Processing Conditions for Low-Salted Squid Jeotkal

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Low-salted and fermented squid products, squid jeotkal was prepared and fermented at 10℃. During fermentation of squid, microbiological and chemical changes were examined. Sensory evaluation was also carried out. After 20 days of fermentation, taste and flavor of the squid jeotkal containing 10% NaCl were proven to be the best; in contrast, the jeotkal with 7% NaCl exhibited the highest sensory score. At the period showing the best flavor and taste, viable cell count reached to $10^8/g$. Throughout the fermentation period, types of microorganisms isolated were significantly different in squid jeotkal of different salinity. In general, protease producer and bacteria producing components of jeotkal-flavor and organic acids more likely contributed to producing the jeotkal of the best quality. pH of the squid jeotkal with 10% salinity maintained under pH 7.0 throughout the fermentation periods; however, in the case of the jeotkal with 7% salinity, pH increased over pH 7.0 after day 25. Similar tendency was observed in the results from VBN and hypoxanthine formation. Total nitrogen was decreased as fermentation proceeded; in contrast, total free amino acids were increased.

Introduction

Jeotkal, one of the most beloved traditional food of Korea used to be prepared in home-made scale until recently. However, the rapid changes in dwelling patterns have made the jeotkal industry compatible with its home-made counterpart. It is confirmed by the steady increase in sales of the ready-made jeotkal. And yet, the large scale process has not been fully developed only to follow the traditional methods. To preserve jeotkal for long period of time, salt has been added to the jeotkal at very high concentration. This causes a lot of health problems such as hypertension and nephritis. For this reason, low salted jeotkal appears to be the attractive product for the consumers sensitive to the health problem. So far, only a few researches have been carried out to reduce the salt concentration in jeotkal(Lee *et al.*, 1982; Mori *et al.*, 1980). Partial substitution of NaCl with KCl was also tried by several researchers(Marsh, 1983; Camirand *et al.*, 1983; Cha and Lee, 1985). However, most of these attempts were proven to have some drawbacks such as reduced shelf life and poor flavor and taste.

To cope with these limitation of jeotkal manufacturing process, squid was selected for model study. Salt concentrations of 3, 5, 7, and 10% were employed and fermentation was carried out at low temperatures rather than room temperature. This process will be more suitable considering the marketing of jeotkal product, which employs cold chain system. By evaluating the changes in microbial population, sensory and chemical properties, we intend to determine the optimal conditions for preparing low salted squid jeotkal with low fermentation temperatures.

Materials and Methods

Preparation of the squid jeotkal

Squid(*Todarodes pacificus*) was purchased from local market as frozen state. The average body length and weight were 30 ± 5*cm*, 130 ± 20*g*, respectively. After removing viscera, squid was sliced at 5*mm* thickness. To 20*kg* of squid, NaCl was added to make the final concentration of 3, 5, 7, and 10% (w/w); 3, 5, 7, and 10% salted squid will be designated as 3, 5, 7, and 10% SSJ, respectively. The salinity of the raw material was 1.0%. Fermentation was carried out at 7℃ and 10℃ for 40 days. During the fermentation, changes in microbial population, sensory and chemical properties were evaluated at five day interval.

Analysis of the squid jeotkal

Moisture was determined on 5*g* of homogenized samples by placing them in a drying oven at 105℃ and determining the weight loss. Structural nitrogen was estimated by semi-micro Kjeldhal method after washing the sample with 0.9% saline. pH and salinity were determined by pH meter and $AgNO_3$ titration, repectively(A.O.A.C., 1984). Volatile basic nitrogen(VBN) was estimated by Conway's method (Miwa and Iida, 1973) using conway unit and free amino acids by using o-phthalodialdehyde(Ryu *et al.,* 1988). Hypoxanthine contents were measured by the Ke and Burns' method(1989). 5.0*g* of each sample was extracted with 0.6M perchloric acid(50 *ml*), and then filtered, the filtrate was neutralized with 4.0N KOH and an aliquot was injected on the HPLC. Separation of Hypoxanthine was performed with an C_{18} column(Finepak SIL-C18, JASCO). The mobile phase and flowrate were 0.01M KH_2PO_4 and 1.5*ml/min*, respectively.

Isolation and identification of microorganisms

Medium for isolation or viable cell count was prepared by adding 1.5% agar and 6.5% NaCl to brain heart infusion medium(BHI medium, Difico, U. S. A.). Each sample was mixed with 6.5% saline water to give 10% by weight and homogenized with waring blender at 10,000 rpm for 90 sec. Aliquots of 0.2*ml* were spreaded on the BHI agar plates and incubated at 20℃. After 40~72*h*, colonies were counted and isolated. The isolated colonies were identified according to the method of Krieg and Holt(1984) and morphorlogical and biochemical test were undertaken by the method for Harrigan and McCance(1976).

Comparative sensory assessment

Sensory evaluation of the squid jeotkal was carried out by the panel of ten members. Five different description were employed to grade the overall quality in terms of flavor, taste, texture, and color: excellent, good, acceptable, unacceptable, and poor. 'Poor' corresponded to 1.0, indicating the beginning of spoilage and 'Excellent' to 5.0. 'Unacceptable' meant that consumers could notice the bad changes in quality of the products. To have a commercial value, grade should be either 'good' or excellent because these products will be displayed and sotred for substantial period of time until consumption.

Statistical analysis

To detect significant differences in the results of the sensory evaluation and other analyses, data were treated statistically using SPSS. The subprogram, ONEWAY, which was specifically designed for a one-way analysis of variance, was employed using a significance level of 0.05. If significant differences were present, Duncun's multiple range test was performed using a significance level of 0.05.

Results and Discussion

Sensory evaluation

Overall quality of the squid jeotkal was evaluated by sensory panel. Sensory evaluation was based on color, texture, flavor, and most importantly, the taste. The grading guidelines for quality evaluation of the squid jeotkal are shown in Table 1. As shown in Table 2, salt concentration of 10% proved to produce the best products at 10℃ in overall quality followed by the 10% salted jeotkal at 7℃. As expected, putrefaction was observed in 3% and 5% salted squid jeotkal(SSJ) even at 7℃. Thus, 7% and

Table 1. Sensory grading guidelines for the jeotkal

Grade	Overall quality	Criteria			
		Color	Texture	Taste	Flavor
5	Excellent	White translucent	Elastic firm	Excellent	Pleasant
4	Good	Translucent	Firm	Good	Good
3	Acceptable	Brownish translucent	Reasonably firm	Acceptable	Acceptable
2	Unacceptable	Opaque	Soft	Unacceptable	Some odor
1	Poor	Green-yellow opaque	Very soft	Poor	Very offensive odor

Table 2. Sensory evaluation of low-salted squid jeotkal fermented for 20 days.

Temperature (℃)	Salinity(%, w/w)			
	3.0	5.0	7.0	10.0
7.0 ± 0.5	1.1^{a}	1.3^{a}	3.1^{b}	3.9^{c}
10.0 ± 0.5	1.1^{a}	1.5^{a}	3.4^{b}	4.5^{d}

* Scores graded by 10 panelists were evaluated by Duncan's multiple range test. Significant differences were detected by different superscripts.

10% SSJ were selected for the further study and fermented at 10℃. Sensory evaluation was carried out every 5 day.

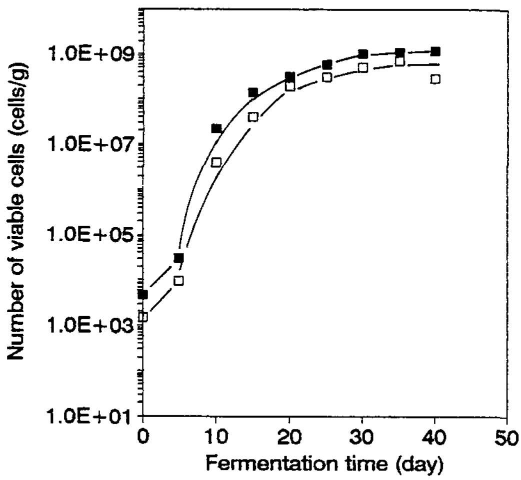

Fig. 1. Changes in viable cell counts of squid jeotkal with 7%(■) and 10% total salinity(□) when fermented at 10℃.

Table 3. Sensory evaluation of low-salted squid jeotkal fermented at 10℃.

Salinity (%, w/w)	Fermentation time(day)					
	10	15	20	25	30	35
7.0	3.2^{c}	3.9^{e}	3.4^{c}	3.1^{b}	2.6^{a}	2.3^{a}
10.0	–	3.7^{c}	4.5^{f}	4.0^{e}	3.8^{d}	3.6^{c}

* Scores graded by 10 panelists were evaluated by Duncan's multiple range test. Significant differences were detected by different superscripts.

Changes in microbial population and sensory assessments

During fermentation of 7% and 10% salted squid jeotkal, changes in viable cell count and sensory assessments were shown in Fig. 1 and Table 3, repectively. Flavor and taste of the jeotkal were found to be the best when viable cell count reached to $10^8/g$. At 10℃ it took 15 and 20 days to reach that number for 7% and 10% SSJ, repectively. 10% SSJ proved to be better than 7% counterpart by sensory evaluation. Moreover, in 10% SSJ viable cell count did not increase to $10^8/g$ until day 40. 7% SSJ, on the other hand, showed increase in viable cell count over $10^8/g$ on day 30 and significant loss of quality was followed.

Significant relationship was observed between sensory evaluation and changes in viable cell count. The index of well fermented jeotkal would be viable cell count of $10^8/g$ and that of $10^9/g$ should be considered as the beginning of quality deterioration for 7% SSJ. This result implies that once well fermented, jeotkal should be stored at low temperature to prolong the shelf life.

Bacteria involving fermentation or squid jeotkal were isolated and identified(Table 4). Four types

Table 4. Morphological and biochemical characteristics of strains isolated from fermented Squid

	Characteristics																	
													Acid from:					
	Shape	Gram	Mot	Cat	Oxi	H_2S	Ind	MR	VP	Cit	Gel	Cas	O/F	Lac	Fru	Dex	Suc	Man	Sal
Staphylococcus xylosus	coccus	+	−	+	−	−	−	−	−	−	−	−	−/+	+	+	+	+	+	±
Micrococcus varians	coccus	+	−	+	−	−	−	−	−	+	−	±	+/−	−	+	+	+	+	−
Unidentified coccus type 1	coccus	+	−	+	+	−	−	−	−	−	−	−	NG	−	−	−	−	−	−
Unidentified coccus type 2	coccus	+	−	+	−	−	−	+	−	−	−	−	NG	−	−	−	−	−	−
Pseudomonas diminuta	rod	−	+	+	+	−	−	−	−	−	−	±	+/−	−	+	+	+	+	+
Flavobacterium odoratum	rod	−	−		+	−	−	−	−	−	−	+	−/−	−	−	−	−	−	−
Pseudomonas sp. D1	rod	−	+	+	+	−	−	−	−	−	−	−	+/−	+	+	+	+	−	−
Pseudomonas sp. D2	rod	−	+	+	+	−	+	−	−	+	+	+	−/−	−	−	−	−	−	−
Acinetobacter calcoaceticus	cocco-bacillus	−	−	+	−	−	−	−	−	+	+	+	+/−	+	+	+	+	−	±
Aeromonas sp. D3	cocco-bacillus	−	+	+	+	−	+	+	−	+	−	−	−/+	+	+	+	+	−	+

Gram; Gram stain, Mot; Motility test, Cat; Catalase test, Oxi; Oxidase test, Ind; Indole test, MR; Methyl-red test, VP; Voges- roskauer test, Cit; Citrate test, Gel; Gelatin liquefaction, Cas; Casein test, O/F; Oxidation and fermentation of glucose, Lac; Lactose, Fru; Fructose, Dex; Dextrose, Suc; Sucrose, Man; Mannitol, Sal; Salicin.

of cocci, four types of bacillus, and two types of coccobacilli were isolated. *Micrococcus varians* was the most dominant coccus throughout the fermentation. Interestingly, *Staphylococcus xylosus* grew well in 10 % SSJ but seldom in 7% SSJ. Type 1 and 2 were unable to be identified and appeared only in the initial period of fermentation. In the case of bacilli, *Pseudomonas diminula, Pseudomonas* spp. D1 and D 2, and *Flavobacterium odoratum* were isolated.

When 7% SSJ was fermented at 10℃, *F. odoratum* and *M. varians* were isolated; as fermentation proceeded, their numbers increased dramatically to the maximum in between day 15 and 20(Fig. 2). In that period the flavor and taste were proven to be the best in 7% SSJ. Besides, *Pseudomonas* spp. D2 and *P. diminuta* were detected in high numbers during the same period. With *F. odoratum* and *M. varians*, they were likey to be involved in jeotkal ripening. *F. odoratum* and *Psudomonas* spp. D2 were protease producer but were not able to utilize sugars to produce acids; on the contrary, *M. varians* and *P. diminuta* were unable to produce proteases but able to utilize sugars. Thus, the unique flavor and taste of the squid jeotkal seemed to be the combined effect of these two kinds of bacteria with different biochemical characteristics.

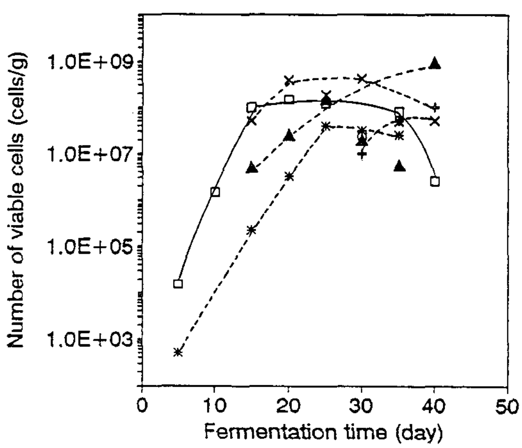

Fig. 2. Changes in microbial population isolated from squid jeotkal of 7% total salinity when fermented at 10℃.
Each microorganism was designated as follows: *, *Flavobacterium odoratump* □, *Micrococcus variars;* +, *Pseudomonas* spp. D1; ▲, *Pseudomonas* spp. D2; ×, *Pseudomonas diminuta.*

Rather different tendency was observed in 10%

SSJ(Fig. 3). *Pseudomonas* spp. D1 and D2, *P. diminuta, M. varians,* and *S. xylosus* were detected in high number during the period between day 20 and 30 when the flavor and taste were the best. *S. xylosus, P. diminuta,* and *M. varians* in particular were thought to mainly involve in fermentation but these three species did not contain proteolytic activities. Hence, Hydrolysis of the muscle protein, however, could be attributed to *Pseudomonas* spp. D2 considering their steady increases in cell numbers during the later period of fermentation.

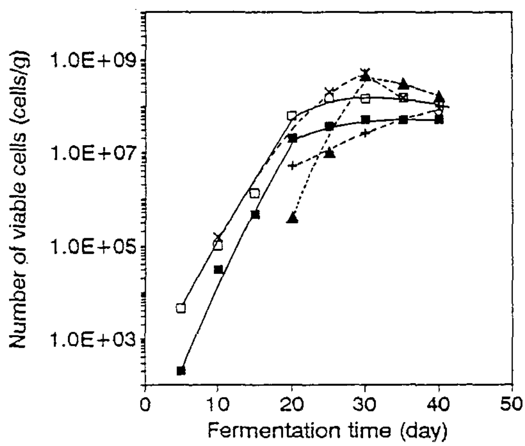

Fig. 3. Changes in microbial population isolated from squid jeotkal of 10% total salinity when fermented at 10℃.
Each microorganism was designated as follows: ■, *Staphylococcus xylosus;* □, *Micrococcus variars;* +, *Pseudomonas* spp. D1; ▲, *Pseudomonas* spp. D2; ×, *Pseudomonas diminuta.*

Changes in pH

Changes in pH during fermentation at 10℃ were monitored(Fig. 4). During the first 15 days, no change in pH was observed for 7% salted jeotkal and then large increase in pH was observed. On day 25, pH came to be over pH 7.0, suggesting possible loss of commercial value of the product. On the other hand, for 10% SSJ, pH was dropped slowly until day 25 followed by substantial increase; however, pH did not go over pH 7.0 even on day 35, suggesting the product still maintains good quality.

When the results of viable cell count and sensory assessments were compared with the pH changes, close relation of pH changes to jeotkal quality became more evident. When viable cell count reached at $10^8/g$, jeotkal turned out to have the best flavor; in addition, pH value was lower than pH 7.0. pH value exceeded pH 7.0 as viable cell count rose to $10^9/g$. For 10% SSJ, viable cell count and pH did not go beyond $10^8/g$ and pH 7.0 throughout the experiment, respectively. Consequently, pH could be used as an indicator to determine the quality of jeotkal; pH 7.0 was the upper limit to keep the good quality.

During the initial period of fermentation, slight decrease in pH was observed regardless of the salinity of jeotkal. In conjunction with biochemical study and their growth curves of bacteria isolated from jeotkal, this phenomena seemed to be partly due to the rapid growth of the bacteria producing acids when utilizing sugars in muscles. In addition, the acids produced might activate proteases which were believed to play an important role in fermentation of jeotkal. To acquire squid jeotkal of excellent quality, total salinity of 10% and fermentation temperature of 10℃ are recommended.

Changes in VBN and Hypoxanthine(Hx)

As shown in Fig. 5, VBN sharply increased after day 20 in the case of 7% SSJ when fermented at

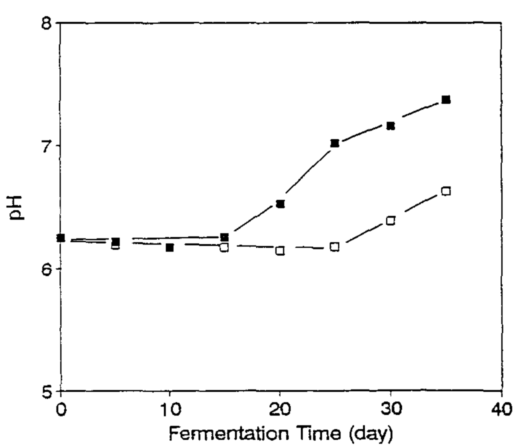

Fig. 4. Changes in pH of squid jeotkal with 7%(■) and 10% total salinity(□) when fermented at 10℃.

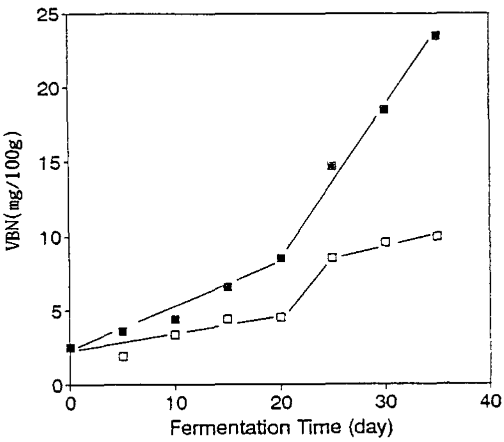

Fig. 5. Changes in VBN of squid jeotkal with 7%(■) and 10% total salinity(□) when fermented at 10℃.

10℃ and reached to 35*mg*/100*g* on day 35(Fig. 5). In contrast, 10% SSJ did not show such a large increase in VBN as 7% counterpart. VBN level of 10 % SSJ did not increase significantly after day 25 and the values remained very close to that of 7% salted one on day 20. According to sensory evaluation, acceptability of 10% SSJ sharply declined after day 25; on the contrary, 10% salted one did not lose its acceptability throughout the test period. Therefore the result of VBN changes show significant relation to sensory evalution. However, to evaluate the commercial value of the squid jeotkal, VBN alone would not be sufficient to represent it.

In this respect, hypoxanthine was selected as a quality parameter for jeotkal grading(Fig. 6). Hypoxanthine formation patterns were similar to VBN patterns. During fermentation at 10℃ Hx levels rose steadily in both samples; however, 7% salted jeotkal showed more dramatic increase than 10% counterpart. Hx levels in 10% salted jeotkal remained below 0.5 *μmolg/g* throughtout the experiment; moreover, even up to day 35, all the samples were pronounced to be acceptable or more than acceptable by sensory evaluation. In contrast, Hx levels in 7% SSJ increased to more than 0.5 *μmolg/g* on day 30; in fact, sample on day 30 obviously lost its commercial value according to sensory evaluation.

As a quality indicator, VBN and Hx contents were compared with the results from sensory assessments. VBN in 7% salted jeotkal remained below 25*mg*/100*g* throughout the experiment; in contrast, Hx levels went beyond 0.5 *μmolg/g* on day 30. Hx levels of 0.5 *μmolg/g* were used to assess the quality of surimi by Ke and Burns(1989) and results from this study well agreed with their standard. For 10% SSJ, VBN and Hx contents remained below 10*mg*/100*g* and 0.5 *μmolg/g* throughout the fermentation, repectively. In the viewpoint of sensory assessments, Hx levels proved to be more acceptable standard than VBN levels.

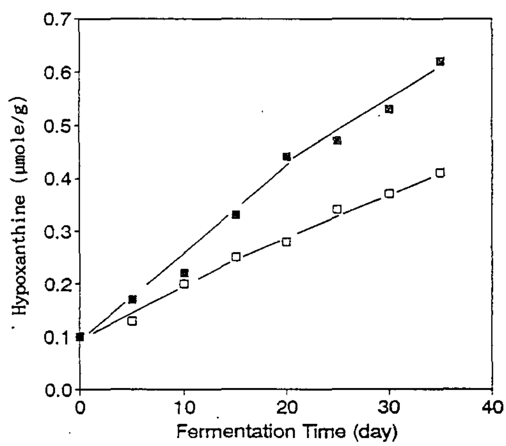

Fig. 6. Formation of hypoxanthine in squid jeotkal with 7%(■) and 10% total salinity(□) when fermented at 10℃. (moisture-free and salt-free basis).

Changes in total free amino acids

Total free amino acids decreased during the initial 5 days and then increased regardless of salinity of the jeotkal(Fig. 7). Free amino acids in muscles of squid seemed to be utilized by the bacteria for growth and then the proteases produced from them resulted in increase of free amino acids. When the flavor of jeotkal was the best(on day 15 and 20 for 7% and 10% SSJ, respectively), peaks were observed in total amino acids curves. Any significant increase in total free amino acids was not observed for 10% SSJ after day 20 but 7% SSJ showed considerable increase after day 20. It was coincided with the fact that protease producer, *Pseudomonas* spp. D3 grew remarkably during the later part of fermentation in 7% SSJ. On the contrary, *Pseudomoans* D3 began to decrease after day 30 in 10% SSJ and *Pseudomonas* D1, a possible acid producer maintain high numbers. The increase in acid producer and decrease in protease producer appeared to be able to maintain the good quality of 10% SSJ.

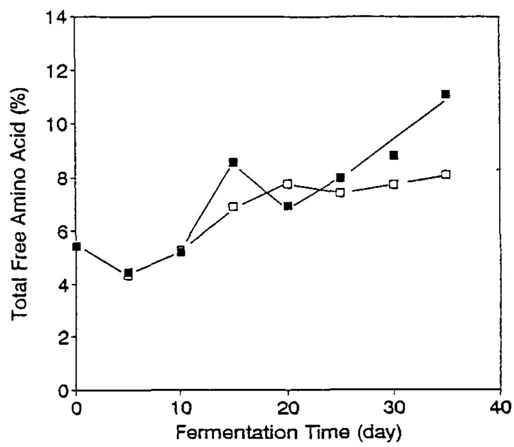

Fig. 7. Formation of total free amino acid in squid jeotkal with 7%(■) and 10% total salinity(□) when fermented at 10℃. (moisture-free and salt-free basis).

Structural nitrogen contents

Structural nitrogen decreased as fermentation proceeded regardless of salinity of the jeotkal(Fig. 8). Thus it confirmed that protease played an important role in jeotkal production. Interestingly Structural nitrogen contents were almost the same for both jeotkals at the time of the best flavor(on day 15 and 20 for 7% and 10% SSJ, repectivlely). Therefore, a certain amount of protein had to be hydrolyzed to give the desirable flavor.

To produce the squid jeotkal of good quality, we recommend the following coditions for large scale process; Jeotkal with total salinity of 10% should be fermented at 10℃ for 20~25 days followed by storing at 3℃ or lower temperatures. In our unpublished data microbial action was slowed dramatically if the jeotkal was stored below 3℃. By using this process, salt concentration and fermentation time can be remarkably reduced; consequently, flavor will be enhanced and the cost of product will be lowered. In addition, during the fermentation of the jeotkal at 10℃. We found that change in microbial population, chemical and sensory properties were closely related each other. Thus, the quality changes in the jeotkal could be assessed by measuring pH or viable cell count. Further research is in progress to provide criteria for evaluating quality of the jeotkal during fermentation.

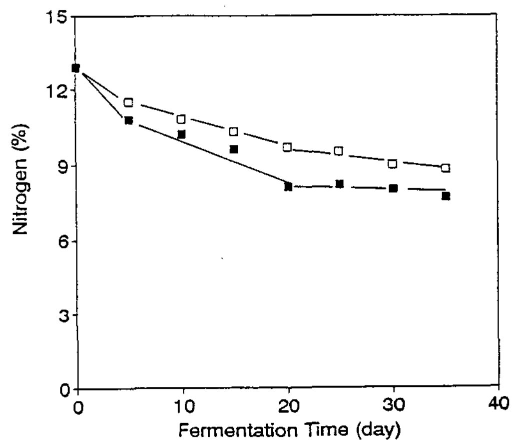

Fig. 8. Changes in total nitrogen of squid jeotkal with 7%(■) and 10% total salinity(□) when fermented at 10℃. (moisture-free and salt-free basis).

Acknowledgement

This paper was supported by the fund from Research Institute of Korean Food and Dietary Culture, MIWON, 1991.

References

A. O. A. C. 1984. Official methods of analysis. 12th ed., Association of official analytical chemists. Washington D. C.

Camirand, W., J. Randall, K. Popper and B. Andich. 1983. Low sodium/high potassium fermented sauces. Food Technol. 37(4), 81~85.

Cha, Y. J. and E. H. Lee. 1985. Studies on the processing of low salt fermented seafoods: 5. Processing conditions of low salt fermented anchovy and yellow corvenia. Bull. Korea Fish. Soc. 18(3), 206～21.

Harrigen, W. F. and M. E. McCance. 1976. Laboratory methods in food and dairy microbiology. Academic Press Co., pp. 21～78.

Ke, P. J. and B. G. Burns. 1989. Recommended objective methods for surimi quality evaluation based on hypoxanthine and free fatty acids formation. Lebensm.-Wiss. u-Technol. 22, 93～97.

Krieg, N. B. and J. G. Holt. 1984. Bergey's manual of systematic bacteriology. Vol. 1. Williams and Wilkins Co., pp. 140～598.

Lee, E. H., K. S. Kim, J. K. Jeon, S. H. Nam and J. G. Kim. 1982. The taste compounds of fermented anchovy. Bull. Nat. Fish. Univ. Pusan, 22(1), 13～18.

Marsh, A. C. 1983. Process and formulations that affect the sodium content of foods. Food Technol. 37(7), 45～49.

Miwa, K. and Iida, H. 1973. Studies on ethylacahol dtermination in "Shiokara" by the microdiffusion method. Bull of the Jap. Soc. of Sci. Fish. 39(11), 1189～1194.

Mori, K., H. Shinano and M. Akiba. 1980. Histological changes of "Ika-shiokara" during the ripening process. Bull. Jap. Soc. Sci. Fish. 46(10), 1287～1292.

Nie, N. H., Hu, C. H., Jenkins, J. G., Steinbrenner, K. and D. H. Bent. 1975. Statistical package for the social sciences. 2nd ed., McGraw-Hill Book Co., New York. p. 675.

Ryu, H., Moon, J. and Lee. K. 1988. Conditions of quantitative analysis for free amino acid in fermented proteins. J. Korean Soc. Food Nutr. 17 (2), 136～143.

Received February 17, 1993
Accepted July 3, 1993

저식염 오징어젓갈 제조 조건

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저식염 오징어젓갈을 제조하기 위하여 10℃에서 발효시키면서 관능적, 미생물학적 및 이화학적 품질 변화를 조사하였다. 염도가 10%인 제품의 경우, 발효 20일 째에 관능적으로 가장 우수한 품질을 나타내었으며 7% 식염제품은 15일 째에 맛과 냄새가 좋았다. 두 제품 모두 이 시점에서 생균수가 $10^8/g$으로 나타났으며 발효 기간에 따라 분리된 균주의 종류는 염농도에 따라 차이가 있었다. 단백질 분해효소 생산균주와 젓갈 고유의 냄새 성분과 산을 생산하는 균주가 함께 젓갈의 품질 형성에 기여하는 것으로 나타났다. pH는 10% 제품의 경우 35일 째까지 pH 7.0 이하를 유지하였으나, 7% 제품은 25일 째부터 pH 7.0 이상이 되었다. 이러한 경향은 VBN과 Hypoxanthine 생성량 등의 결과와 유사하였다. 오징어 젓갈의 총질소량은 발효 시간이 경과함에 따라 감소하였고 이에 따라 총 유리 아미노산은 증가하는 경향을 보였다.