, 270	4.0	19.4
orange juice, heated, 2 min	224, 247	3.8	22.6
orange juice, heated, 4 min	218, 258	3.4	19.8
H_2O control	99, 128		
positive control (NQNO, 0.1 μg/plate)	544		

[a] Freeze-dried samples was resuspended to 5× concentration of fresh juice. Each test was done with 0.2 mL of the suspension. There was no change in pH of the orange juices following heat treatments.

MATERIALS AND METHODS

Preparation of Juices. Commercial juices including several brands of frozen concentrate and reconstituted, pasteurized, refrigerated juices were obtained from local stores. The juices from concentrates were reconstituted immediately before testing following directions on the labels. All fruits used for making fresh juices were purchased from local markets. These juices were prepared with a bench-top juice extractor except for orange juice, which was squeezed out by hand. Fresh-squeezed juices were passed through eight layers of cheesecloth before they were heat-treated. Juices were heated in 20-mL

Table III. Quantitative Plate Tests of Commercial Orange Juices in *Salmonella typhimurium* Strains TA98, TA100, TA102, and TA2637 without Microsomal Activation (Duplicate Values Are Revertants per Plate (1 mL of Juice))

test compound	TA98	TA100	TA102	TA2637
brand A ready to drink orange juice	96, 187	362, 365	754, 764	126, 141
brand B ready to drink orange juice	102, 104	266, 268	735, 837	75, 77
brand C orange juice from concentrate	118, 126	215, 215	678	81, 150
brand D orange juice from concentrate	104, 119	219	661, 854	112, 132
brand E orange juice from concentrate	140	243, 256	779, 902	60, 114
H_2O control	35, 45	148, 161	413, 448	56, 63
positive controls (all with microsomal activation)	aflatoxin 0.4 μg: 1050, >1200	aflatoxin, 0.4 μg: >1200, 1200	danthron, 45 μg: >1500, 1500	emodin, 30 μg: 399, 458

Table IV. Quantitative Plate Tests of Orange Juices in *Salmonella tryphimurium* Strains TA98, TA100, and TA2637 without Microsomal Activation (Duplicate Values Are Revertants per Plate (1 mL of Juice))

test compound	TA98	TA100	TA2637
orange juice, freshly squeezed, unheated	77, 105 (3.9)[a]	310, 324 (1.9)	79, 80 (1.5)
orange juice, freshly squeezed,heated (2 min at 100 °C)	61, 78 (3.0)	305, 313 (1.8)	93, 87 (1.4)
orange juice, commercial from carton (brand C)	64, 72 (2.9)	331, 380 (2.1)	67, 72 (1.2)
H_2O controls	22, 25 (1.0)	167, 173 (1.0)	52, 59 (1.0)
NQNO, 0.1 μg/plate	159, 186	636, 749	198, 203

[a] Values in parentheses are ratios of average revertant counts to H_2O controls.

Table V. Quantitative Plate Tests of Fresh Apple, Grape, and Pear Juices in *Salmonella typhimurium* Strain TA100 without Microsomal Activation (Duplicate Values Are Revertants per Plate (1 mL of Juice))

	TA100	% H_2O control
apple juice, freshly squeezed, unheated[a]	212, 236	118
apple juice, freshly squeezed, heated[b]	220, 240	121
grape juice, freshly squeezed, unheated[c]	364, 418	206
grape juice, freshly squeezed, heated[b]	372, 377	197
pear juice, freshly squeezed, unheated[d]	262, 266[e]	152
pear juice, freshly squeezed, heated[b]	210, 216[e]	122
pear juice, freshly squeezed, heated[b]	200, 230[e]	124
H_2O control	161, 219	100
H_2O control	177, 171[e]	
positive control (NQNO, 0.1 μg/plate)	459	
(NQNO, 0.15 μg/plate)	468, 481[e]	

[a] Prepared from Washington State Golden Delicious Apples. [b] 2 min at 100 °C. [c] Prepared from Thompson white seedless grapes. [d] Prepared from d'Anjou pears. [e] Separate experiment.

screw-cap tubes by immersion in a boiling water bath and then immediately cooled in tap water.

The cooled, freshly squeezed juices were pH-adjusted with 4 N NaOH to 7.4 and then centrifuged for 20 min at 20000*g* (Tables I–III) or 24000*g* (Tables IV and V). One-milliliter samples of supernatant were assayed in the Ames test, except as shown in Table II.

Analysis for Free Histidine and Lysine. Forty microliters of orange juice was applied to the ion-exchange column of a Durum D-500 amino acid analyzer. The content of free amino acids was calculated with reference to a standard mixture of free amino acids (Friedman and Levin, 1989).

Mutagenicity Tests. Assays for mutagenicity were carried out with modifications of the Ames *Salmonella* test (MacGregor and Friedman, 1977; Friedman et al., 1980; Maron and Ames, 1983), as suggested by Ekasari et al. (1986). Specifically, bacteria and test agents were exposed for a 4-h preincubation period in a 37 °C shaking water bath. The incubation mixture contained 0.1 mL of bacterial culture, plus 1 mL of test solution or H_2O control. For the juices listed in Table II, stored and resuspended freeze-dried samples were used. The dried samples were resuspended in 0.3 M phosphate buffer to 5 times the original concentration, and 0.2 mL was added to the incubation tubes, along with 0.8 mL of H_2O. In addition to the bacterial and test liquids, either 0.25 mL of concentrated S-9 mix or 0.1 mL or 0.33 M phosphate buffer (pH 7.4) was added. Buffer strength was increased to 0.5 M for the experiments in Tables IV and V. Following 4 h of preincubation, molten top agar containing histidine/biotin solution was added to exposure tubes for plating: either 2 mL of standard top agar (Table V) or 3 mL containing 6.7 mL of BioHis/100 mL of agar. For Table IV, BioHis was contained in the bottom agar at 7.27 mL/L. The concentrated S-9 mix consisted of 16 mM $MgCl_2$, 66 mM KCl, 10 mM glucose 6-phosphate, 8 mM sodium diphosphate, 116 mM sodium phosphate (pH 7.4), and S-9 at 0.2 mL/mL of mix. Aflatoxin, nitroquinoline *N*-oxide (NQNO), emodin, and danthron were used as positive controls.

RESULTS AND DISCUSSION

Tables I–IV, which summarize data from different experiments, show that (a) revertant frequencies without microsomal activation in the presence of fresh orange juice are 2–3 times greater than those of the H_2O controls; (b) heating from 0.5 to 30 min at 100 °C did not significantly alter the level present in fresh juice; (c) the observed increases in revertant count were similar for all four bacterial strains tested; and (d) with five commercial brands tested in four strains, the values were similar to those obtained by heating juice from freshly squeezed oranges. Thus, in no case does the response appear greater than that observed with fresh juice.

When the same experiments were carried out in the presence of S-9 mix, background lawns on the plates were abnormal, containing an abundance of barely visible pinpoint colonies. It is difficult to state whether these are true revertants. Visually, they do not appear to be. The pinpoint colonies were too small to be read at 0.2-mm size threshold by an automatic colony counter (Artec 980) and were also too small to carry out confirmatory streaking experiments. Thus, no data from S-9 activation are presented in the tables.

Mutagenic response, however, was confirmed by restreaking randomly sampled revertant colonies from TA98 plates exposed to fresh orange juice, heated fresh orange juice, commercial orange juice from a carton, and an H_2O control. Since all of the orange juice derived colonies grew on minimal glucose plates lacking histidine, the results suggest that the original orange juice response is a true reversion, i.e., mutagenesis in TA98.

One factor that may increase the apparent, but not actual, mutagenicity in the *Salmonella*/microsome assay is the presence of free histidine. For example, van der Hoeven et al. (1983) report that additional histidine up to 20 μg/plate resulted in plates with dense background "lawns" and irregular patterns of revertant colonies.

To assess the fate of free histidine and lysine in orange juice (Kuneman et al., 1988) following heat treatment,

we measured the content of these two amino acids in fresh and heat-treated orange juices by amino acid analysis. Table II shows that the amount of both amino acids decreased after heat exposure. Thus, after heat treatment for 4 min, the histidine level changed from 6.6 to 3.4 μg/mL. The corresponding change in lysine level was from 27 to 20 μg/mL. These findings imply that fresh orange juice did not contain sufficient free histidine to affect the mutagenic count in the *Salmonella* assay. Generally since both the histidine and lysine levels decrease after heat exposure, the net mutagenic count may arise from a decreased influence of histidine and an increased influence of mutagenic browning products derived from free lysine, histidine, or other amino acids (Gazzini et al., 1987; MacGregor et al., 1989).

Another factor that could increase the mutagenicity of juices is the heat- and glucosidase-promoted hydrolysis of nonmutagenic flavone glucosides to the mutagenic aglycones, such as quercetin, kaemferol, or limocitrin. However, this is an unlikely explanation for the present results because the strain specificity and activation requirements characteristic of flavone mutagenicity (MacGregor, 1984, 1986) were not observed in the present studies.

To find out whether other fruit juices behave in a manner similar to orange juice, we measured the number of revertants produced by unheated and heated freshly prepared apple, grape, and pear juices. Table V shows that (a) for apple juice the number of revertants differed only slightly from the control (H_2O) value and did not change with heating; (b) for grape juice the number of revertants was about twice that of the H_2O control and also did not change with heating; and (c) for pear juice the number of revertants was about 1.5 times greater than the H_2O control and decreased somewhat with heating.

CONCLUSIONS

Our observations do not confirm the dramatic increase in mutagenicity reported for orange juice exposed to heat treatment (Ekasari et al., 1986). The data in Table II were obtained with concentrated resuspended, freeze-dried orange juice similar to that used by Ekasari et al. (1986). Because these results did not differ from those obtained with fresh orange juice, all further studies were done with fresh juices. It should also be noted that Ekasari et al. (1986; Figure 1) cite a maximum effect at 0.2 mL of sample, obtained after resuspending freeze-dried orange juice to a concentration 5 times that of fresh orange juice. In terms of concentrations, our results with 1-mL samples of fresh orange juice (Tables I, III, and IV) are therefore equivalent with the 0.2-mL samples.

Our limited studies also indicate that heating at 100 °C for 2 min (Table IV) does not seem to change the apparent mutagenic activities in bacteria induced by apple, grape, and pear juices.

The results of this and earlier studies suggest the need for further research to differentiate relative contributions of (a) naturally occurring mutagens such as flavonoids, carbonyl compounds, and furans; (b) induced mutagens formed during food processing such as amino acid–carbohydrate browning products (Spingarn et al., 1983; Trammell et al., 1986; Gazzini et al., 1987; Handwerk and Coleman, 1988; MacGregor et al., 1989); (c) antimutagens of known and unknown structure (Shinohara et al., 1988; Stich and Rosin, 1984); and (d) free histidine to the net mutagenic activities in bacteria of fresh and processed fruit juices.

ACKNOWLEDGMENT

We thank J. T. MacGregor for helpful advice.

LITERATURE CITED

Beveridge, T.; Harrison, J. E. Nonenzymatic browning in pear juice concentrate at elevated temperatures. *J. Food Sci.* **1984**, *49*, 1335–1340.

Bjeldanes, L. F.; Chew, H. Mutagenicity of 1,2-dicarbonyl compounds: maltol, kojic acid, diacetyl, and related substances. *Mutat. Res.* **1979**, *67*, 367–371.

De Flora, S.; Benicelli, C.; Serra, D.; Izzotti, A.; Cesarone, C. F. Role of glutathione and N-acetylcysteine as inhibitors of mutagenesis and carcinogenesis. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 3, Chapter 3.

Ekasari, I.; Jongen, W. M. F.; Pilnik, W. Use of bacterial mutagenicity assay as a rapid method for the detection of early stage of Maillard reactions in orange juices. *Food Chem.* **1986**, *21*, 125–131.

Ekasari, I.; Bonestroo, M. H.; Jongen, W. M. F.; Pilnik, W. Mutagenicity and possible occurrence of flavonol aglycones in heated orange juice. *Food Chem.* **1989**, *31*, 289–294.

Friedman, M. *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins*; Pergamon Press: Oxford, England, 1973; Chapter 16.

Friedman, M. Sulfhydryl groups and food safety. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum Press: New York, 1984; pp 31–63.

Friedman, M.; Smith, G. A. Inactivation of quercetin mutagenicity. *Food Chem. Toxicol.* **1984**, *22*, 535–539.

Friedman, M.; Levin, C. E. Composition of Jimson weed (*Datura stramonium*) seeds. *J. Agric. Food Chem.* **1989**, *37*, 999–1005.

Friedman, M.; Diamond, M. J.; MacGregor, J. T. Mutagenicity of textile dyes. *Environ. Sci. Technol.* **1980**, *14*, 1145–1146.

Friedman, M.; Wehr, C. M.; Schade, J. E.; MacGregor, J. T. Inactivation of aflatoxin B_1 mutagenicity by thiols. *Food Chem. Toxicol.* **1982**, *20*, 887–892.

Gazzini, G.; Vagnarelli, P.; Cuzzoni, M. T.; Mazza, P. G. Mutagenic activity of the Maillard reaction products of ribose with different amino acids. *J. Food Sci.* **1987**, *52*, 757–760.

Handwerk, R. G.; Coleman, R. L. Approaches to the citrus browning problem. A review. *J. Agric. Food Chem.* **1988**, *36*, 231–236.

Kuneman, D. W.; Braddock, J. K.; McChesney, L. L. HPLC profile of amino acids in fruit juices. *J. Agric. Food Chem.* **1988**, *36*, 6–9.

Lee, H. S.; Nagy, S. Quality changes and nonenzymatic browning intermediates in grapefruit juice during storage. *J. Food Sci.* **1988**, *53*, 168–172.

MacGregor, J. T. Genetic and carcinogenic effects of plant flavonoids: an overview. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum: New York, 1984; pp 497–526.

MacGregor, J. T. Genetic toxicology of dietary flavonoids. In *Genetic Toxicology of the Diet*; Knudsen, I., Ed.; A. R. Liss: New York, 1986; pp 33–43.

MacGregor, J. T.; Friedman, M. Nonmutagenicity of tetrabromophthalic anhydride and tetrabromophthalic acid in the Ames *Salmonella* microsome mutagenicity test. *Mutat. Res.* **1977**, *56*, 81–84.

MacGregor, J. T.; Tucker, J. D.; Ziderman, I. I.; Wehr, C. M.; Wilson, R. E.; Friedman, M. Nonclastogenicity in mouse bone marrow of fructose/lysine and other sugar/amino acid browning products with in vitro genotoxicity. *Food Chem. Toxicol.* **1989**, *27*, 715–721.

Maron, D. M.; Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* **1983**, *113*, 173–215.

Molnar-Perl, I.; Friedman, M. Inhibition of food browning in amino acid-carbohydrate mixtures, apples, potatoes, fruit juices, and protein-rich foods by sulfur amino acids. 4th International Symposium on the Maillard Reaction, Laussane, Switzerland, Sept 5–8, 1989; Abstract.

Powrie, W. D.; Wu, C. H.; Molund, W. P. Browning reaction systems as sources of mutagens and carcinogens. *EHP, Environ. Health Perspect.* **1986**, *67*, 47–54.

Robertson, G. L.; Samaniego, C. M. L. Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *J. Food Sci.* **1986**, *51*, 184–187.

Shinohara, K.; Kuroki, S.; Miwa, M.; Kong, Z. L.; Hosada, H. Antimutagenicity of dialyzates of vegetables and fruits. *Agric. Biol. Chem.* **1988**, *52*, 1369–1375.

Spingarn, W. E.; Garvie-Gould, C. T.; Slocum, L. A. Formation of mutagens in sugar-amino acid model systems. *J. Agric. Food Chem.* **1983**, *31*, 301–304.

Stich, H. F.; Rosin, M. P. Naturally occurring phenolics as antimutagenic and anticarcinogenic agents. In *Nutritional and Toxicological Aspects of Food Safety*; Friedman, M., Ed.; Plenum: New York, 1984; pp 1–30.

Stich, H. F.; Rosin, M. P.; Wu, C. H.; Powrie, W. D. Clastogenic activity of dried fruit. *Cancer Lett.* **1982**, *12*, 1–8.

Toribio, J. L.; Lozano, J. E. Nonenzymatic browning in apple juice concentrate during storage. *J. Food Sci.* **1984**, *49*, 889–892.

Trammell, D. J.; Dalsis, D. E.; Malone, C. T. Effects of oxygen on taste, ascorbic acid loss and browning of pasteurized, single-strength orange juice. *J. Food Sci.* **1986**, *51*, 1021–1023.

Van der Hoeven, J. C.; Lagerwij, W. J.; Bruggeman, I. M.; Voragen, F. J.; Koeman, J. Mutagenicity of extracts of some vegetables commonly consumed in the Netherlands. *J. Agric. Food Chem.* **1983**, *31*, 1020–1026.

Yamaguchi, T.; Nakagawa, K. Mutagenicity of and formation of oxygen radicals by trioses and glyoxal derivatives. *Agric. Biol. Chem.* **1983**, *47*, 2461–2465.

Received for review June 19, 1989. Accepted November 7, 1989.

Registry No. L-His, 71-00-1; L-Lys, 56-87-1.

Effect of Dietary Fish Oil on ω-3 Fatty Acid Levels in Chicken Eggs and Thigh Flesh

Zhi-Bin Huang,[†] Henry Leibovitz,*[,‡] Chong M. Lee,[‡] and Richard Millar[§]

Department of Food Science and Nutrition, University of Rhode Island, West Kingston, Rhode Island 02892, Department of Aquatic Product Processing, Shanghai Fisheries College, Shanghai, China, and Department of Animal and Veterinary Science, University of Rhode Island, Kingston, Rhode Island 02881

Incorporation of ω-3 polyunsaturated fatty acids into chicken egg, thigh meat, and adipose tissue was studied by feeding laying hens diets containing up to 3% menhaden oil for 4 weeks. Dietary fish oil increased eicosapentaenoic acid (20:5ω3 (EPA)) and docosahexaenoic acid (22:6ω3 (DHA)) in the fatty acid distribution of egg yolk. In thigh meat EPA did not increase while the DHA increased with 3% dietary fish oil. The levels of EPA and DHA in adipose tissue increased with increased dietary fish oil. The ratio of EPA to DHA in egg and thigh meat was inversely proportional to the ratio in fish oil. Organoleptic evaluation showed that eggs and thigh meat remained acceptable with up to a 3% dietary fish oil stabilized with 0.1% ethoxyquin.

Early in the 1960s capsules of fish oil and fish oil fatty acid concentrates were manufactured for heart patients (Stansby, 1982). Through long-term studies, scientists discovered that ω-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) lowered ischemic heart disease (Anonymous, 1981; Ahmed and Holub, 1984). It has been reported that these two fatty acids not only alter membrane lipid composition by reducing the production of thromboxane A2 but also reduce the levels of plasma triglycerides and cholesterol (Peifer, 1967; Sanders, 1985), thus preventing thrombosis and atherosclerosis (Anonymous, 1979; Zapsalis and Beck, 1985). DHA is also known to be a very important fatty acid in the vertebrate nervous system (Ackman, 1980).

Attempts to produce a secondary source of dietary ω-3 polyunsaturated fatty acids have been made by feeding animals fish meal rich in ω-3 fatty acids. When hens were fed fish oil, the fatty acid composition of egg yolk reflected that of the diet (Navarra et al., 1972). Machlin et al. (1962) found that polyunsaturated fatty acids in egg lipid can be readily increased by increasing the level of polyunsaturated fatty acids in the diet. Other reports indicated that dietary lipids could also influence the fatty acid pattern of chicken muscle lipids (Marion and Woodroof, 1963; Miller et al., 1967a). Feeding laying hens with fish oil increased both egg production and hatchability (Gauglitz et al., 1974). The effective level of fish oil in the diet was found to be 2–6% (Stansby, 1967). The results of other studies indicated that fish oil could also promote the growth of chickens (Dansky, 1962; Edwards et al., 1961).

As for flavor taint, laying hens were fed up to 2.5% fish oil without adversely affecting egg flavor (Holdas and

[†] Department of Aquatic Product Processing, Shanghai Fisheries College.

[‡] Department of Food Science and Nutrition, University of Rhode Island.

[§] Department of Animal and Veterinary Science, University of Rhode Island.

0021-8561/90/1438-0743$02.50/0