Some New Observations on the Pathways of Formation of Dimethylamine in Fish Muscle and Liver

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Dimethylamine (DMA) formed at a more rapid rate in frozen Pacific whiting muscle that had been preheated (40–60 °C) than in unheated muscle. DMA formed only during frozen storage and not during the heating cycle, indicating that its formation was not the direct result of enzymic activity on TMAO but rather to the reaction between TMAO and compounds that were formed during the heating cycle. The catabolites of cysteine and Fe^{2+} catalyze the degradation of TMAO to DMA: cysteinesulfinic acid > hypotaurine > taurine. In vitro studies showed that cysteine did not catalyze the degradation of TMAO to DMA, but it accelerated the formation of DMA in fish liver homogenates. EDTA and Fe^{2+} also accelerated the formation of DMA in fish muscle homogenate, and the data suggest that DMA will not form in fish muscle in the absence of Fe^{2+}.

Extensive studies have shown that the origin of dimethylamine (DMA) and trimethylamine (TMA) in fresh and processed fishery products is trimethylamine oxide (TMAO). Unlike TMA whose formation parallels microbial growth, the formation of DMA occurs maximally at temperatures below the freezing point of fish (−5 to −10 °C). Further, the formation of DMA is species dependent, forming primarily in the gadoid species, e.g., cod (*Gadus morhua* Linnaeus), Pacific whiting (*Merluccius productus*), and pollock (*Theragra chalcogrammus*) (Yamada, 1969; Castell, 1971; Castell et al., 1973b). Predominantly the literature ascribes the formation of DMA to the action of endogenous tissue enzyme(s) on TMAO. Presumably these enzyme(s) directly utilize the TMAO as a substrate to form DMA and formaldehyde (Amano and Yamada, 1964a; Tokunaga, 1964, 1970, 1974; Castell, 1971). In spite of the many reports alluding to the presence and isolation of TMAOase enzymes in fish muscle and organs, the only definitive works in this area are those reporting on the preparation of active fractions isolated from fish organs such as pyloric ceca, kidney, and liver (Amano and Yamada, 1964b; Yamada and Amano, 1965a,b; Yamada et al., 1969; Tomioka et al. 1974). Recently, Harada (1975) reported on the purification and properties of an active TMAOase isolated from the liver of *Saurida tumbil*. But even in this work he acknowledged that the isolate was a mixture of at least two zymoproteins that required a cofactor for activation and then concluded that the TMAO-splitting enzyme was not a specific enzyme but rather a portion of a complex enzyme system. Investigations with fish muscle are not nearly as definitive, and evidence for TMAOases in muscle is primarily based on the inference that DMA will not form in muscle that has been heated in excess of 88 °C (Tokunaga, 1964; Castell et al., 1971). In more recent work, Lall et al. (1975) found that if silver hake was preheated to 80 °C, no DMA formed when it was stored for 38 days at −10 °C. They also found that if silver hake was preheated to 35 and 60 °C, DMA formation was slightly inhibited under these same time and storage conditions. It has been clearly demonstrated, however, that TMAO can be degraded to DMA and FA by chemical reactions (Lecher and Hardy, 1948; Sundsvold et al., 1969), and over 20 years ago, Tarr (1958) suggested that the formation of DMA in fish muscle may not be enzymically induced. Recent work at our laboratory (Spinelli and Koury, 1979) showed that DMA rapidly formed in heat-processed dry fish muscle and that several chemical compounds and ionic constituents could easily convert TMAO to DMA.

Additional work is presented in this paper suggesting that DMA can be formed by the reaction of TMAO with endogenous ionic constituents and/or catabolically derived substances formed post-mortem in fish muscle and liver. Data are presented showing that the catabolites of cysteine and Fe^{2+} may be implicated in these reactions.

MATERIALS AND METHODS

Fish. Pacific whiting (*Merluccius productus*), rockfish (*Sebastes ruberimus*), and Dover sole (*Microstomus pacificus*) were obtained from local trawlers and were held at melting ice temperatures prior to use.

Studies To Determine the Ability of Various Compounds To Produce DMA in Fish Muscle Homogenates. *Special Reagents.* 2,2′-Bipyridine, L-amino acids, acid hydrolyzed casein, taurine, hypotaurine, cysteine, cysteinesulfinic acid, cysteic acid, and sodium iodoacetate were obtained from Sigma Chemical Co., St. Louis, MO. EDTA was used in the disodium form, Fe^{2+} as $FeSO_4 \cdot 7H_2O$, Fe^{3+} as $FeCl_3 \cdot 6H_2O$, Cu^{2+} as $CuSO_4 \cdot 5H_2O$, and Ca^{2+} as $CaCl_2$. TMAO was obtained from ICN Pharmaceutical Co., Plainsville, NY. Enzymically hydrolyzed casein hydrolysate (4.9% amino nitrogen) was obtained from Nutritional Biochemical Co., Cleveland, OH. Prior to use, 1% aqueous solutions of each reagent were prepared.

Reaction Conditions. For the data presented in Table I, homogenates were prepared to contain 200 ppm of all reagents shown except for H_2O_2 where a 3% stock solution was prepared and the homogenate was made to contain 5000 ppm of H_2O_2. Samples were frozen in mylar pouches and stored at −5 °C for at least 30 days prior to analyses for DMA: 50 g of the homogenate was blended for 3 min with 75 mL of 6% $HClO_4$ and filtered. The filtrates were neutralized with 50% KOH and appropriate aliquots were analyzed for DMA by the method of Dyer and Mounsey (1945).

Heat Treatment of Pacific Whiting Muscle. Fresh headed and gutted Pacific whiting were used to prepare minced flesh by using a Bibun flesh separating machine (Robert Reiser & Co. Inc., Boston, MA). The minced flesh was packed in 14-lb wax-coated cartons, frozen in a plate freezer, and stored at −20 °F until used.

Slices, approximately 10 × 4 × 1.25 cm, were cut from the blocks of minced fish. Two slices (~125 gm) were placed in individual plastic bags. After being thawed, the

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samples were heated to the desired temperature in a water bath. The temperature in the packages was monitored by placing thermocouples in one package. The samples were heated to 40, 60, 70, 80, and 100 °C and reached equilibrium within 3–10 min. They were held at that temperature for an additional 10 min and then removed and immediately cooled in an ice bath. The samples were stored at 0, −5, −17.7, and −28.8 °C.

Separation of Fish Muscle into Myofibrillar and Sarcoplasmic Proteins. Sarcoplasmic proteins were obtained by centrifuging muscle homogenates for 0.5 h at 8000 rpm in a refrigerated centrifuge (+1 °C). The supernatant fluids were identified as the sarcoplasmic proteins. The solids were resuspended in 0.1 M NaCl (3 parts of NaCl to 1 part of solids) and recentrifuged. The resuspension of the solids in the 0.1 M NaCl was repeated 3 times, and the supernatant fluids were discarded. The solids were identified as myofibrillar proteins.

Reaction of Cysteine and Its Catabolites. *In Solution.* Aqueous solutions of the following concentrations were prepared: TMAO, 0.25 M; cysteine, 0.1 M; cysteinesulfinic acid, 0.1 M; hypotaurine, 0.1 M; taurine, 0.1 M; cysteic acid, 0.1 M; Fe^{2+}, 0.01 M. One milliliter of the cysteine and/or catabolites was added to 5 mL of the TMAO solution. After standing for 24 h at ambient temperature (22 °C), the solutions were analyzed for DMA by the method of Dyer and Mounsey (1945). Where the effect of Fe^{2+} was assessed, 1 mL of the Fe^{2+} solution was added to the reaction mixture.

In Liver Homogenates. Aqueous solutions (10%) of TMAO and L-cysteine·HCl·H_2O were prepared. One milliliter of TMAO and 1 mL of cysteine were added in rapid sequence to 25 g of freshly prepared liver homogenate. The homogenates were reblended (50 mL capacity Waring blender cup) for 10 s, transferred to plastic petri dishes (9 cm), and stored at −5 °C. After each storage interval, 5 g was extracted with 25 mL of 6 N $HClO_4$ for DMA analyses (Spinelli and Koury, 1979), and appropriate dilutions were analyzed for DMA by the method of Dyer and Mounsey (1945).

In Muscle Homogenates. Muscle homogenates were made to contain 200 ppm of either cysteine, hypotaurine, or cysteinesulfinic acid by adding 4 mL of 1% aqueous solutions of these catabolites to 200 g of the homogenates. The effect of Fe^{2+} was assessed by adding 2 mL of a 1% solution/200 g of homogenate. The homogenates and additives were immediately remixed for 5 min in a stainless steel mixing bowl (Hobart Kitchen Aid mixer). The samples were packed in mylar pouches and stored at −5 °C.

RESULTS

Reaction between Various Substances and TMAO to Form DMA. On the basis of findings presented in our previous model-system studies (Spinelli and Koury, 1979) showing that TMAO can be degraded to DMA by in vitro reaction with SO_2 and by EDTA in combination with Fe^{2+} and Sn^{2+}, we added several compounds to Pacific whiting muscle homogenates to determine their effect on the rate of formation of DMA during frozen storage (−5 °C). The compounds used in this experiment were chosen because of their known reactivity with TMAO and inherent chemical characteristics such as oxidation/reduction potential, ion-binding properties, and ability to influence enzymic reactions. The results of this study are tabulated in Table I. It shows that protein hydrolysate accelerated the formation of DMA, but that the amino acids arginine and histidine had no effect. Cysteine, however, exhibited a slight but significant accelerating effect. H_2O_2 and $KBrO_3$ showed an inhibitory effect as did Cu^{2+} and sodium iodoacetate. Ionic constituents in the reduced form such as Fe^{2+} and Sn^{2+} accelerated the formation of DMA, but Fe^{3+} and Ca^{2+} had no effect. Combinations of Fe^{2+} and cysteine accelerated the rate of DMA formation over that observed when these compounds were individually added, but no net effect on the reaction rate was observed when cysteine was combined with Fe^{3+}.

Table I. Effect of Various Compounds on the Production of DMA in Pacific Whiting Muscle Homogenates Stored at −5 °C

compd	effect[a]
protein hydrolysate	+
arginine	±
histidine	±
cysteine	+
H_2O_2	−
$KBrO_3$	−
EDTA	+
phytic acid	+
2,2′-bipyridine[b]	−
Fe^{3+}	±
Fe^{3+} + cysteine	±
Fe^{2+}	+
Fe^{2+} + cysteine	+
Sn^{2+}	+
Ca^{2+}	±
SO_2	+
Cu^{2+}	−
iodoacetate	−

[a] (−) Inhibitory; (+) accelerates; (±) no effect. [b] Tested only in pollock muscle.

The active role of Fe^{2+} in the formation of DMA became more evident when the effects of the ion-binding compounds, EDTA, phytic acid, and 2,2′-bipyridine, were examined. EDTA and phytic acid accelerated DMA formation, but 2,2′-bipyridine inhibited its formation. This apparent inconsistency can probably be explained by the difference in the modes of Fe^{2+} binding. 2,2′-Bipyridine forms an iron complex (Treadwell and Hall, 1948), whereas EDTA and phytic acid form an iron chelate that is accompanied by the loss of an electron and significant change in the potential of the system (Dow Chemical Co., 1959). These observations are related to and consistent with those of Craig et al. (1961), who proposed a mechanism for the rearrangement of tertiary amino acids via metallic complexing of iron and various organic acids such as tartaric, citric, and aspartic.

The strong inhibitory effect of Cu^{2+} and iodoacetate tends to support the contention that the pathway of DMA formation is enzymically induced as both of these compounds are powerful enzyme inhibitors.

Effect of EDTA and Fe^{2+} on the Formation of DMA in Fish Tissue. *Species Differences.* Shown in Table II are the rates of formation of DMA in the muscle homogenates of three different species of fish containing added Fe^{2+}, EDTA, and combinations of Fe^{2+} and EDTA. Fe^{2+} and EDTA accelerated the formation of DMA in all the species during storage. Significantly more DMA was produced in the gadoid species, Pacific whiting, than in the nongadoid species, rockfish and Dover sole. The combination of Fe^{2+} and EDTA, however, clearly exerted a greater net effect in Dover sole and rockfish than in Pacific whiting. In this respect, it is interesting to note that with Pacific whiting the combination of Fe^{2+} and EDTA had no greater effect of DMA formation than did EDTA, whereas significantly more DMA was produced in the homogenate containing only the added Fe^{2+}. This would indicate that Fe^{2+} is limiting in the reaction(s), converting TMAO to DMA. Analyses of the three hom-

Table II. Effect of EDTA and Fe^{2+} on the Production of DMA When Added to Muscle Homogenates of Pacific Whiting, Dover Sole, and Rockfish

species	DMA, mg/100 g, after 0 day	14 days	70 days
Pacific whiting control	0.50	2.86	5.85[a]
Pacific whiting + EDTA	0.47	6.50	16.25[a]
Pacific whiting + Fe^{2+}	0.63	19.50	22.10[a]
Pacific whiting + Fe^{2+} + EDTA	1.27	11.05	16.25[a]
rockfish control	0.08	0.14	0.30
rockfish + EDTA	0.08	0.54	2.96
rockfish + Fe^{2+}	0.08	0.55	0.78
rockfish + Fe^{2+} + EDTA	0.08	4.16	11.70
Dover sole control	0.03	0.03	0.12
Dover sole + EDTA	0.03	0.28	1.04
Dover sole + Fe^{2+}	0.03	1.07	1.43
Dover sole + Fe^{2+} + EDTA	0.03	3.25	8.23

[a] 30 days of storage.

Table III. Formation of DMA in Sarcoplasmic and Myofibrillar Protein Extracted from Pacific Whiting Muscle and Stored at −5 °C

protein fraction	DMA, mg/100 g, after 0 day	14 days	30 days
sarcoplasmic	0.33	7.15	7.48
myofibrillar[a]	0	0.39	0.87
myofibrillar[a] + EDTA[b]	0	1.87	2.60
myofibrillar[a] + Fe^{2+}[c]	0	2.93	2.96

[a] 250 mg of TMAO/100 g of myofibrillar (wet) protein added to all samples. [b] 200 ppm of disodium EDTA added. [c] 200 ppm of Fe^{2+} added.

ogenates for total Fe content showed that there were no significant differences between the species: Dover sole 2.9 ppm, rockfish 3.6 ppm, and Pacific whiting 3.1 ppm. On the basis of our observation that Fe^{3+} (singly or in combination with cysteine) did not influence the rate of DMA formation in muscle, we would conclude that Fe contained in Pacific whiting muscle is present in a reactive Fe^{2+} form, whereas in rockfish and Dover sole it was present in unreactive (complexed) or Fe^{3+} form.

Reaction Site. Pacific whiting muscle was separated into its sarcoplasmic and myofibrillar fractions to determine the respective rates of DMA formation in these two fractions. The data in Table III show that less than 1.0 mg of DMA/100 g was found in the myofibrillar fraction, whereas 7.48 mg of DMA/100 g was found in the sarcoplasmic fraction. About 35–40% less DMA was found in the myofibrillar fractions containing added EDTA and Fe^{2+} than was found in the sarcoplasmic fraction, indicating that the myofibrillar proteins are not implicated in the transformation of TMAO to DMA. In this respect, the data also show that the TMAO-splitting fraction(s) in the muscle are of relatively low molecular weight. If they are bound to cellular fractions as suggested by Dingle (1977), either they maintain their activity in this form or the bond is easily broken by solutions of low ionic strength.

Heat Treatment and Its Effect on DMA Formation during Frozen Storage. Pacific whiting homogenates were heated to 40, 60, 70, 80, and 100 °C prior to storage at temperatures of 0, −5, −17.7, and −28.8 °C. The results presented in Figure 1 show that significantly greater amounts of DMA were formed at all storage temperatures in the samples heated to 40 and 60 °C than in the unheated control samples. Since no DMA formed during the heating cycle, it would be unrealistic to believe that the production of DMA during frozen storage results from direct enzymic activity. A more reasonable explanation is that DMA

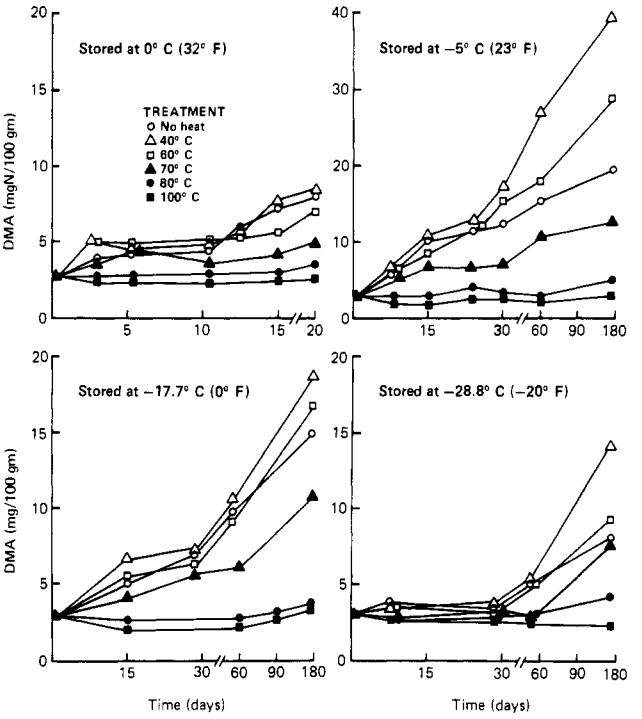

Figure 1. Formation of DMA in Pacific whiting homogenates that had been heated to 22, 40, 60, 70, 80, and 100 °C prior to freezing and storage at −5 °C.

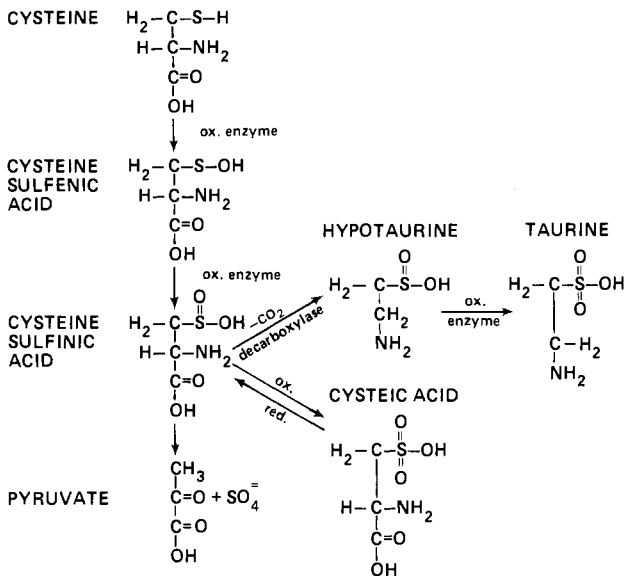

Figure 2. Catabolism of cysteine.

formation results from indirect enzymic activity, i.e., enzymically produced reactive substances are formed during the heating cycle that subsequently react with TMAO during storage. The higher concentrations of DMA formed at the higher storage temperatures are plausibly related to the concentration of the reactants that occur during freezing. Sussman and Chin (1966) showed that the liquid phase in frozen cod is 28 and 6%, respectively, at −5 and −28.8 °C.

Reaction between TMAO and the Catabolites of Cysteine: In Vitro Reactions. In several species of fish, the most abundant nonprotein nitrogen constituent in the muscle is taurine (Love et al., 1958; Mackie and Ritchie, 1974). Although its formation and function in the muscle is not clear, it has been demonstrated that in the muscle it can be derived as a catabolite of cysteine as shown in Figure 2 (Harrow and Mazur, 1966).

Table IV. Reaction of TMAO with Cysteine and Catabolites of Cysteine: Effect of Fe^{2+} on Reaction Rates

compd	Fe^{2+}	formation of DMA	reaction rate
cysteine	present	–	no reaction
	absent	–	no reaction
cysteine-sulfinic acid	present	+	very fast
	absent	+	slow
hypotaurine	present	+	fast
	absent	–	slow
taurine	present	+	slow
	absent	–	no reaction
cysteic acid	present	–	no reaction
	absent	–	no reaction

Table V. Formation of DMA in Pacific Whiting Muscle Homogenates Containing Fe^{2+}, Cysteine, Hypotaurine, and Cysteinesulfinic Acid and Stored at –5 °C

treatment	DMA, mg/100 g, after 0 day	14 days	35 days
cysteine			
control	0.5	3.1	6.0
cysteine	0.5	4.3	9.6
Fe^{2+}	0.7	9.1	13.0
cysteine + Fe^{2+}	0.6	11.0	18.2
hypotaurine			
control	0.6	3.8	9.9
hypotaurine	0.6	3.9	10.7
Fe^{2+}	0.6	13.7	18.9
hypotaurine + Fe^{2+}	0.7	14.3	22.1
cysteinesulfinic acid			
control		3.4	8.3
cysteinesulfinic acid		4.9	13.5
Fe^{2+}		9.1	15.0
cysteinesulfinic acid + Fe^{2+}		7.2	18.2

Experiments were done to determine whether TMAO and the catabolites of cysteine reacted to form DMA and whether Fe^{2+} influenced the rate of reaction. The results given in Table IV showed that although cysteine and cysteic acid did not react with TMAO, some of its catabolites did produce DMA as a reaction product at these respective rates: cysteinesulfinic acid > hypotaurine > taurine. When Fe^{2+} was added, the reaction rate was increased.

Effect of Adding Cysteine and Catabolites to Fish Muscle and Liver Homogenates. When cysteine, cysteinesulfinic acid, and hypotaurine were added to Pacific whiting muscle homogenates, significantly greater amounts of DMA were produced during storage than in the control sample (Table V). The homogenates containing cysteinesulfinic acid contained over twice as much DMA as the control samples, whereas the samples containing added cysteine and hypotaurine respectively contained ~40–50% more DMA than the control. Fe^{2+} accelerated the reaction rates in all the samples to which it was added, again indicating that Fe^{2+} plays a primary role in the formation of DMA in fish muscle.

The addition of cysteine to Pacific whiting and rockfish liver homogenates resulted in the production of large quantities of DMA after only 7 days of storage. The results given in Table VI show that after 7 days of storage, 8% of the TMAO in rockfish liver had been converted to DMA. In the Pacific whiting homogenates ~60% of the TMAO was converted to DMA. Although the Pacific whiting liver homogenates possessed the ability to convert TMAO without the added cysteine, the addition of cysteine doubled the reaction rate. Rockfish liver homogenates, however, had little or no ability to convert TMAO to DMA in the absence of cysteine. These data indicate

Table VI. Formation of DMA in Pacific Whiting and Rockfish Liver Homogenates Containing Added Cysteine and TMAO and Stored at –8 °C for 2 and 7 Days

species	DMA, mg/100 g, after 2 days	7 days
rockfish		
control	0	0.11
control + TMAO	0.08	0.26
control + TMAO + cysteine	6.24	9.90
Pacific whiting		
control	0.60	0.78
control + TMAO	6.8	31.2
control + TMAO + cysteine	7.1	67.6

that if DMA is being produced in the homogenates via an indirect enzyme pathway, the enzymic systems that catalyze the catabolism of cysteine are more active in the livers of rockfish than in Pacific whiting. That is, what may be taking place is that the more reactive catabolites are accumulating in the Pacific whiting liver homogenates, allowing for their subsequent reaction with TMAO. The same phenomenon may be occurring in muscle and this area requires further investigation.

DISCUSSION AND CONCLUSIONS

The above experiments definitely show that the formation of DMA in frozen fish can be derived by pathways not requiring the presence of TMAO-splitting (TMAOases) enzymes. The fact that DMA formed in much greater quantities in muscle homogenates that had been preheated to 60 °C than in unheated samples strongly suggests that reactive substances were formed during and possibly after the heating cycle and that these, in turn, reacted with TMAO to yield DMA. The mechanism leading to the formation of these compounds is probably enzymic in nature and accounts for the general belief that DMA is formed by direct enzymic conversion of TMAO to DMA. Our results with preheated Pacific whiting could be in slight disagreement with those of Lall et al. (1975). In their experiments, they stored silver hake samples for ~38 days at –10 °C, whereas we stored our samples at three different temperatures for 180 days and found that DMA formed most rapidly after 30–60 days of storage. Experiments showing the ability of the catabolites of cysteine to degrade TMAO to DMA provide support to the possibility that the origin of DMA in fish muscle is the end product of indirect enzyme activity. The data show that although cysteine does not react in vitro to produce DMA, it will accelerate the formation of DMA when added to fish muscle and liver homogenates. The addition of cysteine catabolites such as cysteinesulfinic acid and hypotaurine to muscle homogenates shows that they exert a greater influence on DMA formation than cysteine and rules out the possibility that cysteine is merely acting as an enzyme cofactor. Pacific whiting liver has the ability to degrade TMAO to DMA, but this ability is practically absent in rockfish liver. With the addition of cysteine, however, DMA rapidly forms in rockfish liver. The comparatively large differences in the amounts of DMA found in Pacific whiting and rockfish livers would tend to rule out the possibility that DMA is formed by the reaction between Fe^{3+} and cysteine: cysteine + Fe^{3+} → cystine + Fe^{2+}; cysteine + Fe^{2+} → DMA + FA (Vaisey, 1956).

It is significant to note that if Fe is removed from the system by complexing compounds such as 2,2′-bipyridine, the formation of DMA is inhibited, showing that Fe^{2+} is involved in the formation of DMA, regardless of any described mechanism of the reaction. Fe^{2+} will in itself or

in the presence of chelators (EDTA and/or phytic acid) accelerate the formation of DMA. These observations are consistent with those of Craig et al. (1961), who discussed tertiary amine oxide rearrangement in the presence of Fe^{2+}, Fe^{3+}, and organic constituents, and Ferris et al. (1967), who found that Fe^{2+} catalyzed the degradation of TMAO but that Fe^{3+} did not. In this respect, no means (except severe heating) has been found to inhibit the formation of DMA in frozen gadoid muscle. On the contrary, several accepted food additives such as EDTA and SO_2 accelerated its formation—the same may be true for some antioxidants. Because the presence of DMA has been related to fish quality problems in frozen fish (Babbitt et al., 1972; Castell et al., 1973a) and potential problems of public health significance (Singer and Lijinsky, 1976; Ember, 1980), more work is required to define its formation and, in particular, its inhibition in processed fishery products.

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Identification of Methionine-Rich Polypeptides in Peanut (*Arachis hypogaea* L.) Seed

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Peanut seed protein was extracted from the defatted meal with a buffer containing 2 M NaCl, 0.01 M Tris-HCl (pH 8.2), and 0.002% (w/v) NaN_3. The protein extract was then separated into 10 fractions by gel filtration on a Sephacryl S-300 column. Amino acid analysis of the protein eluants from the column showed 1.4%, 2.9%, and 1.2% methionine in fractions V, VI, and VII, respectively. In addition to methionine, fractions VI and VII were found to be rich in cystine (10.75% and 6.52%, respectively). Methionine-containing proteins were also identified by labeling the seed proteins with [^{35}S]methionine, 18 weeks after planting. The [^{35}S]methionine incorporation data was found to be consistent with the methionine content of the fractions which also showed the highest amount of radioactivity in fraction VI. Gel electrophoresis revealed that all the proteins of fraction VI contained [^{35}S]methionine radioactivity. However, the amount of radioactivity varied among the polypeptides of fraction VI. Autoradiographs of two-dimensional polypeptide maps from fraction VI indicated the presence of four major and two minor radioactive polypeptides with different isoelectric points and molecular weights.

Peanut seeds have an excellent potential as a source of plant protein for incorporation into a variety of foods (Dechary and Altschul, 1966; Johnson and Lay, 1974). However, like other legumes, peanuts are also low in sulfur-containing amino acids like methionine and cystine. Many studies have shown that the nutritional value of peanut proteins (arachin and conarachin) differs considerably because of higher amounts of methionine, lysine, and cystine in the conarachin fraction than in the arachin fraction (Sauberlich et al., 1948; Horn and Blum, 1956; Woodham and Dawson, 1968; Basha and Cherry, 1976). These observations suggest that identification of cultivars with higher amounts of conarachin would lead to an improvement in the nutritional level of peanut proteins. Limited exploratory research has shown that there are genotypes within the species *hypogaea* with a better balance of nutritionally important amino acids than that

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