Korean J. Food & Nutr. Vol. 26. No. 4, 678~684 (2013)
http://dx.doi.org/10.9799/ksfan.2013.26.4.678

THE KOREAN JOURNAL OF
한국식품영양학회지
FOOD AND NUTRITION

Influences of Squid Ink Added to Low Salt Fermented Squid on Its Changes in Lactic Acid Bacteria

†Sung-Cheon Oh
Dept. of Food & Pharmacy, Daewon University College, Jecheon 390-702, Korea

저염 오징어 젓갈의 숙성 중 오징어 먹즙 첨가가 젖산균의 변화에 미치는 영향

†오 성 천
대원대학교 제약식품계열

국문요약

오징어 먹즙을 첨가하지 않은 저염 오징어 젓갈은 숙성 발효에 따라 숙성 초기에 젖산균 총수가 급속히 증가하고, *Leuconostoc*이 증가하여 숙성 적기에 최대량을 나타내고 이후 서서히 감소하였다. *Lactobacillus*는 숙성 적기 이후의 총 균수의 대부분을 차지할 만큼 균수가 많아지므로 숙성 적기 이후 품질에 관여하는 것으로 보인다. *Streptococcus*와 *Pediococcus*는 숙성 적기까지 완만하게 증가하다가 이후 급격히 감소하였다. Yeast는 숙성 중기 이후에 검출되고, 숙성 말기에 급격히 증가하므로 부패에 주로 관여하는 것으로 보인다. 오징어 먹즙을 첨가한 저염 오징어 젓갈의 숙성발효에 따른 젖산균의 변화를 보면 총 균수는 숙성 중반까지 증가하다가 중반 이후 약간 감소하였으나, 최대치에 도달하는 기간은 무 첨가군에 비하여 길었고, 젖산균 중 *Leuconostoc*, *Streptococcus* 및 *Pediococcus*는 숙성 후반까지 증가하였다가 후반에 감소하는 반면에, *Lactobacillus*는 숙성 후반까지 계속 증가하였다. Yeast는 숙성 초기에는 거의 증가하지 않다가 숙성 중반 이후 증가하였는데, 이러한 경향은 무 첨가군과 유사하였으나 젖산균의 수가 무 첨가군에 비하여 적었다. 오징어 먹즙을 첨가한 저염 오징어 젓갈에서 모든 균들의 수가 감소되어 오징어 먹즙이 균들의 성장을 억제하는 것으로 확인되었다.

Key words: lactic acid bacteria, low salt fermented squid, squid ink, yeast, ripening

Introduction

Fermented squid is a fermented seafood product which contains unique flavor caused by a reaction between protease produced by microorganisms and during the ripening stage. The various microorganisms in fermented seafood contribute to preservability as well as flavor and color of the product (Shinano et al. 1975).

Research proved the proliferation distribution of *Micrococcus* and *Staphylococcus* using the microorganisms separated and identified from 10%, 15%, and 20% salt concentration fermented squid experimental groups and also determined *Staphylococcus* is a dominant strain in the ripening stage regardless of the salt concentration although higher salt concentration reduces the bacterial count in an extract (Mori et al. 1979; Mori et al. 1983).

Study reported *Bacillus, Corynebacterium, Lactobacillus, Moraxella, Micrococcus, Acinetobacter, Staphylococcus, Streptococcus*, and extra have been homogenized from fermented squid and *Staphylococcus aureus* has been detected from fermented squid 4-7% of low salt concentration (Fujii et al. 1991; Fujii 1984; Matsubara et al. 1994).

Study reported that squid intestine contributes an important role in forming a special mycoflora in fermented squid (Yamazaki

† Corresponding author: Sung-Cheon Oh, Dept. of Food & Pharmacy, Daewon University College, Jecheon 390-702, Korea. Tel: +82-43-649-3470, Fax: +82-43-649-3470, E-mail: osc5000@mail.daewon.ac.kr

et al. 1992) and another study claims yeast or *Pseudomonas* are related with the ripening of fermented squid (Shinano et al. 1975; Hur et al. 1995). Study reports existence of lactic acid bacteria in fermented seafood including fermented squid. A fish sauce produced in Southeast Asia contains lactic acid, acetic acid, and pyroglutamic acid that result from lactic acid fermentation by acid forming microorganisms. This report also shows lactic acid bacteria are the main *Bacillus* during the ripening of fermented seafood. Shiokara which is Japanese fermented squid contains 10^6-10^7 amounts of *Lactobacillus* and fermented shrimp contains 10^2-10^4 of *Lactobacillus* in condition of 10^4-10^6 total plate counts (Itoh et al. 1985). Identification of lactic acid bacteria from low salted fermented squid shows *Lactobacillus farciminis, Lactobacillus corniformis, Lactobacillus confusus* and *Lactobacillus plantarum* and these 4 strains grow favorably at 25℃ (Morishita et al. 1995). During the ripening of 7% and 9% salt concentration fermented squid, *Staphylococcus xylosus, Micrococcus varians, Pseudomonas diminula, Flavobacterium odoratum* existing in fermented squid had been detected and homogenized and also counted their total plate count change (Kim et al. 1993; Kim et al. 1995). Studies are being conducted about the production of squid *Sikhae* which is another product *(*Kim et al. 1994; Lee et al. 1996). A trait of microorganisms related with fermented squid ripening is the existence of many micrococcus types but recent reports say that lactic acid bacteria have a big part in low slat fermented squid and reducing the amount of salt for health purposes is trending.

The purpose of this study is to contribute to an improvement in quality of fermented squid. The research analyzed the total bacterial count when 5%, 7% and 9% salt concentration fermented squid and 4% squid ink-added fermented squid are ripened at 20℃ and 10℃ and the change in lactic acid bacteria when 5% salt concentration squid as well as squid with 4% squid ink added are ripened at 10℃.

Materials and Methods

1. Sample Preparation

Squid (*Todarodes pacificus*) was used as a raw material for fermented squid. As Fig. 1, after defrosting the frozen squid at 4℃, meat excluding the intestines, head, legs and fins, which were removed, was used. Low salt fermented squid was made according to the Table 1 composition. Squid with no added ink and squid with 4% added ink were each ripened at 10℃ and 20℃. The ripened squid at 10℃ was used at an interval of one week and the squid at 20℃ was used at an interval of 4 days as analysis samples.

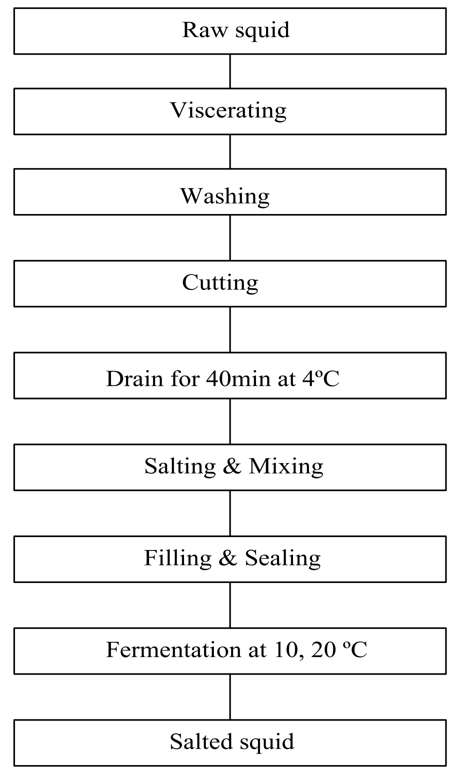

Fig. 1. Flow diagram of preparation of salt fermented squid.

Table 1. The compositions of salt squid samples with squid ink before fermented

Fermentation temperature (℃)	Composition (%)		
	Squid meat	Squid ink	Sodium chloride
10	95	0	5
	93	0	7
	91	0	9
	91	4	5
	89	4	7
	87	4	9
20	95	0	5
	93	0	7
	91	0	9
	91	4	5
	89	4	7
	87	4	9

2. Proximate Composition Analysis

General ingredients were measured following the A.O.A.C. method. That is, high pressure drying method by heating for water, Micro-kjeldahl method for crude protein, Soxhlet extraction process for crude fat, and direct ashing method was used for ash.

3. Total Plate Count Analysis

The sample fermented squid was moved into a blender cup and homogenized for 3 minutes at 15,000 rpm. Then it was diluted in 10 levels using 0.1% peptone water and after choosing 3 suitable levels the sample was incubated at 30℃ for 48 hours using the streak-plate method. Plate count agar (Difco Lab.) was used as a medium for counting total plate count number, as in Table 2.

4. Measurement of Number of Lactic Acid Bacteria

10 g of sample from fermented product from the early stage to ripening was applied 90 ㎖ of sterilized distill water and ground aseptically. Then it was homogenized after being shaken for 10 minutes using a shaking incubator and diluted 10 times.

This diluted solution was smeared in a medium depicted in Table 3 and cultured for 48 hours at 30℃. The total number of microorganisms was the number of colonies formed in plate count agar and the growing strains in the selected medium were counted (Lee et al. 1992; Cha et al. 1988; Shigeo & Toshio 1988).

Table 2. The contents of plate count agar medium for viable cell counts

Composition	Content
Tryptone[1)]	5.0 g
Yeast extract[1)]	2.5 g
Dextrose[1)]	1.0 g
Agar[1)]	15.0 g
Tween 80	1.0 g
L-Cystine	0.1 g
Distilled water	1,000 ㎖

[1)] Made by Difco Chemical Co. The pH value medium was adjusted to 7.0 at 25℃ and the medium was sterilized at 121℃ for 15 min.

Table 3. Compositions of media used in microbial studies

(Unit: per liter)

Lactobacillus	
	LBS broth + acetic acid + sodium acetate + agar
Leuconostoc	
	PES medium (phenyl ethyl alcohol-sucrose agar medium)
Streptococcus, Pediococcus	
	m-Enterococcus agar
Yeast	
	Potato dextrose agar

Lactobacillus was counted after acetic acid and sodium acetate were added to the LBS broth while PES broth was used for *Leuconostoc.* After adding 2,3,5-triphenyl tetrazolium chloride to m-enterococcus agar and reduction, if the colony appeared red, it was counted as *Streptococcus* and for a white colony, *Pediococcus.* Yeast was counted using potato dextrose agar.

5. Statistical Analysis

For statistical processing, using Statistical Packages for Social Science (ver. 10.0, SPSS, Chicago, IL, USA), significant difference less than 5 percent (P<0.05) for the average value was researched. The study results indicated the average value and the standard deviation (Choi & Kim 2011).

Result and Discussion

1. Proximate Composition of Raw Squid

General ingredients of the raw squid used in the study are according to Table 4. The raw squid's water content, crude protein, crude fat, crude ash and carbohydrate content were each 78.0, 18.2, 1.2, 1.7, 0.8%. Because only salt was added to the intestine-removed squid during production of fermented squid, fat content is low and protein content is relatively high. This could be affected by the area and time when the squid was caught and this result is similar to that of Lee & Kim (2012).

2. Changes in Total Plate Count

Fig. 2 shows the measurement of total plate count in low salt fermented squid of different salt concentrations ripened at

Table 4. Proximate composition of raw squid (%)

Components	Moisture	Crude protein	Crude fat	Ash	Carbohydrate
Raw squid	78.0±0.2	18.2±0.1	1.2±0.1	1.7±0.1	0.8±0.0

10℃. In the case of the 5% salt concentration sample, total plate count rapidly increased after he first week of ripening slowing down to a gradual increase until the fourth week when maximum total plate count was maintained at 10^8 (CFU/g). For the 9% salt concentration sample, total plat count showed a tendency to increase until the third week. After ward there was a small increase but mostly the total plate count maintained at 10^6-10^7 (CFU/g). Increase in salt concentration resulted in lower total plate count, meaning it inhibits growth of microorganisms.

Fig. 3 shows total plate count in low salt fermented squid with 4% squid ink added ripened at 10℃. The squid ink added to low salt fermented squid resulted in lower total plate count, meaning squid ink inhibits growth of microorganisms.

Fig. 4 shows total plate count in low salt fermented squid of different salt concentrations ripened at 20℃. In the 5% salt concentration sample total plate count rapidly increased on the fourth day, slowed down until the eighth day when it showed maximum total plate count of 10^8 (CFU/g) then decreased. In the 9% salt concentration sample the maximum total plate count showed at 10^7 (CFU/g) on the twelfth day of ripening.

Fig. 5 shows the results of sensory evaluations of bacteria in low salt fermented squid with 4% squid ink added ripened

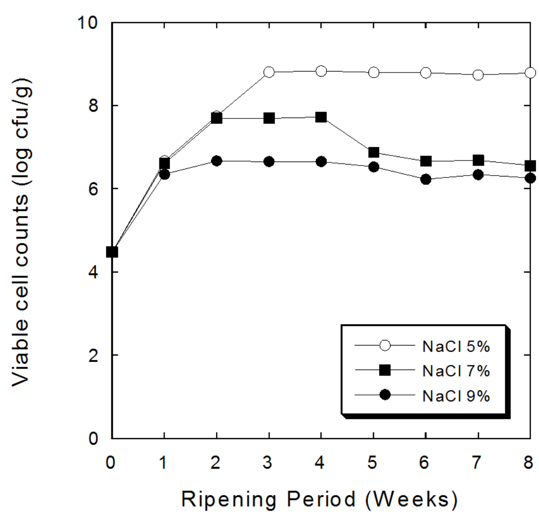

Fig. 2. The viable cell counts of salt fermented squid during fermentation at 10℃.

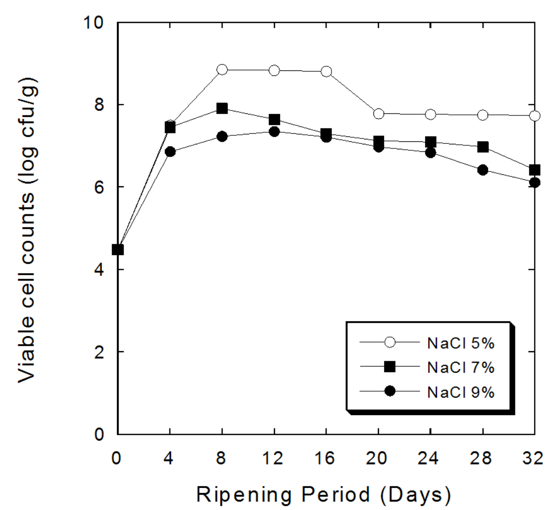

Fig. 4. The viable cell counts of salt fermented squid during fermentation at 20℃.

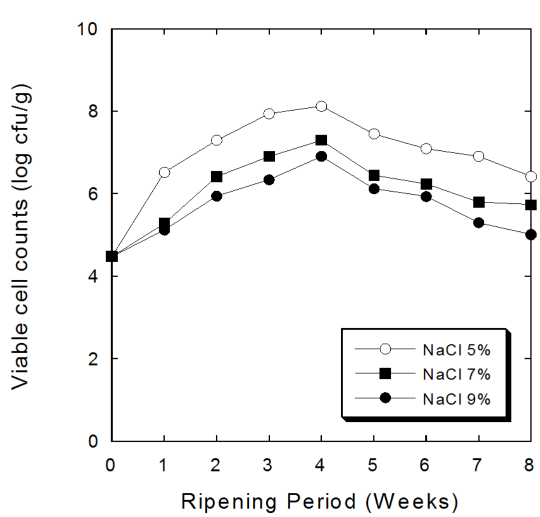

Fig. 3. The viable cell counts of salt fermented squid with 4% squid ink during fermentation at 10℃.

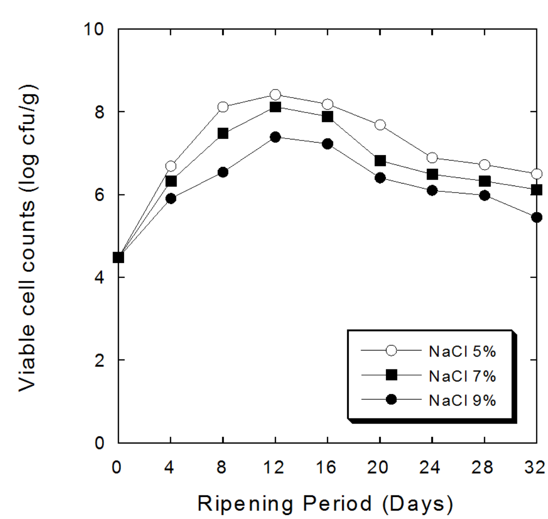

Fig. 5. The viable cell counts of salt fermented squid with 4% squid ink during fermentation at 20℃.

at 20℃ carried out in 4 day intervals. In the 5% salt concentration sample the optimum stage of ripening was observed on the twelfth day. In the 9% salt concentration sample the maximum total plate count appeared on the eighth day but that number was low and it is assumed it cannot increase any larger.

According to these results, in the without addition of the squid ink sample low salt concentration and high ripening temperature resulted in higher total plate count.

However depending on the salt concentration the types of bacteria are largely different and therefore organically there will be differences.

3. Changes in Lactic Acid Bacteria

Because fermented squid cannot be perfectly sterilized the quality of the squid depends on the microorganisms involved during the ripening process. The change in microflora when the 5% salt concentration sample was ripened at 10℃ is depicted in Fig. 6 and the change in the squid ink added sample is shown in Fig. 7.

In all samples *Leuconostoc* rapidly increased in the beginning stage of ripening, forming an important part of the total plate count while maximum count was achieved at the optimum stage of ripening. In the case of the 5% salt concentration sample bacterial count increased quickly compared to the ink-added sample. Therefore squid ink is assumed to suppress the growth of microorganisms. *Leuconostoc,* which increased rapidly during the beginning stage, rapidly decreased during the optimum stage of ripening.

Lactobacillus was detected in small amount in the beginning stage but increased rapidly after the optimum stage, forming most of the total plate count. This leads to the conclusion that it contributes to the quality of fermented squid after the optimum stage rather than the beginning stage.

In the 5% salt concentration sample the total plate count reached 10^8 (CFU/g) in just three weeks and *Streptococcus* and *Pediococcus* increased to 10^7 (CFU/g) in five weeks. When decomposition occurs after the optimum stage yeast rapidly increases so it can be assumed that yeast is related to decomposition after optimum stage.

In the case of the ink-added sample the change in microorganisms showed a similar course; however a delay in optimum stage could be observed. In all samples *Leuconostoc* rapidly increased during the beginning stage, forming an important part of the total bacterial count while in the middle stage *Lactobacillus* constituted as the prominent microflora.

In a study for Japan's representative fermented squid, Ika-shiokora, *Lactobacillus farciminis,* which is a kind of lactic acid

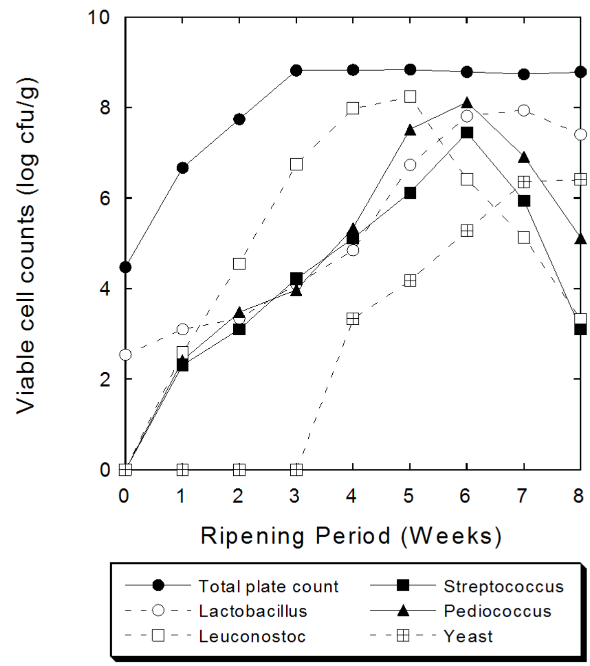

Fig. 6. Changes in lactic acid bacteria of 5% salt fermented squid during fermentation at 10℃.

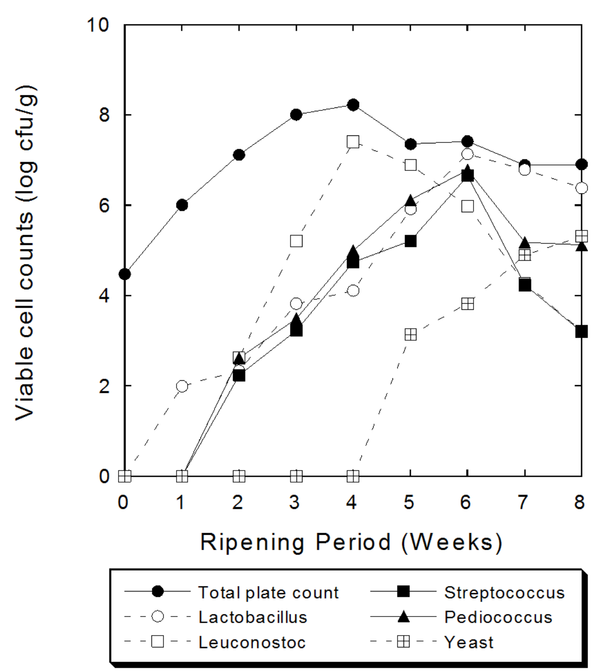

Fig. 7. Changes in lactic acid bacteria of 5% salt fermented squid with 4% squid ink during fermentation at 10℃.

bacteria, was the most dominant bacteria during the 5℃ low temperature ripening process. In 25℃ high temperature ripening *Lactobacillus confusus*, *Lactobacillus farciminis* and *Staphyllococcus epidermidis* were the most dominant. The study claimed to have segregated *Lactobacillus* and that *Lactobacillus* is the most dominant bacteria (Morishita et al. 1995).

Microorganisms such as *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Pediococcus* are lactic acid forming bacteria and therefore Gram positive bacteria. In this study the changes of these microorganisms were compared according to temperature and salt concentration. The reason these Gram positive bacteria increase in number during the beginning stage of ripening but decrease later is because the lactic acid formed by these bacteria causes the pH to decrease, restricting growth of bacteria.

Summary

This study measured the change of lactic acid bacteria during the ripening fermentation process of low salt fermented squid with no squid ink added. All study groups showed increase of *Leuconostoc* and rapid growth of total plate count at the beginning stage of ripening and the maximum microbial count showed at the optimum stage of ripening which gradually reduced after the optimum stage. It is believed that *Lactobacillus* occupied the major part of the total plate count after the optimum stage of the squid fermentation, and it was related to the quality after the optimized ripening stage. *Streptococcus* and *Pediococcus* were gradually increased until the optimum stage of the ripening, and then decreased rapidly. Yeasts were detected in the middle stage of the fermentation and rapid increase was shown after the last stage of the fermentation which suggests that yeasts participate in putrefaction of the low salt fermented squid. The change of lactic acid bacteria observed during the ripening fermentation of low salt fermented squid with squid ink added was that the total plate count increased until ripening middle stage but showed a tendency to slightly reduce after the middle stage. The length of time to reach the maximum value was longer than the no treatment groups. Among the lactic acid bacteria, *Leuconostoc*, *Streptococcus* and *Pediococcus* has increased until the middle stage of the ripening while *Lactobacillus* constantly increased to the end part of the ripening. Yeasts had no increasing in the early ripening stage, but after middle of the ripening, it started to increase. That kind of tendency was similar to the case of no treatment groups. However, the amount of lactic acid bacteria tended to be less than no treatment groups. The tendency of decreasing number of all bacteria in low salt fermented squid with squid ink added shows squid ink restricts the growth of all bacteria.

References

A.O.A.C. 1970. Official Methods of Analysis, 11th ed. Association of Official Analytical Chemists

A.O.A.C. 1980. Official Methods of Analysis. 13th ed. Association of Official Analytical Chemists

Cha YJ, Lee KH, Chang DS. 1988. Characterization of the strong proteolytic bacteria isolated from low salt fermented anchovy and of protease produced by that strain. *Bull Korean Fish Soc* 21:71

Choi SH, Kim SM. 2011. Quality properties of fermented squid viscera product with *Aspergillus oryzae* koji and its seasoning. *J Korean Soc Food Sci Nutr* 40:94-101

Fujii T, Suzuki K, Sugihara K, Okuzumi M. 1991. Changes in numbers and floral composition of foodborne and sanitary relating bacteria in squid "Shiokara" with low salt concentration. *J Tokyo Univ Fish* 78:1

Fujii T. 1984. Chemical composition and microflora of fish sauce "Shotturu". *Bull Jap Soc Sci Fish* 50:1061

Hur SH, Lee HJ, Kim HS, Choi SH, Kim YM. 1995. Processing conditions of low-salt fermented squid and its flavor components. 3. Characterization of protease produced by *Pseudomonas* D2 isolated from squid *Jeotkal. J Korean Soc Food Nutr* 24:636

Itoh H, Hadioetomo R, Nkkuni S, Okada N. 1985. Studies on lactic acid bacteria in fish sauces (part 1). Chemical composition and microflora of fish sauces. *Rept Natl Food Res Ints* 47:23

Itoh H, Hadioetomo R, Nkkuni S. Okada, N. 1985. Studies on lactic acid bacteria in fish sauces (part 2). Identification of salt-tolerance and acid-producing bacteria from fish sauces. *Rept Natl Food Res Ints* 47:31

Kim SM, Cho YJ, Lee KT. 1994. The development of squid *Sikhae* in Kang-Nung district. 2. The effects of fermentation temperatures and periods on chemical and microbial changes, and the partial purification of protease. *Bull Korean Fish Soc* 27:223

Kim YM, Jeong YM, Hong JH. 1993. Processing conditions for low salted squid *Jeotkal*. *Bull Korean Fish Soc* 26:312

Kim YM, Lee WJ, Jeong YM, Hur SH, Choi SH. 1995. Processing conditions of low salt fermented squid and its flavor components. 2. Effect of temperature, salinity and pH on the growth of bacteria from isolated low salt fermented squid. *J Korean Soc Food Nutr* 24:631

Lee CW, Ko CY, Ha DM. 1992. Microfloral changes of the lactic acid bacteria during *Kimchi* fermentation and identification of the isolates. *Kor J Appl Microbial Biotechnol* 20:102-109

Lee KG, Kim SM. 2012. Quality changes in low-salted squid *Jeot-gal* during fermentation and determination of shelf-life. *J Korean Soc Food Sci Nutr* 41:687-694

Lee NH, Oh SW, Kim YM. 1996. Biochemical changes in muscleprotein of squid *Sikhae* during fermentation. -Effect of temperature and moisture content-. *Korean J Food Sci Technol* 28:292

Matsubara M, Fujii T, Itoh Y, Okuzum M. 1994. Microbial contributions on ripening of squid shiokara. *Nippon Suisan Gakkaishi* 60:265

Mori K, Shinano H, Akiba M. 1979. The aerobic bacteria in the ripening process of "Ika-Shiokara". *Bull Jap Soc Sci Fish* 45:771

Mori K, Shinano H, Akiba M. 1983. Studies on the microorganisms in salted and ripened squid meat product - IV. Microflora of Ika-shiokara" inoculated with several kinds of bacteria. *Rep Fac Fish Hokkaido Univ* 34:355

Morishita K, Otakasaka W, Yamazaki K. Kawai Y, Inoue N, Shinano H. 1995. Isolation and characteristics of lactic acid bacteria in commerical "Ika-Shiokara". *Fisheries Science* 61:371

Morishita K, Otakasaka W, Yamazaki K. Kawai Y, Inoue N. 1994. Chemical an microbiological characteristics of commerical "Shiokara". *Rep Fac Fish Hokkaido Univ* 45:100

Shigeo M, Toshio O. 1988. Selective media for enumerating lactic acid bacteria groups from fermented pickles. *Nippon Shokuhin Kogyo Gakkaishi* 35:610

Shinano H, Sato M, Akiba, M. 1975. Studies on the microorganism in foods. I. Distribution of yeasts in fermented and salted foods. *Rep Fac Fish Hokkaido Univ* 26:207

Shinano H, Sato M, Akiba, M. 1975. Studies on the microorganisms in foods. I. Distribution of yeasts in fermented and salted foods. *Rep Fac Fish Hokkaido Univ* 26:207

Yamazaki K, Kitamura F, Inoue N, Shinani H. 1992. Effect of squid liver on microflora of "Ika-Shiokara". *Nippon Suisan Gakkaishi* 58:1971

접　　수 : 2013년 9월 30일
최종수정 : 2013년 10월 25일
채　　택 : 2013년 11월 7일