27330-46-7; 2,4-dimethyl-3-thiazoline, 60755-05-7; 2,4,5-trimethyl-3-thiazoline, 60633-24-1; 4,5-dimethyl-2-ethyl-3-thiazoline, 76788-46-0; thiazole, 288-47-1; 2-methylthiazole, 3581-87-1; 2-acetylthiazole, 24295-03-2; 2,5-dimethylthiazole, 4175-66-0; 2-methyl-5-ethylthiazole, 19961-52-5; 4,5-dimethyl-2-acetylthiazole, 7531-76-2; 2,4,5-trimethylthiazole, 13623-11-5; 2-thiazolyl ethyl ketone, 43039-98-1; 1,2,3-trithia-5-cycloheptene, 13005-82-8; pyrazine, 290-37-9; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2-methyl-5-ethylpyrazine, 13360-64-0; 2-methyl-6-ethylpyrazine, 13925-03-6; 2,3,5-trimethylpyrazine, 14667-55-1; 2,5-dimethyl-3-ethylpyrazine, 13360-65-1; 2,6-dimethyl-3-ethylpyrazine, 13925-07-0; 2,6-diethyl-3-methylpyrazine, 18138-05-1; 2,4,5-trimethyloxazole, 20662-84-4; 2,3-dimethylpiperidine, 5347-68-2; 2,4,6-trimethyl-1,3,5-dithiazine, 94944-51-1.

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Effect of Solar Drying on Vitamin D_3 and Provitamin D_3 Contents in Fish Meat

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Vitamin D_3 (cholecalciferol) and provitamin D_3 (7-dehydrocholesterol) in fish meat were analyzed by high-performance liquid chromatography, and their contents in solar-dried dried fish meat were compared with those in fresh one. Moreover, the effects of peroxides and freshness on the nutritive constituents in fish meat were examined. Vitamin D_3 in fish meat was reduced by solar drying from 7.4 (mackerel) and 5.2 (saury) $\mu g/100$ g to less than detection limits (1.4 $\mu g/100$ g). However, no marked changes in provitamin D_3 contents by the processing were observed. The vitamin D_3 was unstable against sunlight, but it was not affected by peroxides and freshness. These results suggest that vitamin D_3 in fish meat is not produced by solar radiation and sunlight plays a large part in the reduction of vitamin D_3.

It has long been known that a deficiency of vitamin D leads to rickets in children and osteomalacia in adults. In recent years, the number of patients with diseases peculiar to the aged, such as osteoporosis, has tended to increase with the advancing average span of human life. Recently, it was reported that the plasma 1α,25-$(OH)_2$-D_3 levels in osteoporosis patients decreased as the characteristic symptoms progressed (Heaney, 1977). Moreover, Orimo et al. (1982) indicated that relief of lumbago and an increase in bone density were produced by the administration of 1α-OH-D_3 to the patients. These findings suggest that vitamin D plays a very important role in the bone disease.

In foods vitamin D is a natural component and results from fortification with a source of vitamin D_2 or vitamin D_3. A relatively small number of natural foods, such as fungi (Takeuchi et al., 1985), eggs (Parrish, 1979), and fishes (Higashi et al., 1961; Yamakawa et al., 1963; Takeuchi et al., 1984a), contain nutritionally significant quantities of vitamin D. There are many processed foodstuffs, especially in fishes. Considering the relation between the health of aged humans and the foods they consume, it is important to determine the changes in vitamin D content of fish meat by processing. However, there are few studies of vitamin D contents in the processed foodstuffs of fishes.

The present study was designed to determine the effect of solar drying on the contents of vitamin D_3 and provitamin D_3 in fish meat and to clarify the effects of sunlight, peroxides, and freshness on the nutritive constituents.

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MATERIALS AND METHODS

Preparation of Samples. The fresh mackerels (average weight 402 g) and sauries (average weight 132 g) caught in the Pacific Ocean in February, 1986, were obtained from a fish market. To determine the effect of solar drying on vitamin D_3 and provitamin D_3 contents, mackerels and sauries of six fishes each were used. These fishes were cut

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to butterfly fillet, and three fishes each were directly exposed to the sun for 6 h. The other fishes as controls (fresh) were kept in a dark place to avoid sunlight. The dried fishes prepared for this experiment were equivalent to the quality of the goods on the market. The edible portions of dried and fresh fishes were subjected to the analysis of vitamin D_3 and provitamin D_3 by high-performance liquid chromatography (HPLC). In order to clarify the factors affecting vitamin D contents in fish meat, experiments showing the effects of sunlight and peroxide on the nutritive constituents in the crude fat and the effect of freshness on the constituents in fish meat were performed. The crude fat was extracted from mackerels according to the Bligh and Dyer method (1959). The crude fat (15 g) in a beaker (50 mL) was directly exposed to the sun for 6 h, and the control was kept in a dark place in the same manner as the fish samples. Moreover, the crude fat in beakers was permitted to stand 17 days in a dark room kept at 22.0 ± 0.5 °C. The peroxide value and the contents of vitamin D_3 and provitamin D_3 in the fat were measured, and their time courses were elucidated. To investigate the relationship between the degree of freshness and the contents of vitamin D_3 and provitamin D_3, the edible portion of mackerels was minced and made uniform in an electric-powered meat chopper. The minced meat was incubated at 37 °C for 4 and 8 h. According to sensory evaluation, the sample of 8 h entered the early phase of spoilage. The contents of vitamin D_3 and provitamin D_3 in these samples were determined by HPLC.

Determination of Vitamin D_3 and Provitamin D_3. The crude fats of fish meat (50 g) were extracted by the method of Bligh and Dyer (1959). The extracted fats were saponified in KOH–ethanol (containing 0.2 g of L-ascorbic acid) with room temperature and darkness for 24 h. The unsaponified fraction was extracted with hexane, washed with distilled water, and dried with anhydrous sodium sulfate. After the fraction was distilled to dryness under reduced pressure below 40 °C, the vitamin D_3 and provitamin D_3 were dissolved in 1 mL of methanol. The methanol solution (20 μL) was subjected to HPLC analysis.

HPLC was performed on a Jasco TRIROTAR-II liquid chromatograph with a JASCOSIL SS-10-ODS-B column (ambient). Detection of vitamin D_3 and provitamin D_3 was carried out with use of a Jasco UVIDEC-100-II ultraviolet spectromonitor (265 nm, 0.04 AUFS). The mixture of acetonitrile and methanol (80:20) was used as the mobile phase, flowing at a rate of 1 mL/min. The standard mixture of vitamin D_2 (ergocalciferol), vitamin D_3 (cholecalciferol), provitamin D_2 (ergosterol), and provitamin D_3 (7-dehydrocholesterol) was separated satisfactorily, and calibration curves plotting the peak area were linear. The identities of peaks on the chromatogram of samples were established by the retention times of each standards (Suzuki et al., 1987). The results are reported as the mean plus/minus standard deviation, and significant differences between experiment and control were determined by the Student's t-test (Snedecor and Cochran, 1967).

Measurement of Peroxide Value. Peroxide value was determined by the iodometric method (AOAC, 1984). The crude fats of mackerel were dissolved in glacial acetic acid–chloroform, and KI was added. This solution was titrated with a 0.01 N solution of thiosulfate using 1% starch solution as an indicator, and the peroxide value was calculated.

RESULTS AND DISCUSSION

HPLC chromatograms of the unsaponified fraction in fresh and solar dried mackerel are shown in Figure 1. Only a peak corresponding to provitamin D_3 was recognizable in the edible portion of dried mackerel, whereas peaks corresponding to vitamin D_3 and provitamin D_3 in the fresh mackerel were observed. Moreover, no peaks of vitamin D_2 and provitamin D_2 were found in these samples. From these peak areas in the chromatogram we calculated the quantities of vitamin D_3 and provitamin D_3 in the samples. The effect of solar drying on the vitamin D_3 and provitamin D_3 contents in mackerel and saury is expressed with values in Table I. Vitamin D_3 in both mackerel and saury samples was reduced by solar drying below the detection range. However, no marked differences in the provitamin D_3 contents of both mackerel and saury between fresh and dried fishes were observed ($P > 0.05$).

Recently, it was reported that vitamin D_2 in Shiitake (*Lentinus edodes*), one fungi, is photochemically produced from provitamin D_2 and elevated by solar radiation during

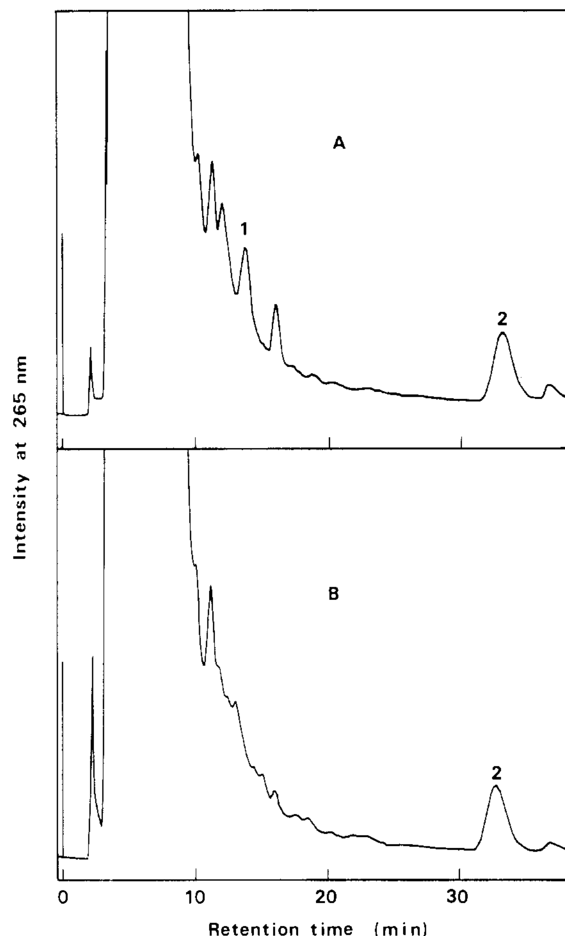

Figure 1. High-performance liquid chromatograms of vitamin D_3 (1) and provitamin D_3 (2) in the edible portion of fresh (A) and solar-dried (B) mackerel.

Table I. Effect of Solar Drying on the Vitamin D_3 and Provitamin D_3 Contents[a,b] in Mackerel and Saury Muscles

fish meat[c]	vitamin D_3 mean	vitamin D_3 SD[d]	provitamin D_3 mean	provitamin D_3 SD[d]
fresh mackerel	7.4	4.5	78.1	10.9
dried mackerel	ND[e]*		56.3	29.4
fresh saury	5.1	3.3	18.1	5.1
dried saury	ND[e]*		15.7	6.7

[a] Micrograms/100 g. [b] Each value represents the mean and standard deviation for three samples. [c] For the preparation, see Materials and Methods. Water contents of the fresh and dried mackerel were 60.1 ± 1.7 and 55.7 ± 5.0%, respectively, and those of the fresh and dried saury were 59.3 ± 4.7 and 52.9 ± 3.1%, respectively. [d] SD = standard deviation. [e] Less than 1.4 μg/100 g in this sample. Key: *, $P < 0.05$ compared to the fresh samples.

Table II. Effect of Sunlight on the Vitamin D_3 and Provitamin D_3 Contents[a,b] in Crude Fat Extracted from Mackerel Muscle

	vitamin D_3		provitamin D_3	
crude fat[c]	mean	SD[d]	mean	SD[d]
exposure	18.6*	2.7	103.2	23.9
control	70.9	7.7	105.7	6.7

[a] Micrograms/100 g. [b] Each value represents the mean and standard deviation for triplicate analyses. [c] For the preparation, see Materials and Methods. [d] SD = standard deviation. Key: *, $P < 0.05$ compared to the control.

Table III. Effect of Incubation at 37 °C on Vitamin D_3 and Provitamin D_3 Contents[a,b] in Mackerel Meat

	vitamin D_3		provitamin D_3	
hour	mean	SD[c]	mean	SD[c]
0	7.9	1.1	33.8	7.4
4	7.4	1.4	34.3	5.0
8	7.9	0.9	35.1	10.8

[a] Micrograms/100 g. [b] Each value represents the mean and standard deviation for triplicate analyses. [c] SD = standard deviation.

farming (Takeuchi et al., 1984b). On the other hand, our results indicate that vitamin D_3 contents in fish meat are not produced from provitamin D_3 and they are reduced by the treatment of solar drying. The discrepancy between these results may be caused by the differences in the stability and state of vitamin D between fish meat and Shiitake.

The effect of sunlight on the vitamin D_3 and provitamin D_3 contents in the fat of mackerel meat is indicated in Table II. Vitamin D_3 contents in the fat decreased clearly by the exposure to sunlight for 6 h, while no significant changes in the provitamin D_3 contents by the same exposure were found ($P > 0.05$). The data show that vitamin D_3 in the fat is unstable against sunlight, and it is not produced from provitamin D_3 by sunlight. These results are good agreement with the case of solar drying of fish meat. Time courses of the vitamin D contents and peroxide value in the fat permitted to stand under the condition of 22.0 ± 0.5 °C are illustrated in Figure 2. The peroxide values in the fat at 9 and 17 days increased definitely. But, no essential changes in both vitamin D_3 and provitamin D_3 contents were observed. These results suggest that there is no relation between the vitamin D_3 content and peroxide value, and peroxide does not play an important role in the phenomenon such as reduction of vitamin D_3 contents in fish meat.

Effects of incubation at 37 °C on vitamin D_3 and provitamin D_3 contents in mackerel meat are shown in Table III. No significant changes in the vitamin D_3 and provitamin D_3 contents were recognized within the incubation of 8 h, entered upon the early phase of spoilage ($P > 0.05$). This result suggests that vitamin D_3 and provitamin D_3 in fish meat are not affected by the microorganisms and enzymes, which are varied in numbers and activities with advancing spoilage of fish meat, respectively.

In conclusion, this study provides the first information that vitamin D_3 in fish meat is not produced and that it is reduced by solar radiation, despite the fact that provitamin D_3 is rich in the meat. Furthermore, our results suggest that sunlight, not peroxide and freshness, plays a large part in the reduction of vitamin D_3. Additional studies are needed to determine the increase and decrease in vitamin D_3 contents of fish meat by other processing. These findings will be useful for improving the diet of aged humans.

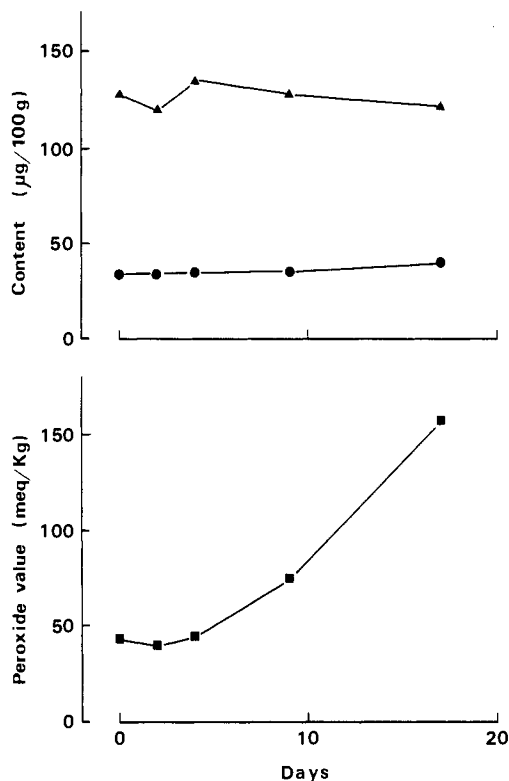

Figure 2. Time courses of vitamin D_3 (●) and provitamin D_3 (▲) contents and peroxide value (■) in the crude fat extracted from mackerel muscle.

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Browning and Protein Polymerization Induced by Amino–Carbonyl Reaction of Ovalbumin with Glucose and Lactose

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Ovalbumin (OVA) was stored with glucose or lactose in a solid system. Though there was no major difference in the decrease of the free amino group between OVAs stored with glucose and lactose, the browning of the OVA–glucose system was much stronger than that of the OVA–lactose system. Removal of free glucose in the early storage of the reaction strongly suppressed the browning reaction, while the addition of glucose to lactosylated OVA promoted it. Glucose induced protein polymerization more strongly than lactose, and the polymerization proceeded even after free glucose was removed during storage. These results suggested that the presence of free glucose in the storage system was an important factor for browning but not for protein polymerization and that the Amadori rearrangement compound formed in the OVA–glucose system was smoothly converted into aldehyde compounds; however, the Amadori compounds formed in OVA–lactose and 4-*O*-methyl-D-glucose systems were stabilized by the protecting effect of the galactopyranoside and methyl group, respectively, on 4-OH of glucopyranosides.

Browning, protein insolubilization, and reduction of protein nutritional value are well-known to result from the protein–sugar Maillard reaction. The glycosylation was studied on lysine residues in protein such as RNase (Baynes et al., 1984) and collagen (Monnier et al., 1984). However, there are only a few reports comparing the Maillard reactions of various sugars with protein amino groups. Bunn and Higgins (1981) reported that the rate of Schiff base formation of various sugars with protein under physiological conditions depended on the extent of carbonyl formation rather than the ring structure. We previously reported that in a solid system a decrease in protein amino group induced by the reaction with glucose was not so different from that induced by the reaction with galactose, whereas browning and protein polymerization of the protein–galactose system proceeded more strongly than that of protein–glucose system (Kato et al., 1986).

Lactose is a major sugar in milk, and the Maillard reaction in some dairy products has been investigated from nutritional and biochemical viewpoints (Möller, 1981; Lee et al., 1979). The studies evaluating the Maillard reaction during processing and storage in lactose-hydrolyzed milk products induced much greater protein quality losses than in unhydrolyzed milk. This difference might be attributed to the difference in Maillard reaction between glucose–galactose–protein and lactose–protein systems (Burvall et al., 1977). However, the comparison of the reactivities of lactose and glucose with protein have not yet been investigated under the same condition.

The experiments reported here were designed to investigate browning and polymerization of protein occurring during Maillard reaction of ovalbumin–glucose and ovalbumin–lactose systems.

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MATERIALS AND METHODS

Preparation of Maillard-Reacted Protein. Ovalbumin (OVA) was prepared from fresh egg white of White Leghorn hens by the ammonium sulfate method (Marshall and Neuberger, 1972). Glucose and lactose were purchased from Waco Co., Ltd. (reagents of superfine grade).

The mixtures of ovalbumin and glucose (OVA–glu) (1:1, w/w) or lactose (OVA–lac) (1:2, w/w) were dissolved in distilled water, and the solution was adjusted to pH 7.5 with dilute NaOH. These mixtures were freeze-dried and kept in desiccators for various periods (0–26 days) at 50 °C and 65% relative humidity (RH) maintained with saturated KI solution. As controls, ovalbumin and each sugar were individually maintained for 26 days in a similar manner.

Separation of Free Sugars from OVA–Sugar Systems. Two-day-stored OVA–glu and 4-day-stored OVA–lac samples in which protein amino groups were blocked at almost the same rate were filtrated with Sephadex G-50, and free sugars were removed from the ovalbumin–sugar mixtures. These protein–sugar adducts without free sugars were adjusted to pH 7.5 with dilute NaOH solution. The freeze-dried samples were stored for another 1–3 weeks at 50 °C and 65% RH. Ovalbumin–glucose or –lactose complexes were abbreviated as OVA–G and OVA–L, respectively.

Electrophoresis. Sodium dodecyl sulfate ($NaDodSO_4$) polyacrylamide gel electrophoresis (8% acrylamide) (SDS-PAGE) was performed by the method of Laemmli (1970).

Browning. Browning of OVA–sugar mixtures (1 mg/mL protein concentration) was measured by absorbance at 420 nm. The insoluble protein–sugar mixtures were solubilized by hydrolysis with Nagase (2 Penn Plaza, New York, NY) for 2 h at 37 °C, and the absorbance was recorded.

Analytical Methods. Free amino groups were measured by the fluorometric method using fluorescamin (Rade) according to Böhlen et al. (1973). The fluorescence

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