AN ABSTRACT OF THE THESIS OF

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Title: MEASUREMENT OF DIMETHYLAMINE AND TRIMETHYL-

AMINE IN DUNGENESS CRAB, CANCER MAGISTER

Abstract approved: _____
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A rapid gas liquid chromatographic method was adapted to pro-cessed Dungeness crab for the determination of basic and neutral volatiles. Methylamine, dimethylamine, and trimethylamine were measured in processed Dungeness crab for the first time. Generally, the range for trimethylamine was 42 ppm for frozen crab to 700 ppm for iced crab. Low levels of dimethylamine were found in iced and frozen samples whereas higher levels (33-71 ppm) were found in can-ned and iced-canned samples. Ammonia, methyl sulfide, acetalde-hyde, acetone, diacetyl, benzene, and toluene were also identified in processed Dungeness crab for the first time.

Measurement of Dimethylamine and Trimethylamine
in Dungeness Crab, <u>Cancer</u> <u>magister</u>

by

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MEASUREMENT OF DIMETHYLAMINE AND TRIMETHYLAMINE IN DUNGENESS CRAB, CANCER MAGISTER

INTRODUCTION

The development of the colorimetric estimation of trimethyla-

mine (TMA) (Dyer, 1945; Dyer and Mounsey, 1945) resulted in the

studies of the formation of TMA during refrigerated storage of fish.

Dyer's method proved satisfactory for the estimation of TMA in iced

fish; however, in some frozen fish species much more dimethylamine

(DMA) than TMA was produced. Several workers (Hashimoto and

Okaichi, 1957; Tozawa, et al., 1970; and Castell et al., 1970) recog-

nized that DMA, the main methylamine produced in frozen gadoid

species, contributed significantly to the colorimetric estimation of

TMA. Japanese workers suggested that K_2CO_3 be replaced by KOH

in the picric acid procedure to eliminate the error due to DMA.

Tozawa et al., (1970) found that KOH derived values agreed with gas

liquid chromatographic (glc) values.

Several glc systems have been employed and some have yielded

satisfactory results. Accurate quantitation of methylamines has posed

a problem due to tailing on most supports. Approaches to elimination

of the tailing problem have been deactivation of the support by washing

with methanolic NaOH (James et al., 1952), coating the support with

KOH (Smith and Radford, 1961), or using an inert support such as

graphitized carbon black (Di Corcia et al., 1970). Tetraethylene-

pentamine (TEP) was used to deactivate Chromosorb W, but resolution

of methylamines was poor (Sze and Borke, 1963). Excellent resolu-

tion of methylamines and ammonia has been obtained with TEP deac-

tivated carbon blacks (Di Corcia et al., 1970). In this study, columns

of 0.5%, 2%, 5%, and 10% TEP on 40/60 mesh Graphon were employed

in the separation of methylamines and ammonia. Resolution was com-

plete for 0.5% and 2% columns; however, increasing the liquid phase

decreased the retention time of the amines. This phenomenon sug-

gested a support deactivation role of TEP in the chromatographic

process.

Miller et al., (1972) used TEP coated Graphon columns (Di

Corcia et al., 1970) and an alkali flame ionization detector (AFID)

to determine methylamines in fish. In this method ground fish was

placed in a vial together with 10% NaOH to liberate the amines from

their salts. Equilibrium vapor analyses (1 ml) were performed and a

linear detector response with known amounts of amine hydrochlorides

(HCl) was demonstrated. Miller et al., (1972) also adapted the on-

column trapping technique of Morgan and Day (1965) to the analysis

of methylamines in fish and reported lower limits of detection of 10

ppb and 50 ppb for TMA and DMA respectively.

The occurrence of amines in fish has been utilized as an index of fish quality (Dyer, 1945; Beatty, 1938). Beatty and Gibbons (1937) established that 94% of TMA in iced cod originated from trimethylamine oxide (TMAO) by bacterial reduction. Thus, the TMA level provided an indicator of bacterial deterioration in iced sea fish. Methylamine (MA) and DMA were also formed in small amounts by bacterial action (Beatty and Collins, 1940). Shewan (1938) proposed the measurement of DMA as a spoilage test. More recently Castell et al. (1955) have suggested that DMA rather than TMA be used as a quality indicator in frozen cod and haddock. Beatty (1937) and Castell et al., (1955 and 1958) correlated TMA levels with acceptability of cod and haddock.

Tarr and Ney (1949) reported several cases in rockfish where TMA levels were high and bacterial counts were low. Castell et al., (1970) concluded that the decline in freshness does not necessarily coincide with increase in bacteria. He demonstrated that (TMAO) can be reduced to TMA by the addition of cysteine and ferrous iron. Yamada (1969) reported that TMA levels differ according to fish type, fishing grounds, handling conditions, storage conditions and time of year caught.

Nitrosamines have received considerable attention due to their potential role in the induction of human cancers (Lijinsky et al., 1972;

Magee, 1972; Wolfe and Wasserman, 1972). Nitrosamines are formed

from secondary amines under human stomach conditions (Sen et al.,

1969; and Sander et al., 1968). Fiddler et al., (1972) reported the

formation of N-nitrosodimethylamine (DMNA) from tertiary amines at

elevated temperatures. Ender et al., (1967) reported DMNA formation

from hydrochlorides (HCl) of MA, DMA, and TMA and from TMAO at

elevated temperatures and the yield from primary and tertiary amines

was less than from the secondary amine.

The formation of TMA in fish has been shown to be due chiefly

to bacterial action by a TMAO reductase. Watson (1939) studied the

mechanics of TMA formation and produced the following generaliza-

tion.

$$AH_2 + TMAO \longrightarrow TMA + A + H_2O$$
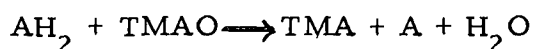
Optimum conditions of the reaction have been reported to be $37\text{-}40^\circ$ C

at pH 7.0-7.5 (Castell and Snow, 1951). An endogenous enzyme that

reduces TMAO to TMA has also been reported (Kawabata, 1953).

Several studies have been conducted in an attempt to explain the

formation of DMA in fish. Although DMA has been produced in sterile

Atlantic cod (Beatty and Collins, 1940) an endogenous enzyme has not

been confirmed. Hattori (1940) heated TMAO at $160\text{-}200^\circ$ C and pro-

duced DMA and formaldehyde (FA). Although these are severe con-

ditions DMA has been formed in sardine, salmon, and cuttlefish

processed at 113° C for 1 hr (Ohta, 1958). Atlantic herring was

heated for 1 hr at 120° C to produce DMA (Hughes, 1959). Vaisey

(1956) coverted TMAO to DMA and FA by the action of ferrous iron

and cysteine. TMAO may also be converted to DMA and FA under acid

conditions or in the presence of a catalyst which is able to transfer

electrons (Amano and Yamada, 1965).

Gangal and Magar (1967), using Dyer's method, measured TMA

nitrogen in canned Indian crab Scylla serrata and reported it to vary

from 1.5 to 21.3 ppm. TMA nitrogen in claw meat was greater than

in body meat. TMA nitrogen also increased steadily over a period of

9 months in canned crab.

Gangal and Magar (1963) studied the effects of freezing crab meat

Scylla serrata and Neptunus pelagicus in polyethylene bags. TMA

nitrogen values varied from 1.9 to 11.5 ppm and they tended to in-

crease and then decrease during storage at -30° C.

The purpose of this study was to measure by glc the amounts of

methylamines in Dungeness crab under various processing and storage

conditions.

EXPERIMENTAL

Sample Preparation

Live Dungeness crabs, Cancer magister, were obtained from a commercial source on the Oregon coast. Crabs butchered by debacking were bled, cooked for 13 min in boiling water, and samples were prepared as follows: (canned) vacuum sealed in 303 X 212 cans filled with 15° Sal brine containing 1 g of citric acid per 16 oz of brine and thermally processed at 118° C for 65 min; and (frozen) vacuum sealed in 303 X 212 cans and frozen at -23° C. Whole crabs were cooked in 1% salt water and samples were prepared as follows: (iced) iced at 1° C; (iced-frozen) iced 6 days at 1° C, vacuum sealed in 303 X 212 cans, and frozen at -23° C; (iced-canned) iced 6 days at 1° C, vacuum sealed in 303 X 212 cans, and thermally processed at 118° C for 65 min after the addition of 15° Sal brine containing 1 g of citric acid per 16 oz of brine; (iced-frozen leg and body meat) iced 12 days at 1° C, vacuum sealed in 303 X 212 cans, and frozen at -23° C after separating the leg meat from the body meat. Raw samples were picked from fresh butchered crab. Three commercial samples from a local supermarket were evaluated for comparative purposes.

GLC Column Preparation

Known amounts of 40/60 mesh Graphon (Cabot Corp., Boston,

Mass.) obtained by sieving and liquid phase (TEP) dissolved in dichloromethane were added to a petri dish. Excess solvent was evaporated under a stream of nitrogen and the remaining solvent was evaporated in an oven at 36° C. The dried packing was resieved and columns were packed and conditioned for 16 hr at 70° C with a nitrogen flow rate of 20 ml/min.

Drying Column Preparation

A drying column was prepared to prevent on-column trapping of water (Heatherbell et al., 1971). Anhydrous K_2CO_3 was ground and packed into a 10 cm X 4 mm i.d. stainless steel tube and the column was conditioned for 16 hr at 130° C with a nitrogen flow rate of 10 ml/min at room temperature.

Standards Preparation

MA:HCL, DMA:HCL, and TMA:HCL were dried over NaOH in a vacuum desiccator. Isopropylamine (IPA), the internal standard, was purified by distillation. Each was quickly weighed by difference and brought to volume with .N HCL.

Heaspace Analysis of Basic Volatiles

Analyses were accomplished by modifying the on-column trapping technique developed by Morgan and Day (1965) with the addition of a

water trap developed by Heatherbell et al. (1971). A taller cap on the entrainment vial was used to prevent occasional leakage encountered with standard caps.

Thermally processed crab was drained for 30 sec and frozen crab was thawed in 16° C water. Approximately $5 \pm .02$ g of crab was weighed into a screw-capped vial (Kimble No. 60957 size No. 1) and a small amount of Dupont Antifoam 60 was added with 100 μl of IPA. Five ml of 10% NaOH was added and the vial was immediately sealed. Samples were entrained at 60° C for 10 min with a nitrogen flow rate of 10 ml/min at room temperature.

A Varian Aerograph 1200 flame ionization detector (FID) gas chromatograph (gc) was employed in the determination of basic volatiles. The conditions were: column, 5.4 m X 2 mm i.d. stainless steel packed with 2% TEP on 40/60 mesh Graphon; column temperature, 60° C isothermal; injector, 180° C; detector, 265° C; and a nitrogen flow rate of 20 ml/min at room temperature.

Peak height (h) times adjusted retention time (t'_r) was used to quantitatively determined amines in crab according to the following formula where (F) is the calibration factor.

$$\frac{h \times t'_r \text{ sample}}{h \times t'_r \text{ internal standard}} = F \frac{\text{mass sample}}{\text{mass internal standard}}$$

An F & M Model 810 gc was employed in the separation of basic volatiles for tandem gc-mass spectrometer (ms) examination. The

conditions were: column, 5.4 m X 2 mm i.d. stainless steel packed with 2% TEP on 40/60 mesh Graphon; column temperature, 60° C isothermal; injector, 208° C; detector, 242° C; and a helium flow rate of 20 ml/min at room temperature.

A splitter directed 17% of the gc effluent to the FID and the remainder to the ms. A heated micro-volume switching valve (Carle Instruments Inc., Fullerton, Calif.) and a membrane separator connected the gc to the ms. The valve allowed diversion of air and water from the ion source of the ms.

Mass spectra were obtained using an Atlas CH-4 ms. Scans were made from m/e 12 to m/e 250 in 3 sec. The ms operating conditions were: filament current, 20 μA; electron voltage, 70 eV; multiplier voltage, 1.6 kV; analyzer pressure, 6.5×10^{-7} torr; and accelerating voltage, 3.0 kV.

Determination of Neutral Volatiles

Neutral volatiles were examined in fresh cooked crab by the same on-column trapping technique as used for basic volatiles except NaOH was replaced with water (4:1 w/w water to sample). The sample was mixed in a Waring blender for 1 min prior to analysis. The conditions for tandem gc-ms were: column, 3 m X 2 mm i.d. stainless steel packed with 20% 1, 2, 3-Tris (2-cyanoethoxy) propane on 60/80 mesh Celite 545; column temperature, 40° C isothermal; injector,

78° C; detector, 190° C; helium flow rate, 26 ml/min at room temper-
ature; filament current, 20 µA; electron voltage, 70 eV; multiplier
voltage, 1.6 kV; analyzer pressure, 8×10^{-7} torr; and accelerating
voltage, 3.0 kV.

RESULTS AND DISCUSSION

Basic Volatiles

The amine values for stored crab samples are summarized in Table 1-2 and presented graphically in Figures 1.-8. The amine values for zero time were omitted; however, in raw crab no amines were detected. The lower limit of detection was 340 ppb, 95 ppb and 24 ppb for MA, DMA, and TMA respectively.

The chromatogram of basic crab volatiles is illustrated in Figure 9. Peaks A, B, C and D: NH_3, MA, DMA and TMA respectively were confirmed by relative retention times of authentic compounds and by mass spectral data.

In general, the TMA levels in iced (Figure 1), frozen (Figure 2), and iced-frozen (Figure 3) increased and decreased with storage. This phenomenon was reported by Keay and Hardy (1972); they hypothesized the results as anomalous due to intrinsic variation between fish. Another interpretation for the decrease in amine content would be exhaustion of precursor followed by either reaction or volatilization of the amine. Ammonia production may induce loss by volatilization since an increase in pH would favor the conversion to the more volatile free base.

Iced crab after 13 days contained the highest concentration of TMA. Since TMA production was probably due to bacterial reduction,

Table 1. Amine Content of Stored Crabmeat in PPM.

Days Stored	Iced			Iced-Frozen			Frozen			Canned			Iced-Canned		
	MA	DMA	TMA	MA	DMA	TMA	MA	DMA	TMA	MA	DMA	TMA	MA	DMA	TMA
1	*	2.7	200												
7				2.7	5.4	270	*	4.2	42						
10	*	7.4	250	*	2.3	370	*	5.7	160						
13	*	9.7	700	*	9.3	460	*	6.3	210						
16	24	25	310	7.7	17	510									
20	7.3	10	190	10	19	390							2.3	35	140
29							3.0	5.6	230	2.7	33	130	2.4	42	160
36										1.9	33	150			
59							4.7	5.9	240	3.6	51	110			
66				9.3	13	290							1.4	71	290
89							7.4	6.2	280	1.1	33	140			
96													3.3	50	130
97				8.0	10	200									
119							7.2	5.5	84	4.1	50	160			
129				2.2	5.0	120							2.7	67	230

* None Detected

then it was not surprising that the highest TMA values occurred in
iced crab.

Frozen crab contained high levels of TMA but rather low levels
of DMA. This pattern is found in most frozen fish except the gadoid
species where DMA levels exceed TMA levels (Castell et al., 1971).
One possible explanation for the production of TMA could be that an
endogenous enzyme reduced TMAO (Yamada, 1969).

Levels of TMA production in iced-frozen crab were between
levels found in iced and frozen crab. TMA production continued at
essentially the same rate during frozen conditions through
16 days. This seemed peculiar, since one would anticipate a decrease
in the rate of TMA production upon freezing.

TMA and DMA levels of iced-frozen leg and body meat (Table 2)
differed little from each other except for TMA levels after 37 days of
storage. Castell et al., (1971) reported more DMA production in dark
than in light muscle of cod. Our data suggests this relationship also
applies in crab although the difference is not as dramatic as was demon-
strated in cod.

Table 2. Amine Content of Iced-Frozen Leg and Body Meat in PPM.

| Days | Iced-Frozen Leg | | | Iced-Frozen Body | | |
Stored	MA	DMA	TMA	MA	DMA	TMA
37	5.1	8.9	200	3.3	6.0	150
97	4.9	7.2	120	3.9	4.2	120

Canned and iced-canned crab (Figures 4 and 5) contained appreciably higher levels of DMA than the other samples. Hattori (1940) reported that DMA is formed by thermally processing TMAO; however, DMA continued to be produced after thermal processing in this study. Chemical reduction was the most probable route of formation since microorganisms and tissue enzymes would have been inactivated. TMA levels in canned crab were relatively stable over storage as compared to the other crab samples.

Three commercial samples of frozen crab were found to contain: 0.0, 0.0, and 2.3 ppm MA; 1.7, 2.4, and 5.9 ppm DMA; 310, 300, and 340 ppm TMA. These values were in the same range as those found in the frozen samples prepared in our laboratory. Commercially canned crab was not available for analysis.

Although TMA levels have been used as an index of quality (Beatty and Gibbons, 1937; Tarr, 1938; Shewan, 1939; Ronold and Jakobsen, 1947; Castell and Triggs, 1955), it would be difficult to conclude whether TMA levels would be of value in predicting crab quality as sensory evaluations were not included in this study. The increase and subsequent decrease in TMA content in iced crab during storage would indicate that the use of TMA values as indices of crab quality is questionable. In all probability TMA values would have to be used in conjunction with other methods, such as sensory evaluation, to be useful in predicting crab quality (Yamada, 1969). In canned crab, DMA rather than TMA, might prove to be the more useful indicator.

The formation of nitrosamines from amines in foods has received considerable attention in recent years (Fiddler et al., 1972; Ender and Čeh, 1968; Sen et al., 1968; Ender, 1966). Secondary amines have been considered the more important nitrosamine precursors and in this connection canned crab would be a dietary source of DMA. The fact that secondary amines can react with nitrite in the stomach to produce nitrosamines has been established (Sander et al., 1968; Sen et al., 1969).

Fiddler et al., (1972) have recently reported a comparison of DMNA formation from DMA and TMA at elevated temperatures. DMNA was formed from TMA but in lesser amounts than from DMA. It would be difficult to assess the potential hazards of TMA as a precursor for DMNA as the conversion of TMA to DMNA has not been studied under human stomach conditions. If TMA can be converted to DMNA under human stomach conditions, then the toxicological implications of the dietary contribution of TMA from crab as well as other fish species would have to be evaluated.

Neutral Volatiles

Methyl sulfide, acetaldehyde, acetone, diacetyl, benzene, and toluene were confirmed by relative retention times of authentic compounds and by mass spectral data. Propanal and ethanol were tentatively identified by relative retention times.

Methyl sulfide was confirmed in all but one cooked sample and none was detected in raw muscle. The precursor is probably heat labile. Several sea fish have been shown to contain a heat labile dimethyl sulfide precursor, dimethyl-β-propiothetin (Mendelsohn and Brooke, 1968). The concentration of methyl sulfide remained relatively constant regardless of processing and storage. It is probable that the decline in freshness of crab is not accompanied by a significant loss of methyl sulfide.
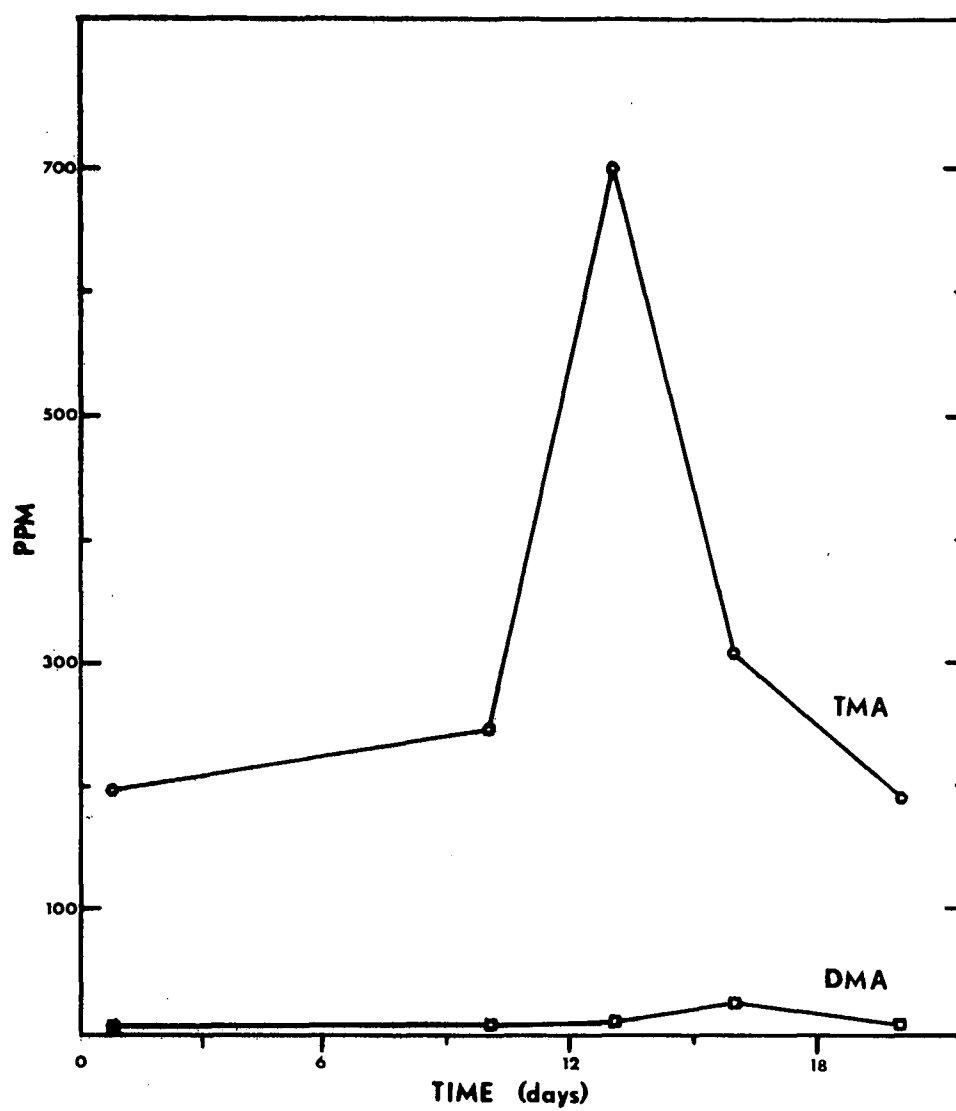
Figure 1. TMA and DMA content of iced crab.
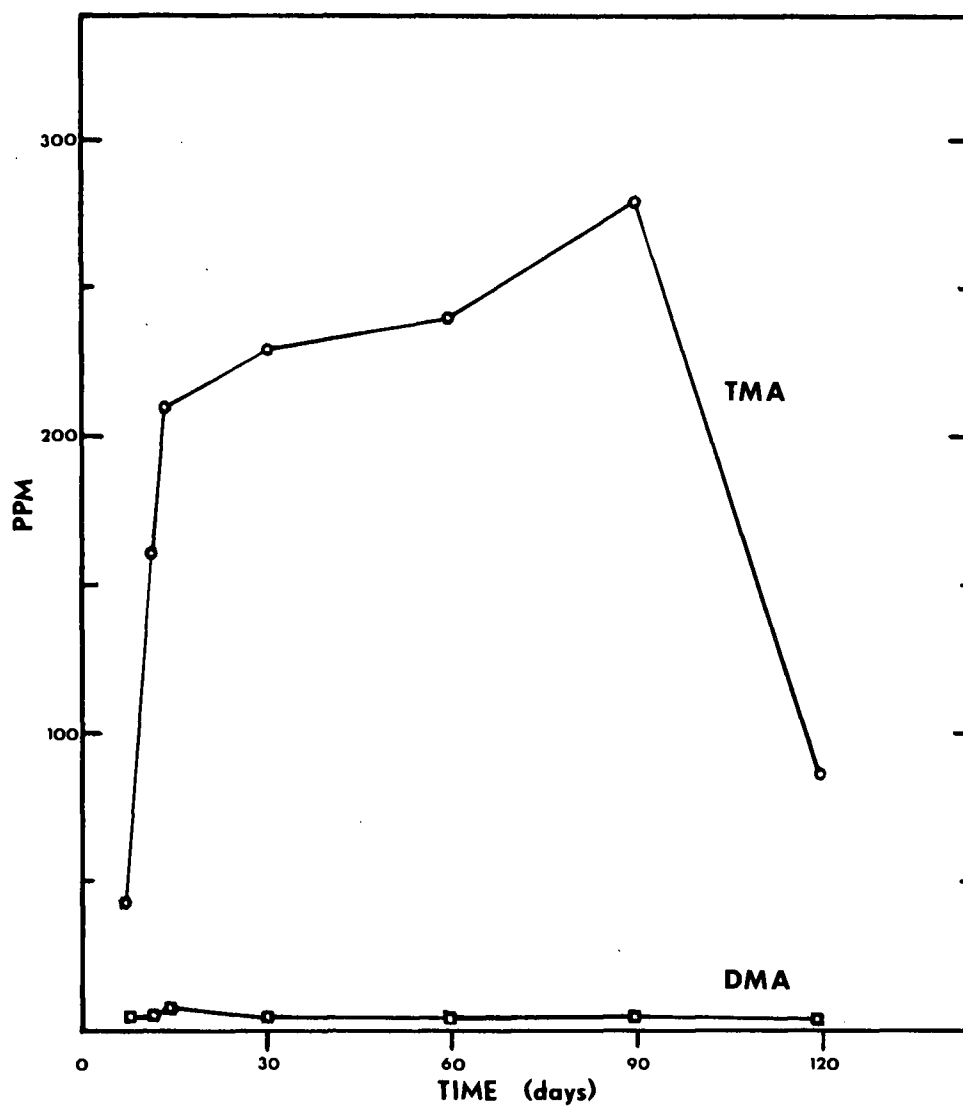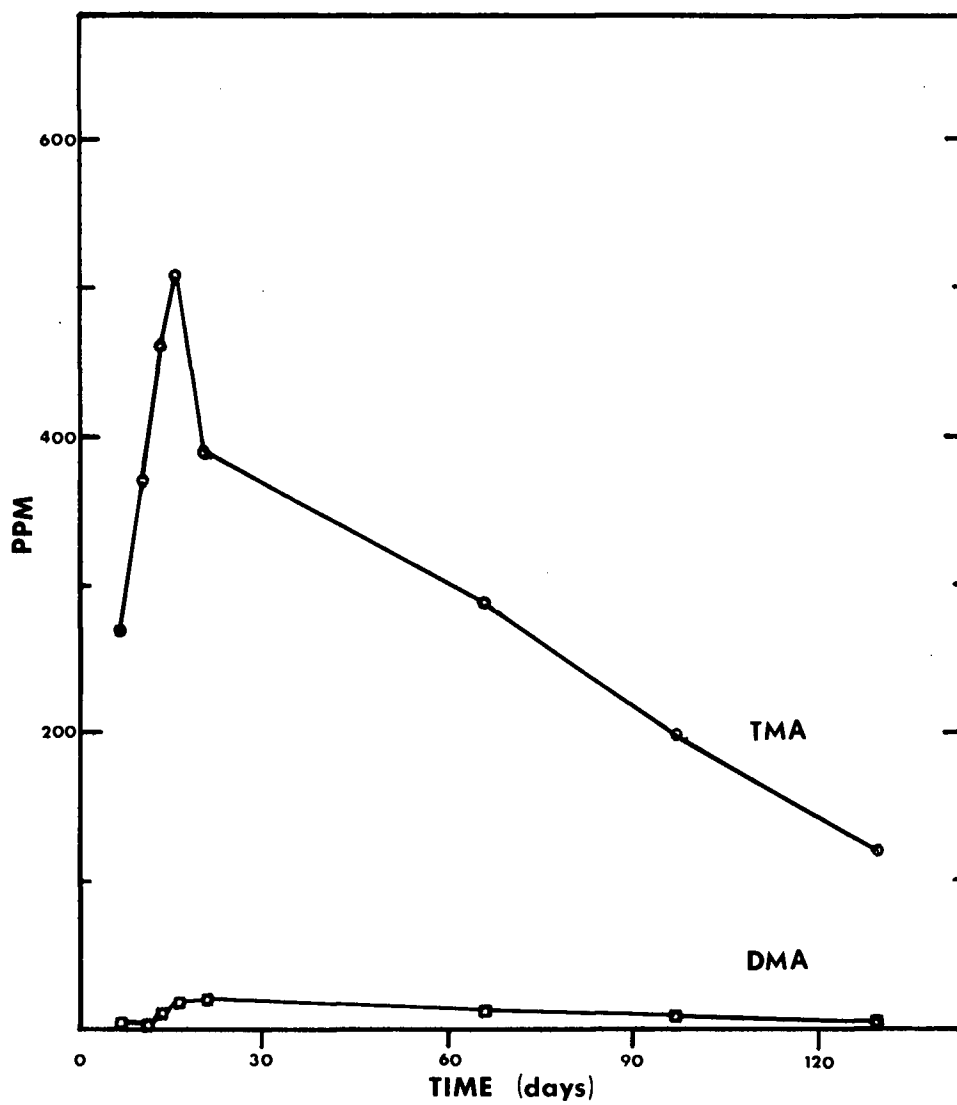
Figure 2.   TMA and DMA content of frozen crab

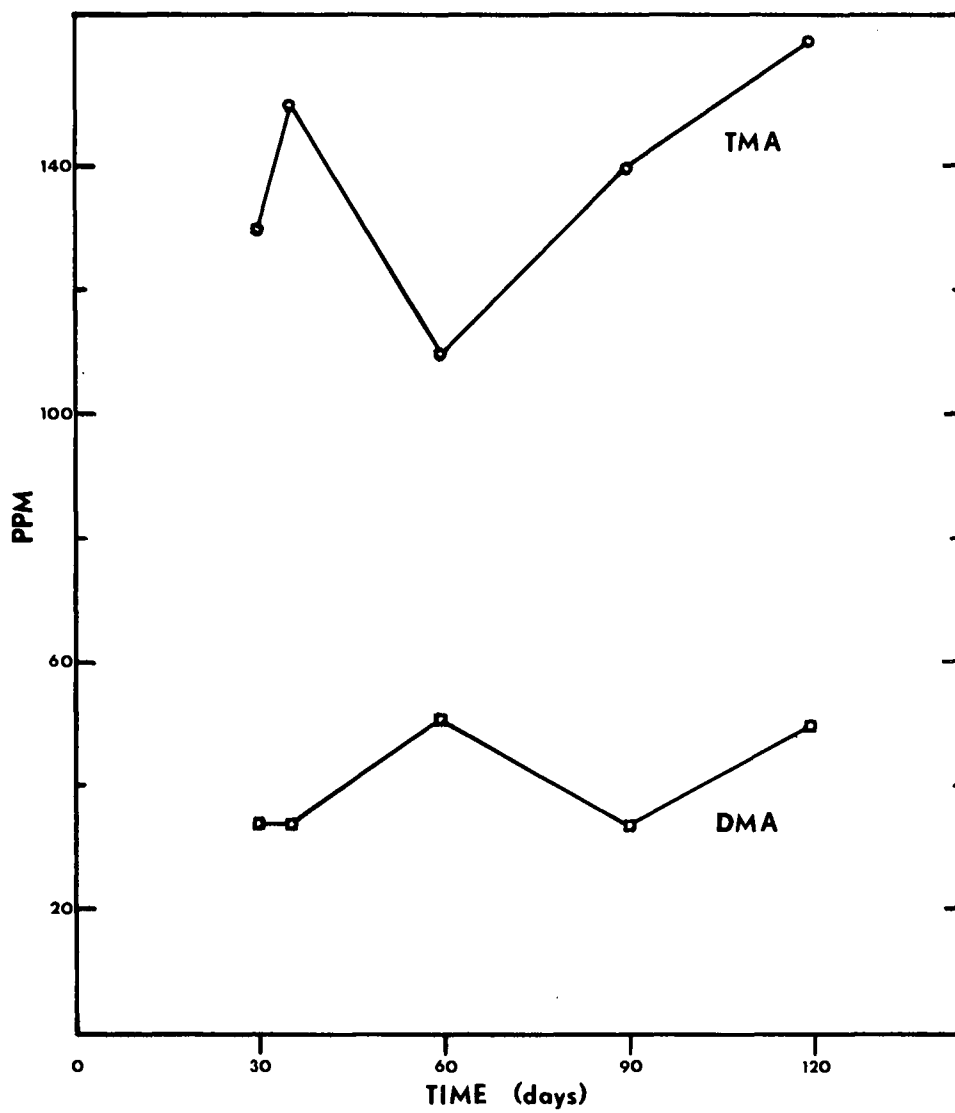Figure 3.   TMA and DMA content of iced-frozen crab

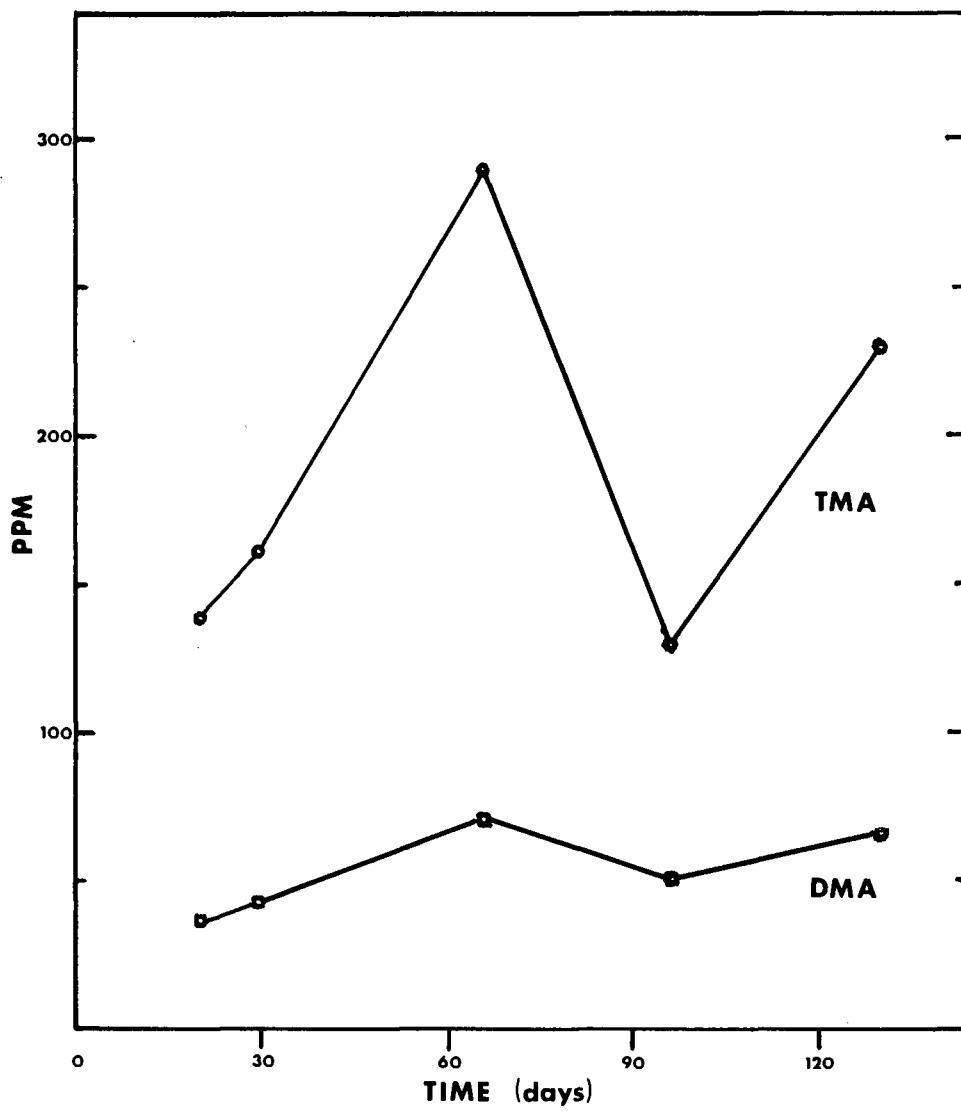Figure 4. TMA and DMA content of canned crab

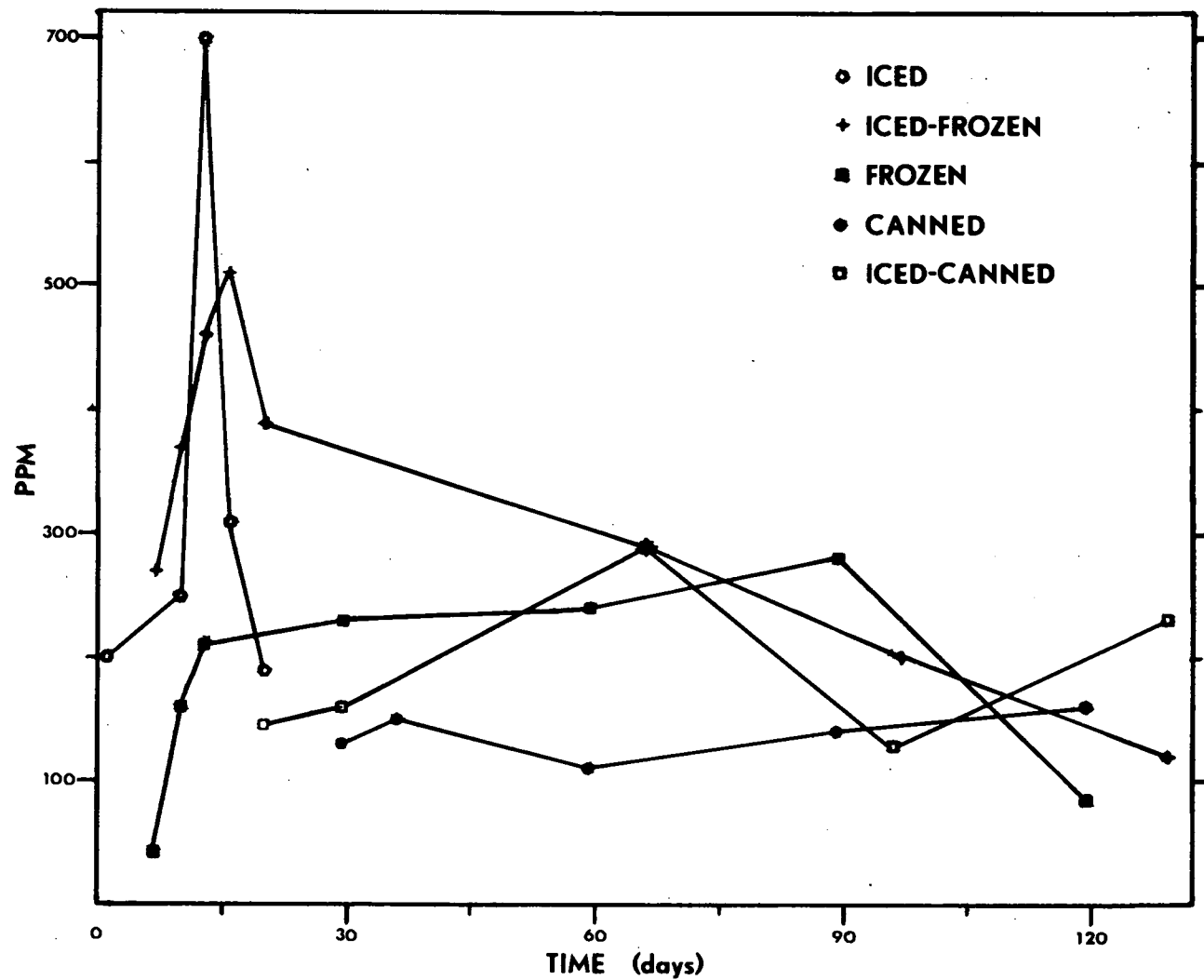Figure 5. TMA and DMA content of iced-canned crab
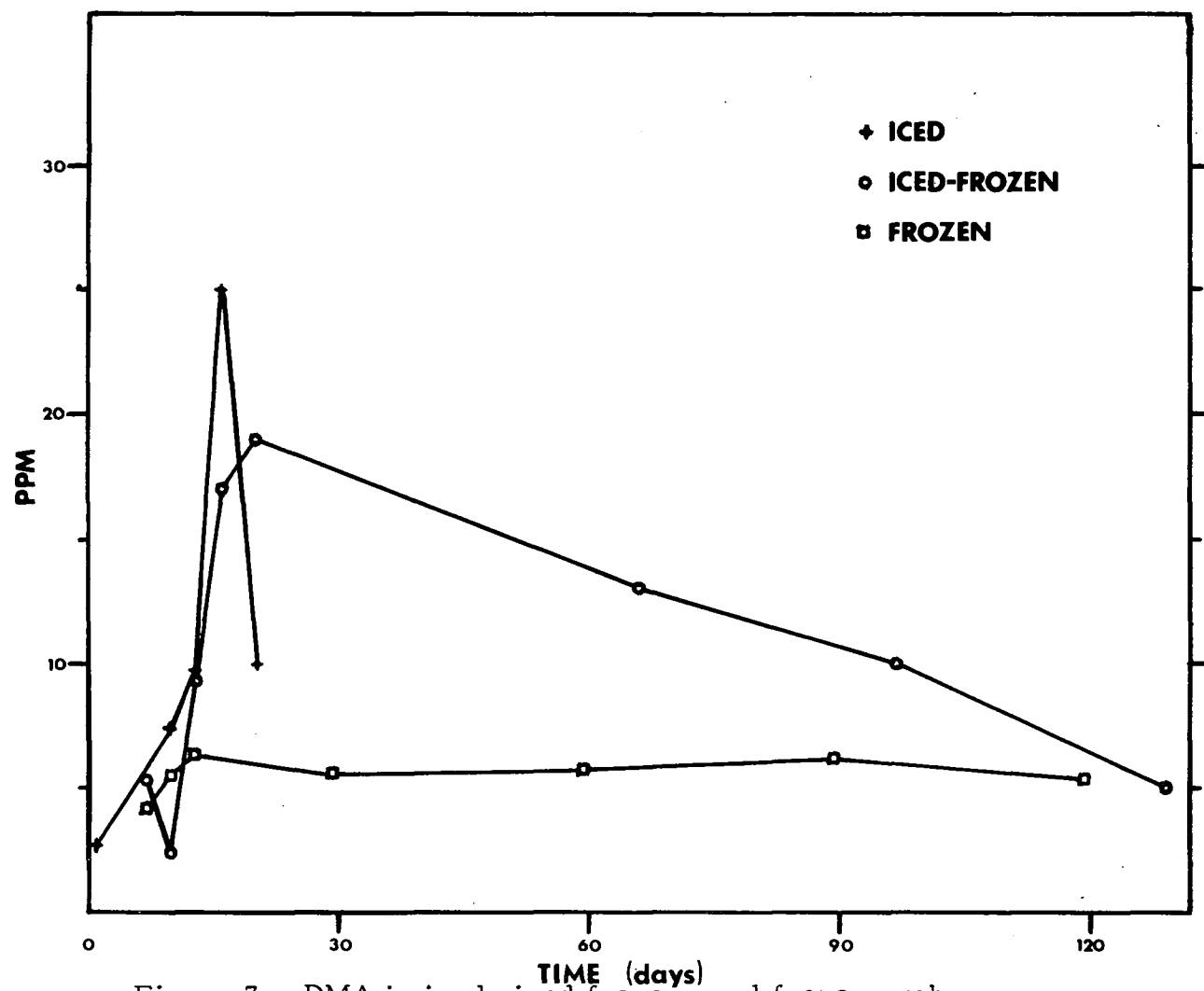
Figure 6. TMA in processed crab

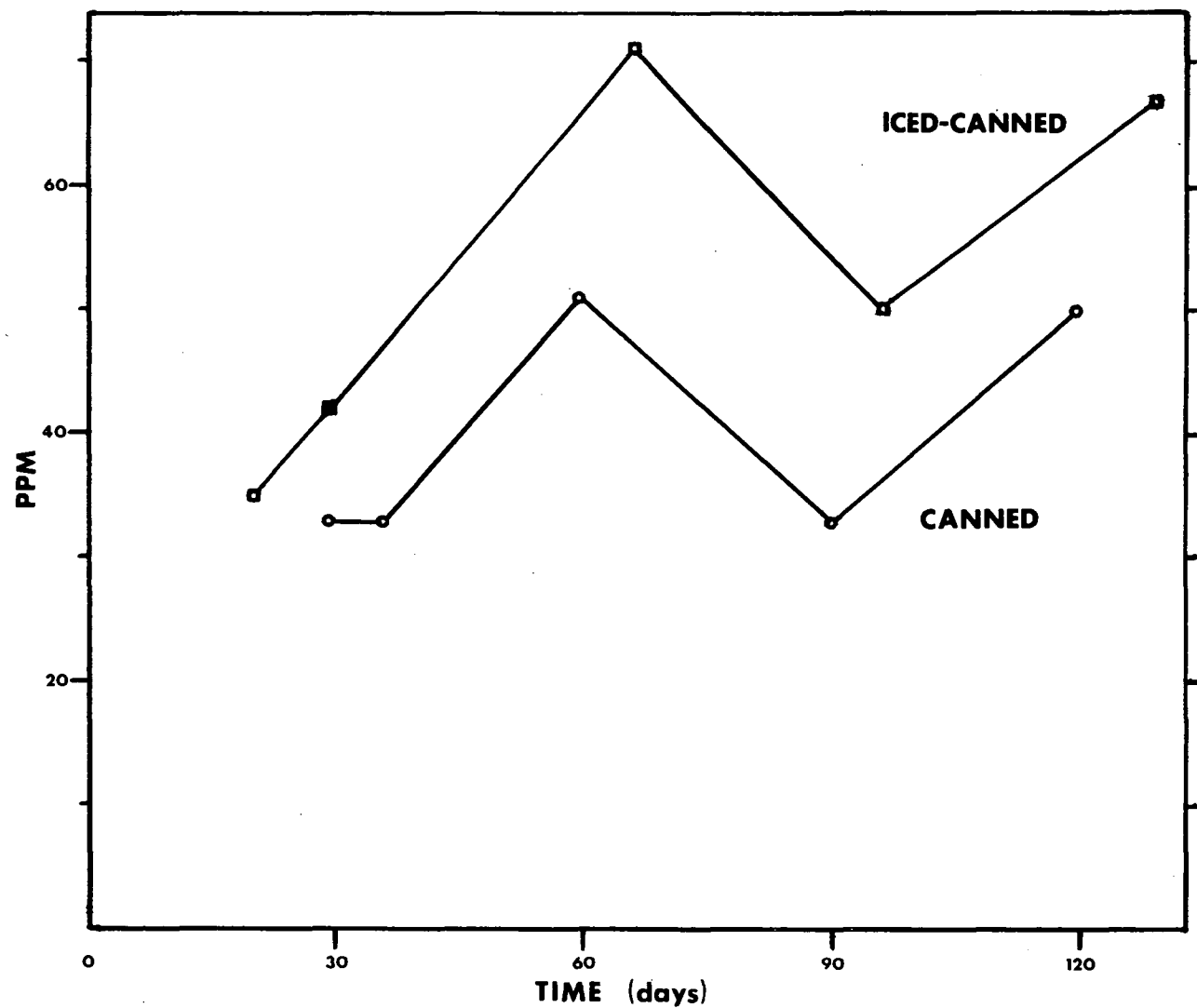Figure 7.   DMA in iced, iced frozen, and frozen crab
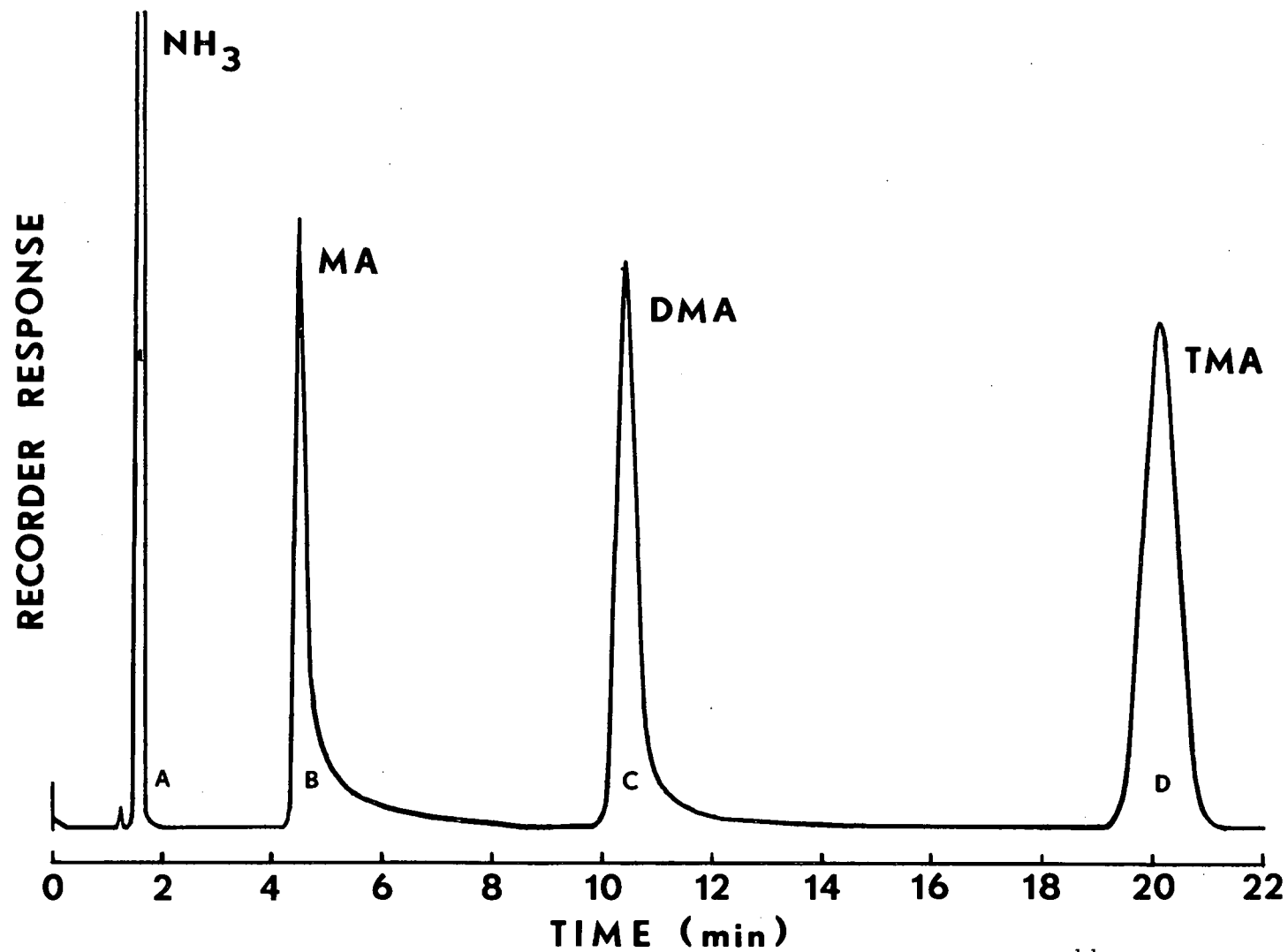
Figure 8. DMA in canned and iced-canned crab

Figure 9. FID response to volatile amines. Column: 2% TEP on Graphon. 4×10^{-11} amps/mV full scale sensitivity

BIBLIOGRAPHY

Amano, K. and K. Yamada. 1965. The biological formation of formaldehyde in cod flesh. London, Fishing News Ltd. p. 280.

Beatty, S. A. 1938. Studies of Fish Spoilage I. The origin of trimethylamine produced during the spoilage of cod muscle press juice. Journal of the Fisheries Research Board of Canada 4: 63-68.

Beatty, S. A. and V. K. Collins. 1940. Studies of Fish Spoilage II. Dimethylamine production in the spoilage of cod muscle press juice. Journal of the Fisheries Research Board of Canada 5: 32-35.

Beatty, S. A. and N. E. Gibbons. 1937. Measurement of spoilage in fish. Journal of the Biological Board of Canada 3: 77-91.

Castell, C. H., M. F. Greenough, R. S. Rodgers, and A. S. Mac Farlane. 1958. Trimethylamine values of fillets cut from graded fish. Journal of the Fisheries Research Board of Canada 15: 701-716.

Castell, C. H., W. Neal, and B. Smith. 1970. Formation of dimethylamine in stored frozen sea fish. Journal of the Fisheries Research Board of Canada 27: 1685-1690.

_____ 1971. Production of dimethylamine in the muscles of several species of gadoid fish during frozen storage especially in relation to the presence of dark muscle. Journal of the Fisheries Research Board of Canada 28: 1-14.

Castell, C. H. and J. M. Snow. 1951. Reduction of trimethylamine oxide by bacterial enzymes. Journal of the Fisheries Research Board of Canada 8: 195-206.

Castell, C. H. and R. E. Triggs. 1955. Spoilage of haddock in the trawlers at sea: The measurement of spoilage standards of quality. Journal of the Fisheries Research Board of Canada 12: 329-341.

Di Corcia, A., D. Fritz, and F. Bruner. 1970. Use of graphitized carbon black for linear gas-liquid-solid chromatography of polar low boiling compounds. Analytical Chemistry 42: 1500-1504.

Dyer, W. J. 1945. Amines in fish muscle I. Colorimetric detection of trimethylamine as the picrate salt. Journal of the Fisheries Research Board of Canada 6: 351-358.

Dyer, W. J. and Y. A. Mounsey. 1945. Amines in fish muscle II. Development of trimethylamine and other amines. Journal of the Fisheries Research Board of Canada 6: 359-367.

Ender, F. 1966. N-nitrosodimethylamine, the active principle in cases of herring meal poisoning. Fourth Internationalen Tagung der Weltgesellschaft für Buiatrik 4: 1-7.

Ender, F. and L. Čeh. 1968. Occurrence of nitrosamines in food-stuffs for human and animal consumption. Food and Cosmetics Toxicology 6: 569-571.

Ender, F., G. N. Havre, R. Madsen, L. Čeh, and A. Helgebostad. 1967. Studies on conditions under which N-nitrosodimethyla-mine is formed in herring meal produced from nitrite-preserved ham. Zeitschrift für Tierphysiologie, Tierernährung und Futtermittelkünde 22: 181-189.

Fiddler, W., R. C. Doerr, J. R. Ertel, and A. E. Wasserman. 1971. Gas-liquid chromatographic determination of N-nitrosodimethyla-mine in ham. Journal of the Association of Official Agricultural Chemists 54: 1160-1163.

Fiddler, W., J. W. Pensabene, R. C. Doerr, and A. E. Wasserman. 1972. Formation of N-nitrosodimethylamine from naturally occurring quaternary ammonium compounds and tertiary amines. Nature 238: 307.

Gangal, S. V. and N. G. Magar. 1963. Freezing of crabmeat. Food Technology, p. 1573-1578.

_____ 1967. Canning and storage of crabmeat. Food Technology 21: 397-400.

Hashimoto, Y. and T. Okaichi. 1957. Detection of trimethylamine and trimethylamine oxide. Bulletin of the Japanese Society of Scientific Fisheries 23: 269.

Hattori, Y. 1940. Trimethylamine oxide in meats and fishes: mechanism of the formation of formaldehyde and dimethylamine. Eisel Iho 53:149-172.

Heatherbell, D. A., R. E. Wrolstad, and L. M. Libbey. 1971. Carrot volatiles. 1. Characterization and effects of canning and freeze drying. Journal of Food Science 36:219-224.

Hughes, R. B. 1959. Trimethylamine oxide and volatile amines in fresh, spoiling, and cooked herring fish. Journal of the Science of Food and Agriculture 10:431-436.

James, A. T., A. J. P. Martin, and G. H. Smith. 1952. Gas-liquid partition chromatography: The separation and micro-estimation of ammonia and the methylamines. Biochemical Journal 52:238-242.

Kawabata, T. 1953. Trimethylamine reductase: Reduction of Trimethylamine in the dark muscle of pelagic migratory fish under aseptic conditions. Bulletin of the Japanese Society of Scientific Fisheries 19:505-512.

Keay, J. N. and R. Hardy. 1972. The separation of aliphatic amines in dilute aqueous solution by gas chromatography and application of this technique to the quantitative analysis of tri- and dimethylamine in fish. Journal of the Science of Food and Agriculture 23: 9-29.

Lijinsky, W., E. C. Keefer, R. J. Van de Bogart, and E. Conrad. 1972. Nitrosation of some teritiary amines and biological implications. Journal of the National Cancer Institute 49:1239-1249.

Magee, P. N. 1972. Possibilities of hazard from nitrosamines in industry. Annual Occupational Hygiene 15:19-22.

Mendelsohn, J. M. and R. O. Brooke. 1968. Radiation, processing, and storage effects on the head gas components in clam meats. Food Technology 33:1162-1166.

Miller, A., R. A. Scanlan, J. S. Lee, and L. M. Libbey. 1972. Quantitative and selective gas chromatographic analysis of dimethyl- and trimethylamine in fish. Journal of Agriculture and Food Chemistry 20:709-711.

Morgan, M. E. and E. A. Day. 1965. Simple on-column trapping procedure for gas chromatographic analysis of flavor volatiles. Journal of Dairy Science 48:1382-1384.

Ohta, F. 1958. Carbonyl compounds in fish as related to the deterioration II. Thermal production of formaldehyde in fish meat. Bulletin of the Japanese Society of Scientific Fisheries 24:338-341.

Ronald, A. and F. Jakobsen. 1947. Trimethylamine oxide in marine products. Journal of the Society of Chemical Industries 66:160-166.

Sander, J., F. Schweinsberg, and H. P. Menz. 1968. Spontaneous formation of carcinogenic nitrosamines in the stomach. Hoppe Seyler Z. Physiological Chemistry 349:1691-1697.

Sen, N. P., D. C. Smith, and L. Schwinghamer. 1969. Formation of N-nitrosamines from secondary amines and nitrite in human and animal gastric juice. Food and Cosmetics Toxicology 7:301-307.

Sen, N. P., D. C. Smith, L. Schwinghamer, and J. J. Marleau. 1969. Dimethylnitrosamine and other N-nitrosamines in foods. Journal of the Association of the Official Agricultural Chemists 52:47-52.

Shewan, J. M. 1938. Trimethylamine formation and the viable bacterial population of spoiling fish muscle. Report of the Food Investigation Board of 1937, p. 75-78.

_____ 1939. Spoilage of fish. Report of the Food Investigation Board of 1938, p. 79-87.

Smith, E. D. and R. D. Radford. 1961. Modification of gas chromatographic substrates for the separation of aliphatic diamines. Analytical Chemistry 33:1161-1163.

Sze, Y. L. and M. L. Borke. 1963. Separation of lower aliphatic amines by gas chromatography. Analytical Chemistry 35:240-242.

Tarr, H. L. A. 1938. Trimethylamine formation in relation to the viable bacterial population of spoiling fish muscle. Nature 142:1078.

Tarr, H. L. A. and P. W. Ney. 1949. Effort of flesh acidity on bacterial numbers and trimethylamine in spoiling fish. Fisheries Research Board of Canada; Progress Reports of the Pacific Coast Stations, 78: 11-14.

Tozawa, H., K. Enokibara, and K. Amano. 1970. Effect of dimethyla mine on the value of trimethylamine determined by Dyer's method. Bulletin of the Japanese Society of Scientific Fisheries 36: 605-610.

Vaisey, E. B. 1956. Nonenzymatic reduction of trimethylamine oxide to trimethylamine, dimethylamine, and formaldehyde. Journal of Biochemical Physiology 34: 1085-1090.

Watson, J. W. 1939. Studies of fish spoilage: The bacterial reduction of trimethylamine oxide. Journal of the Fisheries Research Board of Canada 4: 252-265.

Wolfe, I. A. and A. E. Wasserman. 1972. Nitrates, nitrites, and nitrosamines. Science 177: 15-19.

Yamada, K. 1969. Post mortem breakdown of trimethylamine oxide in fishes and marine invertebrates. Bulletin of the Japanese Society of Scientific Fisheries 34: 541-551.