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# Studies on the Odour of Canned Fish (Part 6)

## Volatile Basic Components in the Canned Edible Portion of Short-neck Clam, *Tapes japonica*

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The edible portion of short-neck clam was put up in cans and stored for two months at room temperature.

The volatile basic fraction in the canned contents was obtained by a steam distillation method. Volatile bases in the fraction were extracted with ethyl ether, and then the ether extract was analyzed by gas chromatography as well as paper chromatography. The results obtained are as follows,

1. Ammonia and *n*-propylamine were detected in the ether extract by gas chromatography. Peak area of *n*-propylamine increased with the period of storage.

2. Only one spot was observed in paper chromatogram, and it was identified as *n*-propylamine.

## I Introduction

Volatile acidic and basic components in fresh[1], and steam-boiled meat[2] of several kinds of fish were investigated previously. Besides, in the previous papers, the pattern of increasement of volatile acidic and basic components in canned mackerel[3] and cuttlefish[4] meat in the course of storage was reported, and it was shown that the increasement of peak area of *n*-propylamine in gas chromatogram was the most noteworthy.

The present paper deals with the volatile basic components in the canned edible portion of short-neck clam which was stored for periods up to two months after canning. Some investigations of the volatile bases in the canned meat were carried out by gas chromatographic as well as paper chromatographic analysis.

NISHIBORI et al reported on the volatiles which were produced from the flesh of boiled shellfish. They detected trimethylamine as a volatile basic component in boiled short-neck clam[5], trimethylamine and ammonia in boiled shijimi (*Corbicula japonica*)[5], and ammnoia in boiled oyster[6], respectively.

## II Experimental

### 1. Sample

Short-neck clam was purchased at Kagoshima Central Wholesale Market.

2. Producing process for the canned products of edible portion of short-neck clam

1) Cooking of short-neck clam. Stand for three minutes in boiling water.
2) Shucking.
3) Filling. Pack 150 g of the shucked short-neck clam in a can (sake-hira No. 2, lacquered).
4) Addition of brine solution. Add a ladleful brine (2.5% table salt, 0.15% citric acid solution, pH 3-4. About 50m*l*).
5) Sealing. Seal under reduced pressure with 〈Toyo-seikan〉 model 5 sanitary vacuum seamer.
6) Retorting. Heat at 8 lbs (112.7°C) for 60-70 minutes.
7) Cooling. Cool in running water.

Canned products were then stored at room temperature until these were needed for the following experiments.

3. Preparation of volatile basic fraction

450g of canned short-neck clam meat was minced three times. Can juice and 250 m*l* of distilled water were added to the minced meat. The combined sample was submitted to steam distillation.

Five liter of distillate was caught in 100 m*l* of 0.1 N hydrochloric acid solution and the distillate was evaporated to about 10 m*l* under reduced pressure. After saturation with sodium chloride, the condenced distillate was added ethyl ether and a small amount of solid sodium hydroxide. The mixture was shaken vigorously, and ethyl ether layer was taken out. The extraction procedure with ethyl ether was repeated five times. The slight moisture in the ether extract was eliminated with dried sodium sulfate. The ether extract was moderately evaporated to about one m*l* with heating bath, and then the extract was submitted to gas chromatography directly.

4. Gas chromatography for the volatile basic compounds

A Shimadzu Model GC-IB gas chromatographic unit, with a thermal conductivity detector installed, was used. The detector cell was operated at 200℃. The U-shaped column of 4 mm inside-diameter and 225cm long was packed with triethanolamine or polyethyleneglycol 6000 sodium butylate. The flow rate of carrier gas (helium) was adjusted to 45 m*l* per minute for TEA, and 50m*l* per minute for PEG 6000 Na-butylate, respectively. The operating conditions of the unit are mentioned in detail as the footnote of Fig. 1.

In order to identify the peaks in gas chromotogram, the retention time was compared with that of authentic samples.

5. Paper chromatography

〈Toyo Roshi〉 No. 51 filter paper for paper chromatography was used. Sample

solution subjected to paper chromatography was the same as that for gas chromatography. Solvent system consisted of *n*-butanol-acetic acid-water (5:4:1, v/v/v). The paper was in equilibrium with the solvent mixture before the run was started. Ascending chromatography was used, and standard substances were run in every chromatogram. When the solvent had run a convenient distance, the paper was removed and dried at room temperature. The positions of basic compounds were demonstrated by spraying with nynhydrine.

## III Results and Discussion

Gas chromatograms of volatile basic fractions obtained from the can contents during two months storage are shown in Fig. 1.

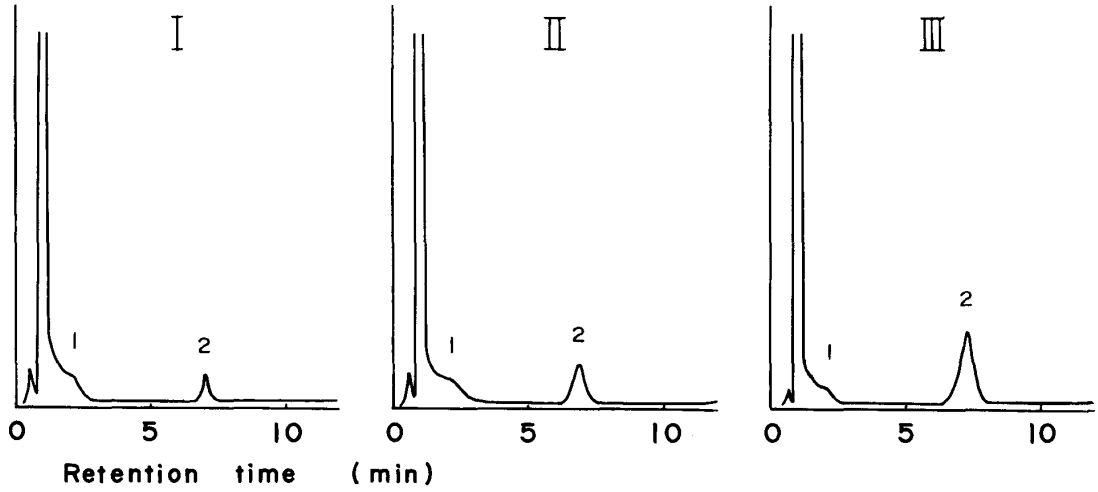


Fig. 1. Gas chromatograms for the volatile basic fractions obtained from the canned edible portion of short-neck clam.

I; Can was stored for one week after canning, and then the contents were analyzed by gas chromatography.

II; Can was stored for one month after canning.

III; Can was stored for two months after canning.

Volume of the ether extract injected to chromatograph; 10$\mu l$.

Peak number 1; Ammonia. 2; *n*-Propylamine.

Conditions for gas chromatography;

Column; 4 mm$\phi$ × 2.25 m.
Packing; TEA.
Liquid phase; 25 %
Support; Shimalite.
Mesh; 60/80.
Carrier gas; Helium.
Flow rate; 45 m$l$/min.
Column temp.; 90℃.
Detector; TCD.
Detector temp.; 200℃.
Rang; 4 mV.
Injection temp.; 150℃.

In every case, ammonia and *n*-propylamine were detected, and it was noted that the peak area of *n*-propylamine in chromatograms increased with the period of storage. Peak area of *n*-propylamine of the sample which was stored for one month multiplyed into about 1.5 times that of the sample stored for one week, and that of the sample stored for two months was even at nearly three times. On the other hand, the peak area of ammonia changed little with the period of storage. These observations on ammonia and *n*-propylamine are in common with the cases of canned mackerel[3] and canned cuttlefish[4].

When PEG-6000 Na-butylate was used as a column packing, no peak was observed in chromatogram except that of *n*-propylamine.

Rf value for ten species of standard amines subjected to paper chromatography are shown in Table 1. When the sample was examined by mean of paper chromatography, only one spot was observed, and the Rf value was 0.42. The value was a little lower than that of standard *n*-propylamine, 0.46. But, usually when a mixture of several components is subjected to paper chromatography, Rf value of each one tends to be slightly lower than that of a single pure corresponding component. Consequently, the low Rf value is likely due to the impureness of the sample solution. So, the spot was identified as *n*-proylamine.

Table 1. Rf value for several authentic basic compounds.

| Authentic basic compound | Rf value | Authentic basic compound | Rf value |
|---|---|---|---|
| Methylamine | 0.31 | Diethylamine | 0.51 |
| Dimethylamine | 0.33 | *iso*-Butylamine | 0.58 |
| Ethylamine | 0.37 | *n*-Butylamine | 0.59 |
| *n*-Propylamine | 0.46 | *iso*-Propylamine | 0.62 |
| Trimethylamine | 0.49 | Triethylamine | 0.71 |

Paper; 'Toyo Roshi' No. 51. The paper was developed by ascending chromatography. Solvent system; *n*-Butanol:acetic acid:water(5:4:1, v/v/v). Color reagent; Nynhydrine.

## References

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