STUDIES ON THE PETROLEUM ODOUR IN CANNED CHUM SALMON

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Contents

I. Introduction

Several years ago, on account of their petroleum odour, about 5% of canned chum salmon (*Oncorhynchus keta*) packed in the Bering Sea and the northern Pacific Ocean failed to pass the export inspection of the Japanese Canned Foods Inspection Association. Not only in canned goods, but also in frozen chum salmon which was exported to the U.K., after being processed for potted salmon, the petroleum odour has been found. In the last one or two years, the petroleum odour has been sometimes found in canned chum salmon. Amongst fruit products, it has been said that canned pineapple or orange marmalade sometimes produces the petroleum odour. However, the problem of the petroleum odour has not yet been solved.

It is important to solve this problem on the petroleum odour for sake of the canning industry.

The author has studied the problem from the beginning of the outbreak. There has been little literature on the petroleum odour in the canned chum salmon. In England, Prichard[1] analyzed the frozen and canned chum salmon having the petroleum odour, but he failed to find the cause.

Using the expression "abnormal odour", McMillin[2] studied the smell in canned chum salmon. But he did not find the chemical components of the smell. As above noted, there were few published reports on the petroleum odour in canned chum salmon, so the author began to study the detection of the odour components and at the same time tried to obtain the opinions of technicians at the canning factories in order to determine the first step in the study.

The chemical components of the odour in canned foods differ according to the kinds. In the canned chum salmon, there is a peculiar odour. Tanikawa *et al.*[3] have detected the odorous constituents of the canned chum salmon. Especially when electric gilt tin plate cans which were wholly coated inner side, were used for the packing of chum salmon, a study was made of the chemical components of an abnormal odour so-called "burned odour" which has been sometimes found. Further, when frozen chum salmon was used for the production of canned chum salmon, the "refrigeration odour" was found in the canned product. The author and Tanikawa[4] have found piperizine, formic acid, formaldehyde, δ-aminovaleric aldehyde and hydrogen sulfide as the chemical components of such abnormal odour. This was due to the peculiar fishy smell of chum salmon and to the odour which was generated during the refrigeration. Especially the formation of this abnormal odour was influenced by the falling of the freshness before the processing.

The petroleum odour which is the topic of this paper is not found from canned chum salmon manufactured during the whole period of the fishing season, but is found only during the middle fishing season within some certain fishing grounds shown in Fig. I-1.

First the author collected informations concerning the petroleum odour of the canned chum salmon.

The information given by the canning technicians at floating canneries is

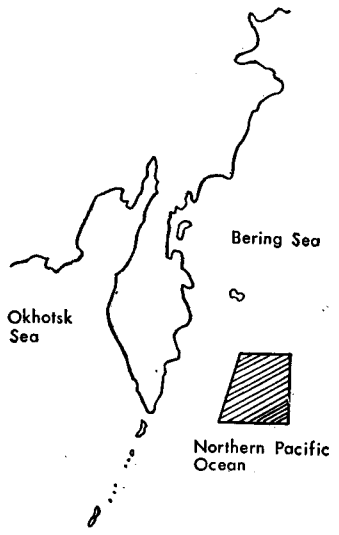

Fig. I-1. Fishing grounds where chum salmon having petroleum odour have been caught

summarized as follows:

(1) At first the expression "abnormal odour" has been used, which has become "petroleum-like odour" or "gasolin-like odour".

(2) The raw material used in production of canned salmon having the petroleum odour is almost restricted to chum salmon; such odour is not found in red, silver, and king salmon.

(3) The period during which the petroleum odour is generated is restricted to the weeks from the middle of June to the end.

(4) The petroleum odour is rarely found in raw chum salmon meat, but it is found when the meat is roasted or boiled, especially remarkably in the belly meat.

(5) In case of the content of the canned chum salmon, the petroleum odour is remarkable in the floating oil in juice, subcutaneous fat layer, dark muscle ("chiai"-meat), belly meat and back fin.

(6) The lap seam part of a tin container in which the petroleum odour is generated, is remarkably discolored (blackening).

(7) The petroleum odour is found in the canned chum salmon of which containers are electric gilt tin plate cans (coated on entire inner side with plastic resin), but not in hot-dipped tin plate cans or in cans of which the cover and bottom are hot-dipped tin plate and the can body is electric gilt tin plate (coated with plastic resin).

(8) The petroleum odour also is found in canned chum salmon of which the raw material is transported from the northern Pacific Ocean to Japan under freezing.

(9) The petroleum odour can be found by the inspectors for export and

by general consumers, especially by women.

(10) During the canning procedure on the floating cannery the contamination caused by machine oil or gasolin is not noticeable as is also true in normal year.

As stated above, the cause of the generation of the petroleum odour is unknown thanks to the complexity of the phenomena.

In order to investigate the cause and the mechanism of the occurrence of the petroleum odour in canned chum salmon, a series of the studies was carried out from various stand points.

Acknowledgement

Before going further the author wishes to acknowledge his indebtedness to several persons for their supporting of his work.

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II. Detection of the Petroleum Odour by Means of Steam Distillation

As stated above, canned chum salmon having the petroleum odour can be distinguished from normal canned chum salmon by skilled inspectors or keen women.

Next, attempts to ascertain the chemical components of the petroleum odour by means of steam distillation of the canned content will be described.

1. Detection of chemical components of the petroleum odour in various kinds of canned salmon

(1) *Samples*

Samples used were several kinds of abnormal canned salmon having the petroleum odour (chum and chum neck), of normal canned salmon (chum, red, red neck, pink, pink neck), and frozen chum salmon meat which were caught in the northern Pacific Ocean (in this lot it is considered probable that the petroleum odour is caused by the canning), and were refrigerated at −30°C on the floating cannery, and transported from the floating cannery to a land storing house. This frozen salmon was used within 30 days' storing after the catch.

(2) *Experimental items and methods*

One *kg* each of all samples was steam-distilled and 50 *cc* each of ca. *l* of the distillates obtained was tested as to the presence of aldehydes, phenols, organic bases, and volatile acids, as usual.

Table II-1. Results of qualitative test of the distillate by steam distillation of various kinds of canned or frozen salmon meats

Sample	Part	Aldehydes	Ketons	Phenols	Organic bases	Volatile acids
Canned chum having the petroleum odour	Meat	++	±	+	β-phenethylamine, Piperidine, Cadaverine	+
	Liquid	++	−	−		
	Skin	+	−	−		
Canned chum neck having the petroleum odour	Meat	+	−	±	+	+
	Liquid	++	−	±		
	Skin	+	−	±		
Canned red	Meat	±	−	−	+	+
	Liquid	−	−	−		
	Skin	±	−	−		
Frozen chum	Meat (back)	±	−	−	+	±
	Meat (belly)	±	−	−		
	Viscera	±	−	−		
	Skin	±	−	−		
Canned red neck	Meat	−	−	±	+	+
	Liquid	−	±	−		
	Skin	±	±	−		
Canned pink	Meat	±	±	±	++	±
	Liquid	−	−	±		
	Skin	±	±	±		
Canned pink meat	Meat				+	+
	Liquid					
	Skin					
Canned chum (normal)	Meat				+	+
	Liquid					
	Skin					

(Note) ++: Significant positive +: Positive
±: Slight positive −: Negative

(3) *Results of experiment*

Results obtained are shown in Table II-1.

As seen in Table II-1, there is no difference in respect to the presence of ketons, phenols, organic bases and volatile acids, excepting aldehydes, between the canned salmon having the petroleum odour and that without the odour. As to aldehydes, there are observed distinct differences between the two sorts of cans. Accordingly, it is considered that there is some relation between the petroleum odour and aldehydes.

2. Amounts of aldehydes in various kinds of canned salmon

(1) *Sample*

Samples employed were the same as in the section **1.**

(2) *Experimental method*

The quantitative determination of aldehydes was made following Ripper's method[5].

The amounts of aldehydes in the various kinds of canned salmon and frozen chum salmon are shown in Table II-2.

Table II-2. Amounts of aldehyde in the meats of various kinds of canned or frozen salmon

Sample	Canned chum (petroleum odour)	Frozen chum	Canned red	Canned red neck	Canned pink	Canned pink neck	Raw fresh pink	Canned chum (normal)
Aldehyde (*mg%*)	0.23	0.18	0.17	0.15	0.19	0.18	0.07	0.19

As seen in Table II-2, the amount of aldehydes in the canned chum salmon having the petroleum odour is 0.23 *mg%* which is larger than that in other samples. But the amount of aldehydes in the normal canned chum salmon is 0.19 *mg%* which is almost equal to that in canned red salmon or in canned pink salmon which have not the petroleum odour. As seen in the qualitative result described in the previous section **1**, it is clear that the amount of aldehydes is larger in the canned chum salmon having the petroleum odour.

The reason why there is a large amount of aldehydes in the canned chum salmon having the petroleum odour is not yet known.

III. Extraction and Fractionation of Chemical Components of the Petroleum Odour by Organic Solvents and Detection of Hydrocarbons in the Canned Chun Salmon by Means of a Detecting Tube

In the previous chapter II, the author described his attempts by steam distillation to obtain the chemical components of the petroleum odour in the canned chum salmon. But the attempts were in vain. It was clarified that the amount of aldehydes was larger in the canned chum salmon having the petroleum odour than in the normal canned chum salmon.

1. The extraction and fractionation of chemical components of the petroleum odour by means of organic solvents

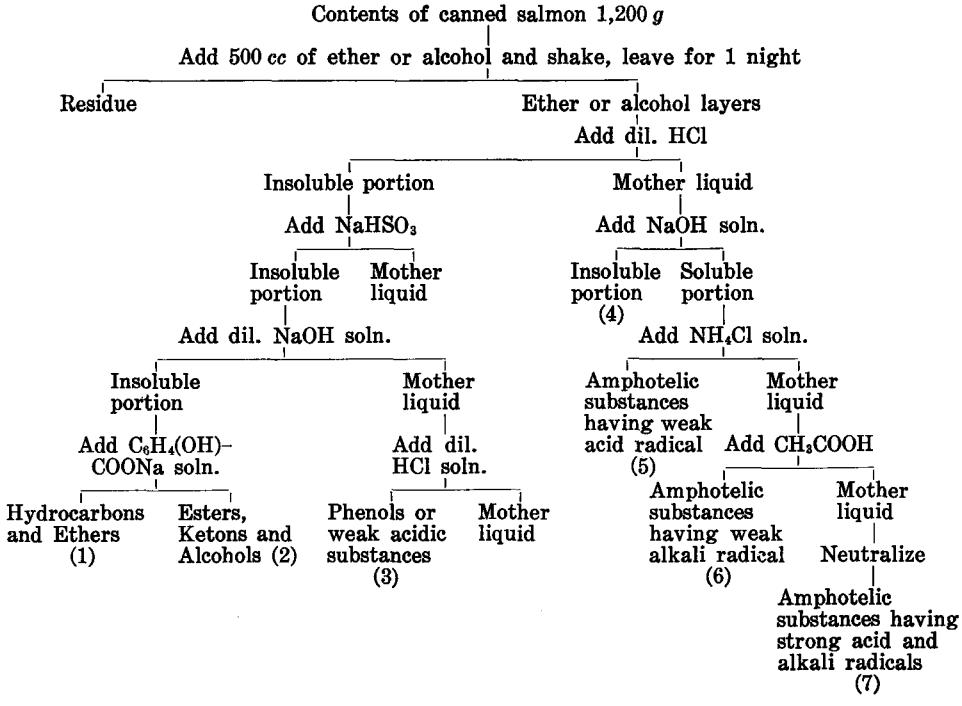
Attempts were made to ascertain the chemical components of the petroleum odour of chum salmon by use of organic solvents, because the chemical components will be extracted by organic solvents without changes by heating.

(1) *Experimental method*

As samples, 1200 *g* each of canned chum salmon having the petroleum odour, normal canned red and canned pink salmon were employed.

The samples were extracted fractionately by organic solvents following the treatments shown in Table III-1.

Table III-1. Method of extraction and fractionation of the components in various kinds of canned salmon meat by means of organic solvents



(2) *Results of experiment*

The extracted and fractionated components obtained by use of ethyl ether or ethyl alcohol were determined qualitatively with the results shown in Table III-2.

As seen in Table III-2, the chemical components extracted by ethyl ether or ethyl alcohol are almost the same as those obtained by the steam distillation. When each fractionated extractives was examined organoleptically, the petroleum-like odour could not be detected in all cases except in the fraction of neutral substances (hydrocarbon-fraction and ether-soluble fraction). In the fraction of neutral substances the odour was the most similar to that of petroleum.

Table III-2. Results of qualitative test of the components extracted

Fraction / Sample	Hydrocarbons, & Ethers (1)	Esters, Ketons, & Alcohols (2)	Phenols, & Weak acidic substances (3)	Bases (4)	Amphotelic substances having weak acid radical (5)	Amphotelic substances having weak alkali radical (6)	Amphotelic substances having strong acid and alkali radicals (7)
Canned chum (petroleum odour)	+	±	−	+	±	−	±
Canned red	±	−	−	+	±	−	±
Canned pink	+	±	−	+	±	−	±

The statement in the introduction that the petroleum odour is organoleptically detected most strongly in the floating oil in the juice of canned chum salmon having the petroleum odour will suggest a relationship between body fat of chum salmon and the odour. In view of this information, the fact that the petroleum odour is detected in the fraction of neutral substances will suggest a relation between the petroleum odour and hydrocarbons, or a relation between hydrocarbons and body fat of chum salmon. From this point, the principal component of the petroleum odour will be suggested to occur in body fat or to be due to hydrocarbons generated during the processing of the cans.

At any case, it seems evident that the principal component exists in the neutral substances.

2. Detection of hydrocarbons in the various kinds of canned salmon

In order to discover the relation between hydrocarbons and the petroleum odour, the author tried to detect hydrocarbons by a detector and by gas chromatography.

(1) *Detection of hydrocarbons by a detector*

The author and Tanikawa[4] have previously detected the chemical components of odour in chum salmon in cold storage (so called "freezing smell") by a detector (a detecting tube in which various kinds of absorbents were stuffed).

Attempts were made to detect hydrocarbons in canned chum salmon having the petroleum odour.

Samples used were as shown in Table III-3. In case of frozen chum salmon, after defrosting, the skin and the bone were removed, and only the meat was used as the sample. Five hundred *g* of each sample was put into a flask of 1 litre content, which was connected with a detector in which an absorbent was stuffed. As the absorbent, silica gel was used. It was soaked in 1% palladium sulfate solution and dried again; the detector was a glass tube of 4 *mm* inner diameter and 150 *mm* length.

One end of the glass tube was connected with the flask in which the sample was put and the other end was connected for two hours with a vacuum pump in order to cause the chemical components of the odour to pass into the detector. The hydrocarbons will react with palladium sulfate in silica gel and cause coloration to occur if they are present in the odour substance.

The result obtained is shown in Table III-3.

As seen in Table III-3, hydrocarbons were detected in all canned salmon sample used, but not in frozen chum salmon.

From those results, hydrocarbons are considered to be generated secondarily during the processing into canned products.

Table III-3. Presence hydrocarbons in various kinds of canned or frozen salmon found by the detector

Sample	Canned chum (petroleum odour)	Canned chum neck (petroleum odour)	Frozen chum	Canned red	Canned red neck	Canned pink	Canned pink neck
Hydrocarbons	+	+	±	+	+	±	±

3. Detection of hydrocarbons in canned salmon by gas chromatography

That the hydrocarbons are detected in all cans of salmon, suggests that more or less amount of the hydrocarbons forms a part of the petroleum odour.

In order to ascertain the kind of hydrocarbons in each canned salmon sample, gas chromatography was employed.

(1) *Experimental method*

As samples, canned chum salmon having the petroleum odour, and canned red salmon and pink salmon having no petroleum odour were used. Each 500 *g* of samples was put into a flask. The gas phase in the flask was collected into a silingie of capacity of 10 *cc*, and it was poured into the sampling chamber of the Shimadzu's GA-II Type gas chromatograph apparatus.

As a fixed phase, D.N.P. (dinony phthalate) or D.M.F. (dimethyl formamide) was separately used in order easily to separate the chemical components. As carrier gas helium was used, of which the flowing velocity was 50 *cc/min.*, gas pressure was 0.15 kg/cm^2, bridge current 210 *mA*, chart speed was 10 *mm/min.*, temperature of column was 10°~15°C.

(2) *Results of experiment*

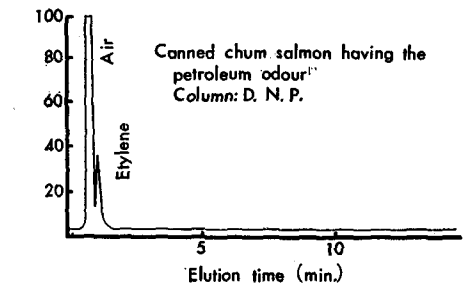
The results obtained are shown in Fig. III-1 and Fig. III-2.

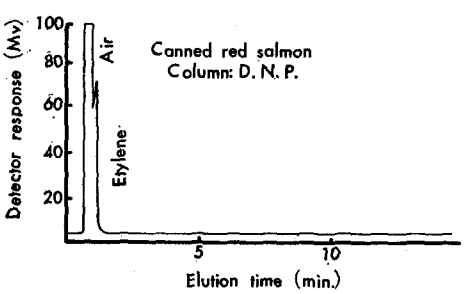
As seen in Fig. III-1 and Fig. III-2, from each kind of canned salmon irrespective of having or not having the petroleum odour, ethylene was detected as one kind of the hydrocarbons.

From this fact and the result described in the previous section **2**, it is considered that ethylene is perhaps generated by heat-decomposition during the processing of salmon meat in cans. The amount of the ethylene found in every can is almost identical. Therefore, ethylene is not considered to be a particular component of the petroleum odour in canned salmon.

According to the detection by gas chromatography, there is only a kind of hydrocarbons *viz.*, ethylene. Naphthenes or paraffin of fuel petroleum is not detected.

Therefore, the petroleum odour in cans is not considered to be generated by hydrocarbons.





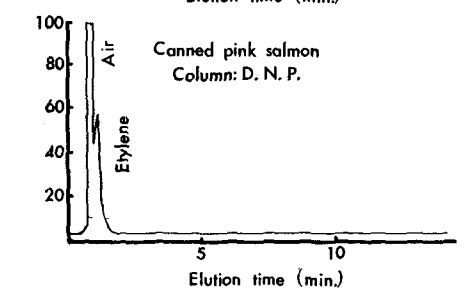
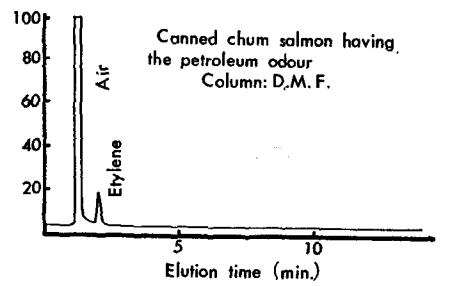
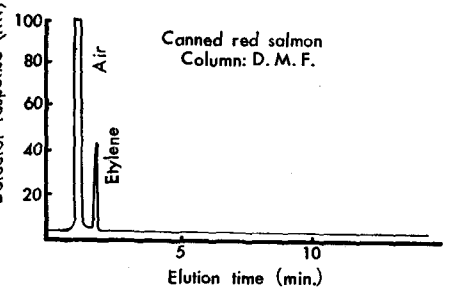


Fig. III-1. Gas chromatographic separation of hydrocarbons in various kinds of canned salmon (Fixed phase: D.N.P.)





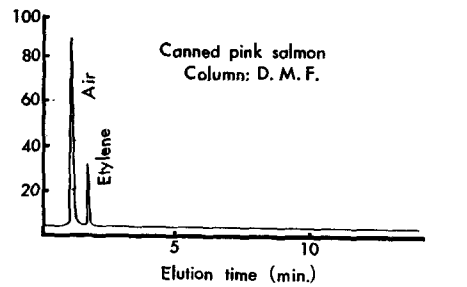


Fig. III-2. Gas chromatographic separation of hydrocarbons in various kinds of canned salmon (Fixed phase: D.M.F.)

IV. Relation between Salmon Oil in Cans and the Petroleum Odour

The petroleum odour is felt in floating oil in canned chum salmon. When chum salmon is roasted or boiled, the petroleum odour is easily noted in belly part of the fish body which is rich in fat content. Thus, there seems some relation between salmon body oil and the petroleum odour.

Next, the author studied this relation as described in this chapter.

1. Chemical properties of floating oil in the various kinds of canned salmon

(1) *Experimental method*

Twenty cans each of chum salmon having the petroleum odour and canned red and pink salmon having no petroleum odour were opened. After the opening of the cans, juice portions were collected and kept separate according to the

kind of cans. Each juice was taken into a separating funnel and the oily portion was separated.

The oil thus separated was used for estimation of acid, saponification and iodine values. The estimation of amount of unsaponifiable matter was carried out by Wilkie's method[6].

(2) *Experimental results*

Results obtained are shown in Table IV-1.

Table IV-1. Chemical properties of the floating oil in various kinds of canned salmon

Sample	Acid value	Saponification value	Iodine value	Unsaponifiable matter (%)
Canned chum (petroleum odour)	0.8	72.9	82.2	0.86
Canned chum neck (petroleum odour)	1.2	71.9	69.0	0.72
Canned red	0.8	72.6	79.1	0.75
Canned red neck	0.7	71.9	81.1	0.51
Canned pink	0.9	70.2	87.7	0.47
Canned pink neck	0.6	75.8	71.7	0.66

As seen in Table IV-1, there seems to be no relation between the specific numbers of acid, saponification and iodine values on the one hand, and the generation of the petroleum odour on the other. The amount of unsaponifiable matter is larger in canned chum salmon and canned chum neck meat than in canned red salmon (of which the most of the unsaponifiable matter is coloring matter) or in canned pink salmon. The iodine value of the floating oil in the various kinds of canned salmon is lower than that of raw fresh salmon fat[7].

2. Detection of hydrocarbons in unsaponifiable matter of floating oil in canned chum salmon having the petroleum odour

In the unsaponifiable matter of marine animal oil, there are coloring matter, hydrocarbons, vitamin A and D and cholesterol[8]. Among those chemical compounds, hydrocarbons including pristan and zamen, are said to be the cause of abnormal odour in skipjack oil[9].

Here, the author tried to detect hydrocarbons in unsaponifiable matter in canned chum salmon having the petroleum odour.

(1) *Experimental method*

As sample, 0.5 *g* each of unsaponifiable matter obtained from the floating oil in canned chum salmon having the petroleum odour as described in the previous section was employed for gas chromatography.

Therein, as fixed phase, D.N.P. (dinonyl phthalate) and D.M.F. (dimethyl formamide) were used respectively. As carried gas, helium was used. The flowing speed of the carrier gas was 60 *cc/min.* The pressure was 0.15 kg/cm^2. The bridge current was 210 *mA*. The chart speed was 10 *mm/min.* The temperature of the column was 10°C.

(2) *Results of experiment*

Results obtained are shown in Fig. IV-1.

As seen in Fig. IV-1, neither any other hydrocarbons nor even pristan and zamen which are found in skipjack oil, is detected in unsaponifiable matter of floating oil in canned chum salmon having the petroleum odour.

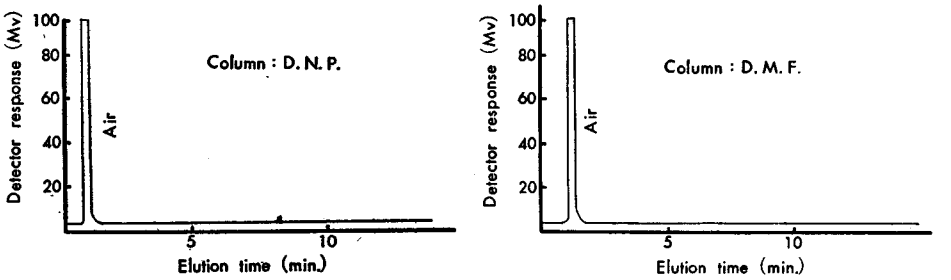


Fig. IV-1. Gas chromatographic separation of hydrocarbons in unsaponifiable matter of the floating oil in canned chum salmon having the petroleum odour

From those results, the petroleum odour is not considered to be due to the hydrocarbons in the unsaponifiable matter in the canned chum salmon.

The reason why the iodine value of the floating oil in various kinds of canned salmon is lower than that of fat in raw salmon may be due to the processing.

3. Change in specific numbers of acid, saponification and iodine values of salmon oil and detection of hydrocarbons in head space gas of the cans, during the processing

Here, the author tried to estimate the change in specific numbers of acid, saponification and iodine values of oil and fat of raw salmon during the processing.

(1) *Experimental method*

As samples, frozen chum and red salmon were used. The extraction of fat and oil from those salmon fish bodies was done by Watts and Peng's method[10].

About 10 *cc* each of the extracted oil was filled respectively into E.T. cans (completely coated inner side) and white cans (hot-dip tin plate) and the cans were seamed. The seamed cans were processed respectively at 5, 8, 10 and 15 *lbs*-pressure for 80 minutes. After the processing, a small hole was opened on the surface of the can-cover, and the gas in the head space was detected by gas chromatography to discover any presence of hydrocarbons. Then, after the can-cover had been completely opened, the oil was used for estimation of acid, saponification and iodine values.

The conditions of the detection by gas chromatography were the same as those described above.

(2) *Results of experiment*

Change in the specific numbers of acid, saponification and iodine values of oil of chum and red salmon which were separately processed in E.T. cans and white cans at various temperatures are shown in Table IV-2; the results of gas

Table IV-2. Changes in specific numbers of acid, saponification and iodine values of oil in canned salmon in the processing

Sample	Pressure of processing (*lbs.*)	E. T. can			Hot-dip can		
		Acid value	Saponification value	Iodine value	Acid value	Saponification value	Iodine value
Canned chum	0	0.8	171.9	133.1	0.8	171.9	133.1
	5	0.8	163.7	130.5	0.8	165.8	131.4
	8	1.0	118.3	113.6	0.8	111.2	120.5
	10	1.7	71.9	81.1	1.5	78.9	93.1
	15	2.2	70.4	80.2	1.8	80.3	83.2
Canned red	0	0.4	182.9	138.0	0.4	182.9	148.0
	5	0.3	170.4	134.7	0.6	167.7	136.6
	8	0.4	113.6	110.5	0.8	108.5	107.7
	10	0.8	72.6	79.1	0.8	73.9	81.3
	15	1.5	63.8	75.5	1.2	64.8	77.6

chromatographic detection of hydrocarbons are shown in Fig. IV-2.

As seen in Table IV-2, the specific numbers of acid, saponification and iodine values of oil processed vary from those of fresh raw salmon oil. With the increasing of processing temperature the changes in specific numbers of acid, saponification and iodine values become more remarkable.

As to the changes according to the kinds of salmon, the change in red salmon oil becomes more remarkable than that in chum salmon oil. This is perhaps due to the fact[11] that red salmon oil is rich in content of unsaturated fatty acid which is easily decomposed.

No difference in the change is observed between the kinds of cans used as containers.

As seen in Fig. IV-2, when chum salmon oil and red salmon oil are heated over 10 *lbs*-pressure, ethylene is detected in both kind, but not when they are heated at 8 *lbs*-pressure. The detection of ethylene accords with the result obtained in the previous chapter. The generation of ethylene from fat or oil takes place generally at high temperatures (250°~300°C), but hardly at low temperatures. So the generation is not considered to take place at the processing temperature (10 *lbs*-pressure, 115°C). But if the oil is heated under the presence of some catalyzer, ethylene may be generated[12]. In the canned foods, the fat and oil will be heated in a vacuum under the presence of protein and some mineral salts which form a kind of colloidal state with water to become a catalyzer, so ethylene will be generated during the processing. Here, ethylene found in canned salmon is considered to have generated from salmon oil during th processing. It is said[13] that if ethylene is mixed with carbon monoxide, the petroleum odour will result. Here, the author has compared the smell of the mixture of ethylene and carbon monoxide with the petroleum odour in the canned chum salmon. The smell of the mixture is near the petroleum pitch, but differs from the petroleum smell in canned chum salmon.

In consequence, the petroleum odour is not considered to come from ethylene which is generated from salmon oil during the processing.

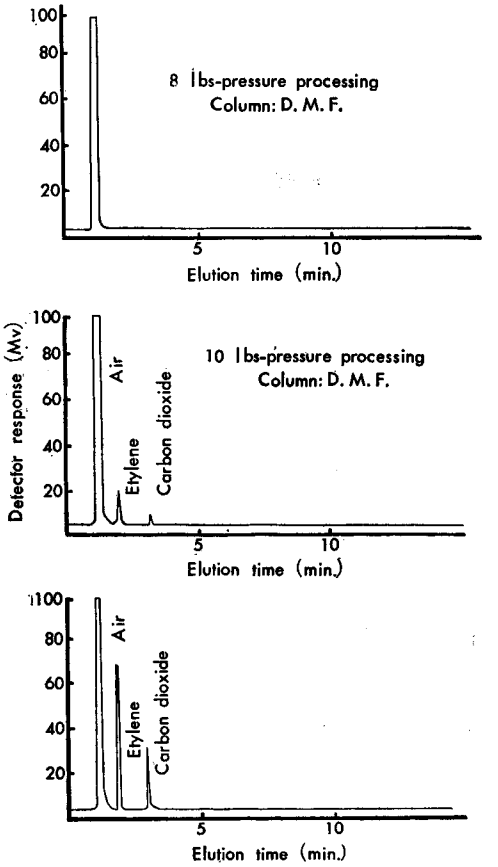


Fig. IV-2. Gas chromatographic observation of hydrocarbons produced from oil in canned chum and red salmon during the processing

V. Detection of Volatile Components in Various Kinds of Canned Salmon by Means of Paper Chromatography and Gas Chromatography

In order to find the chemical components of the petroleum odour in some canned chum salmon, the author tried a distillation by steam and an extraction by organic solvents. By those experiments a part of the components of the petroleum odour was found, but the main component was not obtained. This is perhaps due to the minute amount of the main component.

Under the conception that the petroleum odour is not a single component, but consists of various volatile elements, the author identifies the chemical components in various kinds of canned salmon by gas chromatography and paper chromatography in order to compare those components.

In this chapter, the author describes his attempts by means of gas and paper chromatography detecting chemical components in non-condensed fraction in the steam distillate obtained from canned contents.

1. Volatile components in head space gas of cans of various kinds of canned salmon

As the petroleum odour has been organoleptically observed at the moment of the opening of the cans, if the odour is present in canned salmon, the chemical components in the head space gas must be detected by gas chromatography.

(1) *Experimental method*

As samples, canned chum salmon having the petroleum odour, canned pink salmon, and canned red salmon were employed. A method for collection of odorous constituents of the gas in head space of canned product, was recommended by Stahl[14]. But the author collected the gas by the method as follows. The various kinds of salmon cans were heated at 115°C for 10 *mins.* in order to evaporate the gas into the head space from the inner part of the contents, and the gas was collected separately in a gas collector by opening a small hole on the surface of the can-cover. In each case the amount of the gas was about 20 *cc.*

The collected gas was used for gas chromatography. The apparatus was the same as that described in the previous chapter IV.

The conditions were as follows: Sample volume was 10 *cc*, column was D.O.P., carrier was hydrogen, the current speed was 50 *cc/min.*, pressure was 0.15 *kg/cm*^{2}, bridge current was 210 *mA*, chart speed was 10 *mm/min.*, column temperature was 10°~15°C.

(2) *Results of experiment*

The results are shown in Fig. V-1.

As seen in Fig. V-1, in the gas in head space of the various kinds of canned salmon, there are observed many kinds of chemical components.

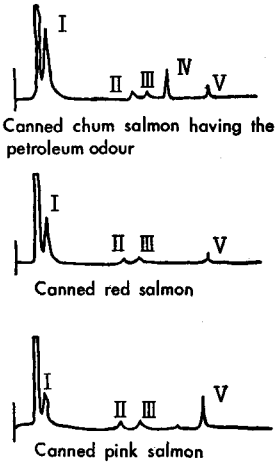


Fig. V-1. Gas chromatographic observation of the gas in head space of cans of various kinds of canned salmon

It is noticeable that the component (IV) is remarkably abundant in canned chum salmon having the petroleum odour, but little in other samples of canned salmon having no petroleum odour.

The chemical properties of the component (IV) will be discussed later. The author considers that the component (IV) is one of the main components having an intimate relation with the petroleum odour.

2. Reexamination of the collecting of the petroleum odour from canned chum salmon by steam distillation

Although the particular component (IV) was detected by gas chromatography as described in the previous section, in the canned chum salmon having the petroleum odour, its chemical characteristics were not determined.

Here, in order to analyze the chemical components, the author attempted to reexamine the method of collecting the components of the petroleum odour. In the steam distillation which was carried out as described in the previous chapter, the collection of the distillate having the petroleum odour seems to be insufficient, because the non-condensed fraction which perhaps contains the petroleum odour, is not obtained.

Then, the author devised means to collect the non-condensed fraction by making salts.

(1) *Experimental method*

The steam distillation was carried out by the same manner as described above. The non-condensed gas was received in solutions of 5% $HgCl_2$, $CuCl_2$, $ZnCl_2$, $Ba(OH)_2$, $PbCl_2$, $MnCl_2$, and CH_3COOPb from a cooler of the steam distillating apparatus.

(2) *Results of experiment*

The formation of precipitates in each salt solution from the non-condensed gas in various kinds of canned salmon is shown in Table V-1.

Table V-1. The formation of precipitates in some salt solutions from non-condensed gas in steam distillation of various kinds of canned salmon

$HgCl_2$	$CuCl_2$	$ZnCl_2$	$Ba(OH)_2$	$PbCl_2$	$MnCl_2$	CH_3COOPb
+	−	−	−	−	−	−

As seen in Table V-1, the non-condensed gas forms a visible precipitate only in $HgCl_2$ solution, but not in other salt solutions. Thus, there seems to be some components in the non-condensed gas reacting with $HgCl_2$.

After drying of the precipitate obtained by $HgCl_2$, various reactions were tested. The precipitate was discovered to have sulfur as one of its components. Thus, there seems to be present some sulfide which forms a double salt with $HgCl_2$. As this sulfur-containing compound is not precipitated by other salts, it is suggested to an organic sulfide, rather than an inorganic sulfide such as hydrogen sulfide.

The m.p. of this sulfide with $HgCl_2$ was 156°~159°C. When it was fused

with solid sodium hydroxide and sodium nitroprusside was added, it turned red color; the sulfur-containing compound was suggested to be thio-alcohol or thio-ether.

Next, the author by use of the steam distillation has tried to estimate the amount of the organic sulfide in the non-condensed gas in various kinds of canned salmon or frozen salmon meat.

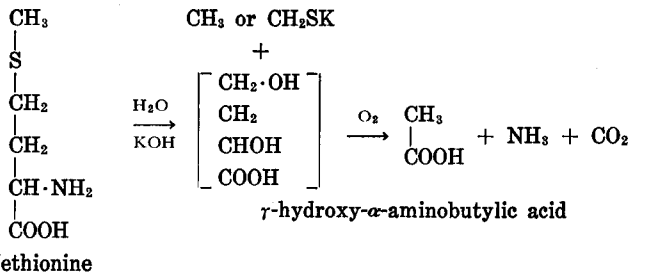
Table V-2. Yields of the precipitate in mercuric chloride solution

Sample	Numbers of cans	Amounts of tin content (*g*)	Amounts of precipitate in $HgCl_2$ (*g*)	Yields of precipitate (*mg%*)
Canned chum having the petroleum odour	40	8,000	0.15	1.87
Canned red salmon	40	8,000	0.10	1.24
Canned pink salmon	40	8,000	0.10	1.24
Frozen chum salmon		3,000	0.05	1.66
Frozen red salmon		2,000	0.03	1.50

As seen in Table V-2, the yields of the precipitate are small in all samples. Furthermore, the qualitative detection of the sulfide was negligible. The amount of the precipitate formed in $HgCl_2$ solution is larger in the canned salmon having the petroleum odour than in other canned salmon having no petroleum odour. The amount of the precipitate is slightly larger in frozen chum salmon than in frozen red salmon. Comparing canned salmon meat with frozen salmon meat, the precipitate formed from canned chum salmon is larger (about 0.2 *mg%*) than that from frozen chum salmon meat in the amount; on the contrary, that from canned red salmon is less (about 0.25 *mg%*) than that from frozen red salmon meat. In such quantitative differences, any relation between the amount of the sulfide and the petroleum odour is not distinct.

Thio-alcohol of organic sulfur-containing compounds, according to Onitake[15], is formed from methionine or methylmercapto compounds, and methyl mercaptan is also formed by heating from γ-methylmercapto-α-propionic acid.

According to Tsuchiya[16], the decomposition of methionine is carried on as follows:



As seen in the scheme of the decomposition, at γ-position of methionine hydrolysis occurs, or a double decomposition is caused between methionine and potassium hydroxide, then methyl mercaptan and γ-hydroxy-α-aminobutylic acid

are formed. γ-hydroxy-α-aminobutylic acid is oxidized to acetic acid, ammonia and carbon dioxide. If there is an excess amount of water in the decomposition described above, hydrogen sulfide is formed with methyl mercaptan.

3. Detection of thio-alcohol (methyl mercaptan)

In order to estimate qualitatively the sulfur-containing compound in the non-condensed gas, the author tried to detect methyl mercaptan following Tsuchiya[16].

Methyl mercaptan is decomposed in alkaline solution by heating as follows:

$$2CH_3SH + 2KOH = (CH_2)_2S + 2H_2O$$

Tsuchiya[16] has tried to detect qualitatively methionine or its derivatives by proving the presence of methyl mercaptan, on the basis that methyl mercaptan reacts with isatin-H_2SO_4 and forms green color.

(1) *Experimental method*

The preparation of the samples was carried out as shown in Table V-3. For the detection of the presence of methyl mercaptan, an apparatus as shown in

Table V-3. Samples employed for the detection of the presence of methyl mercaptan

Sample	Remarks
A	*dl*-Methionine
B	Dimethyl sulfide
C	The precipitate formed in mercuric chloride solution from the gas-phase of steam-distillation of the contents in digestive organ of chum salmon caught in 29th, June
D	The precipitate formed in mercuric chloride solution from the distillate of steam-distillation of the contents in digestive organ of chum salmon caught in 29th, June
E	,, in 3rd, July
F	,, in 25th, July
G	Hot alcohol extracted material from digested squid by digested enzymes of chum salmon caught in 29th, June
H	Ether extracted material ,,
I	The precipitate formed in mercuric chloride solution from the distillate of reduced pressure distillation of frozen chum salmon meat having the petroleum odour
J	Hot alcohol extracted material from canned chum neck meat
K	,, from canned pink salmon meat
L	The precipitate formed in mercuric chloride solution from cold alcohol extracted material of canned chum neck meat
M	,, from ether extracted material of canned chum neck meat
N	The precipitate formed in mercuric chloride solution from hot alcohol extracted deproteinized material of canned red salmon
O	,, canned pink salmon
P	,, canned chum salmon having the petroleum odour
Q	,, canned chum salmon having no petroleum odour
R	,, canned chum salmon having strong petroleum odour

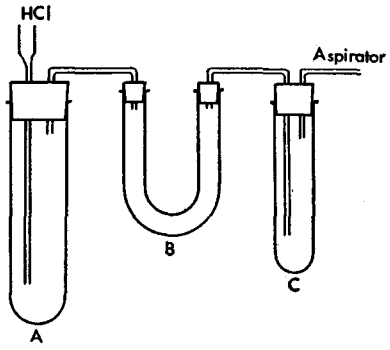


Fig. V-2. An apparatus for the detection of methyl mercaptan
A: Large test tube containing sample
B: U tube in which lead acetate is added
C: Test tube containing isatin-H_2SO_4

Fig. V-2 was employed.

Two or 3 *g* of the dried sample described in Table V-3, was put into a large sized test tube (A), in which granulated potassium hydroxide was put in twice the weight of the sample, and the mixture was heated to fuse. After fusing, the mixture was left to cool, 5 *cc* of conc. HCl was added to acidify. The preparation was aerated in the test tube (A) and the formed gas passed into a test tube (C) in which isatin-H_2SO_4 solution had been poured. Isatin-H_2SO_4 solution was prepared by dissolving 0.02 *g* of isatin in 100 *cc* of conc. H_2SO_4 solution. By the reaction of the formed gas and isatin-H_2SO_4, methyl mercaptan and hydrogen sulfide were formed.

Since hydrogen sulfide is absorbed completely in lead acetate which is stuffed in a U tube (B), if methyl mercaptan is present in the gas formed, the green color should be appeared in the test tube (C).

(2) *Results of experiment*

The results of this attempt to detect presence of methyl mercaptan are shown in Table V-4.

Table V-4. Results of detecting methyl mercaptan in various kinds of samples

Sample	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Jadgement	++	−	+−	+−	−	−−	−	−	−	+−	−	++	+	−−	−	++	+−	+

(Note) −−: Native color of Isatin-H_2SO_4 was not changed.
− : Native color was changed to yellow.
+−: Native color was changed to yellowish green.
+ : Native color was changed to light green.
++: Native color was changed to deep green.

As seen in Table V-4, the presence of methyl mercaptan is remarkable in canned chum salmon having the petroleum odour. The presence of methyl mercaptan is observed in the content of the digestive organs of chum salmon caught in the season corresponding to that of processing of canned product in which the

petroleum odour occurs. If the content of the digestive organs is extracted with ether or alcohol, and is tested, the more remarkable color reaction for methyl mercaptan may appear.

From the results, it is known that there is a remarkable amount of thio-alcohol (methyl mercaptan) in canned chum salmon having the petroleum odour or in the content of the digestive organs of the chum salmon used as the raw material, and that there is little in canned red salmon and pink salmon having no petroleum odour.

4. Detection of thio-ether (alkyl sulfide)

It has been made clear that thio-alcohol, such as methyl mercaptan, is present in the non-condensed gas distilled from the canned chum salmon having the petroleum odour.

Here, the author tried to detect thio-ether in addition to thio-alcohol.

A simple detecting method was devised for thio-ether, by which thio-ether was detected qualitatively by joint use of paper chromatography and gas chromatography.

(1) *New simple method for detecting thio-ether*

It is possible to detect thio-ether by color reaction after the sulfur atom is free in molecule of thio-ether in paper chromatography. This is true because the sulfur atom combines firmly with the alkyl radical, and the liberation of the sulfur must be done by fusing with potassium hydroxide at high temperature. As heating at high temperature must be avoided, thio-ether must be changed to its derivatives or addition products with heavy metal salts, which are effective to detect color reaction. From those considerations, the author attempted to make a double salt of thio-ether and $HgCl_2$, which is developed on a piece of filter paper by the use of water as a solvent. After developing, the spot was sprayed with silver nitrate solution which was acidified with nitric acid, to make silver chloride. Then the excess of silver nitrate attached to filter paper was washed with water. After washing, the paper was dried and was sprayed with potassium chromate solution as a revealing agent, and the position of the spot was determined by observation of yellowish red color formed. The separation of thio-ether from canned salmon was brought about by the method of steam distillation as shown in Fig. V-3.

In Fig. V-3, a sample is put into a flask (B) and the evaporated gas containing the petroleum odour is collected by steam distillation in a receiver (C). Thio-ether in the non-condensed gas is passed through a cooler (G_2), a trap (H) and a calcium tube (I), by means of which water is removed, and then is collected in receivers (D_1, D_2, D_3) each containing saturated $HgCl_2$ solution.

In each receiver, thio-ether forms a double salt with $HgCl_2$ making a white precipitate.

Although the time of steam distillation varies according to the kinds of samples, it is finished within 40~60 *mins.* (F) is an aspirator, which drives out the non-condensed gas. If the aspiration strength is too strong, the non-condensed gas will escape without reacting with the $HgCl_2$ solution. It is sufficient to adjust aspiration to the degree to cause bubbling two times per

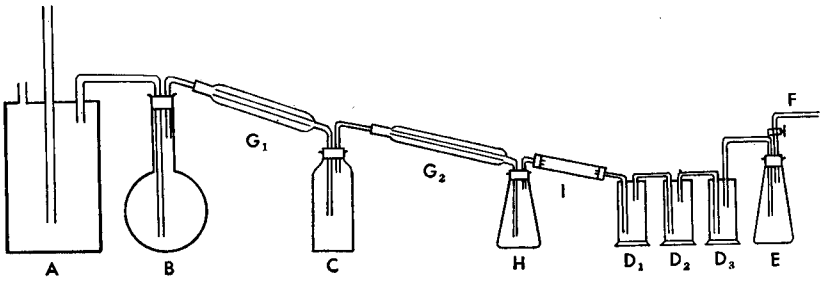


Fig. V-3. An apparatus for steam distillation
A: Steam boiler, B: Steam-distillation flask, C: Receiver, D_1, D_2, D_3: Traps containing saturated $HgCl_2$ soln, E: Trap, F: Aspirator, G_1 G_2, G_3: Cooler, H: Trap, I: Calcium tube.

second in each receiver, (D_1), (D_2) and (D_3).

About 0.2 *g* of sodium hydroxide is put into a test tube, and is melted by heating. Into the melted sodium hydroxide, about 0.2 *g* of the salt of thio-ether obtained by steam distillation is added. Since sulfur which is freed from the salt by this reaction changes to sodium sulfur turns to S″ ion. One drop of this solution is taken onto a filter paper and dried. After drying, the spot is sprayed with 0.5% sodium nitroprussid. S″ ion reacts with sodium nitroprussid in alkaline, and shows red color.

$$Na_2S + Na_2[Fe(CN)_5NO] = Na_4[Fe(CN)_5S] + NO$$

The double salt of thio-ether with $HgCl_2$ is insoluble in water, but soluble gradually in hot alcohol. As the developing solvent of the double salt for paper chromatography, alcohol can not be used singly. So a mixture of alcohol and a large amount of water is employed as the developing solvent. Two-tenths *g* of the double salt of $HgCl_2$ obtained from steam distillation is dissolved in hot alcohol. This is used for paper chromatography.

On an end of a piece of filter paper, one drop of it is put and developed by the solvents as shown in Table V-5 in an air-tight vessel according to one-dimensional paper chromatography. The developing solvent ascends about 30 *cm* at room temperature (about 20°C) during 4 hours. After the developing, the filter paper is dried and sprayed with 0.2% silver nitrate solution acidified with nitric acid. The sprayed paper is washed sufficiently with water to remove the excess of silver nitrate. The double salt forms silver chloride which is not washed out from the paper. As revealing reagent, 2% K_2CrO_4 is sprayed. As silver nitrate remains on the filter paper, the whole surface of the paper shows reddish brown. Around a spot which is the double salt of $HgCl_2$ with thio-ether, is shown a mixed color (*e.g.* yellowish red) of reddish brown formed by mercuric chromate and white formed by silver chloride, which differs distinctly form reddish brown of the back ground.

Some kinds of double salts of $HgCl_2$ with thio-ether were treated by the paper chromatography method described above. The results obtained are shown in Table V-5.

Table V-5. Rf obtained by paper chromatography on double salts of mercuric chloride with thio-ether

Solvents / Thio-ethers	90% phenol	0.2% NH_4OH: phenol (1:5)	Formalin	*n*-butanol: acetic acid: H_2O (4:1:2)	Chloroform contained H_2O
Dimethyl sulfide	0.44~0.45	0.59~0.60	0.79~0.81	0.77	0.63~0.65
Diethyl sulfide			0.77~0.78	0.74	0.64~0.62
Allyl sulfide	0.48~0.51	0.56~0.57	0.77~0.79	0.25~0.28	0.68~0.69

As seen in Table V-5, all the developing solvents, excepting a mixture of *n*-butanol, acetic acid and water, can be hardly used for the separation of thio-ether because Rf values of spots developed by them are too close. As developing solvent, a mixture of *n*-butanol : acetic acid : water (4 : 1 : 2) shows a better result, but others such as mixtures of *n*-butanol and hydrochloric acid; acetone and hydrochloric acid; methanol and hydrochloric acid; acetone and water; *n*-butanol, methanol and water etc. do not.

(2) *Detection of thio-ether by means of gas chromatography*

In the previous experiment, the detection of thio-ether by paper chromatography was possible, but sometimes the spots revealed by paper chromatography were not distinct.

Accordingly attempts were made to ascertain clearly the presence of thio-ether by means of gas chromatography.

Ryce[17] has tried to detect organic sulfides by gas chromatography, and obtained a chart shown in Fig. V-4. He employed the following conditions: carrier gas was helium; temperature was from room temperature (about 20°C) to 150°C; fixed phase was T.C.P. (tricresyl phosphate).

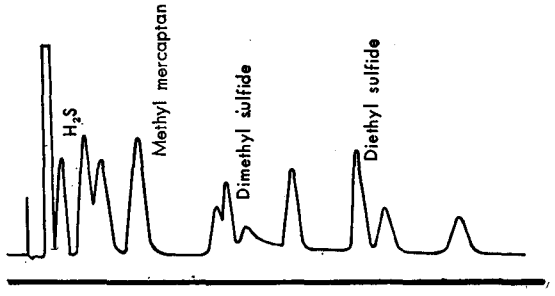


Fig. V-4. Gas chromatographic separation of various kinds of organic sulfides (Ryce[17])

Double salts of $HgCl_2$ with dimethyl sulfide, ethyl sulfide or allyl sulfide were used as samples for gas chromatography. The employed conditions were as follows: fixed phase was D.O.P.; temperature was 80°C; carrier gas was helium.

Data obtained are graphed in Fig. V-5.

As seen in Fig. V-5, thio-ethers can be detected by the use of their solid double salts in gas chromatography. Additional condition employed was current

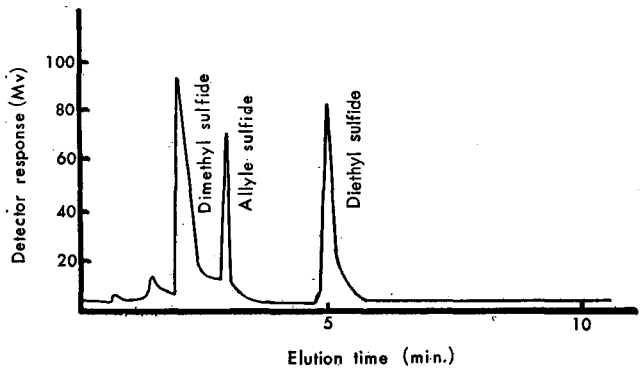


Fig. V-5. Gas chromatographic separation of double salts of mercuric chloride and thio-ether

speed of carrier gas (helium) was 40~60 *cc/min.*

(3) *Detection of thio-ether in the non-condensed gas obtained by steam-distillation from the contents of various samples of canned salmon and frozen salmon meat*

Several kinds of thio-ether were found to be detectable by paper and gas chromatography as above mentioned. In this experiment, the author tried to detect thio-ether in the non-condensed gas obtained by steam distillation from various kinds of canned salmon and frozen salmon meats.

Such the non-condensed gas was reacted with $HgCl_2$ solutions. The precipitate formed was examined by means of paper chromatography and gas chromatography as described in the previous section.

The results obtained by paper chromatography are shown in Table V-6.

Table V-6. Paper chromatographical results on the detection of thio-ethers in non-condensed gas phase in various kinds of canned and frozen salmon

Sample	Rf value
Dimethyl sulfide	0.68~0.71
Diethyl sulfide	0.74
Allyl sulfide	0.63~0.65
Canned chum salmon having the petroleum odour	0.08, 0.68
Canned pink salmon	0.08, 0.67
Frozen chum salmon having the petroleum odour	0.08, 0.69
Frozen red salmon	0.07, 0.68

As seen in Table V-6, two spots are revealed, *e.g.*, Rf 0.08 and Rf 0.67~0.69, from all samples used. A spot having 0.67~0.69 Rf value corresponds to dimethyl sulfide. From those results, it is clear that thio-ether is present in all of canned or frozen salmon meat with or without the petroleum odour and that it is dimethyl sulfide. Nonaka[18] has said that the petroleum odour in canned chum salmon resembles some sulfides such as allyl sulfide. But in the present author's

experiment, allyl sulfide was not discovered.

In order to ascertain the cause of the petroleum odour in canned chum salmon, determination of the amount of organic sulfide was undertaken by gas chromatography.

The results obtained are shown in Fig. V-6.

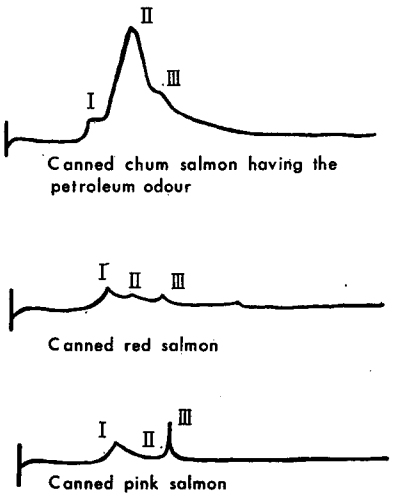


Fig. V-6. Gas chromatographic separation of thio-ethers in various kinds of canned salmon

As seen in Fig. V-6, the amount of organic sulfide is larger in canned chum salmon having the petroleum odour than in canned red salmon and pink salmon having no petroleum odour. This difference is important in connection with an explanation of the cause of petroleum odour.

In Fig. V-6, the amount of component (II) is the largest in canned chum salmon. On the other hand, the amount of component (I) is larger than that of component (II) in canned red salmon or pink salmon.

By gas chromatography under the same condition use being made of pure reagents of sulfide, component (II) was ascertained to be dimethyl sulfide whilst component (I) was methyl mercaptan. That is to say, the major part of the gas in canned chum salmon may be considered to be dimethyl sulfide; contrarily that in canned red salmon is methyl mercaptan.

In Fig. V-6, based on the results of gas chromatography, allyl sulfide is again not detected.

5. Volatile components excepting the $HgCl_2$-reactable components in the head space of canned chum salmon having the petroleum odour

In the previous section **1**, it was ascertained that component (IV) may be an organic sulfide such as dimethyl sulfide.

(1) *Experimental method*

Into head space of 5 cans each of canned chum salmon having the petroleum

odour and canned red salmon and pink salmon having no petroleum odour, saturated $HgCl_2$ solution was poured through a small hole which was immediately soldered. The cans were heated at 115°C (10 *lbs*-pressure) for 10 minutes in order to increase the inner pressure. The gas which rushed out by the opening of a small hole in the cans under water in a tank was received in a collector. The chemical components of this gas were detected by gas chromatography. The conditions and apparatus were the same as those described in the previous section.

(2) *Results of experiment*

Results obtained are shown in Fig. V-7.

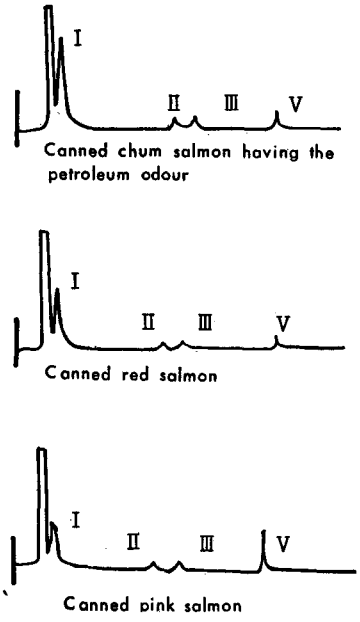


Fig. V-7. Odorous constituents in head space gas, excepting the mercuric chloride reactable component, of various kinds of canned salmon found by means of gas chromatography

As seen in Fig. V-7, component (IV), obtained from the various canned materials as shown in the previous section **1**, disappears. This may have happened because that component (IV) perhaps reacts with $HgCl_2$ and becomes a non-volatile substance. From this fact component (IV) is considered to be an organic sulfide.

6. Estimation of the amount of thio-alcohol (methyl mercaptan) and thio-ether (dimethyl sulfide) in various kinds of canned salmon

It is known that organic sulfide is present in various kinds of canned salmon, and that the amount of dimethyl sulfide is larger in canned chum salmon having the petroleum odour than in canned red salmon and pink salmon having no

petroleum odour, but the amount of methyl mercaptan in both cases does not differ so much amount of dimethyl sulfide.

(1) *Experimental method*

Here, the author will describe his estimation of the amounts of dimethyl sulfide and methyl mercaptan.

Use being made of various kinds of canned salmon, at the time of steam distillation the non-condensed gas was received in a collector containing 4% mercuric acetate was filtered through Toyo filter paper No. 5a, then was washed with water. The washed precipitate was dried in a desiccator containing P_2O_5, and weighed. To the distillate in the collector containing benzol, saturated $HgCl_2$ solution was added. The double salt of $HgCl_2$ with dimethyl sulfide was precipitated. The precipitate was washed, dried and weighed[19].

The amounts of methyl mercaptan and dimethyl sulfide are shown in Table V-7.

Table V-7. Amounts of methyl mercaptan and dimethyl sulfide in various kinds of canned salmon

Sample	Methyl mercaptan (*mg*/250 *g* of meat)	Dimethyl sulfide (*mg*/250 *g* of meat)
Canned chum salmon having the petroleum odour	1.3	2.1
Canned red salmon	0.4	0
Canned pink salmon	0.6	0.1
Canned chum neck having the petroleum odour	1.4	1.8

As seen in Table V-7, the amount of dimethyl sulfide in canned chum salmon having the petroleum odour is about 1.52 *mg*%, on the other hand its amount in canned red salmon or pink salmon is 0.15 *mg*% or 0.28 *mg*%, respectively. That is to say, the amount of dimethyl sulfide in canned chum salmon having the petroleum odour is about 6~10 times as great as its amount in canned red salmon or pink salmon.

On the other hand, the amount of methyl mercaptan in canned chum salmon having the petroleum odour is 0.27 *mg*% whilst its amount in canned red salmon and pink salmon is 0.12 *mg*% and 0.11 *mg*%, respectively. The difference in the amount of methyl mercaptan in both cases is less than that of the dimethyl sulfide.

The results obtained suggest that the component of the petroleum odour is an organic sulfide, thio-ether or thio-alcohol.

VI. Isolation of a Precursor of Dimethyl Sulfide found in Canned Chum Salmon having the Petroleum Odour

It has been observed that there is a large amount of dimethyl sulfide in canned chum salmon having the petroleum odour; so it perhaps has some relation with the petroleum odour.

It has been found by many investigators[20)21)22)] that dimethyl sulfide is contained in marine algae. As a result of studies, the transposition of dimethyl sulfide from marine algae to fish meat is considered to be easily carried out.

The boiling point (b.p.) of dimethyl sulfide is 37°C, and the presence of the component is detected by its peculiar odour (*e.g.*, odour of roasted laver, "Yaki-nori", a Japanese food). In raw fresh chum salmon the peculiar odour of dimethyl sulfide is not felt. This perhaps is because in raw fresh chum salmon dimethyl sulfide is not present in free state, but is fixed with other components from which dimethyl sulfide is formed by heating. This is probably explained from the fact that when chum salmon is roasted or boiled, the petroleum odour is noticeable.

From this point, the author assumed that some precursor of dimethyl sulfide is present in raw fresh chum salmon meat and that it becomes dimethyl sulfide by heating; he undertook to detect the precursor in chum salmon meat.

1. Alcohol extracted material (A. E. M.) from frozen chum salmon

(1) *Sample*

Chum salmon caught in the northern Pacific Ocean in the middle fishing season (during which the cans having petroleum odour were frequently manufactured) and in the final fishing season (during which the cans having the petroleum odour were rarely found) were respectively viscerated on the deck of a mother-ship; the body and entrails were separatly frozen at −30°C and were brought to the author's laboratory. After defrosting, the skin and bones were removed from the body, and meat was obtained.

Three *kg* of the defrosted meat was crushed in the Waring Blendor. To the crushed meat 4.5 *l* of absolute ethyl alcohol was added and the mixture was kept at a cool place (0°C) for a week in order to extract chemical components. The extract was condensed to about 15 *cc* of syrup state under a vacuum at 40°~50°C. The syrup was brown, then it was used as the sample.

(2) *Experimental method*

Each 0.5 *cc* of the sample was poured into separate test tubes having 10 *cc* of buffer solutions of sodium phosphate and sodium citrate, of which pH values were 6.0, 6.5, 7.0 and 7.5, respectively. After heating at 100°C for 20, 40 and 60 minutes, respectively, the presence of dimethyl sulfide was detected by gas chromatography in the same way as in the previous article. The conditions were as follows: carrier gas was helium, current speed was 60 *cc/min.*, pressure was 0.15 kg/cm^2, bridge current was 180 *mA*, temperature of the column was 80°C, span was 1.8 *mA*.

(3) *Experimental result*

In fraction of alcohol-extractive, in which dimethyl sulfide was detected after heating, the presence of the component is shown in Table VI-1.

As seen in Table VI-1, when the alcohol-extractive of chum salmon meat obtained in the middle of the fishing season is heated at 100°C for above 40 minutes in alkaline side of pH 7.5, it produces dimethyl sulfide. On the other hand, regarding chum salmon meat caught at the end of the fishing season the formation of dimethyl sulfide is not detected by heating at 100°C in either the

Table VI-1. Formation of dimethyl sulfide in the case of heating of A.E.M. in the solutions of various pH values

Sample	pH / Heating thme	6.0	6.5	7.0	7.5
Chum salmon (middle season)	20 *min.*	−	−	−	±
	40	−	−	−	+
	60	−	−	±	+
Chum salmon (final season)	20	−	−	−	−
	40	−	−	−	−
	60	−	−	−	−

acidic or alkaline side.

From those results, it is suggested that there is some component from which dimethyl sulfide is formed by heating (at 100°C) in chum salmon meat caught in the middle fishing season.

As follows, the author tried to detect various qualitative reactions for a precursor, from which dimethyl sulfide may be formed.

2. Chemical characteristics of a precursor substance from which dimethyl sulfide is formed by heating, in a alcohol-extract of frozen chum salmon meat caught in the middle fishing season

(1) *Sample*

By the same method as described in the previous section, defrosted chum salmon meat was extracted with absolute alcohol. The extract was concentrated at 40°~50°C under a vacuum. The concentrated matter thus obtained was brown-paste. This paste was dried in a vacuum desiccator, and brown solid matter (A.E.M.) was obtained.

(2) *Qualitative tests*

The brown solid matter (A.E.M.) was acidic for litmus; identified reactions as organic substance and the presence of nitrogen, sulfur and chlorine were positive.

If A.E.M. was heated in 6 N solution of HCl at 100°C for 20 hours, the reaction for sulfur became remarkable. If A.E.M. was heated in dil. H_2SO_4 solution (1 : 1), and neutralized, and if solid sodium acetate, glacial acetic acid and phenyl hydrazine were added to the neutralized matter, then osazon or phenyl hydrazon was formed. Molish reaction was negative. The test for amino acids was positive. When 0.5% ninhydrin solution was added to the neutralized A.E.M., and heated, the material showed violet color. When ammonia was added to A.E.M. and heated, the odour of dimethyl sulfide resulted. When sodium hydroxide solution was added to A.E.M. and the odour generated was absorbed by saturated $HgCl_2$ solution, a double salt of $HgCl_2$ with dimethyl sulfide was formed, of which m.p. was 157°~158°C. When Reinecke's salt ($NH_4Cr(NH_3)_2(SCN)_4$) solution was added to A.E.M., some preciptate was formed.

The excess of Reinecke's salt ion was removed by the addition of silver sulfate, and the excess of silver sulfate was in turn removed by the addition of barium chloride. The residual solution was evaporated, then platinum chloride was added to the residue making platinum salt. Then metalic silver powder was added, and silver chloride and metalic platinum formed were removed. The filtrate was evaporated to a dry-matter which was considered to be the chloride of the main substance of A.E.M. This chloride was treated with picric acid and repeatedly recrystallized to make picrate. This picrate was optical inactive. Next the picrate was hydrolyzed with HCl and the decomposed matter was estimated by elementary analysis; a material of the chemical composition of ($C_5H_{11}O_2ClS$) was obtained, of which m.p. was 134°C. When S-benzyl thiuronium chloride was added to the picrate, crystalline salt was formed, in which –COOH radical was identified. The picrate did not act on 2, 4-dinitrophenyl hydrazine. From this fact the presence of –OH or –CHO radicals were not considered. According to the results obtained above, the main substance of A.E.M. was considered to resemble to dimethyl-2-carboxyethyl sulfonium chloride $(CH_3)_2\overset{+}{S}(\overset{-}{Cl})\cdot CH_2\cdot CH_2\cdot COOH$ (dimethyl-β-propiothetin) which was isolated from *Polysiphonia fastigiata*, a kind of marine alga, by Challenger and Simpson[23].

The A.E.M. obtained from chum salmon meat caught at the end of the fishing season did not show the facts as above mentioned.

From the above results, it is considered that dimethyl-β-propiothetin (D.M.P.T.) has an intimate relation with the petroleum odour.

The D.M.P.T. is perhaps transported to chum salmon meat from marine algae.

3. Synthesis of dimethyl-2-carboxyethyl sulfonium chloride

It has been ascertained that A.E.M. of chum salmon meat caught in the northern Pacific Ocean in the fishing season, during which the cans having petroleum odour were frequently manufactured, resembles dimethyl-2-carboxyethyl sulfonium chloride (dimethyl-β-propiothetin) (D.M.P.T.) in chemical characteristics.

Here, the author describes his effort to discover whether the two substances are the same or not.

The author synthesized D.M.P.T. according to Challenger *et al.*[23], and compared the synthesized D.M.P.T. with the substance obtained from A.E.M. in chemical characteristics.

An equivalent volume each of β-bromopropionic acid and dimethyl sulfide was mixed, and heated at 55°~60°C for 6~8 hours with reflux condensor. During the heating, the mixture was violetish brown liquid, and after the cooling it became solid. The solid was washed with dried ether to removed unreacted matter. The residue was recrystallized with absolute ethyl alcohol. The m.p. of the crystal was 112°~114°C which accorded with the m.p. of ethyl sulfonium bromide, when the crystal was reacted with silver chloride, the m.p. of chloride formed was 134°C.

As to the m.p. of the substance obtained from A.E.M. compared with that of the synthesized sulfonium compound, the data are shown in Table VI-2.

Table VI-2. The melting points of the substance obtained from A.E.M. and of synthesized sulfonium compounds

Sample \ Derivative	Chloride (°C)	Bromide (°C)	Picrate (°C)
Dimethyl-β-propiothetin	134	112~114	132
A.E.M.	134	112~113	131.5
A.E.M.+dimethyl-β-propiothetin; mixed m.p.	132~133	112~113	131~132

As seen in Table VI-2, the m.p. of the substance obtained from A.E.M. was almost the same as that of the synthesized compound. Therefore, the substance is considered to be dimethyl-2-carboxyethyl sulfonium.

4. Detection of chemical characteristics of the substance obtained from A.E.M. by paper chromatography

It was suggested that A.E.M. contains the substance which is a kind of carboxylic acid containing sulfur. Here, the author tried to ascertain the chemical characteristics of this substance by means of paper chromatography. A portion of the same alcohol-extract obtained in the previous section was passed through Amberlite IRC-45 (of which column was 1 *cm* × 20 *cm*); the elution was concentrated under a vacuum. The concentrated matter was a solid of greenish brown. One or 2 drops of dist. water was added to a part of the solid matter, and it was detected by paper chromatography after Chargaff's method[24]. One-half *g* of the rest of the solid matter was put into a test tube and heated in an oil bath having a reflux condenser at 135°C for one hour. After cooling, 5 *cc* of methanol was added into the test tube. Then fatty acids in the thermo-decomposed product were detected by paper chromatography after Fink and Fink's method[25]. According to Chargaff's method, one dimensional ascending was employed by using 65% pyridine as developing solvent and by spraying 1% ninhydrin as revealing reagent. According to Fink and Fink's method, one dimensional ascending development was employed by using water-saturated *n*-butanol as a developing solvent and by spraying 2% ferric chloride as a revealing reagent. In both paper chromatographies, Toyo filter paper (No. 50) (1×50 *cm*) was used.

Results obtained by paper chromatography are shown in Figs. VI-1 and VI-2.

In Fig. VI-1, are shown the Rf values of methionine and 3-amino-3-carboxypropyl dimethyl sulfonium compound. As seen in VI-1, Rf value of this substance was different from those of the other two compounds, but agreed with that of the synthesized dimethyl-2-carboxyethyl sulfonium compound.

Fig. VI-2 shows the chromatogram of fatty acids formed by the thermo-decomposition of the substance. Namely, fatty acid detected is propionic acid only.

Prichard[1] has also isolated sarcolactic acid from frozen chum salmon meat having the petroleum odour. That acid is very similar as a compound to propionic acid. Cantoni[26] has obtained propionic, acrylic and acetic acids. According to

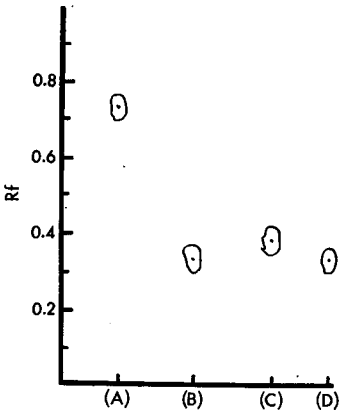


Fig. VI-1. Paper chromatography of several sulfonium compounds
A: Methionine
B: Sample
C: 3-amino-3-carboxy-propyl dimethyl sulfonium
D: D.M.P.T.

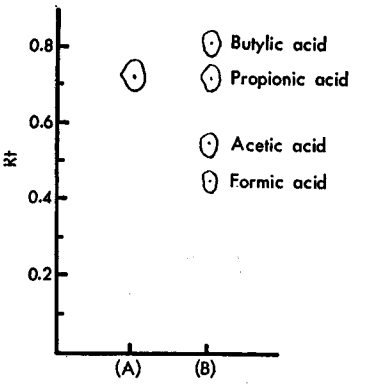


Fig. VI-2. Paper chromatography of several fatty acids
A: Sample
B: Standard fatty acids

Cantoni, acrylic acid is considered to be formed by the hydrolysis of D.M.P.T. following equation (I), but the present author has considered that the thermo-decomposition of D.M.P.T. follows equation (II).

$$(CH_3)_2\overset{+}{S}\cdot CH_2\cdot CH_2\cdot COOH \longrightarrow (CH_3)_2S + CH\cdot CH\cdot COOH + \overset{+}{H} \qquad (I)$$

$$(CH_3)_2\overset{+}{S}\cdot CH_2\cdot CH_2\cdot COOH \longrightarrow (CH_3)_2S + CH_3\cdot CH_2\cdot COOH \qquad (II)$$

5. Thermo-decomposition of the substance obtained from A.E.M. of chum salmon meat having the petroleum odour

According to the results obtained in the previous section, the substance is comparatively unstable against alkali and against heating. This fact has an important significance for the processing of canned chum salmon.

(1) *Experimental method*

Here, the author describes his observations of the thermo-decomposition of the substance in various pH values. One-half *g* each of A.E.M. was put into electric tin plate cans of which the inner sides were coated or hot-dip tin plate white cans. In each can, 20 *cc* each of phosphate-citrate buffer solutions having pH values 5.5, 6.0, 6.5, 7.0, 7.5 were poured; the cans were closed by a seamer under normal pressure or by a vacuum-seamer under 10 inch, heated at 5, 10 and 15 *lbs*-pressure for 80 minutes, and then cooled.

The gas in the head space of each can was collected in an injector through a small hole made on the cover of the can. In this case, air mixed at the time of collection was identified on a chart of gas chromatography.

The gas was tested for dimethyl sulfide by gas chromatography as described in the previous chapter.

Results obtained are shown in Table VI-3. When a small portion of Reineck's

Table VI-3. Thermo-decomposition of the substance obtained from A.E.M. in various kinds of containers during processing (Judged by the formation of dimethyl sulfide)

Tin container	Seaming method	pH / Processing press. (*lbs.*)	5.5	6.0	6.5	7.0	7.5
E.T. can	Vacuum seaming	5	−	−	−	−	+
		10	−	−	−	±	+
		15	−	−	−	±	+
	Normal pressure seaming	5	−	−	−	−	±
		10	−	−	−	−	±
		15	−	−	−	±	±
Hot-dip can	Vacuum seaming	5	−	−	−	−	+
		10	−	−	−	±	+
		15	−	−	−	±	+
	Normal pressure seaming	5	−	−	−	−	±
		10	−	−	−	−	±
		15	−	−	−	±	±

+: Remarkable formation of dimethyl sulfide
±: Slight formation of dimethyl sulfide
−: No formation of dimethyl sulfide

salt solution was poured into each can through a small hole, the amount of the precipitate formed was estimated. These results accorded nearly with the Table VI-3.

In Table VI-3, as to relation between the degree of thermo-decomposition of the substance and kinds of cans (E.T. cans or hot-dip cans), there is no difference between the kinds of cans. This is perhaps due to the reason that dimethyl sulfide reacted with neither tin nor iron which were the material of the cans, differing from hydrogen sulfide or mercaptan. Under the normal pressure in cans no thermo-decomposition is observed in the case of heating at 5, 10 and 15 *lbs*-pressures. Under the vacuum pressure in cans, the thermo-decomposition occurs by heating at 10 *lbs*-pressure for 80 minutes. From those observations, the vacuum pressure in cans is considered to have some relation with the degree of thermo-decomposition.

The thermo-decomposition occurs easily in the alkaline side above pH 7.0, but difficulty in acidic than that. In general, the pH value of the content of canned chum salmon is 6.2~6.4, but becomes alkaline with falling of freshness of salmon as the raw material. Therefore, unfresh salmon should be avoided as the raw material for canning in order to avoid the petroleum odour formation.

From those results, there can be considered to exist some relation between pH value of raw salmon or the degree of vacuum in cans and the thermo-decomposition of D.M.P.T., the evolution of dimethyl sulfide having intimate relation with the petroleum odour.

VII. Isolation of Dimethyl-β-Propiothetin from the Contents in Digestive Organs of Salmon

It is obvious that dimethyl sulfide which is the main constituents of the undesirable petroleum odour, is evolved by decomposition of dimethyl-β-propiothetin in frozen chum salmon meat. Challenger and Simpson[23] have reported that dimethyl-β-propiothetin (D.M.P.T.) is contained in a marine algae, *Polysiphonia fastigiata*. Accordingly, it may be considered that D.M.P.T. in frozen chum salmon meat is derived from marine plants.

If D.M.P.T. comes from the baits of chum salmon, the substance should be contained in the contents of the digestive organs of chum salmon.

The author tried to isolate D.M.P.T. from the contents of the digestive organs of various kinds of salmon.

1. Isolation of D.M.P.T. from the contents in digestive organs of some kinds of salmon

The contents in digestive organs of red, chum and pink salmon which were caught in the northern Pacific Ocean in different fishing season, were collected, respectively. Those contents were frozen at −30°C, immdiately after the collection. They were brought in frozen state to the laboratory; after having been defrosted, the contents were used for the experiment.

The method of isolation of D.M.P.T. was the same as that described in the previous section (VI) 2,

The results obtained on isolation of D.M.P.T. from the contents in digestive organs of various kinds of salmon, are shown in Table VII-1.

From the results shown in Table VII-1, the amount of D.M.P.T. in the contents in digestive organs of chum salmon caught in the middle fishing season is the highest, *e.g.* 0.77 *mg%*, and in earlier (May 25th) and later (July 20th) seasons is 0.28 *mg%* and 0.16 *mg%*, respectively. Therefore, the amounts of

Table VII-1. The amounts of D.M.P.T. in the contents in digestive organs of various kinds of salmon caught in different fishing season

Sample (Contents in digestive organs)	D.M.P.T. (*mg%*)	Date of fishing
Chum	0.28	May 25th
	0.77	June 20th
	0.16	July 20th
Red	Trace	June 6th
	Trace	June 25th
	Trace	Aug. 2nd
Pink	Trace	June 6th
	0.16	June 20th
	Trace	July 20th

Table VII-2. Amounts of D.M.P.T. in the contents in digestive organs of various kinds of salmon caught in different fishing season (Gravimetric determination by addition of Reinecke's salt)

Sample (Contents in digestive organs)	D.M.P.T. (*mg%*)	Date of fishing
Chum	0.47	May 25th
	1.07	June 20th
	0.36	July 20th
Red	0.00	June 6th
	0.02	June 20th
	0.00	July 2nd
Pink	0.03	June 6th
	0,19	June 20th
	0.00	July 20th

D.M.P.T. which are taken into the digestive organs of chum salmon, are considered to be the largest in the middle of the usual fishing season, and to become small before and after this season.

In the case of red salmon, only a trace amount of D.M.P.T. is isolated from the contents of digestive organs throughout the whole fishing season. The amount of D.M.P.T. in digestive organs of pink salmon caught in the middle season, was 0.16 *mg%*; while in earlier or later fishing season, is little.

Moreover, this fact was ascertained by the next experiment.

From the same material as described above, 200 *g* each portions was put into separate beakers with twice the volume of water, and were blended sufficiently to a slurry. The slurry was allowed to stand for 40 minutes at room temperature (20°±2°C), then was separated into liquid and solid portions by centrifugation (3,000 r.p.m., 10 *mins.*). To the liquid portion thus obtained was added 2% Reinecke's solution; after the precipitate formed had been weighed, the amounts of D.M.P.T. were estimated.

The results obtained are shown in Table VII-2. It is understood that the values shown in Table VII-2, are slightly larger than those in Table VII-1, although both one almost the same in the tendency of change.

From the results of the above described experiments, there seem to exist some correlations between the formation of the petroleum odour and the amounts of D.M.P.T. of the contents in digestive organs.

Methyl sulfonium compounds including D.M.P.T. have been isolated from *Strept. brevicaulis*[27] and *Penicillium notatum*[28]. From parsley, cabbage, turnip greens, turnips, pepper, carrot, onion and lettuce, one of the methyl sulfonium compounds, 3-amino-3-carboxypropyldimethylsulfonium salt $(CH_3)_2\overset{+}{S}\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH$, has been isolated[29]. In view of these facts, the methyl sulfonium compounds are supposed to be distributed widely in many plants, and are thought to be a biologically important substance as an intermediate product of natural occurrence of methionine. It has been reported that methyl sulfonium compounds are not produced in animal tissues, but are synthesized in plants[23]. Considering these facts, D.M.P.T. isolated from chum salmon meat is not produced in chum salmon body, but is taken in from such food as plankton. From the fact that the isolation of D.M.P.T. from the contents in digestive organs of chum salmon having the petroleum odour, it can be presumed that D.M.P.T. moves into the chum salmon body through the bait.

The details of biochemical change in D.M.P.T. after being taken into salmon body, will be described in following chapter.

2. The species of plankton in digestive organs of some kinds of salmon

It is obvious that the existence of a lot of D.M.P.T. in the contents of digestive organs, may explain the production of the petroleum odour. Then, why do the amounts of D.M.P.T. in the contents of the digestive organs differ in each of various kinds of salmon? Besides, why do the amounts of D.M.P.T. differ in the contents of digestive organs in the same kind of salmon caught in different fishing seasons?

With respect to these questions, the author considered that the kinds of

baits intaken by salmon differ according to the kinds of salmon, and that they afford the differences of the amounts of D.M.P.T. in the contents of digestive organs of salmon.

Then, the author observed the kinds of contents in digestive organs in several kinds of salmon caught in different fishing seasons.

The materials used for the experiment were the same as described in the previous section.

The frozen materials were defrosted at room temperature (20°±2°C), and a small portion of the material was put into a petri dish, then was suspended in

Table VII-3. Species of plankton contained in digestive organs of several kinds of salmon

Salmon	1958	1959	1960	Period of the fishing season
Chum	*Euphasia* (13%) *Copepod* (80%) Jellyfish (2%) Fish (5%)	*Euphasia* (20%) *Copepod* (70%) *Pteropod* (5%) *Myctophid* (5%)	*Myctophid* (3%) *Amphipod* (10%) *Pteropod* (7%) *Copepod* (80%)	From May 20th to June 6th
	Euphasia (5%) *Amphipod* (7%) Jellyfish (3%) *Pteropod* (85%)	*Pteropod* (75%) *Copepod* (20%) *Amphipod* (5%)	*Pteropod* (80%) *Copepod* (10%) *Amphipod* (8%) *Euphasia* (2%)	From June 14th to July 6th
	Calanus (65%) *Amphipod* (33%) Squid (2%)	*Amphipod* (40%) *Copepod* (50%) *Myctophid* (10%)	*Copepod* (65%) *Amphipod* (30%) *Euphasia* (8%) *Pteropod* (2%)	From July 7th to August 10th
Red	*Copepod* (70%) *Amphipod* (25%) *Pteropod* (1%) *Myctophid* (4%)	*Copepod* (60%) *Amphipod* (35%) *Euphasia* (5%)	*Myctophid* (8%) *Copepod* (80%) *Amphipod* (12%)	From May 20th to June 6th
	Copepod (50%) *Amphipod* (45%) *Euphasia* (4%) Squid (1%)	*Copepod* (60%) *Amphipod* (25%) *Euphasia* (10%) Squid (5%)	*Copepod* (80%) *Pteropod* (2%) *Euphasia* (8%) *Amphipod* (10%)	From June 14th to June 25th
	Copepod (40%) *Euphasia* (20%) *Amphipod* (40%)	*Copepod* (60%) *Euphasia* (20%) *Amphipod* (20%)	*Euphasia* (25%) *Amphipod* (30%) *Copepod* (40%) Squid (5%)	From July 7th to August 10th
Pink	*Euphasia* (17%) *Copepod* (50%) *Myctophid* (3%) *Amphipod* (30%)	*Copepod* (63%) *Amphipod* (29%) *Myctophid* (8%)	*Copepod* (62%) *Amphipod* (30%) *Myctophid* (8%)	From May 20th to June 6th
	Copepod (50%) *Amphipod* (45%) *Pteropod* (5%)	*Copepod* (58%) *Euphasia* (36%) *Pteropod* (6%)	*Pteropod* (8%) *Copepod* (60%) *Amphipod* (26%) Jellyfish (6%)	From June 14th to June 25th
	Copepod (50%) *Amphipod* (25%) *Euphasia* (25%)	*Copepod* (58%) *Amphipod* (25%) *Euphasia* (10%) Fish (7%)	*Pteropod* (3%) *Copepod* (53%) *Amphipod* (32%) Jellyfish (12%)	From July 7th to August 10th

water. Determination of the kinds of plankton was done by the observation with the naked eye and with low power microscope (×60).

One hundred *g* of the material were added to twice the weight of water. The mixture was centrifuged at 500 r.p.m., 1,000 r.p.m. and 2,000 r.p.m. for 5 minutes, respectively. Thus owing to the differences of specific gravities of each plankton, the quantitative separation of the contents was carried out.

The data for the years of 1958, 1959 and 1960 on the species of plankton in digestive organs in several kinds of salmon, were collected and shown in Table VII-3.

As seen in Table VII-3, the contents in digestive organs are *Myctophid, Copepod, Euphasia* and *Amphipod* in all of red salmon collected throughout whole fishing seasons. While, in chum salmon, most of the contents are *Pteropods* (*Clione limacina* and *Limacina helicina*) from earlier to middle fishing season. *Copepod* and *Amphipod* replace *Pteropods* in later part of fishing season.

In general, the kinds of contents in digestive organs of red and pink salmon caught throughout the fishing season, are considered to be in agreement.

Ito[30] has summarized the kinds of the contents in digestive organs of various kinds of salmon as shown in Table VII-4, and stated that the kinds of food of chum salmon differ significantly from those of other salmon. The appearance of the stomach is larger than that of other salmon. Chum salmon do not take in a large amount of food at once so much as to dilate the stomach as in pink salmon. The conditions of the contents in digestive organs of chum salmon at the time of inspection show them to be generally well digested. From those results, Ito has concluded that the food taken by chum salmon is easy to digest, or enzymes in digestive organs of chum salmon possesses strong digestive power.

Table VII-4. Kinds of contents in digestive organs of various kinds of salmon classified by Ito[30]

Salmon	Food
King (*O. tschawytscha*)	Squid, *Myctophid*
Silver (*O. kisutch*)	Squid, *Myctophid*
Red (*O. nerka*)	Squid, Fish, *Euphasia, Amphipod, Copepod*
Chum (*O. keta*)	*Pteropod*, Jellyfish, *Amphipod, Copepod*
Pink (*O. masou*)	Squid, Fish, *Myctophid, Copepod, Euphasia, Amphipod*

Saito[31] had survey the resources of the Aleutian fishing grounds and has reported that they are rich in zoo-planktons with a little of Atka-mackerel larva and squid. Among the zoo-planktons, *Thysanoessa, Calanus* and *Parathemisto* occupy the main portion.

The data obtained by the present author almost agree with those by Ito[30] and Saito[31]. From those results, chum salmon slightly differs from the other salmon in respect to the points of food and the ability to digest food. These differences between chum and other salmon may give rise to the differences in the amounts of D.M.P.T. in the contents of digestive organs in various kinds

of salmon.

With respect to the problem why chum salmon differ from other salmon in the point of food, one of the reasons is that there are differences in favourite food among various kinds of salmon as stated by Uchida[32]. But the problem should be solved in the field of physiology of fish.

As above stated, the facts that an abundant quantity of *Pteropod* is taken into chum salmon, and that especially the amounts of *Pteropod* in their digestive organs are the largest in chum salmon caught in the middle fishing season, suggest there are some relations between the petroleum odour and the chemical components of *Pteropod*.

VIII. Influence of the Chemical Constituents of the Bodies of Plankton in the Northern Pacific Ocean on the Petroleum Odour Formation

Dimethyl-β-propiothetin (D.M.P.T.) in the contents of salmon digestive organs is considered to be absorbed into the salmon body as a nutrient. In the contents of the digestive organ and meat tissue of chum salmon caught in the middle fishing season, the amount of D.M.P.T. are larger. From the results obtained as described in the previous chapter, the fact is considered to be due to the fact that a certain part of plankton in the contents contains a large amount of D.M.P.T.

The species of plankton in digestive organs of chum and red salmon were described in the previous chapter. It is also obvious that in chum and red salmon, the kinds of food eaten are different. Further, even in chum salmon, the kinds of food eaten differ according to the fishing season.

In the contents of digestive organ of chum salmon caught in the middle fishing season, *Pteropods* (*Clione limacina* and *Limacina helicina*) were found abundantly.

Then, the author undertook to investigate the chemical constituents of plankton in the northern Pacific Ocean and their connection with the petroleum odour formation.

1. General chemical components of plankton in the northern Pacific Ocean

The food of various kinds of salmon includes such animal planktons as *Copepods*, *Pteropods* and *Amphipods*. The chemical components thereof are absorbed into the salmon body through the intestine as nutrients.

The author undertook to carry out an experiment to determine the general chemical components and the amounts of D.M.P.T. in various kinds of plankton.

Plankton used for the experiment were caught in the northern Pacific Ocean in the salmon fishing season. The construction of a plankton-net employed for collection of samples was as follows; mouth diameter 2 *m*, canvas 1 *m*, 2 *m/m* stramin 2 *m*, 2 *m/m* Müller gauze 1 *m*. The collection of samples was done by vertical haul from 200 *m* depth to the surface of water. Being brought onto the floating cannery, the plankton were immediately frozen at $-30°C$ and stored. The frosted plankton was defrosted at $20°\pm2°C$ in laboratory, and then classified

into the species by the method as described in the previous chapter. The plankton after removal of excess of water, were employed in the experiment to determine the general chemical components.

The method of analysis was as follows; water content was determined by drying, the amounts of crude protein by micro-Kjeldahl method, and the amounts of ash by burning after estimation of the water content.

The amount of D.M.P.T. was determined by weighing the precipitate formed by addition of 2% Reinecke's solution into the water extracts of plankton.

The results obtained are shown in Table VIII-1.

Table VIII-1. Amounts of general chemical components in various kinds of plankton

Plankton	Water content (%)	Crude fat (%)	Crude protein (%)	Ash (%)	D.M.P.T. (*mg*%)
Copepod (Calanus plumchrus)	39.66	31.08	24.94	2.73	0.00
Amphipod (Parathemisto japonica)	44.83	32.00	18.87	3.46	0.00
Pteropod (Limacina helicina)	40.67	28.85	22.41	5.32	2.15

As seen in Table VIII-1, the water contents are almost the same among the various kinds of plankton. The amount of crude protein was the largest (24.94%) in the *Copepod* (*Calanus plumchrus* V.) and the smallest (18.87%) in the *Amphipod* (*Parathemisto japonica*).

It is notable that the ether-extracted materials (amounts of crude fat) show very high value in every plankton. The amounts of crude fat in every plankton are over 28%, especially in the *Amphipod* (*Parathemisto japonica*), the amount is about 32%, and the value measures up to one-third of the total amounts of the components. In the ether-extracted matters, there is contained a part of the pigment of the plankton.

The red-colored substance of the *Copepods* (*Calanus plumchrus, Metridia lucens, Calanus cristatus, Eucalanus bungii*) could be dissolved into the ether-extracted materials, and the color of the materials was found to be similar to that of red salmon meat. From this fact, the color of salmon is considered to have some relation to the color of the *Copepods* consumed.

The amounts of ash are the highest in *Pteropod* among the various kinds of plankton, and that of *Limacina helicina* was 5.32%. Why the *Pteropod* contain such a large quantity of ash, is considered to be because their shells contain abundant amounts of inorganic matters. Calcium phosphate in the shell of the *Pteropod* (*Limacina helicina*) is absorbed into chum salmon body, then volatile phosphoric compounds are generated by reductive decomposition in the tin container at the time of canning. The volatile phosphoric compounds have a relation to the petroleum odour as described in following chapter.

The amounts of ash in *Copepods* and *Amphipods* are between 2.73% to 3.46%.

The amounts of D.M.P.T. in *Copepods* and *Amphipods* are negligiblly small, while in the *Pteropods* it is 2.15 *mg*%. This finding and the fact that the amount

of D.M.P.T. was 1.07 *mg*% in the contents of digestive organs of chum salmon caught in the middle fishing season of which the species of the contents are mostly *Limacina helicina,* suggest that D.M.P.T. in *Limacina helicina* may be transferred into the chum salmon body.

With respect to why *Limacina helicina* contains much D.M.P.T. differing from *Copepods* or *Amphipods,* it is yet unknown. One, however, should remember that as the natural occurrence of D.M.P.T. is in plant tissue, then the fact that abundant contents of D.M.P.T. are found in *Limacina helicina* may be because large quantities of various kinds of diatoms or marine alga are taken by the plankton.

2. Enzymatic cleavage of chemical components in Pteropod (*Limacina helicina*) by digestive enzymes in chum salmon

It is obvious that *Limacina helicina* contains large quantities of dimethyl-β-propiothetin (D.M.P.T.). It is thought to be necessary to clarify the digestion and behavior of D.M.P.T. which has taken into the digestive organ of chum salmon.

Accordingly, the next experiment was carried out on an enzymatic cleavage of chemical components of *Limacina helicina* by digestive enzymes in chum salmon with observation of the evolution of dimethyl sulfide.

(1) *Sample*

Limacina helicina employed for the experiment, were collected in the northern Pacific Ocean in early June. The material was frozen and kept at −30°C until used for the experiment.

(2) *Method*

Fifty *g* of *Limacina helicina* was blended in a mortar, then was put into a large test tube to which was added 10 *cc* of the solution containing enzymes extracted from the digestive organs in chum salmon. Next, to the mixture 60 *cc* of sodium phosphate buffer solution (pH 6.8) and 20 *cc* of toluene were added. Then the test tube prepared was plugged tightly, and allowed to stand at 37°C. After being left, at a definite interval, the content of the test tube was offered to the experiment as next described.

To determine the amounts of volatile basic nitrogen (V.B.-N), Conway's method was employed[33].

The amount of amino acid-N was determined by Pope-Stevens' method. The amount of D.M.P.T. was determined by the method described in the previous section use being made of 30 *g* of sample.

Detection of dimethyl sulfide was done by means of gas chromatography as described in the previous chapter V.

The composition of the buffer solution, pH 6.8, was 0.0128 M sodium phosphate and 0.123 M magnesium sulfate.

In order to prepare the enzyme, frozen stored stomach and intestine of chum salmon caught in the northern Pacific Ocean in early August, were defrosted within 10 days of storing. After removal of the contents, 50 *g* of the digestive organ was blended in the Waring Blendor under cooling. From slurry of the digestive organs, the solid portion was removed by centrifugation (3,000 r.p.m.,

10 *mins.*), then upper supernatant obtained was filtered. The filtrate was employed for the experiment as the enzyme solution.

(3) *Results and discussion*

The results obtained are shown in Table VIII-2 and Fig. VIII-1.

Table VIII-2. Changes in the chemical components in the *Pteropod* (*Limacina helicina*) by digestive enzymes of chum salmon

Leaving time (*hrs.*)	V.B.-N (*mg%*)	Amino acid-N (*mg%*)	D.M.P.T. (*mg%*)
0	10.3	86.6	0.05
2	11.5	80.4	0.05
4	10.8	105.3	0.05
6	20.4	144.1	0.04
8	27.8	168.2	0.05
18	56.6	260.0	0.04
24	68.1	271.9	0.04

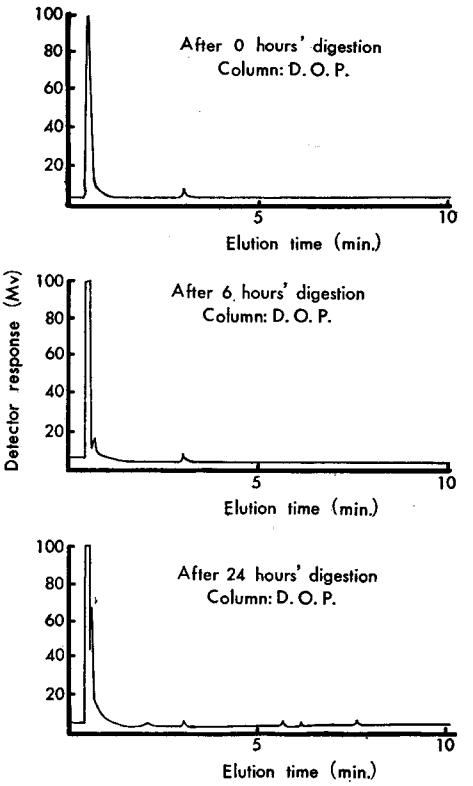


Fig. VIII-1. Gas chromatographic detection of dimethyl sulfide during digestion of the *Pteropod* (*Limacina helicina*) by the digestive enzymes in chum salmon

As seen in Table VIII-2, the amount of D.M.P.T. is 0.05 *mg%* or 0.04 *mg%*, and is almost unchangable during the digestion. From Fig. VIII-1, it is evident that dimethyl sulfide is not formed before or after the digestion.

Changes in the amounts of V.B.-N and amino acid-N prove that *Limacina helicina* is surely digestible by the action of the natural enzymes of chum salmon.

These results suggest that D.M.P.T. in *Limacina helicina* would not be decomposed by the enzymes in digestive organ of chum salmon.

Challenger *et al.*[23] have pointed out the presence of D.M.P.T.-decomposing enzyme in *Polysiphonia fastigiata*. Dubnoff *et al.*[34] have also reported that there exists the transmethylase of D.M.P.T. in liver and kidney tissues of hog, rat and guinea pig. From those reports, the D.M.P.T.-decomposing enzyme seems to be distributed widely in nature.

By Dubnoff *et al.*[34], however, even in the same animal, the enzyme is not found in milt, pancreas and meat tissues. Therefore, the D.M.P.T.-decomposing enzyme may not always exist in the digestive organs of chum salmon. D.M.P.T. is little decomposed in the digestive organs of chum salmon, and it may be absorbed into the body through the intestine. McMillin[2] has presumed that the abnormal odour in canned chum salmon may be caused by a chemical composition of the food of salmon. Also the results obtained by the author show that dimethyl sulfide which is a constituent of the petroleum odour in canned chum salmon, is contained in *Limacina helicina* which is a principal food of chum salmon. The behavior of D.M.P.T. after being taken into chum salmon body, will be described below.

3. The activity of digestive enzymes from chum and red salmon

In order to know the activity of digestive enzymes of chum and red salmon, the experiment was carried out.

The method of preparing the enzyme solution was the same as described in the previous section. The mixture of 20 *cc* of the buffer solution, mixture of M/10 sodium citrate and N/10 hydrochloric acid, (pH 1.42, 1.93, 2.97, 3.53, 3.95 and 4.16), 10 *cc* of the enzyme solution and 50 *g* of plankton (*Calanus plumchrus*) collected in the northern Pacific Ocean, was made.

The mixture was blended by means of the Waring Blendor to a slurry to which 20 *cc* of toluene was added. The test tube was plugged and allowed to stand at 37°C for 48 hours.

After standing, the amounts of amino acid nitrogen were determined by the method of Pope-Stevens.

Results obtained are graphed in Fig. VIII-2 and Fig. VIII-3.

As seen in Figs. VIII-2, VIII-3, there is no difference between chum and red salmon in the opt. pH of the digestive enzyme, that is, the opt. pH is 2.97. The value is in agreement with that obtained by Oshima and Sasaki[35] who have studied pepsin in gastric juice of trout, and by Oya and Nakai[36], who have studied protease in the stomach of red trout.

It is said that salmon actively searches for food, in general, during the off-shore migration, but approaching to shore it hardly, and the activity of the digestive enzyme is gradually decreased. This is understood clearly from these

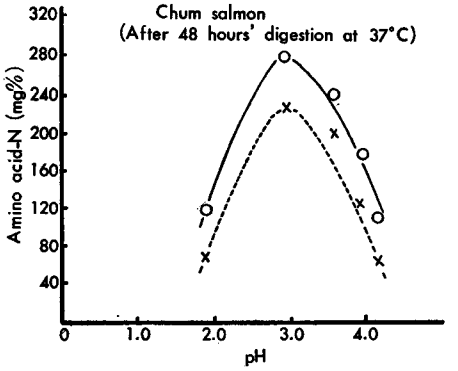


Fig. VIII-2. Optimum pH of the digestive enzymes from chum salmon.

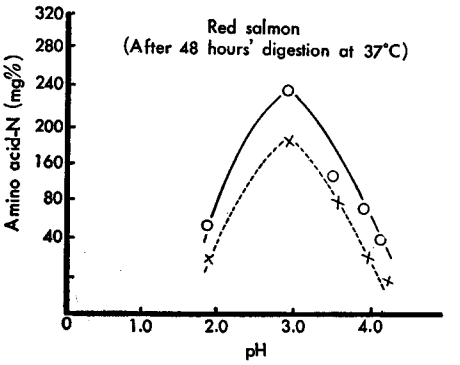


Fig. VIII-3. Optimum pH of the digestive enzymes from red salmon.

—○— Digestive enzyme of the salmon caught in middle period of fishing season.
---×--- Digestive enzyme of the salmon caught in latter period of fishing season.

figures. Also these figures show that the activity of the digestive enzymes is larger in chum salmon than in red salmon. It is suggested that the accumulation of the components of food into the body is more rapid in chum salmon than in other salmon.

From the results mentioned above, it is presumed that the accumulation and the metabolism of the components of food are the most active in chum salmon.

4. Relation between the generation of plankton in the northern Pacific Ocean and the formation of petroleum odour in chum salmon

It is obvious from the experiment described in the previous section that D.M.P.T. is a precursor of dimethyl sulfide and that it is derived from *Limacina helicina* in the food of chum salmon. Therefore, the pathway from the food of chum salmon to the formation of the petroleum odour seems to be important.

Then, some discussion of the generation of plankton in the northern Pacific Ocean in connection with the formation of the petroleum odour in chum salmon is appropriate.

At first, as stated in the previous chapter, the formation of the petroleum odour in canned chum salmon has attracted attention since 1958. But if the formation of the petroleum odour depends upon the accumulation of D.M.P.T. into chum salmon body from *Limacina helicina*, the formation of the petroleum odour must be found even before 1958. With respect to the point, one presumption is that the raw salmon from which the canned product has formed the petroleum odour, should have eaten a large amounts of *Limacina helicina* in the 1958 canning season. Even in 1958, in earlier and later parts of the fishing season, there could not be found the petroleum odour in canned chum salmon. In other words, in the middle period of the canning season in 1958, a large amount of *Limacina helicina* was to be found in the waters where chum salmon had

migrated at the time when the catches were taken for processing into the canned chum salmon.

Then, in order to ascertain whether that assumption is good or not, data records of oceanographic observation and exploratory fishing in the northern Pacific Ocean[37] during four years from 1956 to 1959 which had been made in the author's Faculty, were evaluated.

The data records showed that the greater part of the species of plankton found in the northern Pacific Ocean from 1957 to 1959, were *Copepod* followed by *Amphipod* and *Sagitta*. *Pteropod* was only 1% of the total. Therefore, from the data, it could not be concluded that the distribution density of *Limacina helicina* in the waters where chum salmon had migrated was high in 1958. In the data for 1957, *Limacina helicina* was found in the lot of plankton collected in the waters of 51°14′ N, 167°16′ E in the middle of fishing season of chum salmon. At that time, chum salmon having the petroleum odour might be caught. Then, it is very interesting to consider whether the waters *Limacina helicina* was found agree with those where chum salmon having the petroleum odour were taken. Comparing the data on the distribution of plankton in the northern Pacific Ocean with the observation on the species of plankton in the digestive organs of chum salmon as described in the previous chapter, the two results were not in agreement as to the point that chum salmon had consumed a great amount of *Pteropods* in the middle of the fishing season. This difference was thought to be dependent upon the further fact that the plankton was collected by means of vertical haul at the sampling station in the northern Pacific Ocean, by which procedure even if there might exist a layer of *Pteropod,* only a few of them were collected. On the other hand, if the migration layer of salmon agreed with the floating layer of *Pteropod,* it could be easily considered that there should be an increase in the amount of *Pteropods* taken into salmon body. However, with respect to this point, further study seemed to necessary.

The data records of oceanographic observations and exploratory fishing in the northern Pacific Ocean during the four years from 1956 to 1959, also showed that the water temperatures in 1958, were 0.5°~1.5°C in average higher than those in the usual year. This tendency could be seen in the data on meteorological observation during the four year periods. That is to say, the meteorological conditions of the northern Pacific Ocean waters in 1958 were fairly good. Then, *Pteropods* would generate in larger amount under the conditions than under that in ordinary years.

Amongst the *Pteropoda, Limacina helicina* which has direct connection to the petroleum odour, is one of the typical cold-water plankton, and grows from late April to late May.

There seem to be many factors to generate plankton. One of the factors is temperature of the waters as reported by McMillin[2]. Then, in the data of 1958, the facts that the meteorological conditions were fairly good, and temperature of the waters was higher than that in usual years, may support the presumption that those conditions in the northern Pacific Ocean gave the good condition for generation of *Limacina helicina.* In the oceanographic observations carried on in the waters of the northern Pacific Ocean and Bering Sea in the

two years 1956 and 1957, the distributions of *Pteropod* were investigated.

In 1956, the kinds of *Pteropods* which appeared in a part of northern Pacific Ocean and in the waters to the north of the Aleutian Islands, were *Limacina helicina, Creseis virgula, Clione polita* and *Clione limacina.* In the waters to the north of the Aleutian Islands, the number of *Limacina helicina* was about 4 fold greater than that of *Clione limacina*; the former was made up of 83% adult and 17% larvae, while the latter was found to be only larvae.

In 1957, other than *Creseis virgula* and *Clione polita,* other *Pteropods* appeared in the same waters. Also in 1957, the larva were much more numerous than the adult in *Clione limacina.*

Much greater-than-usual appearance of larva of *Pteropods* might prove that there is the origin in the nearby waters. Further investigations on that point in 1958 showed that the number of *Limacina* were greater in a part of northern Pacific Ocean, and of *Clione* were more in the waters to the north of the Aleutian Islands.

Sverdrup *et al.*[38], Mishima and Nishizawa[39], Koto and Fujii[40] have survayed the marine conditions of the northern Pacific Ocean and a part of the Bering Sea, and reported as follows.

The Black stream ("Kuroshio") which heads north off the east of Japan, diverges near 35°00′ N. One part goes east to become the North Pacific Current. The other divergence goes north-east, and flows into the Kuril Current ("Oyashio") to from the waters called the Subarctic Current. The Subarctic Current, circling from left to right in Alaska Bay, then goes west along the Aleutian Islands. On the way westward a portion of the Alaska Current pours into the Bering Sea from water ways near the Karragai and Tanagai islands. But the main stream of the Alaska Current turns near 53°00′ N to go north. Then the stream moves south along the Kamchatka Peninsula from the Bering Sea to the North Pacific, and becomes the Kuril Current ("Oyashio") which goes south off the east of the Kuril Islands.

With respect to the relation between currents and the distribution of *Pteropod,* in the northern part of the North Pacific, there are *Clione limacina* and *Limacina helicina*; and in waters to the south of the Aleutian Islands, there appear more *Clione limacina* and fewer *Limacina helicina.* On the contrary, in waters to the north of the Aleutian Islands, there appear more *Limacina helicina* and less *Clione limacina.* In northwestern waters, sometimes, there appears *Clio. pyramidata* which is one of the warm-water plankton. As stated above, there are differences between the currents and the distribution of *Pteropod.*

From those facts, it is of much interest that the waters where chum salmon having the petroleum odour is caught, are almost the same waters where more *Limacina helicina* is found to appear, because the formation of the petroleum odour in chum salmon is found in the fish caught wtihin certain limited waters.

A shoal of chum salmon gradually goes west from the northern waters of the North Pacific, continuing the eating as far as to the Kamchatka Peninsula; the *Limacina helicina* does not move themselves, but is moved by the current, by which it goes west along the Aleutian Islands, then is carried north to the Bering Sea by the Alaska Current. Therefore, the migration of a shoal of chum

salmon is always in disagreement with the movement of *Limacina helicina*. From those facts, the catch of salmon having the petroleum odour will be limited in the waters where and in the season when chum salmon can take *Limacina helicina*. Thus it may be considered that the catch is made in waters in the range of 48°00′ N~52°00′ N and 165°00′ E~172°00′ E, and that the season is early June. From these consideration, the change in the marine conditions may result in the change of the season to form the petroleum odour in chum salmon. For example, the canning date when canned chum salmon having the petroleum odour has been produced was in the later part of May in 1960, which date was slightly earlier than in 1958. Then, also in the year when the generated amount of *Limacina helicina* is less comparing with the usual year, the petroleum odour may not be formed even in canned chum salmon.

On the basis of such observations and considerations, it can be said that the formation of the petroleum odour in canned chum salmon may depend upon the amount of *Limacina helicina* which chum salmon taken, and that in the year when larger quantities of *Limacina helicina* are generated, the greater the quantity of canned chum salmon having the petroleum odour would be produced.

IX. Behavior of Dimethyl-β-Propiothetin in Salmon Liver

Maw *et al.*[41] have reported that dimethyl-β-propiothetin (D.M.P.T.) may become a methyl donor as well as betaine and choline in transmethylation. McRorie *et al.*[29] and Challenger *et al.*[23] have also reported that D.M.P.T. may become a methyl donor to homocysteine, and is an "onium"-compound of which energy level is high. Owing to the reason, it is needless to accept energy from A.T.P. which is one kind of high energy compound, in transmethylation from D.M.P.T. to homocysteine. Cantoni[26] has offered an equation to express the reaction as follows;

$$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\overset{+}{S}\cdot R+HS\cdot R_1+H_2 \rightleftharpoons CH_3\cdot S\cdot R+CH_3\cdot S\cdot R_1+\overset{+}{H_3O}:\Delta F_0=0$$

As seen in the above equation, the formation of methionine from homocysteine may occur in living body. The reaction is biologically important from the stand point of the growth of a living animal.

Transmethylation from choline to homocysteine was studied in liver preparations by Dubnoff and Borsook[34]. If the transmethylation from D.M.P.T. to homocysteine occurs in living chum salmon liver, the D.M.P.T. content in the salmon liver will effect directly the growth of the salmon. Amongst plants, the marine algae, *Polysiphonia lanosa*, detached from its host and immersed in tap or distilled water, evolves dimethyl sulfide in a few hours. The evolution of dimethyl sulfide is accompanied by the production of acrylic acid and H^+. The reaction proceeds under the presence of an enzyme[26].

The author has found the presence of D.M.P.T. in the meat and in the contents of the digestive organs of frozen chum salmon having the petroleum odour. From the finding, D.M.P.T. in the salmon meat is supposed to be transferred from the digestive organs. Nutrients including D.M.P.T. are brought to

the liver by the aid of blood from digestive organs. Then, attempts were made to ascertain the behavior of D.M.P.T. in chum and red salmon livers.

1. D.M.P.T. contents in salmon liver

Fresh livers of chum and red salmon which were caught in the northern Pacific Ocean at different periods of the fishing seasons, were frozen on the mother-ship at −30°C, and were brought to the laboratory. After the liver has been defrosted at room temperature (20°±2°C), 50 *g* each portions of the livers was put into beakers with 100 *cc* of water. The mixture was blended by the Waring Blendor to a slurry, then was left at 5°C for 90 minutes. The slurry was centrifuged (3,000 r.p.m., 5 *mins.*), and solid portion was removed. Remaining upper supernatant was filtered through Toyo filter paper No. 5c, and filtrate was obtained. The filtrate was passed through a column of Amberlite IRC-45 (1.5 *cm* × 20 *cm*). The eluate was added to about 5 *cc* of 5% Reinecke's solution, and the preciptate formed was weighed.

Analysis revealed amounts of D.M.P.T. in chum and red salmon livers, as shown in Table IX-1.

Table IX-1. D.M.P.T. contents of chum and red salmon livers

Date of fishing \ Salmon	Chum (*mg%*)	Red (*mg%*)
June 10th	0.77	0.02
June 20th	0.63	0.01
July 3rd	0.34	0.01
July 15th	0.18	0.01
July 30th	0.17	0.01
August 2nd	0.12	0.00

As seen in Table IX-1, the amount of D.M.P.T. in chum salmon liver is larger than that in red salmon. This is due to the fact that as stated in previous chapter (VIII), chum salmon eats large amounts of *Limacina helicina* containing D.M.P.T.

The amount of D.M.P.T. in chum salmon liver through the whole period of the fishing season, shows the highest value at the middle fishing season which corresponds to the time processing of the petroleum odour cans, and lower values at other seasons. On the other hand, in red salmon liver, as to the amount of D.M.P.T., the same tendency is found as in chum salmon. However, the amount of D.M.P.T. in red salmon liver is very small throughout the fishing seasons. A remarkable difference of the amount, therefore, are not found as in chum salmon.

2. Transmethylation in salmon liver

As stated in the previous section 1, in chum salmon liver, there is a large content of D.M.P.T. from which transmethylation to homocysteine may occur under the presence of transmethylase. The formation of methionine in salmon

liver, therefore, would seem to be a considerable reaction.

Hence, an experiment was carried out to learn whether or not transmethylation from D.M.P.T. to homocysteine occurs in the liver of salmon. Fresh liver of salmon which was caught in the northern Pacific Ocean, was frozen at −30°C on the mothership, and transported to the laboratory within two weeks. The liver was defrosted at room temperature (20°±2°C); the liver was blended in the Waring Blendor to a slurry with twice the weight of the buffer solution. The composition of the buffer solution, pH 6.5, was as follows; 0.0128 M sodium phosphate, 0.123 M sodium chloride, 0.05 M potassium chloride, 0.03 M magnesium sulfate. The slurry was centrifuged (2,000 r.p.m., 10 *mins.*), and the supernatant liquid obtained was filtered with Toyo filter paper No. 5c. Thus obtained filtrate was subjected to the experiment.

Into several test tubes, 1 *cc* each of the filtrate and 1 *cc* (25 *mg%*) each of dl-homocysteine solution were poured. To one half lot of the test tubes prepared was further added 1 *cc* (12.5 *mg%*) each of D.M.P.T. solution; to the other lot, was added 1 *cc* of the phosphate buffer to take the place of D.M.P.T.

The test tubes were plugged and were allowed to stand at 38°C for 0.5, 1.0, 2.0 and 3.0 hours, respectively. After a definite interval of leaving, each D.M.P.T.-containing or buffer-containing test tube was subjected to the estimation for methionine analysis.

Quantitative determination of methionine was done by means of the colorimetric method of McCarthy and Sullivan[42].

Methionine formation in chum salmon liver homogenate is shown in Table IX-2 and Fig. IX-1.

Table IX-2. Methionine formation in chum salmon liver homogenate

Leaving time (*hrs.*) / Additions	0	0.5	1.0	2.0	3.0
Homocysteine (a)	2.0	2.7	2.8	3.0	3.0
Homocysteine +D.M.P.T. (b)	2.0	2.8	3.2	3.5	3.5
(b)−(a)	0	0.1	0.4	0.5	0.5
	Amounts of methionine (*mg%*)				

Table IX-3. Methionine formation in red salmon liver homogenate

Leaving time (*hrs.*) / Additions	0	0.5	1.0	2.0	3.0
Homocysteine (a)	3.5	4.2	4.5	2.0	4.5
Homocysteine +D.M.P.T. (b)	3.5	4.5	5.5	5.5	5.5
(b)−(a)	0	0.3	1.0	1.0	1.0
	Amount of methionine (*mg%*)				

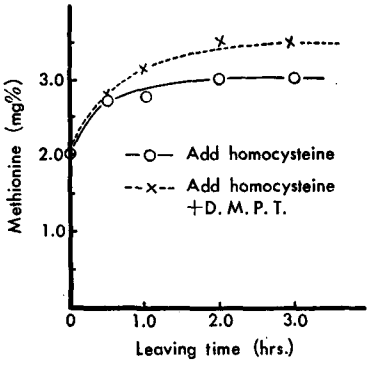


Fig. IX-1. Methionine formation in chum salmon liver homogenate

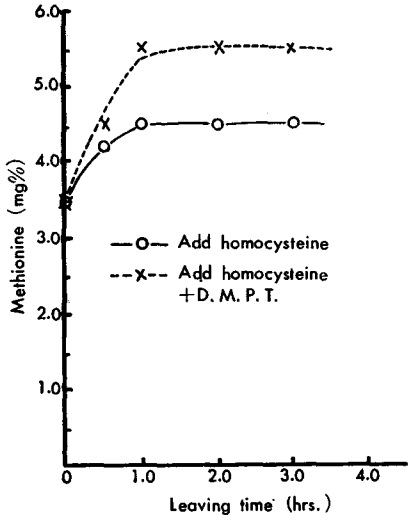


Fig. IX-2. Methionine formation in red salmon liver homogenate

Methionine formation in red salmon liver homogenate is shown in Table IX-3 and Fig. IX-2.

As seen in Tables IX-2 and IX-3, and in Figs. IX-1 and IX-2, it is obvious that transmethylation from D.M.P.T. to homocysteine occurs in chum and red salmon livers.

The reaction is completed within 1 hour at 38°C. Comparing the reaction in the livers of chum and red salmon, methionine formation is larger in the latter than in the former. This suggests that the transmethylation in red salmon is done more actively than that in chum salmon.

Transmethylase activity in red salmon liver is about twice as high as in chum salmon liver.

3. Transmethylation in salmon meat

In the above section **2**, it was ascertained that methionine formation in the salmon liver may take place under the presence of homocysteine and D.M.P.T. Next, an attempt was made to ascertain whether the transmethylation may occur in salmon meat or not.

The samples used were frozen salmon meat as described above.

The sample was defrosted at room temperature (20°±2°C), and back meat was separated. From the meat, an enzyme solution was prepared by means of the procedure described in the previous section **2.** The experimental method was the same as the described in the previous section **2.**

Amounts of methionine formed in chum and red salmon meat homogenates are shown in Table IX-4 and Fig. IX-3.

As seen in Table IX-4 and Fig. IX-3, methionine formation is not found in chum and red salmon meat samples under the presence of D.M.P.T. and

Table IX-4. Methionine formation in chum and red salmon meat homogenates

Salmon	Additions	Leaving time (*hrs.*)				
		0	0.5	1.0	2.0	3.0
Chum	Homocysteine (a)	2.0	2.7	2.3	2.6	2.7
	Homocysteine +D.M.P.T. (b)	2.0	2.8	2.3	2.6	2.5
	(b)−(a)	0	0.1	0	0	−0.2
Red	Homocysteine (a)	2.1	2.4	2.4	2.5	2.4
	Homocysteine +D.M.P.T. (b)	2.1	2.6	2.4	2.4	2.4
	(b)−(a)	0	0.2	0	−0.1	0
		Amount of methionine (*mg%*)				

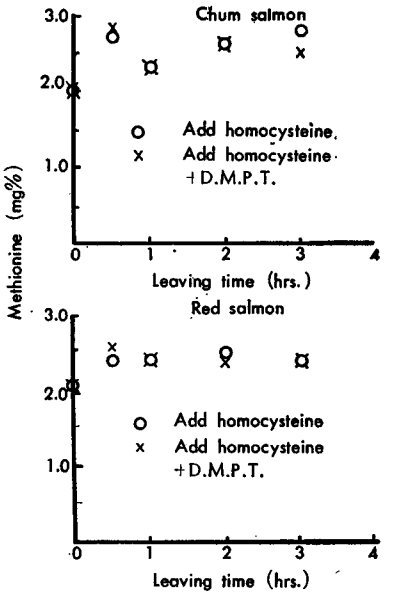


Fig. IX-3. Methionine formation in chum and red salmon meat homogenates

homocysteine. Accordingly, it can be considered that transmethylation would not take place in salmon meat.

4. Optimum pH value of transmethylase in salmon liver

As stated in the previous chapter VI, the stability of D.M.P.T. is less in the alkaline side.

In the case of salmon liver, the activity of transmethylase is considered to be related with the stability of D.M.P.T. Then, an attempt was made to ascertain changes in the activity of transmethylase in solutions of various pH values.

Salmon livers employed were the same as those employed in the previous experiment **2**; also the procedure for treatment of livers, was as same as that in the previous experiment. The buffer solution used was the mixture of sodium phosphate and sodium citrate, of which pH values were 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0, respectively.

One *cc* each of enzyme solution was poured into a test tube, to which 1 *cc* (25 *mg%*) of *dl*-homocysteine, 1 *cc* (12.5 *mg%*) of D.M.P.T. and 1 *cc* of the buffer solution were added.

Into an other test tube, which was tested as a control, D.M.P.T. was not added.

These test tubes were plugged, and allowed to stand at 38°C for 1 hour.

Then the contents of the test tubes were employed for determination of the amounts of methionine.

Results obtained are shown in Table IX-5 (chum salmon liver) and in Table IX-6 (red salmon liver).

Table IX-5. The transmethylase activity of chum salmon liver in solutions having various pH values

pH	5.5	6.0	6.5	7.0	7.5	8.0
Amount of methionine (*mg%*)	0.1	0.5	1.2	1.0	0.7	0.1

Table IX-6. The transmethylase activity of red salmon liver in solutions having various pH values

pH	5.5	6.0	6.5	7.0	7.5	8.0
Amount of methionine (*mg%*)	0.2	0.8	2.5	1.7	0.7	0.2

As seen in Table IX-5 and Table IX-6, the transmethylase activity is the maximum at pH 6.5 in chum and red salmon liver. The activity is rather lowered in pH 7.5. The transmethylase of rat liver is active in the range of values from pH 6.5 to 7.5[41]. However, the transmethylase of salmon liver is active in the range from pH 6.5 to 7.0.

According to the facts that the stability of D.M.P.T. is less in the alkaline side, and that the transmethylase of salmon liver is active in the acidic side, D.M.P.T. decomposition in salmon liver is considered to be caused by transmethylase action under the presence of homocysteine.

Wide distribution of dimethylthetin transmethylase in various organs of animals is suggested by Dubnoff *et al.*[34]. The author has found the presence of the enzyme in salmon liver. The finding may justify a suggestion that dimethyl-β-propiothetin which has been absorbed from digestive organs into the salmon body, may react with homocysteine in liver to form methionine. Methyl transfer from D.M.P.T. to homocysteine occurs under molar equivalent. If more than an equivalent amount of D.M.P.T. in comparison with homocysteine is given to

salmon liver, excess amount of D.M.P.T. will be accumulated in liver or meat tissue. As transmethylase is not contained in salmon meat, enzymatic decomposition of D.M.P.T. does not occur in the meat tissue. The mechanism of the petroleum odour formation seems to proceed by the following pathway: At first, D.M.P.T. which is contained in food, is absorbed from digestive organs into the body, and is brught to the liver by the blood. One portion of D.M.P.T. which has been brought to the liver, is concerned with methionine formation reacting with homocysteine. The remaining of D.M.P.T. is brought to meat tissue where it is accumulated. D.M.P.T. which has been thus accumulated is decomposed by heating to evolve dimethyl sulfide. Thus dimethyl sulfide may generate the petroleum odour.

Methionine formation through transmethylation from D.M.P.T. to homocysteine occurs in red salmon as well as in chum salmon. Then, if the same amount of D.M.P.T. as in chum salmon is taken into red salmon, probably the petroleum odour will generate. However, there are evidences that the kinds of food eaten by salmon differ according to the kinds of salmon, and that the amount of D.M.P.T. in the food of red salmon is less than that of chum salmon.

From these facts, the whole amount of D.M.P.T. which has been taken into red salmon liver, is supposed to be comsumed for transmethylation and to be not carried to the other organs from the liver.

X. Effect of Volatile Phosphoric Compounds in Canned Salmon upon the Petroleum Odour Formation

In the previous chapters from I to IX, it has been made clear that dimethyl sulfide is the main constituent of the petroleum odour, and some further study on the mechanism of the petroleum odour formation has been described.

The author ascertained that in the case of addition of 5 *mg*% of D.M.P.T. into can container with chum salmon having no petroleum odour, the petroleum odour was produced by processing of the content resulting in the same appearance as found in consumer cans. However, D.M.P.T. content in chum salmon having the petroleum odour is 1.07 *mg*%, and is a small amount to make detectable the petroleum odour after heat treatment. Therefore, another odours constituent from dimethyl sulfide seems to be an accelerator for evolution of the petroleum odour.

The author has often found the presence of volatile phosphoric compounds in the odourous constituents of "refrigeration odour" in cold storage warehouse, and has recognized the comparatively high ash content of *Limacina helicina* as described in the previous chapter VIII, of this study. The chemical properties of sulfur atom which is contained in the molecule of dimethyl sulfide, resembles as those of a phosphor atom.

Then, an attempt was made to ascertain the relation between the petroleum odour and volatile phosphoric compounds.

1. Detection of volatile phosphoric compounds in various kinds of canned salmon by means of a detector

In order to know whether various kinds of canned salmon contain volatile phosphoric compounds or not, detection and identification of the compounds by means of a detector was undertaken.

Sample salmon cans were chum and chum neck having the petroleum odour and red, red neck, pink and pink neck having no petroleum odour. Those salmon cans have been processed on a floating-cannery in the northern Pacific Ocean. Frozen chum salmon having the petroleum odour was also employed for the experiment.

Immediately after opening the can container, 500 *g* of the content was put into a flask which was then plugged with a gum stopper. The odour constituents in the flask were aspirated and induced to a glass-made detector tube in which a mixture of silica gel, mercuric oxide and cupric sulfate was contained. Aspiration was continued for about 1 hour.

The results obtained in detection the volatile phosphoric compounds in various kinds of canned salmon, are shown in Table X-1.

Table X-1. Volatile phosphoric compounds in various kinds of canned salmon detected qualitatively by means of a detector tube

Sample	Canned chum having the petroleum odour	Canned chum neck having the petroleum odour	Frozen chum	Canned red	Canned red neck	Canned pink	Canned pink neck
Volatile phosphoric compounds	+	+	±	−	−	−	−

As seen in Table X-1, the volatile phopshoric compounds are found in canned salmon having the petroleum odour, but not found in canned salmon having no petroleum odour. That is to say, volatile phosphoric compounds are considered to be included in the petroleum odour as a kind of odorous constituents.

The smell of volatile phosphoric compounds including reduced phosphine, is like that of the petroleum odour. From such observations, there seems to exist some relation between the petroleum odour and volatile phosphoric compounds.

2. Redox potentials of the contents of various kinds of canned salmon

As found in the previous section 1, volatile phosphoric compounds are contained in canned salmon having the petroleum odour. The potential of the inner part of the content of canned chum salmon is considered to be reductive, because the volatile phosphoric compounds are the substances to be formed under reductive condition.

In order to ascertain the potential of the content of canned salmon, the author undertook an experiment for estimating the redox potentials of the contents of various kinds of canned salmon.

Samples employed were the same as those used in the study described in the

previous section 1. Redox potential (Eh) was estimated by a potentiometer which made by the Shimadzu Manufacturing Co.

The results obtainde are shown in Table X-2.

Table X-2. Redox potential of various kinds of canned salmon

Sample \ Part	Liquid	Meat
Canned chum neck having the petroleum odour	−0.6759	−0.6904
Canned chum having the petroleum odour	−0.6904	−0.6765
Canned red	−0.6579	−0.6313
Canned pink	−0.6401	−0.6405
Canned red neck	−0.6217	−0.6041
Canned pink neck	−0.5879	−0.6175
Canned chum without petroleum odour (packed in white can)	−0.5910	−0.6110

As seen in Table X-2, redox potential is reductive in all cases of canned salmon used. However, it is not possible to prove a relation between the petroleum odour and redox potential. If in raw salmon for canning there is contained a precursor of the volatile phosphoric compounds, the volatile phosphoric compounds may be produced after the processing of the canned product.

3. Amounts of volatile phosphoric compounds in various kinds of canned salmon

In order to find the amounts of volatile phosphoric compounds in various kinds of canned salmon, an experiment was undertaken.

As the sample, canned chum salmon having the petroleum odour was employed. For the samples of the control test, red and pink canned salmons were classified into groups as having weak, middle or strong smell of the petroleum odour.

Immediately after opening the canned product, 100 *g* of the material was weighed and put into a flask. The flask was plugged with gum stopper, then conjoined to a washing bottle with glass tube. In the washing bottle, sodium hypochlorite solution was contained. The washing bottle was conjoined to an aspirator. By aspiration, the volatile phosphoric compounds in the flask were induced into the washing bottle, and reacted with sodium hypochlorite. Aspiration was continued about 1 hour. Volatile phosphoric compounds being reacted with sodium hypochlorite were changed to $Mg_2P_2O_7$ by adding magnesia mixture. Thus the amounts of phosphor were obtained by measuring the weight of $Mg_2P_2O_7$. Amounts of volatile phosphoric compounds in various kinds of canned salmon are shown in Table X-3.

As seen in Table X-3, the stronger the smell of the petroleum odour becomes in canned chum salmon, the higher the amount of volatile phosphoric compounds found in the content become. On the other hand, a trace amount of volatile phosphoric compounds is contained in canned red and pink salmon. Accordingly, it can be considered that volatile phosphoric compouds may become a constituent of the petroleum odour.

Table X-3. Amounts of volatile phosphoric compounds in various kinds of canned salmon

Sample	Amounts of volatile phosphoric compounds (*mg%*)	Detection by detector
Canned chum (having weak petroleum odour)	0.75	−
Canned chum (having the petroleum odour)	0.84	±
Canned chum (having strong petroleum odour)	1.86	+
Canned red	0.09	−
Canned pink	0.09	−

4. Amounts of volatile phosphoric compounds in chum salmon meats of various degrees of freshness

Generally speaking, deterioration of freshness of fish meat results in increase of the amount of volatile basic nitrogen. In such a case, increase of the amount of volatile phosphoric compounds also occurs.

The author undertook an experiment in order to know changes in amounts of volatile phosphoric compounds in chum salmon meat as a raw material for canning, during deterioration of the freshness of the material.

Material for this experiment was caught in nearby waters of Hokkaido, and was brought to the laboratory in good freshness. After removal of head, viscera and bone from the salmon body, only meat portion was allowed to stand at room temperature (20°±2°C). During that deterioration, the amounts of V.B.-N and volatile phosphoric compounds in the meat were estimated. The method for quantitative estimation of volatile phosphoric compounds is the same as described in the previous section **3**.

Amounts of volatile phosphoric compounds in chum salmon meat of various degrees of freshness, are shown in Table X-4.

As seen in Table X-4, the amounts of volatile phosphoric compounds in chum salmon meat increase with the deterioration of the freshness. From the

Table X-4. Amounts of volatile phosphoric compounds in chum salmon meat of various degrees of freshness

Sample \ Estimating items	V.B.-N (*mg%*)	Amounts of volatile phosphoric compounds (*mg%*)	Detection by detector	Odour
Chum	10.0	0.84	−	
	20.0	0.96	−	
	30.0	1.25	±	
Frozen chum	20.0	1.00	±	
	30.0	1.50	±	Slight petroleum odour

results, in case of employment for canning material of chum salmon which has deteriorated, the smell of the petroleum odour in the canned product will become stronger with lowering the freshness of the raw material. As described above in Chapter VI, dimethyl sulfide will easily evolve from D.M.P.T. according to the deterioration of the freshness of raw salmon.

Therefore, deterioration of the freshness of chum salmon as the raw material of the canning may offer a condition for the formation of the petroleum odour.

5. Amounts of volatile phosphoric compounds in the different parts of salmon body

In order to ascertain the amounts of volatile phosphoric compounds in the different parts of some kinds of salmon, an experiment was undertaken.

The samples used were the frozen salmon as described above. Freezing storage period was about 1 month. The material was defrosted at room temperature (20°±2°C) in the laboratory, then was separated into back, belly and neck meats and skin, bone and viscera parts. Amounts of volatile phosphoric compounds in the meats and the parts were estimated by means of the method described above in the section **3** of this chapter.

Amounts of volatile phosphoric compounds in various parts are shown in Table X-5.

Table X-5. Amounts of volatile phosphoric compounds in various parts of salmon body (*mg%*)

Parts / Salmon	Back meat	Belly meat	Neck meat	Skin	Bone	Viscera
Chum	0.02	0.02	0.03	0.00	0.75	0.05
Red	0.03	0.02	0.02	0.02	0.68	0.04
Pink	0.02	0.02	0.02	0.00	0.70	0.02

As seen in Table X-5, amounts of volatile phosphoric compounds are large in the bone part of every kind of salmon, and range from 0.68 to 0.75 *mg%*. The contents in the meat are small, and are less in amount than that in the viscera part. Therefore, volatile phosphoric compounds are considered to be produced principally from bone.

6. Relation between amounts of volatile phosphoric compounds in salmon meat and heat processing of canned product

From the results described in previous sections, it is presumed that volatile phosphoric compounds may be produced from a precursor after post-mortem change in raw salmon.

Accordingly, an attempt was made by an experiment to clarify the relation between amounts of volatile phosphoric compounds in fresh salmon meat and heat processing.

The material and the procedure employed were the same as those used in the previous section **4**. Salmon body was separated into two portions of back

meat and bone. Each portion was packed into 1/2-pound consumer size can (E.T. whole-coated-inner side), and was processed at 5 *lbs*-pressure (180.4°C) and 10 *lbs*-pressure (115.2°C) for minutes, respectively. The contents of the canned salmon were employed to estimate amounts of volatile phosphoric compounds. Relation between amounts of volatile phosphoric compounds in salmon meat and heat processing is shown in Table X-6.

Table X-6. Relation between the amounts of volatile phosphoric compounds in canned salmon meat (*mg%*) and temperatures of heat processing

Temp. / Parts / Salmon	5 *lbs.* (108.4°C)		10 *lbs.* (115.2°C)	
	Back meat	Bone	Back meat	Bone
Chum	0.02	0.84	0.04	1.12
Red	0.02	0.71	0.03	1.12
Pink	0.02	0.75	0.03	0.97

As seen in Table X-6, amounts of volatile phosphoric compounds in salmon meat increase with raising of the temperature of heat processing. Therefore, the petroleum odour may be felt more and more strong with raising of the heat processing temperature. The results obtained also proves that the amount of volatile phosphoric compounds is larger in bone part than that in meat part.

Accordingly, it can be considered that large quantities of a precursor of volatile phosphoric compounds are contained in the bone part of the salmon.

7. Quantitative distribution of non-volatile phosphoric compounds in the different parts of salmon body

A precursor of volatile phosphoric compounds produced from the bone of salmon is considered to be inorgnaic phosphate. Amongst inorganic phosphates, many derivatives including non-volatile phosphoric compounds seem to be contained in salmon body. Then the author undertook an experiment to learn amounts of non-volatile phosphoric compounds in the various parts of salmon body.

The sample and the procedure employed were the same as described in section **4**. The salmon body was separated into four portions; back, belly, neck and bone parts. From each portion, acid soluble-, lipids-, nucleic acid- and protein-phosphors were fractionated by the method of Schneider[43]. The phosphors were quantitatively determined by Fiske and Subbarow's method[44].

Amounts of non-volatile phosphoric compounds found in various parts of the salmon body are shown in Table X-7.

As seen in Table X-7, inorganic-phosphor (acid soluble-phosphor) content is very large in the bone, but is very small in others. Also its content in the bone of chum salmon is larger than that in the bone of red or pink salmon. Lipids-phosphor content is higher in both the belly and neck parts, that is, found to be high in the oil-rich part of salmon body. Nucleic acid-phosphor content is almost the same level in every part of the meat of salmon body. Protein-phosphor content is higher in the protein-rich part in salmon body, and shows an opposite

Table X-7. Amounts of phosphoric compounds in various parts of salmon body

Salmon	Phosphoric compounds / Parts	Acid-soluble-P (*mg%*)	Lipids-P (*mg%*)	Nucleic acid-P (*mg%*)	Protein-P (*mg%*)
Chum	Back	0.06	0.08	0.14	0.19
	Belly	0.04	0.15	0.13	0.15
	Neck	0.07	0.13	0.14	0.30
	Bone	0.55	0.00	0.00	0.02
Red	Back	0.04	0.09	0.14	0.17
	Belly	0.04	0.17	0.17	0.14
	Neck	0.06	0.18	0.12	0.21
	Bone	0.52	0.10	0.00	0.02
Pink	Back	0.04	0.08	0.17	0.17
	Belly	0.03	0.19	0.18	0.14
	Neck	0.07	0.17	0.13	0.25
	Bone	0.48	0.00	0.00	0.00

tendency against lipids-phosphor content. Finally, almost the whole amount of non-volatile phosphoric compounds is presumed to exist in the bone.

8. Origin of non-volatile phosphoric compounds in salmon

In the previous section 7, it became clear that the inorganic-phosphor content is larger in chum salmon than in red and pink salmon. On the other hand, *Limacina helicina* which is one kind of food of chum salmon, contains a large amount of ash. Inorganic phosphoric compounds in a shell of *Limacina helicina* may be accumulated into the chemical composition of chum salmon bone.

Then, an attempt was made to ascertain the consideration by estimating quantitatively the amounts of phosphoric compounds in various kinds of plankton in the northern Pacific Ocean.

The plankton employed was the same as described in the previous chapter VIII. The plankton species were *Calanus plumchrus, Parathemisto japonica* and *Limacina helicina.* Of the ash of the plankton, phosphoric compounds were quantitatively determined by means of the method of Fiske and Subbarow[44].

Results obtained are shown in Table X-8.

Table X-8. Phosphoric compounds in various kinds of plankton

Plankton / Phosphoric compounds	Acid-soluble-P (*mg%*)	Lipids-P (*mg%*)	Nucleic acid-P (*mg%*)	Protein-P (*mg%*)	Total-P (*mg%*)
Limacina helicina	0.55	0.10	0.12	0.07	0.84
Calanus plumchrus	0.42	0.09	0.08	0.08	0.67
Parathemisto japonica	0.40	0.08	0.08	0.09	0.65

As seen in Table X-8, phosphorus content in *Limacina helicina* is larger than that in *Parathemisto japonica* and *Calanus plumchrus,* amounting to about 1.5 times that in the latters. The results obtained may explain the fact that chum salmon excels other salmon in the inorganic phosphor content in the bone.

9. Effect of volatile phosphoric compounds upon dimethyl sulfide of the petroleum odour constituent

The raw fresh salmon (V.B.-N, 8.65 *mg*%) caught in waters nearby Hokkaido was eviscerated, and packed into separate can containers which made of E.T. whole-coated-inner side tin plate. Into separate can containers, samples with 1.0, 0.7, 0.5, 0.3 and 0.1 *mg* each of D.M.P.T. and volatile phosphoric compound such as phosphine were sealed. All of the cans were processed at 10 *lbs*-pressure for 80 minutes. After the cans has been processed and opened, organoleptic inspection was made for detection of petroleum odour.

Effect of the presence of volatile phosphoric compounds upon the petroleum odour is shown in Table X-9.

Table X-9. Effect of volatile phosphoric compounds upon the petroleum odour

Phosphoric com.	Added					Non-added				
D.M.P.T. (*mg*)	1.0	0.7	0.5	0.3	0.1	1.0	0.7	0.5	0.3	0.1
Odour	+	+	+	+	−	+	+	±	±	−

As seen in Table X-9, if 1.0 *mg* and 0.7 *mg* each of D.M.P.T. are added into can container with or without volatile phosphoric compounds, the petroleum odour is detectable. On the other hand, if 0.5 *mg* and 0.3 *mg* each of D.M.P.T. are added into the can container with or without volatile phosphoric compounds, the petroleum odour is detected only in the cans which contained volatile phosphoric compounds. Further, if below 0.1 *mg* of D.M.P.T. is added into the cans, the petroleum odour is not detected in cans either with or without volatile phosphoric compounds

In consequence, it may be stated that the smell of dimethyl sulfide is enlarged by the presence of volatile phosphoric compounds. Therefore, the petroleum odour in canned chum salmon is obviously detectable according to the presence of the volatile phosphoric compounds which is formed from the salmon bone.

XI. General Discussion and Conclusion

In this chapter, the author will discuss generally the results obtained up to chapter X, and will inquire into the factors concerning the cause of the occurrence of the odour during the processing of canned chum salmon.

Generally speaking, the cause of the occurrence of the petroleum odour is not the falling of the freshness of raw chum salmon, nor incomplete processing, but is considered to be the intrinsic ecological peculialities of chum salmon.

The principal chemical component of the petroleum odour in the canned chum salmon is clarified to be dimethyl sulfide.

Dimethyl sulfide has been learned to be one of the chemical compounds in algae, which shows so called "Yaki-nori odour" (odour of roasted laver) according to Hass[20], Obata *et al.*[21] and Katayama[22]. Dimethyl sulfide is also found by Patton[45] as concerned in the odour of powdered milk after γ-ray irradiation.

He has also detected dimethyl sulfide as a chemical component of the odour of American petroleum ether. Thus the distribution of dimethyl sulfide is known to be wide in nature.

The pathway from algae into chum salmon body is probably worth consideration interest, because both sorts of creatures live in the sea. Dimethyl sulfide is present in chum salmon body as dimethyl-β-propiothetin (D.M.P.T.), which is decomposed to the former substance by heating (processing of the canning) (Chapter VI). This decomposition is also affirmed by the formation of the petroleum odour at the roasting of raw chum salmon meat (Chapter I).

D.M.P.T. has been isolated by Challenger *et al.*[23] from *Polysiphonia fastigiata*, a kind of algae. D.M.P.T. is a kind of sulfonium compounds, and its presence in algae has an important significance in considering the pathway of metabolism in the marine creatures.

Here, in order to consider the pathway from algae to chum salmon body, the author has observed on the digestive organs of various kinds of salmon and on the kinds of foods eaten. According to the results obtained (Chapter VII), in the digestive organs of chum salmon, there are large amounts of *Pteropod*, especially *Limacina helicina*. And a large amount of D.M.P.T. is found in the content of the digestive organs of chum salmon. From the studies on the digestibility of plankton which is food for salmon in the northern Pacific Ocean, and on the quantitative determinations of D.M.P.T. in the contents of the digestive organs, there is found in *Limacina helicina* bodies to be a large amount of D.M.P.T., which is not decomposed in the digestive organs of chum salmon.

The fact that there is a remarkably large amount of *Limacina helicina* in the diegstive organs of chum salmon has probably some relation with the occurring season and place of *Limacina helicina* and with the migrating habits of chum salmon.

Limacina helicina lives in cold sea area near the Aleutian Islands, and goes northward with an ocean current. On the other hand, schoals of chum salmon migrate southward to Kamchatka from the Aleutian Islands. When the migration of the schoals crosses the breeding place of *Limacina helicina*, the chum salmon voraciously eats the later. Chum salmon gathered for feeding are also caught easily by fishermen.

As realized later, the period of processing of canned chum salmon having the petroleum odour is restricted to the middle period of the salmon fishing season in the area where *Limacina helicina* breeds.

Especially in the 1958 fishing season, large amounts of canned chum salmon having the petroleum odour were processed. According to the Oceanographical Expeditions from 1956 to 1959 in the northern Pacific Ocean by Hokkaido University, training ship "Oshoromaru", and other investigation ships, the whether was good and the temperature of sea water in the northern Pacific Ocean in 1958 was comparatively high, therefore the breeding ratio of *Limacina helicina* was higher than usual year. D.M.P.T. which is taken into chum salmon through the digestive organs from *Limacina helicina* body transfers to the liver of salmon, in which a part of D.M.P.T. as an onium compounds will play rôle on the biosynthesis of methionine with homocysteine. In the combination of homo-

cysteine with D.M.P.T., there is need of transmethylase, of which the activity is different according to the kind of salmon, for example, the transmethylase in the liver of red salmon is more active than in that of chum salmon. That is to say, the amount of D.M.P.T. decomposed in the liver of chum salmon is clarified to be about a half of that of red salmon.

In case of chum salmon, therefore, the greater part of D.M.P.T. is transferred to muscle tissue without decomposition. This agrees with the fact that the amount of D.M.P.T. is larger in chum salmon muscle tissue than in red salmon.

As seen in the previous observations, D.M.P.T. which is a precursor of dimethyl sulfide (a principal component of the petroleum odour of canned chum salmon) is clarified to be due to *Limacina helicina* which is food for chum salmon, and the behavior of D.M.P.T. in chum salmon body has also become known.

By heating, D.M.P.T. is readily decomposed at a comparatively low temperature (96°C) to evolve dimethyl sulfide. Therefore, under the lower temperature than that of the processing of canned salmon of 10 *lbs*-pressure (115°C) for 85 minutes, D.M.P.T. accumulated in the chum salmon body should be decomposed to evolve dimethyl sulfide. The decomposition of D.M.P.T. proceeds more remarkably in a medium having pH value on the alkaline side. If the freshness of chum salmon falls and the meat becomes alkaline, the decomposition seems to be promoted. Consequently, in order to avoid the formation of dimethyl sulfide, the raw chum salmon as fresh as possible must be used for the canning.

Dimethyl sulfide is a principal component of the petroleum odour in canned chum salmon, but it gives off the odour when its amount is comparatively large. Accordingly, it follows that a certain kind of substance to enlarge the odour of dimethyl sulfide may be present in chum salmon body. The author has sought for such a substance. At last, he has found (Chapter X) that volatile phosphoric compounds obviously increase the intensity of the odour of dimethyl sulfide. The precursors of the volatile phopshoric compounds are non-volatile phosphoric compounds, and are present, in principal, in the bone. Non-volatile phosphoric compounds are supposed to be changed to volatile phosphoric compounds during the canning process, because the atmosphere of inner part of the can container is in vacuum, and is under reductive condition. As the chemical characteristics of sulfur which is a constituent element of dimethyl sulfide resembles that of phosphorus, the petroleum odour in canned chum salmon may be heightened by phosphoric compounds. By the same reasoning, the petroleum odour in canned pineapple or grape fruit marmalade may be due to a substance produced by heating of methyl β-methylsulfonylpropionate[46] which is a homologue of D.M.P.T.

It is difficult to overcome the odour of dimethyl sulfide by other various chemical reagents, because dimethyl sulfide does not react easily with other substances.

It is considered, as to the method for prevention of the petroleum odour, that method to avoid use of raw chum salmon having D.M.P.T., is better than that of prevent the formation of the petroleum odour.

In order to avoid use of raw chum salmon having D.M.P.T. the following

methods are recommended.

(1) The author has tried to detect the presence of D.M.P.T. by means of a simple detector which was devised by him and Prof. Eiichi Tanikawa.

The detector is shown in Fig. XI-1.

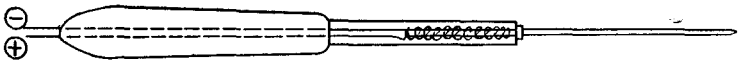


Fig. XI-1. A detector being a kind of electric soldering-iron

As seen in Fig. XI-1, the detector is a kind of electric soldering-iron, of which the end takes the form of a sharp awl.

The awl heated by electricity (at about 150°C) is thrust into tail meat of raw chum salmon for one minute, the awl is taken out and the odour is smelled. If any petroleum odour exists on the awl, the raw chum salmon should not be used for canning. According to an experience on a floating-cannery in the northern Pacific Ocean, some skillful inspectors can distinguish by their olfactory sense the raw chum salmon which will form the petroleum odour after processing.

However, on a large scale, when a great amount of raw fish are unloared at the cannery, it will be difficult to examine each separate fish.

In respect to mass production, there is room for improvement in measures for detection of fish likely to evolve the petroleum odour.

(2) In the digestive organs of chum salmon which must produce the petroleum odour, there is contained a large amount of *Limacina helicina*. In the digestive organs, differing from *Calanus* and *Euphasia*, *Limacina helicina* shows dark green color, and its shell remains for a comparatively long time in the organs without digestion. At the dressing of raw chum salmon, if the contents of the digestive organs are dark green color or have shells of *Limacina helicina*, that chum salmon should be rejected for canning. From the oceanographic investigations, when it is known that *Limacina helicina* is distributed abundantly in salmon-fishing grounds, raw chum salmon caught in such an area should be manufactured into other types of merchandise, because the canned chum salmon manufactured may have the petroleum odour if use is made of raw material caught in such a fishing ground.

(3) As canned chum salmon is processed under a vacuum, there generate variuos kinds of reduced substances, in which are included hydrogen sulfide, volatile phosphoric compounds etc. If hot-dipped tin plate cans are used, the reduced substances may react with the tin or iron of the plate to make a can-odour.

If tin plate cans coated innerside are used, there is no tin or iron to react with reduced substances, so the petroleum odour is smelled as it is.

Therefore, can containers for chum salmon should be hot-dipped tin plate cans or electric tin plate without coating inner side (*e.g.* differential electric tin plate can 100/50).

(4) D.M.P.T. which is a precursor of the petroleum odour is readily decomposed in a medium whose pH value is in the alkaline side; the raw chum salmon for canning should be as fresh as possible.

If the content of the can is acidic, the decomposition of D.M.P.T. is some-

what inhibited. Therefore, one method to prevent the formation of the petroleum odour is to add citric acid or tartaric acid making to pH value of the content about 6.4, of less decomposition of D.M.P.T. on heating.

Conclusion

According to the results of experiments conducted to the present point, the following conclusions will be reached:

(1) The principal chemical component of the petroleum odour in canned chum salmon is dimethyl sulfide.

(2) Dimethyl sulfide is formed from D.M.P.T. accumulated in chum salmon body as a result of heating.

(3) D.M.P.T. accumulated in raw chum salmon originates from *Limacina helicina* taken in as food.

(4) D.M.P.T. is transferred from *Limacina helicina* to chum salmon meat without decomposition in the liver of the salmon; some small part of D.M.P.T. is decomposed in the liver.

(5) The petroleum odour is strengthened by the co-existence of volatile phosphoric compounds.

(6) It is difficult to overcome the petroleum odour in the canned chum salmon, but is possible to cover it somewhat by the use of white tin plate can, or by the avoidance of the use of the raw chum salmon which will form the petroleum odour after the processing: this latter can be done by the use of the detector or by the observation of the content of the digestive organs of the salmon.

Summary

Some canned chum salmon processed on floating-canneries in the northern Pacific Ocean were found at the time of export inspection to have an odour of petroleum.

The author has investigated the chemical components of that petroleum odour and further studied the mechanism of the formation of that odour. Results obtained through the course of the investigation may be summarized as follows:

(1) The formation of the petroleum odour was restricted to canned chum salmon processed on floating-canneries in the northern Pacific Ocean; furthemore, it was restricted as to the fishing season and the fishing ground.

According to the information on the occurrence of the petroleum odour, the conditions of the formation of the odour were different intrinsically from those ever previously found in canned salmon.

(2) In one effort to discover the chemical components of the odour, steam distillation of the various kinds of canned salmon was carried on. However, the results obtained showed that the amount of the components of the odour was too small to detect by that method.

(3) Extraction of chemical components of the odour from the canned chum salmon having the petroleum odour was performed with organic solvents. From

the results, the components of the odour were observed to exist in the neutral fraction by the chemical fractionation. Chemical components contained in the neutral fraction were detected. An important comopnent was identified to be ethylene. But ethylene was not considered to be a cause of the petroleum odour of the can, because ethylene was found also in canned red and pink salmon meats.

(4) The study on the relation between the salmon oil and the petroleum odour was carried out. At first, the chemical properties of the floating oil in various kinds of canned salmon were observed, and it was found that there was a large amount of unsaponifiable matter in canned chum salmon having the petroleum odour. Next, the change of chemical properties of various kinds of salmon oil during the processing was observed. From the results, it became clear that the salmon oil was decomposed partially by the processing, and only ethylene was formed. But as above noted, ethylene was also found in other canned salmon, and no hydrocarbon other than ethylene was found in the unsaponifiable matter. From those facts, hydrocarbon could not be considered a cause of the petroleum odour in canned chum salmon.

(5) Next, the chemical components in the head space of the various kinds of canned salmon were detected, and any unique components was found only in canned chum salmon having the petroleum odour. The steam-distillation of the meat of various kinds of canned salmon was re-examined, in order to ascertain the chemical components in non-condensed gas of the distillate. Consequently, the chemical components of the petroleum odour were obtained in the non-condensed gas of the canned chum salmon; it was found to be a neutral substance having sulfur.

The neutral substance was identified to be dimethyl sulfide. In this experiment, a new method of chromatography for detecting thio-ether was devised. Dimethyl sulfide was ascertained to be a cause of the petroleum odour.

(6) In order to find a precursor of dimethyl sulfide in the chum salmon having the petroleum odour, another series of studies was carried out. As a result, a kind of sulfonium compound was obtained, which was identified to be dimethyl-2-carboxyethyl sulfonium chloride (dimethyl-β-propiothetin) (D.M.P.T.). When D.M.P.T. was added to raw chum salmon, which was processed in the can container, the formation of the petroleum odour was found to occur in the canned chum salmon product. From various points of view, D.M.P.T. was considered to transfer from algae to chum salmon muscle tissue.

(7) In the digestive organs of chum salmon which was caught in the middle period of the fishing season, in which many cans of chum salmon which revealed the petroleum odour were processed, there was a comparatively large amount of D.M.P.T. which was originated from the food.

The contents of the digestive organs of various kinds of salmon were classified. It was found that a large amount of *Pteropods*, which are one kind of zoo-planktons, was contained in the digestive organs of chum salmon caught in the middle period of the fishing season.

(8) The chemical components of bodies of plankton collected in the northern Pacific Ocean were found out. The results proved that *Limacina helicina* which is one kind of *Pteropods*, contains a large amount of D.M.P.T. In the sea water

in the fishing ground where chum salmon having the petroleum odour was caught, there was observed a large amount of *Limacina helicina* germed. The germination was ascertained to have an intimate relation with the sea current and the sea water temperature from the data of oceanographical observations.

(10) The amount of D.M.P.T. present in the liver was larger in chum salmon than in red salmon throughout the whole fishing season, especially in the fishing season during which the canned chum salmon having the petroleum odour was processed. D.M.P.T. was ascertained to be serving as a methyl donor to synthesize methionine from homocysteine. In this case, the transmethylase of chum salmon was ascertained to be remarkably weaker than that of red salmon in the activity. The transmethylase was found in the liver, but not in the muscle tissue of chum and red salmon. The transmethylase of salmon was found to have 6.2 of the optimum pH value, which was more acidic than that of rat and guinea pig. It was presumed that the amount of D.M.P.T., which was transferred to the muscle tissue from the liver without decomposition, was remarkably larger in chum salmon than in red salmon.

(11) The petroleum odour which appeared as a result of the presence of dimethyl sulfide in canned chum salmon was ascertained to be increased by the co-existence of a small amount of volatile phosphoric compounds. Such compounds were ascertained to be formed from the inorganic phosphate in bone, in principal, by heating under reduced conditions.

(12) The mechanism of the formation of the petroleum odour in canned chum salmon was considered to be as follows: Into chum salmon meat, D.M.P.T. was transferred from *Limacina helicina* which was germed activity in a certain period of the fishing season in the northern Pacific Ocean. Most parts of D.M.P.T. were not decomposed in the liver, and were accumulated in the muscle tissue, and then were decomposed to dimethyl sulfide during the processing of the cans. The petroleum odour resulted. In this case, if volatile phosphoric compounds were co-existed, the odour became remarkable.

At last, the methods for prevention of the petroleum odour in canned chum salmon were studied as follows. (a) To use a detector which can know previously that chum salmon used as the raw material may produce the petroleum odour. (b) To inspect the content of the digestive organ in order to know previously that chum salmon may produce the petroleum odour. (c) To cover the petroleum odour by the so called can-odour which is formed by reaction of the content and tin plate. (d) To add citric or tartaric acids making slightly acidic, or to use the raw material having an acidic condition as fresh as possible, because D.M.P.T. is easily decomposed in alkali.

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