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On the Production of Carbon Dioxide in Fish Flesh During the Deterioration of Freshness

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(Figs. 1-2; Tables 1-3)

It is well known that carbon dioxide (CO_2) is produced in fish flesh during putrefaction. According to the research by GALE[1] the quantity of CO_2 thus produced is related to the pH of the medium.

The present work has been undertaken to evaluate the roles of autolysis and microbial actions in producing CO_2 in fish flesh in the course of spoilage. Results of the experiments, as will be described later, indicated that relatively small amount of CO_2 was produced in fish flesh by autolysis, whereas a large amount of CO_2 was produced by the decarboxylation due to microorganisms. Since CO_2 content in fish flesh reached a peak in the vicinity of incipient stage of decay and then tended to decrease slowly, it was suggested that CO_2 content might be of little, if any, significance as an index of the freshness of fish flesh, its usefulness being much limited as compared with such properties as VBN (volatile basic nitrogen) or VRS[2),3)] content.

MATERIAL AND METHODES

The freshest possible raw mackerel (*Scomber japonicus*) were obtained commercially. Their dorsal muscles were minced small with a meat chopper and mixed. Then the mixed muscle was kept in an incubator adjusted to 20°C until the measurement of CO_2 took place.

There are tow methods for determing CO_2 content of a muscle material. One is the gasometric method, and the other is the titrimetric method in which an alkaline solution that has absorbed CO_2 is titrated with an acidic solution. The authors adopted the latter method in this work. By aeration or diffusion CO_2 produced and accumulated in the muscle material was made to be absorbed in a 1% to 5% potassium hydroxide solution. This solution was titrated with 0.2N sulfuric acid (a m*l* needed for this titration) in the phenolphthalein indicator; then, titration was resumed with the same acid solution (b m*l* needed for this titration) in the methyl orange indicator. A blank potassium hydroxide solution, to which CO_2 had not been made to be absorbed, was titrated in the similar mannor with the same acid solution (a' m*l* and b' m*l* respectively). One may calculate the weight of CO_2 contained in a muscle sample by using the following formula:

$$CO_2\ (mg) = 4.4 \times \{(b-a)-(b'-a')\} \times f$$

Where 4.4 is the weight (in mg) of CO_2 equivalent to 1m*l* of 0.2N sulfuric acid, and f is the factor of the 0.2N sulfuric acid.

VBN content was determined in each sample by the usual method with the Conway's apparatus.

RESULTS AND DISCUSSION

One hundred grams of the muscle material was used for each measurement, and the results obtaind were given in Fig. 1 and Table 1.

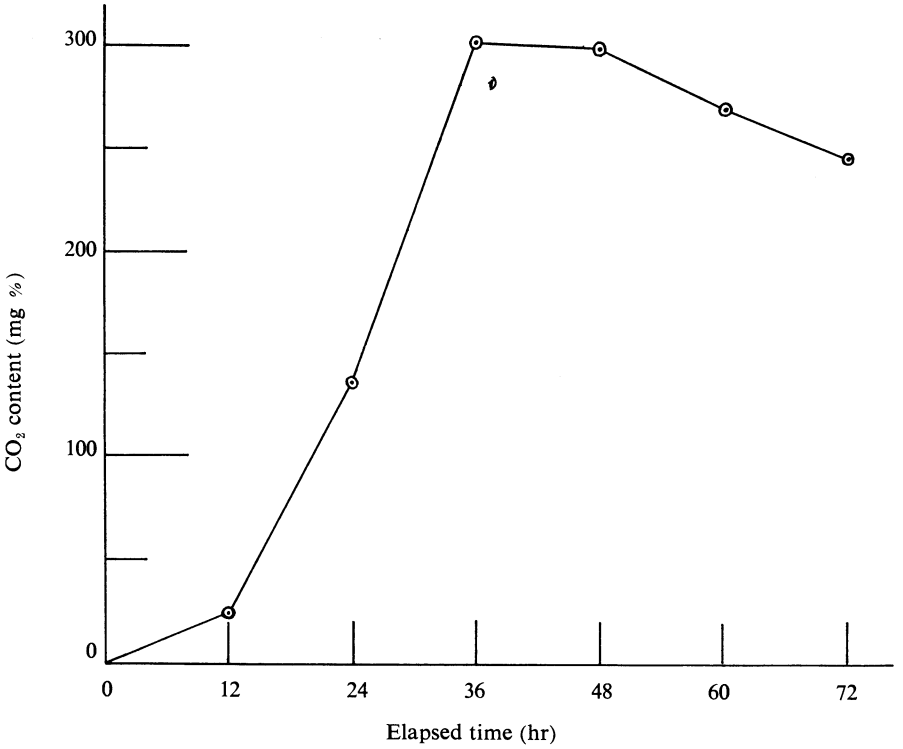

Fig. 1. Production curve of CO_2 in minced mackerel muscle during putrefaction at 20°C.

Table 1. Change in pH and VBN content of the minced mackerel muscle during putrefaction at 20°C

Elapsed time (hr)	0	12	24	36	48	60	72
pH	6.10	6.25	6.40	7.00	7.30	7.50	8.00
VBN (mg%)	13	24	32	64	162	165	240

As shown in Fig. 1, CO_2 content in the muscle increased with progress of time, and reached a peak in 36 hours or after the incipient spoilage stage which was attained

in about 24 hours as seen from Table 1. In view of the results described above, it might be suggested that the production of a large quantity of CO_2 was due to putrefactive microbial actions in the muscle material.

In an effort to determine the microbial effect, an antiseptic (0.5g of thymol plus 5m*l* of toluene) was added to 50g of muscle material. As a possitive control, a second 50g of the material was inoculated with a strain of *Micrococcus* sp. which had been separated from a putrefied fish flesh in the author's laboratory. And as a negative control, a third 50g of the material was treated nothing. The production of CO_2 in these three samples were measured by the foregoing technique, and the resultes obtained were given in Fig. 2 and Table 2.

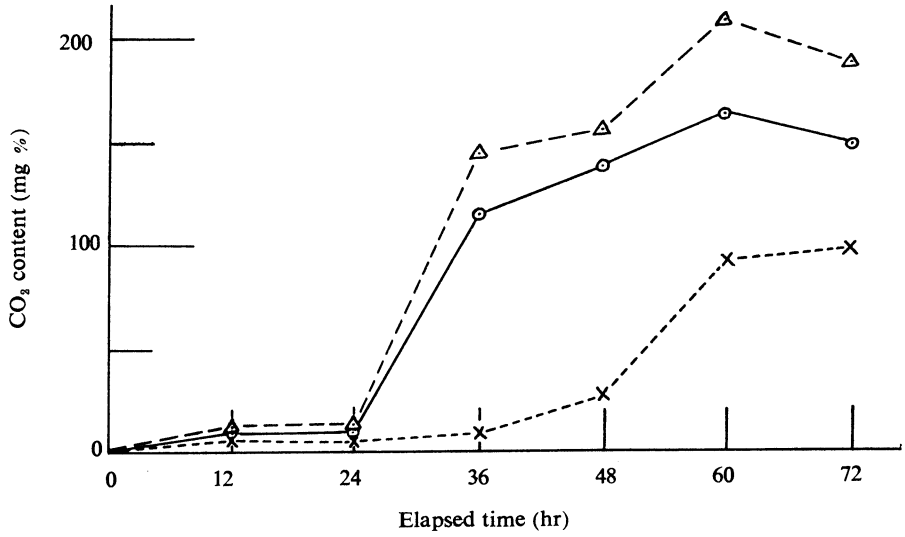

Fig. 2. Production curves of CO_2 in minced mackerel muscle stored at 20°C.
– – –△– – – : Positive control muscle (Inoculated with *Micrococcus* sp.)
——⊙—— : Negative control muscle (Without treatment)
- - - - × - - - - : Antisepticized muscle (With thymol and toluene)

Table 2. Change in pH in three samples of the minced mackerel muscle stored at 20°C

Elapsed time (hr)	0	12	24	36	48	60	72
Positive control muscle	5.8	6.1	6.3	6.9	7.2	7.6	7.8
Negative control muscle	5.8	6.0	6.2	6.8	7.0	7.2	7.6
Antisepticized muscle	5.8	5.8	5.8	6.0	6.2	6.4	7.4

These muscle samples have the same note as in Fig. 2.

In this experiment the antisepticized muscle kept its freshness against microbial actions for 36 hours, and only a small quantity of CO_2 was produced in this sample as shown in Fig. 2.

In contrast, much large amount of CO_2 was produced in the positive control lot. From the above facts, it seems most reasonable to conclude that a significant quantity

of CO_2 was produced in the fish flesh by microbial actions during the deterioration of freshness, while a small quantity of CO_2 was produced by autolysis of the flesh.

It has been reported by GALE[1] that the optimum pH for bacterial decarboxylase is on the acidic side. The foregoing experimental data indicated that the decarboxylase of the crude population of microorganisms which grew in the muscle sample exhibited considerable activity also on the alkaline side of pH. Therefore next experiment was carried out using the muscle material (15g each) adjusted to pH 5.41, pH 6.35, or pH 7.75 with 0.2M phosphate buffer solutions (135 m*l* each).

Table 3. Amount of CO_2 produced and change in pH of the minced mackerel muscle in buffer solutions stored at 20°C

Elapsed time (hr)		0	12	24	36	48	60	72
Buffered muscle to pH 5.41	CO_2(mg%)	0	4	21	71	252	293	230
	pH	5.41	5.41	5.41	5.43	5.80	6.05	6.60
Buffered muscle to pH 6.35	CO_2(mg%)	0	24	66	283	305	353	310
	pH	6.35	6.35	6.35	6.35	6.38	6.58	6.61
Buffered muscle to pH 7.75	CO_2(mg%)	0	12	21	168	231	297	290
	pH	7.75	7.75	7.75	7.75	7.75	7.75	7.76

The experimental results were given in Table 3, which shows that the optimum pH for microbial decarboxylase was on a slightly acidic side. Besides the above, the quantity of CO_2 produced in an acidic medium (pH 5.41) and that in an alkaline medium (pH 7.75) were essentially identical, as far as this experiments were concerned.

As REAY and SHEWAN[4] referred to in their book, decarboxylases are an inducible enzyme. The production of CO_2 in a fish muscle probably depends not only on the optimum pH of the microbial enzymes, but on amount of original substrates in the muscle, therefore, since there were many original substrates such as free amino acids in a putrefying muscle, it seems reasonable that CO_2 should be produced also on the alkaline side of pH.

SUMMARY

Carbon dixoide (CO_2) content in the muscle of the mackerel (*Scomber japonicus*) during putrefaction was measured.

(1) The quantity of CO_2 produced and accumulated in the sample of fish muscle increased quick to some degree in process of time, and then gradually decreased after the complete spoilage of the fish muscle. Accordingly, the CO_2 content of fish muscle might to be of little significance as a better index of the freshness of the fish flesh.

(2) A large amount of CO_2 was produced by microbial actions, while a little amount of the gas was produced by muscle's autolysis.

(3) Though the optimum pH for decarboxylases are on the acidic side, not a small quantity of CO_2 was produced in fish muscle also at an alkaline pH. In this connection,

it was discussed that microbial decarboxylases were inducible enzymes, and that they might therefore produce CO_2 to some extent on the alkaline side if such substrates as free amino acids were present in sufficient amounts, as it was often the case in a putrefying fish flesh.

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魚肉の鮮度低下中における炭酸ガス発生について

浅　川　末　三

魚肉の鮮度と肉中の炭酸ガス (CO_2) 量との関係を見るために，この実験は行われた．

サバ (*Scomber japonicus*) の精肉を用い，鮮度低下過程中のCO_2 量をアルカリ吸収逆滴定の方法で実測した結果，発生 CO_2 量は鮮度低下の初期においては急増したが，腐敗期においては漸減した．従って CO_2 量を以てその魚肉の鮮度指標とすることは良法ではないと論じた．

魚肉中に発生するCO_2 は，無菌および菌接種試験の結果，その大部分が微生物作用により，小部分が筋肉の自己消化作用によるものであることを知った．

なお，微生物の脱炭酸酵素の至適 pH は酸性側にあるとされているが，微アルカリ性の魚肉からも相当量のCO_2が発生した．これについて，微生物の脱炭酸酵素は適応酵素であるから，例えば遊離のアミノ酸などの基質が豊富に存在するならば，微アルカリ性になった魚肉からも CO_2 は発生しうると推論した．