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Post-mortem breakdown of trimethylamine oxide in fishes and marine invertebrates.

By Kinjiro Yamada

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Comments:

Underlined Sections, are sections which are literal and involve chemical or other expressions not familiar to translator, and therefore left as much as possible in original text to convey closest possible meaning.

Some underlined parts are phonetic translations of the Japanese phonetically expressed words and expressions. For instance many English terms are expressed in Japanese phonetically, and makes translation difficult except to the Japanese chemist familiar with phonetic expressions of these terms.

Words in Quotation Marks. These are phonetic translations of Japanese fish names, which for the most part are expressed in Japanese phonetics in the original text. A glossary has been prepared for these words on page 33.

Interchangeable Words The same word is expressed in Japanese to mean: "estimate" "deduce" and "presume." The word to "form" is translatable only as to be "present" in some context.

Japanese Names The pronunciations of Japanese names, especially the given names are arbitrary, and quite often the names can be read in more than one way.

Special Words sound awkward in translation, but have been left that way on purpose. For instance, the word "aquatic life" has been used in translation. This may be translated as "fisheries" or "marine" life but the latter expressions convey certain connotations which may or may not have been desired by the writer.

Words like "species" "types" "varieties" loosely used in the text in many areas. Also the word "species" or "types" have fairly broad and inexact usage in Japanese.

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(January 31, 1968, Filed)

Post-mortem Breakdown of Trimethylamine Oxide in Fishes and Marine Invertebrates

Kinjiro Yamada**

PARAGRAPH (1)

The previous summary(1) outlined the sources and distribution of Trimethylamine Oxide (to be abbreviated TMO) in fishes and dealt with a biological study on TMO formation. The post-mortem breakdown of this substance is an interesting problem, on the one hand from the scientific viewpoint regarding freshness, and on the other hand from a biological standpoint.

(2) TMO in the flesh of fish species is reduced by bacterial enzyme after death into Trimethylamine (to be abbreviated TMA). But in the system of fish species and in the organs as well, there seems to exist a TMO reductase plus a type of enzyme that breaks down TMO by enzyme action, in other words, an enzyme which forms formaldehyde (to be abbreviated FA) and dimethylamine (to be abbreviated DMA). Centred on these two enzymes, we will deal here with the post-mortem breakdown of TMO.

Formation of TMA

(3) TMA in the flesh of fish species increases with the decline in freshness. This appears to have become clear in the period 1920-1930(2-6). The Beatty team(3) established the fact that this increase in TMA is due to bacterial action. In other words, they report that TMA does not increase in the sterile flesh of Atlantic cod Gadus morrhua when left at

low heat or room temperature. In addition, by verifying that the sum of TMO and TMA contained in the flesh of Atlantic cod which had been in storage for varying days is always constant, Beatty(7) made it clear that the parent body of TMA contained in the flesh is chiefly TMO. According to this Beatty report, at least 94% of the TMA formed originates from TMO. But the Ronold team(8) found that in the flesh of Atlantic herring Clupes harengus, C. sprattus of the same herring genus, and Gadus virens flesh of the "madara" genus, although whereas TMO disappears within a few days of storage, TMA continued to increase in substantial quantities.

(4) There are many reports on the formation of TMA in the flesh of fish species through bacterial action. Most of these reports deal with the determination of freshness. Among these reports, the flat-gilled fish species have been studies by Shewan(5,13), the Reay team(9), Elliott(10), Shimizu(11), and Suyama(12); the ocean teleosts have numerous works(3,4,13-45) centred around Canada's Halifax Fisheries Research Board and Britain's Torry Laboratories; and other types of aquatic life have been studied by the Shimizu group(46-48), and the Collins team(49), and also the Lartigue team(50).

(5) page 542 Flat-Gilled Species. The formation of TMA in dogfish species of fish in cold storage has been studied by Shewan(5,13) and the Reay team(9). According to results obtained, a large quantity of TMO exists in the flesh of this shark species, but the formation of TMA is slow, and the quantity formed showed no great difference from that of haddock Melanogrammus aeglefinus or Atlantic herring. Elliott(10 sought to clarify the reason for this with respect to "abura tsunozame" Squalus acanthias. According to this, a large quantity of ammonia is formed in the flesh as the degree of freshness drops, leading to the increased of pH in the flesh,

and this in turn results in the suppression of the action of TMO reductase. But the Shimizu team(11) and Suyama(12) report that in the flesh of hammerhead Sphyrna zygaena, "dochizame" Triakis scyllia, "yoroizame" Dalatias licha, and "abura tsunozame" Squalus acanthias, the pH increases to over eight accompanying the decline in freshness, and a large quantity of volatile amine is also formed. Since it is thought that a large part of this volatile amine is TMA, the results obtained by the Shimizu team(11) and Suyama(12) differ from that obtained by Shewan(5,13), the Reay team(9) and Elliott(10). Furthermore, Suyama(12) notes that TMO almost disappears from the flesh of blue shark Glyphis glaucus accompanying the decline in freshness, and an amount of TMA almost corresponding to the amount of TMO loss is formed. From the foregoing, there appears to be a need for further investigation to determine whether there is a difference between flat-gilled species and the ocean teleosts with respect to the formation of TMA in the flesh accompanying the decline in freshness; and the relationship between the formation of ammonia and the loss in the activity of bacteria TMO reductase.

Ocean teleosts. Generally the TMA increase accompanies the decline in freshness with respect to ocean teleosts. This increase becomes an indicator of freshness(actually of decay) (3-5,13,15,20,32,33,37-39). But there is an opposite opinion to this(17,24). The Beatty team(3) recommended that a specific amount of TMA be used as an index of freshness, having found that a specific amount of TMA usually led to the perception of decay odor in the flesh of Atlantic cod, and although this is indirect, found the overall curve for bacteria increase paralleling the TMA increase curve. But the Tarr team(24) studied the relationship between

TMA amounts and bacteria number during the decaying in the flesh of Ophidion elongatus of the rock trout family plus three other fish species, and reported situations where the bacteria number was small and the TMA production large, as well as where the opposite situation applied. Furthermore, the decline in freshness does not necessarily coincide with the increase in bacteria.(51)

(4) The effectiveness of the method of determining freshness through a specific amount of TMA must, as is the case with other physical and chemical determination methods, be based on the standard of whether the results obtained are functional. Furthermore, it is necessary to be exactly clear regarding the purpose of freshness determination. The purpose of the generally applied freshness determination is first, to make clear whether the degree of freshness is good or not at the time of the determination, second, to estimate the number of days in storage possible with respect to fish species where its degree of freshness is unknown, and third, to estimate the time lapse since the time of catching.

(8) Regarding the first objective, there are many reports with respect to the use of TMA amount as indicator of freshness. The Beatty team(3), taking 100 ml of liquid squeezed from the flesh of Atlantic cod, reports that initial decay odor is noticed when TMA-N rises to about 4-6 mg, complete decay odor at 10 mg, and a strong decay odor at 20-30 mg. Similar to the Beatty team, the Brocklesby team(4) reports that even with dressed fish of "ohiyo" hippoglossus stenolepis kept in cold storage, decay is indicated at 4-5 mg. On the other hand, the Castell team(32, 33,39) studied the relationship between quality and TMA amounts (TMA-N in about 100 g of flesh) in a large number of Atlantic cods and haddocks. According to this result, grade one has 0-1 mg% of TMA-N, grade II has 1-5 mg%, and grade III has more than 5 mg%. Grade I is judged good in freshness, grade II declines in freshness but is considered usuable for

filleting, grade III is extremely poor in freshness, possibly unsuited for filleting and must be used for fish powder. Results obtained by the Castell team(39) were studied statistically by Hoogland(38) who proposed giving a value of over 7 mg% of TMA-N for grade III. This value was later scrutinized by Castell and satisfactory results obtained(40).

(9) The Dyer team(20) and the Shewan team(42) both recognized a high degree of mutual relationship between functional determination and TMA amounts in the case of Atlantic cod and haddock which are extremely closely related fish species. But it is questionable whether the same TMA-N value can be used as freshness indicator in these two fish groups. Recently, the Castell team(44) studied the relationship between quality and TMA amounts in over 3000 instances of the above mentioned two fish groups, and reports that using the same TMA-N value, the latter's (haddock's) freshness is lower than that of the former (Atlantic cod.) In addition, the Shewan team(31,36,42) clarified that the relationship between freshness amounts differs with the catch with respect to Atlantic cod, and the Castell team(44) reports that this relationship differs according to the period (in the fishing season) of the catch.

(10) The relationship between freshness and TMA amounts differs according to fish types, fishing grounds, handling conditions after the catch, storage conditions, condition of the specimens to be tested, etc. Sigurdsson(22) reports that TMA-N is 5-7 mg% in initial decay, and 12 mg% at complete decay with respect to a fish species presumed to be Atlantic herring; and the Horie team(35) established that the TMA amount at decay period differs extremely according to fish species, from investigations on 11 fish species. Also, by comparing fish species

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of Atlantic and Pacific oceans, the Tarr team(24) clarified that the TMA cannot be taken as freshness indicator even in the same fish species if fishing grounds differ. The Dyer team(21) has a study on fish handling conditions after the catch. According to this, the TMA amount in Atlantic cod differs with the storage location in the same fishing boat. With reference to storage conditions, TMA can be a freshness indicator when the storage temperature is over 0 degrees C but not when it is -2 degrees C, as a result of studies by Sigurdsson(22) on what is presumed to be Atlantic herring. In addition, it is reported that TMA is not a freshness indicator with fish that have been treated for anti-decay, or which have been radiation-treated(52,53). The influence on the relationship between freshness and TMA amount by the specimen condition at the time of the determination is reported on by the Castell team(40). Taking Atlantic cod and haddock, they clarified that the relationship between freshness and TMA amount varies between fish in the round or where the insides have been removed, as against those which have been filleted.

(11) From the above studies on TMA, it is possible to understand the difficulty of establishing exactly the physical and chemical method of determining freshness, even where the objective is merely to determine whether freshness is satisfactory or not at the time of the determination. Accordingly, in the actual determination of freshness, the functional test is chiefly used, jointly using TMA and other physical and chemical test methods. In export testing (54) of fresh-frozen mackerel types, where it is not possible to make a functional determination of freshness, the test basis is through the measurement of volatile alkali and the degree of hydrogen ionization. In this case, the TMA measurement method is Conway's

minute quantity scatter method(55), but there are other measurement methods such as the Aconite method(55), Picrate method(56,57), and chromatography method(45). On the other hand, the TMA measurement is not utilized as test standard in the case of frozen mackerel species that has been heat treated. This is probably obvious from the fact that TMO in fish breaks down to form TMA (58-62) in the process of heat application.

(12) With reference to the second objective, namely to estimate the period of storage withstandable for specimens of unknown freshness, there is the Hess(18) report. The results obtained here indicate that when liquid squeezed from cod flesh of unknown freshness is left at 25 degrees C for three hours and the increase in TMA during this period is 75% or more, this specimen can be stored at 0 degrees C for 24-48 hours before decay starts.

(13) No report can be found regarding the third objective, namely to estimate the lapse of time since the catching of the fish. The possibility of establishing exactly the freshness determination method for this purpose is extremely slim.(63)

(14) Other Aquatic animals. There are only a few studies concerning the relationship of freshness and TMA amount in fisheries life apart from the fish types.(46-50) It is reported that the TMA amount method of determining decay is effective in one type of "taraba-ebi" Pandalus sp. which has been in ice storage or refrigerated by sea water(49), but not effective in the case of oysters in ice storage(50).

TMO Oxidase

(15) The formation of TMA in the flesh of fish species is due chiefly to the action of the bacteria, TMO reductase. But as stated previously, there appears to exist at the same time in the fish, outside the flesh

section, an enzyme that is able to reduce TMO.

Bacteria Enzyme. Tarr(64,65) took two species of fresh fish, (16) five species of smoked fish, and separated them into Micrococcus 15 parts, Flavobacterium 4 parts, Achromobacter 6 parts, yeast 5 parts, totalling 30 parts; and reported that of these, 2 parts of Micrococcus and 1 part of Achromobacter possessed the ability to reduce TMO. Later, the Wood team(66) studied the TMO reducing ability of Enterobacteriaceae(74 bacteria types, 650 parts), and the Baird team(67) did likewise with Micrococcaceae (30 bacteria types, 56 parts). According to these studies, much of the bacteria types and bacteria parts belonging to Enterobacteriaceae will reduce TMO but the greater part of that belonging to Micrococcaceae does not have this ability. For this it becomes evident that the existence of aerobic bacteria TMO reductase, including the common anaerobic bacteria, is not too universal. On the other hand, there is a study by the Ando team(68) regarding the TMO reducing ability in abnormal anaerobic bacteria. According to the Ando team, Clostridium botulinum will, regardless of the bacteria type, types A. B. and also E., reduce TMO.

"part" refers to "parts" mentioned above

(17) This bacteria enzyme that participates in the reduction of TMO are called triamine oxidase(69), trimethylamine oxidase(69), or trimethylamine-oxide reductase(71-73), and the naming is thus not uniform. From the fact that this bacteria will activate trialchelamine oxide possessing the generality of $R_3{\equiv}N{=}O$, Tarr(69) named it triamine oxidase. But there has been no later study on the substrate peculiarity of this bacteria so it is not clear whether Tarr's naming is appropriate or not. Furthermore it is also not clear whether this bacteria will induce the oxidizing reaction of TMO into TMA. Accordingly

we will follow the name given by Tomizawa(71-73), and provisionally call this Victoria trimethylamine oxide reductase (to be abbreviated TMO reductase).

(18) Since the characteristics of bacteria TMO reductase and the part played by this bacteria in the reaction mechanics has been studied almost entirely in the bacteria's dormant state, there are many uncertainties. Tarr(65) was unsuccessful in trying to secure cell-free bacteria through its self digesting action, and the Castell team(74) also failed in trying to produce cell-free bacteria. But Tomizawa(71-73) obtained cell-free bacteria through supersonic treatment, and reported that this bacteria in the presence of formic acid (hydrogen yielding body) reduced TMO(73). But the detailed characteristics of this enzyme has not yet been determined.

(19) According to the study of Tomizawa(71-73) who employed Escherichia coli and Proteus vulgaris in dormant bacteria state, TMO reductase appears to be an adaptable enzyme. This has been confirmed by studies of Suyama(12) and the Tsuchiya team(75). Suyama recognized the existence of a receptive-inducing period in "aozame" Isurus glaucus and two other fish species; and the Tsuchiya group reports that the TMO reduction by Micrococcus separated from shark flesh describes a sigmoid curve. But it cannot be stated definitely that bacteria of TMO reductase are all adaptable enzymes. Tarr(65) has noted that the reduction of TMO by Micrococcus proceeds in a straight line. But the Wood team(66) reports that when TMO reducing bacteria belonging to the Enterobacteriaceae group is kept even as long as two years in culture ground free of TMO, its TMO reducing potential is not lost, and denies the formation of the enzyme through adaptation. And Watson(76), although he does not deny the possibilities due to adaptation, reports two divisions in Achromobacter, that is TMO reducing bacteria and non-TMO reducing bacteria.

It is thought that Pseudomonas is also contained in this Achromobacter(77).

Regarding the mechanics of TMA formation due to TMO reductase, there are studies by Tarr(65), Watson(78), Collins(79), Neilands(70) and Tomizawa(71-73). Because of the inducement of TMO reduction in the presence of various hydrogen yielding bodies in his experiments with dormant bacteria, Tarr thought TMO reductase activated the TMO,and the activated TMO is then reduced by dehydrogenized enzyme, which has a low oxidation reduction potential. Separate from this, Watson studied the mechanics of TMO reduction and presented the following generalization as a reaction route:

$$AH_2 + TMO \rightarrow A + TMA + H_2O$$

AH_2 is a hydrogen yielding body, and the principal hydrogen yielding body in TMO reduction through Achromobacter is said to be lactic acid(78,79). But the oxidation of lactic acid through bacteria does not necessarily act in conjunction with TMO reduction.(80). It is not clear from studies by Tarr and Watson that NAD or NADP participates in TMO reduction, but Neilands claims the existence of an intermediate hydrogen conducting body (or intermediate hydrogen conducting type). According to Tomizawa's study, there is thought to be in existence an unknown auxiliary enzyme in TMO reduction. This substance is thought to exist in the yeast producing solution(71-73).

There are studies by Suyama(12) and the Castell team(74) on the optimum temperature for TMO reductase. Suyama reports that the optimum temperature for TMO reductase is about 40 degrees C; the Castell team(74) while finding the optimum temperature over 37 degrees C in Serratia marcescens, Achromobacter (No. 176) and Escherichia coli, finds the value at 25 degrees C in Pseudomenas putrefaciens. While it

is obvious that the optimum temperature differs according to experiment conditions, especially the difference in the length of the reaction period, the reason is not clear for the difference between the bacteria types obtained by the Castell team. Approximately the same values for optimum pH are obtained by researchers, the Castell team(74) reporting 7.0-7.2, the Tsuchiya team(75) approximately, 7.3, and Tomizawa(72,73) 7.0-7.5.

(23) Results from the studies of the substance which impedes the reduction of TMO in its inactive state ~~is~~ are not necessarily unanimous, (65,70-76). For instance Tomizawa(71-73) deduces that TMO reductase is iron enzyme from the fact that TMO reduction is strongly impeded by KCN and a,a'-dipyridyl. Both Tsuchiya(75) and Tarr(65) see toluene impeding the reduction of TMO. But as Tarr(65) points out, it is not clear whether it is toluene or the dehydrogenized enzyme that page 545 impedes the TMO reductase. But as claimed by the Castell team(74), the impeding action of nitrates and nitrites is thought to be due to their competitive action against dehydrogenized enzyme types. The reasons are: the types of bacteria involved when nitrates and nitrites strongly impede TMO reduction are respectively nitrate reductase and nitrite reductase(74); Achromobacter with ability to reduce TMO also reduces nitrates and nitrites(76-78); and a TMO-sparing effect can be seen (76) in the impeding action by nitrates.

(24) As already stated, there is Tarr's study(69) concerning the substrate peculiarity of TMO reductase. Tarr reports that TMO reductase will reduce, besides the TMO, triethylamine oxide and tripropylamine oxide, and form respective appropriate amine, but that TMA is not formed from betaine, choline, acetyl-choline and stacidoline. Among the bacteria, there are some that will form TMA from choline(81), but since

there are bacteria types that cannot form TMA from choline and yet form TMA from TMO(82), it can be said that TMO reductase is a separate type of enzyme from that which forms TMA from choline.

(25) There are views by the Beatty team(80) and Watson(78) regarding the significance of TMO reductase in bacteria physiology. According to the Beatty team, TMO appears to be an important hydrogen receiving body in common anaerobic bacteria. And Watson reaches the same conclusion from the fact that common anaerobic bacteria in Achromobacter reduce TMO in addition to creating a TMO-sparing effect in the presence of particle state enzyme.

(26) Fish Species Enzyme. TMO reductase seems to be present in fishes as well. Kawabata(83) noted the increase of TMA in the blood-containing flesh section of "marusoda" Auxis tapeinosoma and "binnaga" Thunnus alalunga to which preservative and TMO had been added. When this was left at room temperature or 20 degrees C, he also noted the fact that this increase is strongly suppressed by heating and heavy-metals impeding solution; from the foregoing he deduced that TMO reductase is present in the blood-containing flesh. Similar to Kawabata, Suyama(12) found the formation of TMA in the preservative-treated blood-containing flesh of "hoshizame" Mustelus manazo and sting ray Dasyatis akajei. There are studies by the Amano team(84) and the Harada team(85) on TMA formation in the system other than the blood-containing flesh section. In other words, the Amano team and the Harada team both noted a very rapid formation of TMA on the addition of TMO, the first team in the pylorus fold of "madara" Gadus macrocephalus (preservative-treated), and the latter group in the midintestinal section of similarly treated sagittated calamary Todarodes pacificus.

On the other hand, the presence of TMA oxidizing enzyme is reported in the systems of vertebrate animals including fishes(86,88). The Baker team(86,87) established the formation of TMO from TMA in pig liver homogenate, using 14C as tracer. By this study, the Baker team clarified that the formation of TMA from TMO is due to bacterial action (provisionally called TMA oxidizing enzyme), and that the reducing type NADP is required as auxiliary enzyme. It was indicated furthermore that this enzyme is present in the microsome and not in the Mitochondria, and that the enzyme necessary for TMO formation comes from particle state enzyme and not from solution state. Later, the Baker team made many studies on the presence of this enzyme, mostly in the liver of animals. According to these studies, TMA oxidizing enzyme is present in mammals in the kidneys and lungs as well as in the liver. In fish species, it is present in the liver of one flat-gilled species, and the liver of ocean as well as shallow water teleosts, but it is not present in the midintestinal section of octopus, cancer magister of "ichogani" family "tanbi" order, nor in Pacifastacus leniusulus of the shallow-water "zarigani" species. The fact, as stated, that TMA oxidizing enzyme is not present in all aquatic animals which contain TMO would appear to contradict the idea that the presence of TMO in fish species is due to dissolved poison of TMA(1). But in this connection it is necessary to consider the possibility, as pointed out by the Baker team(88), that the reducing type NAD instead of the reducing type NADP participates in the formation of TMO from TMA, and also the possibility that TMO is formed by a different route. Furthermore, it is possible to imagine the existence of TMA oxidizing enzyme in inactive state under certain conditions. It is not clear whether or not the Baker group's TMA oxidizing enzyme is the same TMO reductase

believed to exist in fish species. It is stated that TMO reductase in bacteria form does not induce the formation of TMO from TMA(65).

Formation of DMA

The formation of DMA in fish flesh accompanying the decline in freshness became clear in the period 1938 to 1940. In 1938, Beatty(7) found the presence of DMA in Atlantic cod. The following year, Reay(6) established the presence of a small amount of DMA in the flesh of Atlantic cod which had been kept in ice storage 24 hours. The same year, Shewan(5) found that although DMA is formed prior to TMA in haddock during storage, hardly any DMA is formed in the dogfish type of fish, and estimated that TMO is probably the parent body of DMA. Again in 1940, Shewan(13) reported that the measurement of DMA is effective in the freshness determination of haddock. Although these studies on the detection of DMA in fish species ~~is~~ are limited to the cod family, later studies report the presence of DMA in fishes other than cod and also in aquatic animals outside the fish species.

Fresh Fish Species. As stated before, there are studies on the presence of DMA in fresh fish species by Beatty(7), Reay(6), and Shewan (5,13), but there are other works by the Dyer team(20), the Miyagi team (89), Hughes(61,62), the Yatsuzaka team(90), Miyahara(91), the Amano team(92), the Hayashi team(93), Tokunaga(94,96), and the Yamada team(95). These studies employ the following methods of DMA measurement: through the process of changing DMA into Nitron(7), copper-dithiocarbamate method (5,6-20,92,94-96), ion exchange resin method(89,91), polarography(90), and gas chromatography(61,62,93). The fish species studied were clam species, head-feet species, shellfish species, flat gill species, and

teleost species, more than 45 species in all. Among these, the DMA was clearly detected in the following eight fish species--the cod family, and its closely related Atlantic cod, "madara" Gadus macrocephalus, "suketodara" Theragra chalcogramma(92,94), "komai" Eleginus gracilis(94), haddock(5,20), "kattaibohige" Lepidion oidema(95), "ezoisoainame" Lotella maximowiczi(92,95), and "isoainame" Lotella phycis(95). In these fish species, the DMA content is greater in the internal organs rather than in the flesh(92,94). A substantial quantity of DMA has been detected in the flesh of Atlantic herring(9,61,62), but hardly any in the flesh of closely related "maiwashi" Sardinops melanosticta, "katakuchiiwashi" Engraulis japonica, "konoshiro" Konosirus punctatus and "kibinago" Sprattelloides japonicus(90,91). Besides this, the DMA content has been studied in the flesh of more than thirty other fish species, but detected in any of the fish species. And even in the fish species where it was detected, the amount was so small that the presence of DMA is not a certainty. On the other hand, with respect to aquatic animals outside the fish species, DMA was detected in the flesh of Loligo formosana, thought to be one species of "jindoika"(91), and in sagittated calamary (94), and in the midintestinal section(95) of "gazami" Portunas trituberculatus.

(30) The DMA content in fish species varies with the degree of freshness. This has been made clear by studies of Shewan(5), the Beatty team(97), the Dyer team(20), Hughes(61,62), and the Amano team(98). The Beatty team(97) report that DMA increases in the liquid squeezed from the flesh of fresh Atlantic cod that is left standing, but the increase is not noted if the same liquid is left in a sterile and filtered state. From this, it can be considered that bacteria participates in the formation

Of DMA in fish species but the existence in bacteria state of DMA producing enzyme is not definitely established.. The Reay team(99) concluded that that TMO is not the parent body of DMA from the fact that when several parts of bacteria that produces DMA in fish liquid ~~was~~ were planted in TMO-containing culture grounds, DMA was not produced. On the other hand, Yaisey(100) studied DMA formation using inert bacteria as enzyme specimen, glucose as the hydrogen-yielding body, and TMO as the receiving body, and noted the formation of TMA but not the formation of DMA. Accordingly, the definite establishment of the existence of DMA producing enzyme in bacteria state must await a future study. But it must be considered also that DMA formation in fish species may not only be due to bacteria oxidizing action, but may also be the action of enzyme possessed by the fish species itself. This has been mentioned in the section dealing with FA formation.

Processed Fish Species. Regarding the presence of DMA in (31) processed fish species, there are studies by Ohta(60), Hughes(61,62), Yatsuzaka team(90), the Kusakabe team(101), Miyahara(91), Tokunaga(94,96, 102), and the Murata team(103). Ohta heated the flesh of mackerel, sardine, and "aodai" Paracaesio caeruleus for one hour at 113 degrees C while Hughes heated the flesh of Atlantic herring for one hour at 120 degrees C and both reported the formation of DMA. This fact indicates the possibility of DMA formation during the sterilization process in certain species of canned fish. Actually, DMA has been detected by Ohta in canned mackerel, canned salmon, and canned cuttlefish. Tokunaga has reported the increase of DMA in "suketodara" in frozen storage, and he is investigating the factors that influence this DMA increase such as the degree of freshness of the original fish, preliminary preparation conditions, and additives such as reducing agents. According to the Yatsuzaka team, considerable DMA is contained in salted or dried "maiwashi" Sardinops melanosticta.

Miyahara reports that although DMA is not present in fresh "maiwashi" meat, @ substantial amount is found in dried "maiwashi." Kusakabe has separated DMA from dried cuttlefish in the form of piclin oxygen salt; but Murata did not find any DMA in dried cuttlefish.

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(32)

Formation of FA

In conducting a FA detection in canned crab in 1917, Ishida(104) noted the FA's characteristic show many color reaction in the crab meat, and by precipating in crystal form through urotropin mercuric chloride double salt, he confirmed that this show many color reaction substance was in fact FA. After that, many studies detected FA in canned fish species. (105-114).

(33)

On the other hand, FA has been detected as well in non-heat-treated fresh fish species, as well as salted and other processed fish species (104,108,109,111-118). But in these FA detection studies, since in most cases the specimens were distilled through vapor distillation or direct distillation and tested for show many color FA reaction, there is a danger of FA being formed due to the breakdown of specimen ingredient due to heat application. This danger is even greater in the cases where precipitation is effected by addition of phosphoric acid or sulphuric acid. Also, the Tankard group(115) and Reay(118) point out that even when FA detection is done directly with solution used to draw out water from fish meat, the FA could possibly be formed through the oxidation of the TMA and DMA present in that solution. For this reason, the presence of FA in fresh fish species was passed over for a long time. In 1961, the Amano team(119) investigated the reason for the FA indicative show many color reaction observed in frozen "madara" meat Gadus macrocephalus while on board an Artic vessel, and separated FA in the form of formol dimedone from the

unheated draw out water solution of "madara" flesh. By this it became clear for the first time that FA is present in fresh fish species also.

(34) Fresh Fish Species. The Amano team(92,119) measured the FA content in the systems and organs of "madara" Gadus macrocephalus, and "sketodara" Theragra chalcogramma(92,94) by the Nash method(120,121), and found FA scarce in the skin and flesh, but plentiful in the pylorus fold, stomach, gall bladder, and spleen. This difference can be seen also in "komai"(122) Eligimus gracilis of the same cod family, "kattaibohige"(95) Lepidion oidema of the "chigodara" family which is closely related to the cod family, "ezoisoainame"(92,95) Lotella maximowiczi, "isoainame"(95) Lotella phycis, and "ibarahige" Nematonurus acrolepis(122) of the "sokodara" family. And furthermore, there are reports on FA content in the flesh of the cod species, by Tokunaga(94) in "sketodara" and "komai," by the Fujimaki team(123) in "madara" and "sketodara." Then also, the Yurkowski team(124) separated and identified FA in the form of volatile carbonir compound through thin chromatography in the salted cod species. From these results, it is unmistakable that FA is present in the cod and its closely related species. But whether or not the presence of FA is the special characteristic of this species of fish must be subject to further investigations. The Yamada team(95) reports the presence of no FA in the flesh nor in other parts of the system of "itachiuwo" Brotula multibarbata and "yoroiitachiuwo" Hoplobrotula armata which outwardly resemble the cod species. Opposed to this, Tokunaga(102) has found the presence of a small quantity of FA in the flesh of "ishigarey" Kareius bicoloratus and "kurogarei" Liopsetta obscura while in cold storage. Also the Fujimaki team(123) reports the presence of FA in shrimps and "onagadai." Regarding the presence of FA in aquatic animals outside

the fish species, Tokunaga(94) has reported its presence in the flesh of saggittated calamary, and the Yamada team(95) in the midintestinal section of "gazami" Portunao trituberlatus and "hiratsumegani" Ovalipes punctatus.

(35) Parent Body of Enzyme. The Amano group(92,95) points out that there is an intimate relationship between the formation of FA and DMA, noting that in "madara" Gadus macrocephalus and "suketodara" Theragra chalcogramma, DMA is plentiful in the system and organs where the FA is also plentiful. Also, studying the changes while in storage of FA and DMA in the same fish species(98), he deduced that the formation of both substances was due to enzyme action. The (appropriateness accuracy of this deduction was confirmed by later studies(84,125). This formation enzyme is present ~~is present~~ in the sediment section of ammonium sulphate 0.14 to 0.35 saturated, in the pyrolus fold of "suketodara," Theragra chalcogramma, and if the upper clear section of the salt-reduced solution is added to this sediment, the formation of FA and DMA from TMO is noted(126). The auxiliary enzyme present in this upper clear section of the above mentioned solution is a heat-resisting substance(126).

(36) Vaisey(100) reports the formation of a small quantity of FA and DMA accompanying the formation of a large quantity of TMA when TMO in vitro is reduced by sistain. This reaction is induced by haemoglobin or double iron ion(100). But it is difficult to imagine that FA and DMA are formed in the cod species by this kind of chemical reaction, or that an enzyme inducing this kind of reaction is present in the cod species. The reason for this is that the formation of FA and DMA in cod species is always preceded by the formation of TMA(5,98). Soudan(113,114) claims that TMO is an aldimine of FA and DMA, that the breakdown of TMO takes place in the presence of oxide or an enzyme that takes away the potential thus forming FA and DMA in fish species. But this idea does not explain

the fact that FA and DMA are hardly ever found in the flesh of the shark species which are rich in TMO content(5,13,119) and the fact that both substances are found only in specific fish species.

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(37) TMO is deduced to be the parent body of both FA and DMA in cod species. This is because when TMO is added to the system of cod species, a greater quantity of FA and DMA is produced than when it is not added (84,125,127.) Furthermore, the formation of both substances is thought to be due to the action of the same bacteria. The reason for this is the formation of FA and DMA from TMO in the pylorus fold of "suketodara" Theragra chalcogramma under identical experimental conditions shows the identical optimum temperature and the identical optimum pH(125), and in cod species, the formation of FA is always accompanied by the formation of DMA(84,92,94-96,98,102,119,122,125-127). Yet although FA is detected in the midintestinal section of "hiratsumegani", DMA is not, and on the contrary DMA is found but not FA(94) in the flesh of "masaba," "hokke" and "akagarei." It is difficult to state conclusively at this time that in all fish species generally, the FA and DMA formation is due to the action of the same variety of bacteria. But although the formation mechanism of FA and DMA in cod species is not clear at this time, the fact (126,127) that an extremely small quantity of methyline blue induces the FA and DMA formation is thought to be a clue to the future understanding of the formation mechanism.

(38) Processed Fish Species. As stated already, the presence of FA was clarified by Ishida's study(104) in canned crab, but later FA has been detected in many other kinds of canned fish species. Namely, on canned crab, there are studies by the Dill team(105), the Kimura team(106, 107), the Igasa team(108,109), the Yanagizawa team(110), the Lunde team(111)

and Shimizu(112); and on other canned fish species by Ohta(60), the Dill team(105), the Igasa team(108,109), the Lunde team(111) and Soudan(113, 114). It is deduced that natural FA is contained in some of the raw material for these canned merchandise, but that in others, FA is formed from TMO in the sterlization process during canning. Ohta(60) heated a TMO solution for one hour at 115 degrees C and produced hardly any FA or DMA, but when he added fish meat extract, cysteine, cystin or tryptofuan etc., FA and DMA was unmistabably formed; he also found FA and DMA in prepared (seasoned) mackerel on the market, boiled salmon, and canned seasoned cuttlefish. But according to Hughes(61,62), when Atlantic herring was heated for one hour at 120 degrees C during canning, DMA was formed but not FA. Before this, from the fact that when heat is applied to the flesh of crab, cuttlefish, octopus, etc., the show many color reaction of FA becomes very evident(108,109), Igasa and also Hattori conducted investigations from which they deduced the parent body of FA to be TMO, and that the TMO breaks down during the sterilization process in canning and FA and DMA are formed(117,128-130) by way of hydroxy-trimethylammonium hydroxide, O, N, N-trimethylhydroxylamine, dimethyl-methoxylammonium hydroxide. But this deduction is doubtful. That is to say, the Hattori team(130) duduced the above mentioned formation mechanism of FA and DMA through the reaction-formation substance resulting from the one hour heat application during canning at 180 degrees C temperature which is much higher than the temperature required to kill bacteria in canned goods. On the other hand, the Yanagizawa team(110)has deduced the FA reaction indicating substance's parent body in canned crab to be histigen, but experimental foundation for this conclusion is lacking. In the formation mechanism of DMA that occurs when Atlantic herring is heated to 120 degrees C, Hughes(61) supports Vaisey's(100) raaction. But as stated before, Hughes(61,62) did not detect any FA in the Atlantic herring that had been heated. Recently,

the Koizumi team(131) reports that when sulphurous acid is added to TMO solution and heated in boiling solution state, DMA and a substance presumed to be monomythylamine is formed in addition to TMA. On the other hand, it was reported by Bickerd(132) some time ago that FA, DMA and TMA are formed in the presence of a specific quantity of unsaturated fat acid.

(39) There is Tokunaga's study on the formation of FA in frozen fish (94,96,102). According to this study, the formation of FA and DMA in frozen "suketodara" Theragra chalcogramma meat is due to the action of enzyme which is activated in frozen state.

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Regarding the fish species names of Japanese fisheries, I have followed Matsuhara(133) for the most part, and in the names of other aquatic animals, I have followed Okada(134).

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Glossary of Names of Fish Species not Found in Japanese-English Dictionary

Abura tsunozame	Squalus Acanthias	literal translation: "oil dogfish"
Akagarei		literal translation: "red" flat fish, or sole.
Aozame	Isurus Glaucus	literal translation: "blue" shark. Size: 3 m.
Binnaga	Thunnus Alalunga	species of tuna, Size 1 m.
Chigodara		cod species
Dochizame		shark species
Ezoisoainame	Letella Maximowiczi	literal transl. "Hokkaido beach rock-trout"
Gazami	Portunas trituberculatus	species of horny crab
Hokke		Fish species like trout, food fish. Size 25 cm
Hoshizame		Shark species, Size 50 cm used for processed food
Hiratsumegani	Ovalipes punctatus	crab species
Ibarahige	Nematonurus Acrolepis	literal translation "thorny beard?"
Isoainame	Letella Phycis	lit. transl. "beach rock-trout"
Ichogani		crab species
Ishigarei	Kareius Bicoloratus	lit. transl. "stone flatfish or sole"
Itachiuwo	Brotula Multibarbata	?
katakuchiiwashi	Engraulis Japonica	herring species: 15 cm. small lower jaw. Used for dried fish
Jinda ika	Elegimus Gracilis	cuttlefish species
Kattaibohige	Lepidion Oidema	?
Kibinago	Spratelloides Japonicus	?
Komai	Elegimus Gracilis	?
Konoshiro	Konosirus Punctatus	Herring species. 25 cm.
Kurogarei	Liopsetta obscura	Lit. transl. "black flatfish or sole"
Madara	Gadus macrocephalus	Cod species
Maiwashi	Sardinops Melanosticta	Important sardine species for food 25 cm.
Marusoda	Auxis Tapeinosoma	Bonito species, 40 cm.
Masaba		Mackerel species
Mebachi		Tuna species. 2m. Big-eyed.
Onagadai		Sea bream species "LONG TAILED"
Ohiyo	Hippoglossus	?
Sokodara		Lit. transl. "bottom cod"
Sobenmoso	Peridinium polonicum	Type of seaweed ?
Tarabaebi	Pandalus sp.	Shrimp species.
Tanbi	see text for clue	?
Suketodara	Theragra Chalcogramma	Cod species 60 cm. edible roe.
Yoroiitachiuwo	Hiplobrotula Armata	
Yoroizame	Dalatias licha	Shark species. Lit. trans "armored shark"
Zarigani		Lobster species found in Northern Japan rivers.

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Additional information for "Glossaries" page 33 of Translation

Akagarei	Hippoglossoides	flounder or sole species
Binnaga	Thunnus Alalunga	Albacore
Chigodara	Moridae	Cod species
Dochizame	Triakis scyllia	Shark species
Ezoisoainame	Lotella Maximowiczi	Koro or Incense Burner
Hoshizame	Mustelus manazo	Star spotted shark or Gummy shark
Katakuchiiwashi	Engraulis Japonica	Half mouthed sardine
Marusoda	Auxis Tapeinosoma	Frigate mackerel
Masaba	Scomber Japonious	Common Japanese mackerel
Mebachi	Parathumus obesus	"Big eye"
Ohio	Hippoglossus Stenolepis	Halibut

Note: There are many fish species for which there is no common English name. These are mostly fish caught in Japanese waters.

Supplemental information on Translation: Bulletin of the Japanese Society of Scientific Fisheries.

Page 3 "Sobenmoso" A flagellata type microscopic living matter found in water.

page 16, paragraph 27 "tanbi" brachyurous

page 17, final line of page. head-feet species. This is cephalopoda species of mollusc family

page 21 midpage. carbonir this may be carbonyl

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The name "Yoshiyuki Amano" appears to be same personn as "K. Amano"of title 95.

The name Yoshiyuki may also be read - Keiji
Keishi
or Keiyuki