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Comparison of Determination Methods of Amino Nitrogen in Salt-Fermented Anchovy Sauce

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In other to establish the exact determination method of amino nitrogen (AN) in salt -fermented fish sauces, we determined the AN in fish sauces according to the measuring methods and also investigated the main factors influencing on determination method of AN. AN in salt-fermented anchovy sauce increased linearly as fermentation progressed, and was shown the highest amount measuring by the Formol method, followed by the trinitrobenzene sulfonic acid (TNBS) method and the Copper-salt method. AN concentration in anchovy sauces fermented for 12 months was 88.2% and 77.6% for the TNBS method and the Copper -salt method, respectively, on the basis of Formol method. The ratio of AN/total nitrogen (TN) in anchovy sauce fermented for 12 months was higher than that in commercial anchovy sauces. The determination of AN in anchovy sauce by the TNBS method was not affected by salt concentration, and slightly affected by heating. The effect of MSG on AN contents by Copper-salt method was shown higher than those by the Formol method and the TNBS method. The TNBS method was adaptable to measure the content of AN in fish sauce by this study.

Key words: Amino nitrogen, Anchovy sauce, Formol, Copper-salt, TNBS

Introduction

The salt-fermented fish sauce was produced by salting to raw fish and fermenting in a fermentation tank. Total nitrogen (TN) and amino nitrogen (AN) in salt-fermented fish sauce increased linearly by liquefaction of fish meat as fermentation progressed (Cho et al., 1999a, 2000). AN definitely affects the taste of fish sauce because IMP exists in a small amount in fermented fish sauce (Cho et al., 1999b). The AN in various foods is determined by the Formol method (Levy, 1957), Copper-salt method (Spies and Chamber 1951), and TNBS method (Alder, 1979). The determination principle of AN by Formol method is to measure methylene compounds formed by reaction between amino nitrogenous components and formalin. The Copper-salt method is a spectrophotometric assay, measuring blue color formed by interaction between the amino group of amino acids and copper ion. The TNBS method is to measure brown color formed by substitution reaction between the amino group and the sulfonic group.

In Korea, AN in commercial salt-fermented fish sauce was over 600 mg/100 mL, and is usually determined by the Formol method (KFDA, 1999). The measurement of AN by the Formol method was conducted upon sardine sauce (Kim et al., 1990) and electrodialyzed fish sauce (Oh et al., 1997). There were some papers about the measurement of AN in salt-fermented fish sauces by the Copper-salt method (Lee et al., 1989; Bae et al., 1990; Lee et al., 1984, 1988; Oh et al., 1996). However, it is difficult

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to determine correctly AN in salt-fermented fish sauce by the Copper-salt method and the Formol method, because a salt-fermented fish sauce contains high amount of salt with the addition of additives, which might influence in the measurement of AN in salt-fermented fish sauce. Our objectives were to compare the determination methods of AN in salt-fermented anchovy sauce and to investigate the effect of additives (e.g. salt, heating and monosodium glutamate) on the determination of AN.

Materials and Methods

Materials

Anchovy sauce was manufactured by mixing 25% salt (w/w) to raw anchovy. The sample for analysis was prepared after centrifugation (4,000×g, 30 min) and filtration (pore size 1 μm). Commercial anchovy sauces (8 kinds) were purchased from a local market and treated by the same method as the salt-fermented anchovy sauce during fermentation. Effects of salt were also investigated after the adjustment of salt concentration to 5%, 10%, 15% and 20%, respectively, to desalted filtrate of salt -fermented anchovy sauce with electrodialyzer (Asahi model ME-5, Japan). Heating of the filtrate of salt-fermented anchovy sauce was conducted indirectly in a hot water bath on each occasion (20, 45, 60, 90, 120, 150, and 180 min). The effects of monosodium glutamate (MSG) were investigated after addition of commercial MSG to the filtrate of salt-fermented anchovy sauce.

Methods

Moisture, crude ash and total nitrogen of anchovy sauces were determined according to the AOAC method (1990). pH was measured with a pH meter (Orion model 410A, USA). AN contents in all samples were measured by the Formol method, Copper-salt method and TNBS method, respectively. The Formol method was measured as follow: Samples were diluted with 10 volumes, adjusted to pH 8.5 with 0.1 N NaOH and then titrated with 0.1 N NaOH to pH 8.5 after the addition of 25 mL of neutralized formalin. The Copper-salt method was measured as follows: Sample was made up 50 mL and 2 mL was added to 3 mL of copper solution and centrifuged at 3,000 rpm for 15 min after being stirred for 1 min and let stand for 15 min. AN content was calculated from the L-alanine standard curve obtained by optical density at 620 nm. The TNBS method was measured as follow: 125 μL of the sample was diluted with 500 volume and was added to 2 mL of 0.2125 M sodium phosphate buffer, and 1 mL of 0.1% TNBS reagent. After reaction for 30 min at 50℃, the reaction was terminated with 2 mL of 0.1 M sodium sulfite solution. The AN content was calculated from the L-leucine standard curve obtained by optical density at 420 nm.

Results and Discussion

1. Changes of AN according to fermentation periods

The changes of TN and AN in salt-fermented anchovy sauce according to fermentation periods were shown in Fig. 1. TN increased in proportion to fermentation periods and was 2,197 mg/100 mL on 21 months. This indicated a linear relationship between total nitrogen and fermentation periods as

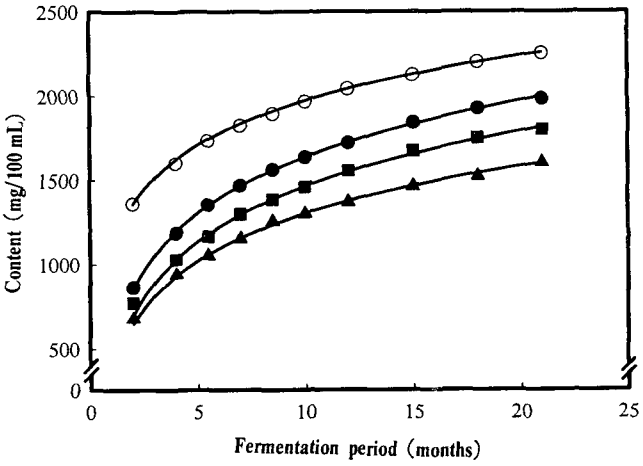

Fig. 1. Change of total nitrogen content (○) and amino nitrogen content determined by Formol method (●), TNBS method (■), and Copper-salt method (▲) in salt-fermented anchovy sauce during fermentation.
Total nitrogen:
$Y=383.09\ (\ln x)+1090.47;\ r^2=0.9992$
Formol method:
$Y=484.62\ (\ln x)+\ 521.32;\ r^2=0.9996$
TNBS method:
$Y=475.11\ (\ln x)+\ 368.69;\ r^2=0.9995$
Copper salt method:
$Y=405.68\ (\ln x)+\ 367.16;\ r^2=0.9998$

follows: Y=383.09 (lnX)+1090.47 (r^2=0.9992), where Y=TN, mg/100 mL; and X=fermentation periods, months.

AN increased almost linearly as fermentation progressed, though the content showed somewhat difference according to measuring methods. Experimental data of the Formol method were fitted to the following equation: Y=484.62 (lnX)+521.32 (r^2=0.9996). The TNBS method indicated the equation as follows: Y=475.11 (lnX)+368.69 (r^2=0.9995). The Copper-salt method indicated the equation as follows: Y=405.68 (lnX)+367.16 (r^2=0.9998). However, the AN by the Formol method were higher than that by the TNBS method and the Copper-salt method. KFDA (1999) regulated the adoption of the Formol method in measuring of AN in fish sauces. In general, fish sauces fermented for 12 months in Korea were marketed and AN in anchovy sauces fermented for 12 months in this study were 88.2% and 77.6% for the TNBS method and the Copper-salt method, respectively, on the basis of Formol method.

Fig. 2 presents the ratio of AN/TN in anchovy sauces as fermentation progressed. The ratio of AN/TN increased almost linearly as fermentation periods. The increase of AN/TN by the Formol method indicated as follows: Y=10.47 (lnX)+58.69 (r^2=0.9985). The TNBS method indicated the equation as follows: Y=11.59 (lnX)+46.70 (r^2=0.9993). The Copper-salt method indicated the equation as follows: Y=8.74 (lnX)+44.94 (r^2=0.9974). The ratio of AN/TN in anchovy sauce fermented for 12 months were 86.4% for the Formol method, 76.1% for the TNBS method and 67.1% for the Copper-salt method. These results demonstrate that AN in anchovy sauce is different significantly according to determination methods. We suggest that the difference of AN in anchovy sauce according to determination methods is caused by the difference of the measuring principle as well as the effect of high salt concentration in fish sauce.

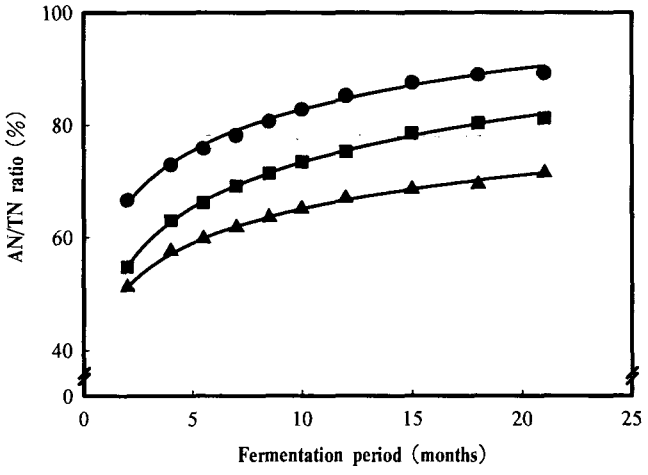

Fig. 2. Change of AN/TN ratio in salt-fermented anchovy sauce determined by Formol method (●), TNBS method (■) and Copper-salt method (▲) during fermentation.
Formol method:
Y=10.47 (lnx)+58.69; r^2=0.9985
TNBS method:
Y=11.59 (lnx)+46.70; r^2=0.9993
Copper salt method:
Y=8.74 (lnx)+44.94; r^2=0.9974

2. AN in commercial anchovy sauces

The contents of AN in commercial anchovy sauces were shown in Table 1. AN in commercial anchovy sauces (635.2~1,375 mg/100 mL by the Formol method, 573.9~1,288 mg/100 mL by the TNBS method, and 520.6~1,157.1 mg/100 mL by the Copper-salt method) were similar to the results of AN in anchovy sauce. In commercial anchovy sauces, AN contents measured by the Formol method was the highest, followed by the TNBS method and the Copper-salt method. However, AN in commercial anchovy sauces by the TNBS method and the Copper-salt method were 90.3~98.1% and 79.1~86.5% respectively, on the basis of the Formol method. AN contents in anchovy sauce fermented for 12 months were 88.2% and 77.6% for the TNBS method and the Copper-salt method, respectively, on the basis of the Formol method (Fig. 1).

The ratios of AN/TN in commercial anchovy sauces were 62.7~76.8% for the Formol method, 58.7~72.5% for the TNBS method, and 52.7~63.1% for the Copper-salt method, and AN/TN in anchovy sauce fermented for 12 months were 86.4% for the Formol method, 76.1% for the TNBS method and 67.1% for the Copper-salt method. The ratio of AN/TN in commercial anchovy sauces were lower than that in anchovy sauce fermented for 12 months. These results imply that it attributes to the addition of brine, MSG, repeated extractive of scrap and analogous fish sauces to salt-fermented an-

Table 1. The contents of total nitrogen (TN) and amino nitrogen (AN) and AN/TN ratio in commercial anchovy sauces determined by Formol, TNBS, and Copper-salt method

(mg/100 mL)

Manufactures	Total nitrogen	Amino nitrogen		
		Formol	TNBS	Copper-salt
A[1]	2,195.4±0.1	1,375.7±0.2 (62.7)[2]	1,288.1±10.6 (58.7)	1,157.1±2.4 (52.7)
E	1,288.0±0.1	864.3±0.2 (67.1)	767.6±10.3 (59.6)	674.5±2.6 (52.4)
F	1,456.0±0.1	902.1±0.2 (62.0)	860.0±25.2 (59.1)	815.4±3.0 (56.0)
G	1,245.6±0.1	868.1±0.3 (69.7)	825.8±14.2 (66.3)	686.5±1.3 (55.1)
H	1,057.9±0.1	735.5±0.1 (69.5)	721.5±15.2 (68.2)	626.5±3.4 (59.2)
J	948.5±0.1	728.6±0.2 (76.8)	687.5±12.3 (72.5)	598.5±2.2 (63.1)
K	882.0±0.1	635.2±0.3 (72.0)	573.9± 9.2 (65.1)	520.6±0.3 (59.0)
N	1,494.4±0.1	952.3±0.2 (63.7)	900.9±10.3 (60.3)	823.3±2.3 (55.1)

[1] Commercial salt-fermented anchovy sauces.
[2] Parentheses were possessed the ratio of amino nitrogen content to total nitrogen content (%).

chovy sauce, as described in our previous paper (Cho et al., 1999b).

3. Effects of salt

Table 2 presents the component compositions before and after desalting of anchovy sauce fermented for 18 months. Salinity and ash content decreased 0.6% and 1.0% from 24.6 and 20.3% after desalting, respectively. Moisture and total nitrogen increased 75.3% and 2.95% from 62.6% and 2.21% after desalting, respectively. These results were similar to the report of Oh et al. (1997). Table 3 show AN contents before and after desalting of anchovy sauce fermented for 18 months at different determination methods. The content of AN in anchovy sauce fermented for 18 months was the highest by the Formol method, followed by the TNBS method and the Copper-salt method. Also, the AN contents in desalted anchovy by electrodialyzer for 4.5 hr were similar to both in the Formol method and the TNBS method, and lower in the Copper-salt method. These results suggest that the AN by the Copper-salt method shows lower

Table 2. The contents of moisture, total nitrogen, ash, pH and salinity in anchovy sauce fermented for 18 months before and after desalting

Desalting	Moisture (%)	Total nitrogen (%)	Ash (%)	pH	Salinity (%)
Before	62.6±0.6	2.21±0.13	20.3±0.9	5.75	24.6
After	75.3±0.6	2.95±0.10	1.0±0.3	6.06	0.6

Table 3. The contents of amino nitrogen determined by Formol method, TNBS method and Copper-salt method in anchovy sauce fermented for 18 months before and after desalting

(mg/100 mL)

Desalting	Formol	TNBS	Copper-salt
Before	2,273.1±0.1	1,792.8±10.3	1,720.6±2.0
After	3,230.1±0.1	3,218.1±10.3	2,924.5±2.0

value due to the following reasons: the majority of glutamic acid and aspartic acid combine to the ratio of one to one with copper ion (e.g. CuA, A=1 amino acid molecule). The increase of AN after desalting was due to the condensation by a decrease of salt with desalting.

The effect of salt, after the addition of salt to the desalted anchovy sauce, was shown in Fig. 3. The AN contents decreased with the increase of salt added in the Formol method and the Copper-salt method, with no difference in the TNBS method. These results suggest that salt concentration in anchovy sauce has a critical influence on determination of AN by the Formol method and the Copper-salt method. We also thought that the TNBS method was affected by dissociated ion (Na^+ and Cl^-) in solution, because substitution reaction between amino group and sulfonic group generate brown with 0.1 M sodium sulfite. We thought that the TNBS method was suitable to determination of AN in salt-fermented fish sauce, because AN content in anchovy sauce by the TNBS method was similar to that by the Formol method after desal-

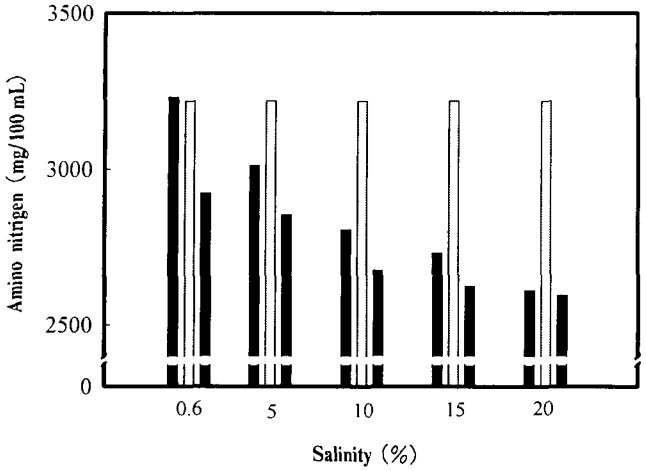

Fig. 3. Effect of NaCl concentration on the content of amino nitrogen contents determined by Formol method (■), TNBS method (□) and Copper-salt method (■) in salt-fermented anchovy sance.

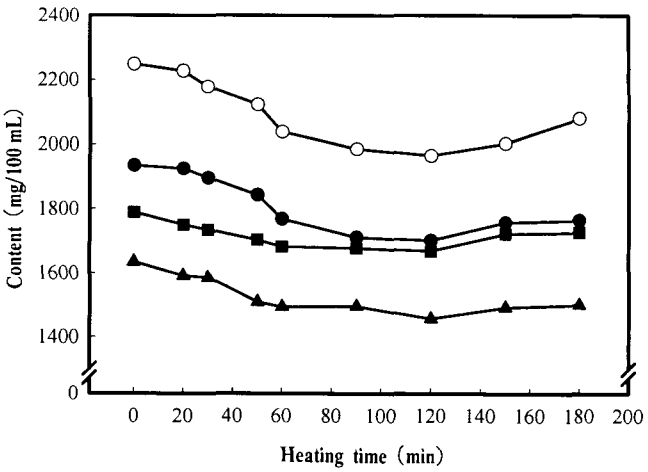

Fig. 4. Changes of total nitrogen content (TN, ○) and amino nitrogen content (AN) determined by Formol method (●), TNBS method (■) and Copper-salt method (▲) in salt-fermented anchovy sauce during heating.

ting, differently from the copper-salt method, and was not affected by salt added after desalting.

4. Effects of heating

Generally, some manufactures have very often marketed products which fitted to a quality standard with addition of brine and repeated extractives to raw salt-fermented fish sauce in Korea (Lim, 2000). Fish sauces (nampla, nuocman, buda, patis, and shottsuru) were produced in south-east Asia and Japan have been marketed after heating of fermented fish sauces for the purpose of preservation (Adames et al., 1985; Saisithi, 1994; Sato, 1990; Lee et al., 1996). Lim (2000) reported that the ratio of AN/TN increased in proportion to extraction times when the scrap of fermented anchovy sauce with saturated brine was heated. Fig. 4 presents the changes of TN and AN when anchovy sauce fermented for 18 months was heated. The TN and AN decreased at the early stage of heating and increased after 120 min. These results imply that the decrease of TN and AN at the early stage attributed to precipitation by interaction of peptides and the increase at a later stage was caused by condensation with heating. The AN, during heating of anchovy sauce, showed a significant difference according to determination methods and was the highest in the Formol method, followed by the TNBS method and the Copper-salt method. Meanwhile, a slight change of AN according to heating time in the TNBS method is due to having no influence of salt condensation with heating as shown Fig. 3.

5. Effects of MSG

Artificial nitrogen compounds (e.g. MSG, IMP), organic acid, and glucose were often added to commercial fish sauces for the purpose of increasing AN content and improvement of taste. Lim et al. (2000) reported that these additives had no influence on changes in color in commercial fish sauce during storage. The AN content, after addition of MSG in anchovy sauce fermented for 18 months, was shown in Fig. 5. The AN in anchovy sauce increased with increasing of MSG. The changes of AN contents showed the most remarkably in the Copper-salt method of three determination methods as MSG concentration. This suggest that the Copper-salt method could not be suitable method in the determination of AN when MSG add to anchovy sauce. So, We suggest that TNBS method in determination AN of anchovy sauce is appropriate method, because TNBS method of three methods was affected the lowest influence on salt, heating, and MSG in salt-fermented anchovy sauce.

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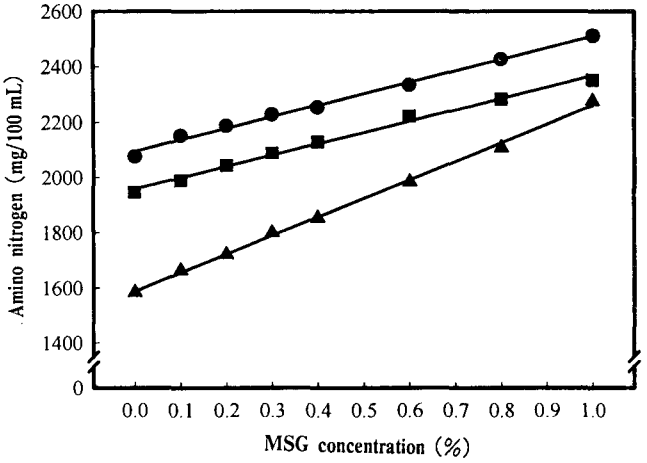

Fig. 5. Change of amino nitrogen content deter-mined by Formol method (●), TNBS method (■), and Copper-salt method (▲) in salt-fermented anchovy sauce by addtion of MSG.
Formol method:
$Y=413.94X+2094.60$; $r^2=0.9969$
TNBS method:
$Y=408.09X+1957.79$; $r^2=0.9966$
Copper salt method:
$Y=671.63X+1587.31$; $r^2=0.9989$

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