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The Effect of Antioxidants on the Fermented Sardine and Taste Compounds of Product

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정어리젓 加工에 있어서의 抗酸化劑 處理 效果 및 製品의 呈味成分

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항산화제처리가 정어리젓 숙성 중의 지질의 산패방지에 미치는 효과 및 정어리젓의 정미성분을 분석하였다 정어리젓을 실온(25±3°C)에서 숙성시켰을 때, 대조구는 숙성 31일째까지 지질의 산화가 급속히 진행되었으나 BHA 및 Tenox—Ⅱ를 처리한 것은 모두 지질의 산패방지 효과가 있었다. 항산화제 처리농도는 BHA, Tenox—Ⅱ 모두 0.01% 첨가한 것보다 0.02% 첨가한 것이 더욱 효과가 좋았다.

ATP 관련물질은 숙성 31일만에 거의 inosine 및 hypoxanthine으로 분해되었다. 그리고, 유리아미노산 중 함량이 많은 것은 leucine, glutamic acid, isoleucine, alanine, valine 및 lysine으로서 전유리아미노산에 대하여 59.4%를 차지하였다. 특히 lysine이 7.2%나 함유되어 있어 영양학적 의의가 크다고 생각된다.

정어리젓 중의 5′-IMP, betaine, TMAO 및 총 creatinine 등은 엑스분에 대하여 1.0%, 0.02% 및 10.8%로서 비교적 적은 양이지만 유리아미노산과 함께 정어리젓의 맛에 보조적인 구실을 할 것이라고 추정된다.

Introduction

Since a long time ago, the pickled fish which has a characteristic taste has been used as one of the fermented foods, salt added to a part or whole body of fish to ferment then kept for a certain period in many Asian countries including Korea.

Yamamoto [1], Lee [2], and Uno *et al.* [3] reported on the effect of temperature and salt concentration in the fermented fish during fermentation. Chung and Lee [4], Lee and Sung [5], and Lee *et al.* [6] also reported the taste compounds of fermented fish. And Song [7] reported the changes of lipid oxidation and free amino acids contents during the fermentation of anchovy.

Recently, the catches of sardine in Korea have been increasing from year to year, as shown in Table 1. But much of the fish has been used for other purpose like fish meal because the degree of freshness of the fish drops rapidly, so it has many problems for the mass consumption as foods.

Therefore, the authors developed the fermented sardine to use the fish effectively and experimented on the effect of antioxidants on the processing of fermented sardine, and analyzed nucleotides and their related compounds, free amino acids, TMAO, TMA, and total creatinine to

Table 1. Annual catches of sardine since 1970 in Korea

Year	1970	1971	1972	1973	1974	1975	1976	1977	1978
Catches(M/T)	101	138	315	3,689	194	3,555	11,154	50,229	53,829

Table 2. Treatment of raw sardine for the fermentation process

Sample No.	Kinds of antioxidant	NaCl content in the raw sardine(%)	Ratio of antioxidant to the raw sardine(%)
C	Control	20	0
B-1	BHA	〃	0.01
B-2	BHA	〃	0.02
T-1	Tenox-Ⅱ	〃	0.01
T-2	Tenox-Ⅱ	〃	0.02

search for the taste compounds of the product.

Materials and Methods

1. Preparation of sample

Specimens of sardine, *Sardinops melanosticta* (body length 13-16cm, body weight 62-76g), caught from the East Sea, were purchased at Busan Cooperative Fish Market in July 1980.

The sardine treated with table salt and other additives as shown in Table 2, was patched up in the glass bottles, and then fermented at room temperature for a certain period. Each fermented sample was blended in a Waring blender and put into polyethylene film bag (tickness 0.03mm). The sample in the bag was frozen immediately and stored at -35℃ until analyzed.

2. Experimental methods

(1) Chemical components, salinity and pH measurements

Moisture, ash, crude, protein and lipid were determined by general methods, total sugar by Somogyi's method[8], salt by Mohr method[9] and pH by pH meter (Fisher Accumet pH meter model 630).

(2) Volatile basic nitrogen (VBN) and amino-nitrogen(NH_2-N)

Volatile basic nitrogen was determined by Conway micro-diffusion method[10] and amino-N by Spies method[11].

(3) Preparation of sample oil

Blended fermented sardine sample was dehydrated by the addition of anhydrous sodium sulfate and then extracted with ethyl ether in a dark place for 2 hours. After filtering, the extracted oil was collected by rotary evaporator at below 40°C.

(4) Acid value and peroxide value

Acid value was determined by general method[9], and peroxide value by Lea's improved method[9].

(5) Thiobarbituric acid (TBA) value

TBA value was determined by Tarladgis' distillation procedure[12].

(6) Sensory evaluation

A panel of 12 experienced testers marked taste, odor, color, texture, and appearance of samples by profile method during fermentation periods.

(7) Nucleotides and their related compounds

a) Preparation of the sample solution

The sample solution was prepared by the methods of Nakajima *et al.*[13] and Lee and Park[14].

b) Fractionation of the nucleotides and their related compounds

Ion exchange resin column: The fraction was collected by the methods of Bergkuist and Dentsch[15], and Nakajima *et al.*[13] using stepwise elution system.

Seperation of inosine and hypoxanthine: Inosine and hypoxanthine were separated by the

methods of Arai and Saito [16], and Seki *et al.* [17]

Absorbance and calculation: The absorbance of the fractionates measured at 260nm using a Shimazu 140−02 spectrophotometer and the molecular extinction coefficient at 260nm was used to calculate the concentration.

Identification of each fractions: Each fraction was identified by comparison with eluted position and the absorbance curve to standard compound.

(8) Free amino acids

An accurately weighed 5 g of sample was blended with 80 ml of 1% picric acid in a homogenizer. The resultant slurry was diluted to 100ml and centrifuged at 4,000 rpm for 15 minutes. A certain amount of supernatant was taken to transfer the Dowex-2 x 8 (Cl^- form, 100-200 mesh) resin column to eliminate the picric acid and the resultant eluate was diluted to 50 ml with distilled water. A 20ml portion of the dilution was taken to be absorbed in Amberlite IR-120 column (H^+ form, 100−200 mesh: ϕ 1.5×5cm) and washed with 150ml of distilled water. The amino acids were eluted by washing the column with 120 ml of 2 N-ammonia water, and the eluate was evaporated to dryness under reduced pressure in a rotary evaporator. The residue was taken up in 25 ml of citrate buffer solution (pH 2.2), then the solution was ampouled and stored at −30°C. Each samples for the determination of free amino acids were analyzed by the method of Spackman *et al.* [18] using the amino acid auto-analyzer (JLC-6AH, No. 310).

(9) Extractive nitrogen (Ex-N)

A 4 to 5g of ground sample was blended with 1% picric solution in a homogenizer for 15 minutes. The resultant slurry was diluted to 100 ml and centrifuged at 4,000 rpm for 15 minutes. A 20ml portion of the supernatant was introduced onto the top of the column of Dowex 2 × 8 (Cl^- form, 200−400 mesh) to eliminate the picric acid, and determind by the semimicro-Kjeldahl method.

(10) Betaine, Trimethylamine oxide (TMAO) and Trimethylamine (TMA)

Betaine: Betaine was determined by the methods of Konosu and Kasai [19], and Focht *et al.* [20]

TMAO and TMA: Extraction solution by the method as in case of betaine was determined by the methods of Sasaki *et al.* [22] Hashimoto and Okaichi, [23] as suggested by Dyer. [21]

(11) Total creatinine

Total creatinine was determined by the method of Sato and Hukuyama. [24] [48].

(12) Omission test

After the fermented sardine was centrifuged at 3,000 rpm for 10 minutes, a certain portion of the supernatant was taken up, poured onto the column of Amberlite IR-120 (H^+ form) to eliminate the amino acids. At the same time these solution and supernatant were prepared for the sensory evaluation to compare with the others after amino acids, nucleotides and their related compounds were eliminated. A panel of 12 experienced testers carried out a sensory evaluation of each samples by scoring method.

Results and Discussion

1. Changes of chemical components, salinity and pH

The contents of moisture, crude lipid, crude protein, total sugar, ash and salt in fermented sardine were listed as shown in Table 3.

Few changes of chemical components were detected in the view point of kinds and concentration of antioxidants during fermenting periods.

The pH of the control increased sharply for 30 days, and then slowly for another 30 days in comparison with the sample treated with antioxidants, while those of fermented sardines treated with antioxidants increased slowly for 60 days. These results are a little higher than those of Lee *et al.* [6], who reported that the pH of salted clam pickle and salted croaker pickle on sale was 5.2 and 6.0, respectively.

2. Changes of volatile basic nitrogen (VBN) and amino-N

The changes of VBN and amino-N in fermented

Table 3. Changes in chemical composition, pH and salinity during the fermention of sardine treated with antioxidant (*g*/100*g*)

	Raw	Fermentation period (days)														
		19					31					60				
		C	B-1	B-2	T-1	T-2	C	B-1	B-2	T-1	T-2	C	B-1	B-2	T-1	T-2
Moisture	77.8	63.5	62.6	62.8	63.5	63.2	63.2	62.3	62.7	63.0	62.8	63.9	63.5	63.7	63.9	64.2
Lipid	3.2	4.9	4.5	4.4	4.7	4.2	4.8	4.6	4.5	4.7	4.5	4.7	4.6	4.4	4.5	4.3
Protein	16.0	14.2	14.7	14.1	14.3	14.0	14.1	14.4	14.1	14.8	14.6	13.4	13.7	13.4	13.8	13.5
Total sugar	0.3	0.4	0.4	0.5	0.5	0.3	0.5	0.6	0.5	0.5	0.5	0.3	0.4	0.4	0.4	0.4
Ash	2.5	17.0	17.6	17.5	17.2	17.7	17.6	17.7	17.8	17.0	16.9	17.5	17.4	17.5	17.0	17.4
pH	6.62	6.07	6.04	6.03	6.09	6.06	6.53	6.12	6.15	6.16	6.18	6.67	6.24	6.30	6.30	6.26
Salinity	—	16.8	17.2	17.0	17.0	17.3	17.3	17.3	17.1	17.0	17.1	17.3	17.2	17.2	17.1	16.8

C: control, B-1: BHA 0.01%, B-2: BHA 0.02%, T-1: Tenox-Ⅱ 0.01%, T-2: Tenox-Ⅱ 0.02%

Table 4. Changes of volatile basic nitrogen (VBN) and amino nitrogen contents during the fermentation of sardine treated with antioxidants (mg/100g)

	Raw	Fermentation period (days)														
		19					31					60				
		C	B-1	B-2	T-1	T-2	C	B-1	B-2	T-1	T-2	C	B-1	B-2	T-1	T-2
VBN	9.7	75.2	60.2	54.1	74.2	73.1	83.0	66.2	60.2	81.4	77.9	93.9	85.6	80.8	92.3	88.1
NH_2-N	47.1	207.2	201.2	184.0	201.8	197.5	230.1	214.1	210.2	224.2	216.8	228.4	225.8	222.8	230.1	223.4

C: Control, B-1:BHA 0.01%, B-2: BHA 0.02%, T-1: Tenox-Ⅱ 0.01% T-2: teno-xⅡ 0.02%

Table 5. The results of organoleptic test during the fermentation of sardine treated with antioxidant

Fermentation days	Sample	Color	Flavor	Texture	Separation of liquid	Commercial quality
19	C	Light reddish brown	fair	hard	slight	inferior
	B-1	Yellowish brown	〃	〃	〃	〃
	B-2	〃	〃	〃	〃	〃
	T-1	〃	〃	〃	〃	〃
	T-2	〃	〃	〃	〃	〃
31	C	Reddish brown	good	good	considerable	good
	B-1	Light reddish brown	(sweet)	〃	〃	〃
	B-2	〃	〃	〃	〃	〃
	T-1	〃	〃	〃	〃	〃
	T-2	〃	〃	〃	〃	〃
60	C	Reddish brown	fair	good	remarkable	fair
	B-1	〃	〃	〃	〃	〃
	B-2	〃	〃	〃	〃	〃
	T-1	〃	〃	〃	〃	〃
	T-2	〃	〃	〃	〃	〃

C: control, B-1: BHA 001%, B-2: BHA 002%, T-1:Tenox-Ⅱ 0.01%, T-2: Tenox-Ⅱ 0.02%

sardine were determined as shown in Table 4. VBN during fermentation increased as fermentation proceeds and this trend agreed with the reports on the changes of VBN in fermented anchovy by Lee and Choe.[25] At the same time, VBN in fermented sardine treated with antioxidant was less than in that the control and in the case of treating with antioxidants BHA less than Tenox-II. VBN in fermented sardine treated with high concentration of antioxidant was less than that with low concentration of antioxidants, too.

Amino-N increased as fermentation proceeds and showed no difference by the kinds and concentration of antioxidants. Amino-N in the control was 230.1 *mg%* in 31 days fermentation, 228.4 *mg%* in 60 days fermentation, and showed no great difference between them. In the case of treating with antioxidants, amino-N has shown to increase more or less with fermentation periods.

But the results of sensory evaluation on these fermented sardine showed that the control and those treated with antioxidants had good taste in 31 days (Table 5). Those results agreed with those of Pyeun *et al.*[26], who reported that fermented anchovy had good taste in 60 and 30 days at 17°C and 27°C, respectively.

3. Changes of acid value, peroxide value and thiobarbituric acid (TBA) value

Fig. 1 shows that the acid value of fermented sardine increased sharply in 19 days, and then slowly after that time. The control had higher acid value than those treated with antioxidants. And the sample treated with 0.01% Tenox-II among various kinds and concentration of antioxidants had the highest acid value and was followed by those of 0.01% BHA, 0.02% Tenox-II and 0.02% BHA in that order. Saruya *et al.*[27] reported that the acid value of salted-dried saury-treated with BHA and Tenox-II increased gradually with fermentation periods and those treated with higher concentration antioxidants had lower acid value.

The peroxide value of the control after 31 days of fermentation was the highest and decreased more or less after then. The peroxide value of that treated with Tenox-II increased slowly up to for 31 days, and then a little decrease was shown. But the peroxide value of those treated with BHA tended to increase slowly up to for 60 days of fermentation. The samples with high concentration of BHA and Tenox-II showed low peroxide value (Fig. 2).

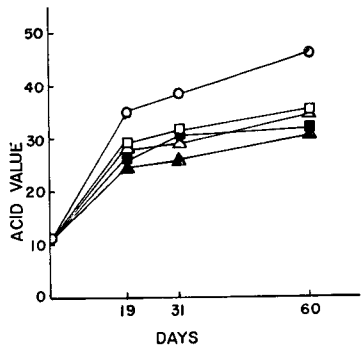

Fig. 1. Changes in acid value during the fermentation of sardine treated with antioxidant.

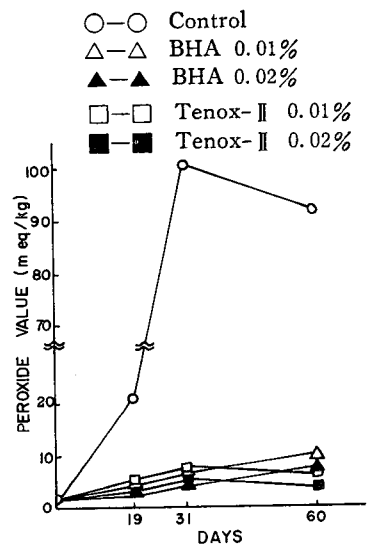

Fig. 2. Changes in peroxide value during the fermentation of sardine treated with antioxidants.

○—○ Control
△—△ BHA 0.01%
▲—▲ BHA 0.02%
□—□ Tenox-Ⅱ 0.01%
■—■ Tenox-Ⅱ 0.02%

Toyama and Saruya [28] reported that when the salted salmon and trout were stored the peroxide value of those treated with 0.015% BHA was more than that of 0.006% BHA. Song [7] reported the peroxide value of fermented anchovy also increased sharply up to for 15 days of fermentation.

The TBA value of the control increased sharply up to for 31 days of fermentation, and then decreased gradually. The peroxide value of fermented sardine treated with antioxidants showed much low in comparison with that of the control, and it showed no great difference up to for 60 days of fermentation. In the case of the samples treated with antioxidants, the samples with 0.02% BHA and 0.02% Tenox-II showed less TBA value than those of 0.01% BHA and 0.01% Tenox-II (Fig. 3).

Suh *et al.* [29] found that when the dressed sardine was put up in the can, the TBA value increased up to for 150 days and then decreased gradually. Song [7] reported that the TBA value of fermented anchovy after 8 days of fermentation reached the peak and showed no great difference up to 3 months. Dakams *et al.* [30] assumed that TBA value decreased after a certain period because the reactivity between TBA and malonaldeyde decreased owing to protein insoluble phenomena by aldehyde.

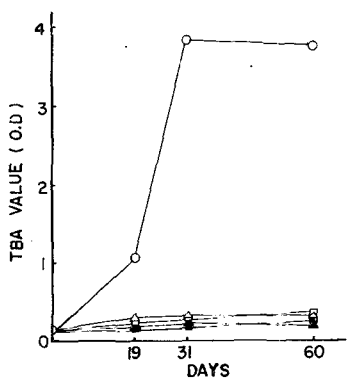

Fig. 3, Changes in TBA value during the fermentation of sardine treated with antioxidants.

○—○ Control
△—△ BHA 0.01%
▲—▲ BHA 0.02%
□—□ TENOX-Ⅱ 0.01%
■—■ TENOX-Ⅱ 0.02%

From the results as above, a conclusion can be reached that fermented sardine after 31 days of fermentation at room temperature (25± 3° C) had better taste than that of 60 days of fermentation (Table 5) and fermented sardine treated with 0.02% BHA as antioxidant showed the least oxidation during 31 days fermentation on the basis of acid, peroxide and TBA values (Fig. 1, 2, 3).

4. The taste compounds

The analysis of the taste compounds in fermented sardine treated with 0.02% BHA after 31 days of fermentation were shown as follows.

1) Nucleotides and their related compounds

The contents of nucleotides and their related compounds were shown in Table 6. Among the contents, hypoxanthine was 67.0 μmole/g, inosine was 35.7 μmole/g, followed by AMP and ADP in that order, and then ATP was 0.1 μmole/g. It was indicated that the most of ATP in original sardine meat were degraded to inosine and hypoxanthine through the main degradation pathway as follows: ATP → ADP→ AMP→ IMP→ Inosine→ Hypoxanthine. It was said that 5′- mononucleotides among nucleotides and their related compounds played an important role in taste of fish [32], and there was a strong natural enhancement of flavors between

Table 6. Contents of nucleotides and their related compounds in fermented sardine after 31 days of fermentation (moisture and salt free base, μmol/g)

Nucleotides and their related compounds	Content
ATP	0.1
ADP	0.9
AMP	0.9
IMP	1.0
Inosine	35.7
Hypoxanthine	67.0

ATP and free amino acids, and AMP and free amino acids. But practically only IMP among nucleotides and their related compounds was considered as the taste compounds because the degradation speed of others was very rapid.

At the present time, the taste of IMP is expected in detail. Fujita and Hashimoto [38] reported that fish meat and frozen products of vertebrata in marine products had plentiful IMP and smoked and canned products contained lots of IMP, but was contained little in plain dried products. Lee[6] reported that 5′- mononucleotides in salted croaker pickle on sale contained much 5′- IMP, and Lee *et al.* [39] reported that great amounts of IMP existed in fermented anchovy. But the taste by IMP itself was not expected because the fermented sardine after 31 days of fermentation had little 5′-IMP contributing to the charateristic taste of fermented sardine by the synergistic taste effect with free amino acids as Hashimoto pointed out [31].

2) Free amino acids

In general, it is said that the amount of some kinds of amino acids in aquatic animal muscle occupies the most part of total amino acids [40-42]. The great portion of free amino acids in the extractives of the fermented sardine was occupied by leucine, glutamic acid, isoleucine, alanine, valine and lysine in turn, and their content was 59.4% of the total free amino acids (Table 7). Lee [14] reported that free amino acids occuping great portion in fermented anchovy were glutamic acid, aspartic acid, and histidine. And Chung and Lee [4] reported there were great amounts of lysine, proline, alanine, glycine, glutamic acid and leucine in fermented small shrimps and they might contribute to the characteristic flavor of fermented small shrimps.

Therefore it is assumed that these free amino acids are the most predominant taste compounds of fermented sardine. And because the essential amino acids in fermented sardine count to about 58.5% of tota free amino acids and especially lysine 7.2% of total amino acids, it is thought that fermented sardine has important meanings from the viewpoint of nutrition in Korea where they eat rice as main foods.

Table. 7. Content of free amino acids in the fermented sardine after 31 days of fermentation

Amino acids	mg%	% to total amino acid	N-mg%
Lys	995.1	7.2	190.7
His	608.1	4.4	164.7
Arg	144.6	1.1	46.5
Asp	685.4	5.0	72.1
Ser	582.7	4.2	77.7
Thr	640.5	4.6	75.3
Glu	1,377.7	10.0	131.2
Pro	229.2	1.7	27.9
Gly	430.7	3.1	80.4
Ala	1,138.0	8.3	178.9
Cys	trace	—	—
Val	1,041.2	7.6	124.5
Met	895.4	6.5	84.1
Ile	1,268.3	9.3	137.4
Leu	2,341.4	17.0	249.8
Tyr	515.8	3.7	39.9
Phe	862.2	6.3	73.1
Total amino acids	13,774.3	100	1,754.2

3) Nitrogenous compounds

The amounts of extractive nitrogen in fermented sardine are listed in Table 8.

It is said that among aquatic animals, invertebrates have plentiful betaine, but it is contained below 0.01% in fish muscle, and this contributes to flesh sweetness [43] [44]. Hayashi [45] reported betaine has contributed to good taste of boiled crab, and it is said betaine in fermented shrimp and squid might contribute to taste with sweet free amino acids and TMAO [45].

TMAO was known to be tasty compound of aquatic animal muscle which have flesh sweet taste. Hayashi *et al* [45] reported TMAO had few relationship with characteristic taste of crab, while Chung and Lee[4] presumed TMAO was important tasty compound, and Lee and Sung [5] assumed TMAO in fermented squid might act as assistant of taste. By the way, because betaine

and TMAO were detected very low content in fermented sardine, it is considered that TMAO and betaine in fermented sardine might act as assistants to taste.

Russel and Baldwin [46] reported creatine was related with bitter and astringent taste of foods. And Konostu *et al.* [47] reported creatine-N consisted of about half of extractive nitrogen in red sea bream, stone flounder and puffer, and about 40% in flounder, jack mackerel, but creatinine-N 2.5% in such fishes.

The content of total creatinine-N in fermented

Table 8. Composition of the extractin fermented sardine after 31 days of fermentation (moisture and salt free base)

Component	mg%	% to Ex-N
Extract-N	4,416.2	—
Nucleotide-N	590.5	13.5
TMA—N	25.9	0.6
TMAO-N	1.0	—
Betaine-N	4.9	0.1
Free amino acid-N	1,754.2	39.7
Ammonia-N	396.2	9.0
Total creatinine-N	475.4	10.8
Recovered-N		73.7

sardine was 10.8% of extractive nitrogen and this result was lower than those of Konosu *et al.* [47] But it is considered to contribute to the characteristic taste of fermented sardine.

4) Omission test

The result of the sensory evaluation of the samples eliminating nucleotides and their related compounds, free amino acids from the broth of the fermented sardine after 31 days of fermentation are given in Table 9. And the numbers are averages of 12 panels by scoring method.

When the sample A was centrifuged, the fermented sardine was given 5 points. The sample B eliminating nucleotides and their related compounds recorded 3 points with better taste than the sample C which eliminated free amino acids, and recorded 2.6 points (Table 9). By the way, the taste of sample D eliminating nucleotides and their related compounds, and free amino acids was lowest of all. From the results, the fact was found that the role of free amino acids such as glutamic acid, lysine, leucine, isoleucine, aspartic acid and histidine in tasting action of fermented sardine was predominant, and a little amounts of 5′-mononucleotides acted as a synergist of taste with these free amino acids. And it was assumed that betaine, creatinine and TMAO played an assistant role in the characteristic taste of fermented sardine.

Table 9. Results of omission test with sardine fermented for 31 days

Sample	Score	Average
A	5 5 5 5 5 5 5 5 5 5 5 5	5
B	3 3 4 2 4 3 3 3 2 4 3 3	3.0
C	3 2 4 2 3 2 3 3 2 3 2 2	2.6
D	1 1 2 1 1 1 2 2 1 2 1 1	1.3

(5:the test of original broth, 0: tasteless)

A : The original broth.

B : The broth from which nucleotides and their related compounds were eliminated by introducing the column of Dower 1×8 (Formic form).

C : The broth from which amino acids were eliminated by introducing the column of Amberlite IR-120(H^+form).

D : The broth from which nucleotides, their related compounds and amino acids were eliminated.

Summary

For the effective utilization of sardine, *Sardinops melanosticta*, one of the major coastal fish in Korea, of which annual catch has been increasing from year to year since 1970, it was processed in form of fermented fish paste. The fish were treated with BHA and Tenox-Ⅱ in concentration of 0.01% and 0.02% to prevent the oxidation of lipid during fermentation and then salted with 20% table salt and fermented at room temperature of 25±3°C. The duration of fermentation necessary for the final product with an

acceptable taste was determined by sensory evaluation by means of profile method.

From the result of sensory evaluation, one month was found to be suitable as the reasonable duration of fermentation.

Both BHA and Tenox-Ⅱ in concentration of 0.02% showed a good preventing effect on the lipid oxidation during fermentation. In case of fermented sardine treated with both antioxidants, lipid oxidation occurred little up to two months, whereas the control showed a remarkable deterioration during one month of fermentation.

Most of the nucleotides in sardine was decomposed from adenosine triphosphate to inosine and hypoxanthine during the fermentation of one month.

The great portion of free amino acids in the extractives of product was occupied by leucine, glutamic acid, isoleucine, alanine, valine and ysine in turn, and their content was 59.4% of the total free amino acids. The amount of essential amino acids was 58.4% of the total free amino acids.

The contents of 5′-IMP, betaine, trimethylamine oxide and total creatinine in the extractives of product were 1.9 μmole/g, 4.9 *mg%*, 1.0 *mg%* and 475 *mg%*, respectively.

According to the omission test, the main constituents of the characteristic taste of fermented sardine could be assumed as free amino acids and a little amount of 5′-IMP.

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