BIOCHEMICAL STUDIES ON OCTOPUS I. TRIMETHYLAMINE AND TRIMETHYLAMINE OXIDE CONTENTS OF OCTOPUS

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I. TRIMETHYLAMINE AND TRIMETHYLAMINE OXIDE CONTENTS OF OCTOPUS

By

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Introduction

It is well known that the high content of trimethylamine oxide in marine fishes (especially in elasmobranchs, 2–5 per cent for dry weight) is one of the most important biochemical characteristics, although recent works appear to show that trimethylamine oxide can occur even in fresh water fishes in much smaller quantities. However, the reports dealing with trimethylamine and trimethylamine oxide contents of molluscs are very scarce except those of Norris and Benoit (1) and of Dyer (2).

In the present report, the authors have examined trimethylamine and its oxide contents of octopus; *O. vulgaris* LAMARCK, *O. fangsiao* D'ORBIGNY and *O. dofleini* WUELKER. The expenses for this study are partly defrayed by the research funds of the Ministry of Education for which the authors express their hearty thanks. The authors also express their gratitude to Prof. Tsuchiya for his kind advice and criticism for the report.

Experimental

Materials: Three species of octopi; *O. vulgaris* LAMARCK, *O. fangsiao* D'ORBIGNY and *O. dofleini* WUELKER landed at port Yuriage and Shiwogama, Miyagi Prefecture, from Nov. 1952 to Feb. 1953.

Methods: (1) Method of extraction.

Fifty milliliters of 20 per cent formaldehyde solution are added to 100 g minced sample. The mixture is stirred and left overnight for extraction. Then it is filtered through two sheets of gauze and the filtrate is analysed.

(2) Estimation of trimethylamine (TMA).

The method for the estimation of TMA is principally based on Dyer's procedure(3), but Conway's microdiffusion technique is used instead of

colorimetry of TMA-picrate at 420 mμ. (4).

Reagents : N/70 $Ba(OH)_2$ solution.

N/150 H_2SO_4 solution containing Tashiro's indicator.

Saturated potassium carbonate solution. 110 g $K_2CO_3 \cdot 2H_2O$, dissolved in 100 g water.

Formaldehyde solution. 10 per cent solution of formaldehyde (commercial formalin, shaken with $MgCO_3$ and filtered) in water.

Procedure : Two ml of the tissue extracts and 1 ml of formaldehyde solution are pipetted into the outer chamber of Conway's unit and 1 ml of N/50 sulfuric acid containing Tashiro's indicator is pipetted into the inner chamber of the unit. The lid of the unit is closed and the whole unit is agitated carefully to mix well. Then 1 ml of the saturated potassium carbonate solution is added to the outer chamber and incubated at 36°C for two hours. During incubation diffused TMA is absorbed in the acid and titrated with standard $Ba(OH)_2$ solution. Blank test is also carried out simultaneously.

(3) Estimation of trimethylamine oxide (TMAO).

Procedure : To the aliquot of the tissue extracts (5–10 ml) in an Erlenmeyer flask, 1 g of Devarda's alloy and 2 ml of 6N hydrochloric acid are added. Then the flask is placed immediately in a boiling water bath for 15 minutes. After cooling, it is used for the estimation of the total TMA by the same procedure as above described. TMAO content is calculated by substraction of the initial TMA value from the total TMA value.

(4) Recovery of TMA and TMAO estimations.

Under the experimental conditions employed, recovery tests were carried out for both TMA and TMAO estimations. (Table 1).

Table 1. Recovery of TMA and TMAO estimations

	Initial concentration	estimated value	Recovery per cent	Factor for correction
TMA	18.55μg-N/ml	18.08μg-N/ml	97.46	1.026
TMAO	133.56	127.08	95.15	1.051

Results : The results obtained are shown in Tables 2, 3 and 4.

Table 2. TMA and TMAO contents of *Octopus vulgaris*

Date	Part	Moisture %	TMA mgN per 100g wet weight	TMA % for dry weight	TMAO mgN per 100g wet weight	TMAO % for dry weight	TMAO /TMA ratio	Remarks
Nov. 1952	Abdominal muscle	82.12	0.38	0.009	11.85	0.35	31	average value of three samples. very fresh. landed at Yuriage.
	Foot muscle (basal)	79.27	0.29	0.006	10.94	0.28	37	
Dec. 1952	Abdominal muscle	80.25	0.48	0.010	9.86	0.26	20	average value of three samples. very fresh. landed at Yuriage.
	Foot muscle (basal)	79.35	0.31	0.006	13.85	0.36	44	
	Foot muscle (terminal)	81.12	0.84	0.019	10.39	0.29	12	
	Gonad*	80.11	0.33	0.007	3.31	0.09	10	
	Digestive tract	79.27	0.46	0.009	1.63	0.04	4	
	Salivary gland**	71.41	0.45	0.006	2.81	0.05	6	
Jan. 1953	Abdominal muscle	82.08	1.18	0.027	10.08	0.30	9	average value of two samples. slightly spoiled. landed at Yuriage.
	Foot muscle (basal)	82.80	0.57	0.014	15.09	0.47	26	
	Foot muscle (terminal)	84.02	2.59	0.068	10.66	0.36	4	
	Gonad*	83.17	0.16	0.004	2.88	0.09	18	
	Digestive tract	81.96	1.22	0.028	3.88	0.11	3	
	Salivary gland**	73.01	nil	nil	1.92	0.04	0	

* Unfortunately all of the samples obtained consisted of the female, so it indicates the ovary.

** posterior salivary gland.

Table 3. TMA and TMAO contents of *O. fangsiao* and *O. dofleini*

Date	Part	moisture %	TMA mgN per 100g wet weight	TMA % for dry weight	TMAO mgN per 100g wet weight	TMAO % for dry weight	TMAO /TMA ratio	Remarks
Feb. 1953	Abdominal muscle	85.46	2.34	0.067	27.58	1.01	17	*O. fangsiao*. average value of three samples remarkably spoiled. landed at Shiwogama.
	Foot muscle (basal)	85.40	1.72	0.049	27.36	1.00	15	
	Foot muscle (terminal)	86.43	2.89	0.089	23.92	0.94	8	
	Digestive tract	83.15	2.78	0.069	6.40	0.20	2	
	Gonad*	80.21	1.17	0.025	9.07	0.245	7	
	Salivary gland**	61.87	1.92	0.021	17.66	0.25	9	
Fed. 1953	Abdominal muscle	87.16	2.56	0.084	40.35	1.68	15	*O. dofleini* one sample. remarkably spoiled. landed at Shiwogama.
	Foot muscle (basal)	85.82	2.66	0.078	21.94	0.83	8	
	Foot muscle (terminal)	85.89	4.60	0.137	31.67	1.20	7	

* Ovary.

** posterior salivary gland.

Table 4. Relationship between TMAO content and weight of octopus

Date	part	Body weight of octopus	TMAO % for dry weight	Remarks
Dec. 1952	Abdominal muscle	3912g (A)	0.22	*O.vulgaris* very fresh.
		1690g (B)	0.23	
		1120g (C)	0.36	
	Foot muscle (basal)	" (A)	0.29	
		" (B)	0.30	
		" (C)	0.48	
	Foot muscle (terminal)	" (A)	0.33	
		" (B)	0.14	
		" (C)	0.44	
Jan. 1953	Abdominal muscle	1140g (A)	0.20	*O.vlgaris* slightly spoiled.
		834g (B)	0.40	
	Foot muscle (basal)	" (A)	0 52	
		" (B)	0.41	
	Foot muscle (terminal)	" (A)	0.24	
		" (B)	0.48	
Feb. 1953	Abdominal muscle	1290g (A)	1.13	*O.fangsiao* remarkably spoiled.
		800g (B)	0 88	
		718g (C)	1.04	
	Foot muscle (basal)	" (A)	1.12	
		" (B)	0.91	
		" (C)	0.98	
	Foot muscle (terminal)	" (A)	1.41	
		" (B)	1.01	
		" (C)	0.44	

Discussion

The metabolism and the biochemical significance of TMAO in molluscs have not yet been elucidated as in fishes. According to Kutscher and Ackermann (5), it is suggested that TMAO is formed as the result of biochemical methylation of the lower degradation products of protein metabolism and of oxidation of these products in marine animal tissues. Because of the scarcity of water supply in marine species, it is impossible to eliminate the all waste nitrogens in the form of ammonia, therfore, TMAO formation is very necessary for the the animal to avoid the danger of ammonaemia. As indicated in Tables 2 and 3, TMAO contents of the octopus integument (foot and abdominal muscles) are about three to ten times higher than that in the visceral parts. It seems likely that TMAO in the integument plays some rôle in osmoregulation and detoxication in octopus. The occurrence of TMAO in varying amounts among the species examined may be largely due to the difference of species rather than due to the variable degree of spoilage of the samples, because the quantities of TMA being converted from TMAO are much smaller compared to the absolute quantities of the original TMAO. Since octopus have no supporting tissue in the body such as calcareous tissue or skeleton, osmotic regulation and elasticity of muscles are necessary factors in keeping the stationary body shape and biological functions in waters. Of three species, TMAO content was highest in *O. dofleini* and lowest in *O. vulgaris*.

In regard to the relationship between TMAO content and body weight of octopus, the general trend that rise of TMAO parallels the increase in body size as in the case of cod, haddock and whiting, could not be observed except in the case of *O. fangsiao* when remarkably spoiled. On the contrary, no significant difference was found between the TMA contents of the muscular and visceral parts in octopus. It is interesting to note that TMA increases remarkably in the terminal parts of the foot and in the digestive tracts which are easily spoiled. As indicated in Table 2, TMAO/TMA ratio decreased with the advance of spoilage of the samples, their correlation can be used as a measure of spoilage in octopus. TMA is detectable organoleptically even at the level of 4mg-N/100g wet sample and 20mg-N level is that of the unpalatable stage. Volatile TMA is one of the unpleasant odours in octopus when spoiled.

Summary

Trimethylamine oxide in the octopus integument (foot and abdominal muscles) is three to ten times higher than that in the visceral parts. It seems likely that TMAO in the integument plays some rôle in osmoregulation and detoxication in octopus. Of three species examined, trimethylamine oxide content was highest in *O.dofleini*, lowest in *O. vulgaris*, being intermediate in *O. fangsiao*.

The variation of its content among the species is due to the difference of species. The trend that the larger octopus has higher content of trimethylamine oxide was observed only in the case of *O. dofleini* when markedly spoiled.

High content of trimethylamine has been found in the terminal parts of the foot and in the digestive tracts where are more easily spoiled than in any other parts. Correlation between the trimethylamine content and the degree of spoilage can be used as a measure of spoilage in octopus. Trimethylamine is one of the spoilage odours of octopus.

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