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Preparation and characteristics of *Haliotis Discus Hannai* Ino (abalone) viscera *Jeotgal*, a Korean fermented seafood

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전복내장 젓갈의 제조 및 품질특성

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Abstract

We prepared *Jeotgal* with *Haliotis Discus Hannai* Ino (abalone) viscera and the studied the physicochemical properties. Abalone viscus was fermented with varying amounts of salt for 60 days in order to prepare for the *Jeotgal*. During the fermentation, we measured the change of pH, volatile basic nitrogen (VBN), amino nitrogen (AN) and protease activity. After the fermentation, we examined the composition of free amino acids and sensory evaluation. The pH decreased with the fermentation, which was not significant (from 5.5 to 6.5). After the fermentation, the highest VBN was 96.7 mg/g, while the highest AN value was 406.3 mg/g. Unlike VBN and AN, the protease activity increased and reached the highest activity at the 30th day, and then decreased afterward. Based on the results, it was deduced that higher salinity restrained the fermentation and lowered the VBN, AN and protease activity. The total free amino acids of abalone *Jeotgal*, which were analyzed after the fermentation, (62.75 mg/g) was more than twice the amount in the abalone viscera before the fermentation (30.37 mg/g). We prepared abalone viscera *Jeotgal* and studied the characteristics for the first time. This will provide us with useful information for future related researches.

Key words abalone viscera, *Jeotgal*, VBN, AN, free amino acid, sensory evaluation

Introduction

Jeotgal, prepared by various seafood, such as shrimp, oysters, shellfish, fish eggs and also fish intestines, is a salted fermented food in Korea and many other countries. The manufacture method of *Jeotgal* varies depending on the main ingredients, regions, and personal preferences but the basic procedures are the same. Traditionally, the seafood is treated with 20% to 30% of salt as the main ingredients. The fermentation is allowed from several months to even several years, so that to form the favored taste that *Jeotgal* owns (1). *Jeotgal* is mainly used in the pickling kimchi as a condiment or as a side dish alone. *Jeotgal* is valued for its distinctive taste as well as its high nutritional content (2). In recent years, the health promoting potential of *Jeotgal*, include the antioxidant activity, angiotensin I converting enzyme inhibition potential and the probiotics, have been investigated and discussed (3-5). On the other hand, the manufacture procedures and the main ingredients of *Jeotgal* are also under development as more and more novel *Jeotgal* have been proposed (6-8).

Abalone, *Haliotis Discus Hannai* Ino, is an important aquatic economic species widely cultured in East Asia, Australia, America and many other regions. The production of abalone has increased greatly in the recent two decades with the introduction of farm aquaculture. It is estimated the world total output of abalone is more than 30,000 metric tons per year (9). Generally, the visceral part of abalone is not regarded as edible by the producers as well as customers.

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The viscera is treated as by-product and most of them is discarded directly or used as fish silage (10,11). However, the nutritional and healthy value of the visceral part of abalone should not be overlooked since more and more researchers have paid great attention to the bio-activities and health promoting ability of the abalone viscera. Sun L *et al.* (12) discussed the antioxidant activities of a sulphated polysaccharide obtained from the abalone viscera. Ping K and Qiukuan W (13) studied the potential application of the enzyme extract of abalone viscera. Several other articles mentioned the antioxidant potential of polysaccharide extracted from abalone viscera (14,15). The application of abalone viscera as the food ingredient has also been researched (16). Our previous study has discussed the anti-skin-aging activities of extract from abalone viscera (17). The results of all the researches indicate that abalone viscera possess very high nutritional and functional value, suggesting its more widely application possibility.

In this study, we prepared abalone viscera *Jeotgal* and determined the optimum manufacture conditions. We also examined the physicochemical properties of the proposed *Jeotgal*. At last, the response of the customers to this abalone viscera *Jeotgal* was investigated. Since this is the first research that discussing the abalone viscera *Jeotgal*, it will provide the basis for the future related researches.

Materials and methods

Preparation for the abalone viscera *Jeotgal*

Abalone was obtained from the aquatic market of Wando-gun in February 2012. All the abalone was shucked and eviscerated. After that, the abalone viscera was gathered, homogenized (HMF-3260S, Hanil Electric, Seoul, Korea) for 30 min under room temperature and stored at -20℃ in refrigeratory until use. After thawing, 1 kg of abalone viscera and 130 g, 150 g, 180 g, 200 g and 250 g of solar salt (Taepyung Salt Co., Sinan-gun, Korea) was mixed respectively in a plastic vat. The plastic vat was sealed and placed for fermentation and maturation at the temperature of 10℃ for 60 days.

General composition of abalone viscera

Moisture content, crude protein content, crude lipid, ash and carbohydrate content were investigated according to methods described in the AOAC (1990). Conversion factor for the examination of protein content was 6.25.

pH

The *Jeotgal* was pretreated before the pH was examined. First, the sample was homogenized with 4 times (v/w) of distilled water in a mixer (HMF-3260S, Hanil Electric) for 5 min. The sample was then transferred to a centrifuge tube for centrifugation at 300×g for 20 min (1736R, Hanil Electric). The supernatant was filtered using a filter paper and the filtrate was measured for the pH variation during the *Jeotgal* fermentation period using a pH meter (730P, Isotec. Inc., Miamisburg, OH, USA).

Volatile basic nitrogen (VBN)

The VBN was determined by the Conway's method in a conway unit with some modifications (18). The sample (10 g) was mixed with equal amount of distilled water and twice amount of 10% trichloroacetic acid solution and grinded in a mortar for 5 min. After grinding, the sample was filtered and the remnant was grinded and washed with 5% trichloroacetic acid solution. All the sample and solution was filtered and transferred to a 50 mL volumetric flask to which 5% trichloroacetic acid solution was used to fill. Pretreated sample (1 mL) was extracted and transferred to the outer room of the conway unit with 1 mL of 1% H_3BO_3 solution in the inner room. Saturated K_2CO_3 solution (1 mL) was mixed carefully with the sample solution in the outer room and the conway unit was sealed and incubated at 37℃ for 1 hr. The mixture in the outer room was neutralized with 0.02 N H_2SO_4 solution until the H_3BO_3 solution turned to pink color. VBN value was calculated in the following equation.

$$\text{VBN (mg/g)} = 0.28\times(V_1-V_2)\times5000/S$$

In the equation: V_1 is the volume (mL) of 0.02 N H_2SO_4 solution in the experiment;

V_2 is the volume (mL) of 0.02 N H_2SO_4 solution in the control experiment in which equivalent amount of distilled water was used in place of sample solution;

0.28 is the result of 14 (molecular weight of nitrogen) multiply with the concentration of H_2SO_4 solution, 0.02;

S is the weight of the sample.

Amino nitrogen (AN)

AN was estimated with the Formol method (18). The sample was grinded and mixed with 50 mL of distilled water. The filtrate (25 mL) of the sample was added with 25 mL of formaline solution (30 mL) adjusted to pH 8.4 by 0.1

N NaOH and 30 mL of distilled water. The solution was titrated using 0.1 N NaOH to pH 8.4.

AN value was calculated in the following equation.

AN (mg/g) = (A-B)×280/S

In the equation: A is the volume (mL) of 0.1 N NaOH solution used in the titration in the experiment;

B is the volume (mL) of 0.1 N NaOH solution used in the titration in the control experiment in which equivalent amount of distilled water was used in place of formaline solution;

280 is the result of dilution factor (50 mL/25 mL) multiply with 14 (molecular weight of nitrogen) and divide the concentration of NaOH 0.1 N.

S is the weight of the sample.

Protease activity

The protease activity of the abalone viscera *Jeotgal* was determined by the protocol of Anson (19). The crude enzyme extract was prepared with grinded sample (6 g) extracted in 100 mL distilled water for 1 hr before filtration. The crude enzyme extract (0.5 mL) was mixed with 2 mL of 0.6% casein solution (pH 7.2) used as the substrate and the react was carried out at 30℃ for 10 min. The reaction was stopped by adding 2.5 mL of 0.4 M trichloroacetic acid (TCA). After filtering, 1 mL of the filtrate was mixed with 5 mL of 0.4 M Na_2CO_3 and 1 mL of folin solution and the reaction was proceed for 20 min. The absorption was measured at 660 nm for amino acid liberation. One unit of protease activity was defined as the amount of enzyme to liberate 1 μM tyrosine under the experimental conditions.

Analysis for free amino acid composition

The free amino acid was extracted with twice of 5% trichloroacetic acid solution for 24 hr. Once extracted, the solution was centrifuged at 10,000×g for 10 min. The supernatant was neutralized by three times of 0.05 M hydrochloric acid and then diluted with five time distilled water. All the samples were filtered with 0.45 μm membrane filter and the free amino acid composition was analyzed by the Hitachi L-8800 amino acid analyzer (Tokyo, Japan) according to the method described previously (20,21).

Sensory evaluation

The sensory evaluation was undertook by a panel composed by 40 students in the food engineering department after they have received related education. Characteristics include color, taste, flavor and overall acceptability were evaluated and all the characteristics were scored from 1 (extremely unacceptable) to 5 (extremely acceptable) (22). The control groups were oyster *Jeotgal*, abalone meat *Jeotgal* and changran *Jeotgal* (aged and seasoned intestine of Alaska pollack) were all purchased from the same aquatic products market. The total score of each sample got represented its quality and likeness by the consumers.

Results and Discussions

General composition of abalone viscera

The general composition of abalone viscera before the fermentation was measured. Moisture, protein, lipid, ash and carbohydrate content of abalone viscera (results listed in table 1) was influenced by several factors such as the abalone species, harvested regions, harvested time, feed, abalone age and other factors, as have been studied by several previous articles (23-26). Our research result confirmed with what have been published that moisture content ranged approximately 70%~80%, protein content ranged approximately 1.0%~5.0%, lipid content ranged approximately 0.6%~1.5%, ash content ranged approximately 2.0%~5.0% and carbohydrate content ranged approximately 16%~20%. The nutrition contained in the abalone viscera not only endow abalone viscera *Jeotgal* with the distinct flavor but also provides an appropriate media for the microorganisms involved in the following fermentation.

Table 1. The general nutritional components of abalone viscera (%)

Moisture	Protein	Lipid	Ash	Carbohydrate
72.74±0.05	3.24±0.46	0.82±0.03	4.06±0.05	19.14±0.37

pH variation

The pH variation during the fermentation was showed in the Fig. 1. As we can see in the figure, at the beginning of the fermentation (0 day) the pH scale of all the *Jeotgal* was 5.88~6.03 which fell into the normal pH scale for shellfish (5.5~6.5). During the fermentation the pH fluctuated from 5 to 6. The influence of the salinity to the pH of the *Jeotgal* was not believed in this study even though the *Jeotgal* added with 13% of salt presented higher (5.96) pH than the other *Jeotgal* added with more salt at the end of the fermentation. Generally, the pH variation is caused by the microorganism activity during fermentation. Hence, the pH variation is

influenced by all the factors that have impact on the growth of the microorganism. In this study, the different amount of salt added to the abalone viscera may have affected the growth of the microorganism but the impact did not revealed in the pH variation.

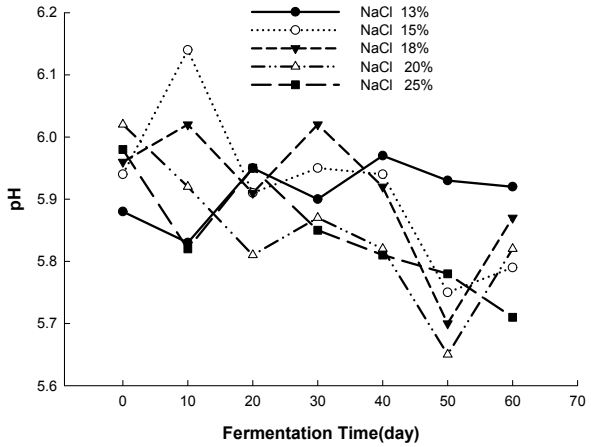

Fig. 1. pH variation during the abalone viscera *Jeotgal* fermentation.

Volatile basic nitrogen (VBN)

The influence of different salinity is more significant on the VBN change during the fermentation process as revealed in the Fig. 2. VBN is widely used as the indicator of fish and meat quality (27,28). While in this study, VBN is more a factor for fermentation than the freeness index since at the end of the fermentation the VBN of the *Jeotgal* exceeded the rejection value for most of the free fish (29). At the beginning of the fermentation, the VBN value ranged from 3.7 mg/g to 20.3 mg/g and as the fermentation proceeded the VBN value increase accordingly. At the end of the fermentation (60 days) the effect of the salinity on the VBN was more clearly. Low salt adding Jeotgal showed higher VBN value (96.7 mg/g) than other Jeotgal with higher salinity. The VBN value decreased in accordance with the increase of the salinity. *Jeotgal* added with 25% salt at the end of the fermentation presented (37.4 mg/g) less than a half of that of the Jeotgal added with 13% salt (96.7 mg/g). The difference of the VBN value among the different Jeotgal groups is attributed to the difference amount of salt added. Since the high salinity would inhibited the growth of the microorganism involved in the fermentation so that the release of the volatile basic nitrogen substances.

Amino nitrogen (AN)

Amino nitrogen (AN) is related with the hydrolysis of the protein contained in the viscera. With the fermentation, protein was either decomposed by the autolysis or the effect of the microorganism happened during the fermentation (30, 31). Also AN is usually believed to confer the special flavor and aroma to *Jeotgal*. The AN result of this study was presented in the Fig. 3 from which we can see that the AN increased with the increased fermentation time. Also at the same time, the effect of salinity on the AN value was indicated from the figure. In a previous study, it was found that high salinity reduced the AN compared with the low salinity in the *Jeotgal* fermentation (32). In our study, the *Jeotgal* added with 13% of salt released higher level of AN than all other *Jeotgal* groups during the 20 and 40 days. And at the end of the fermentation, *Jeotgal* added with 25% of salt showed the lowest AN compared with the other groups. The difference of AN may also be attributable to the different salinity, but the exactly mechanism need further study and investigation.

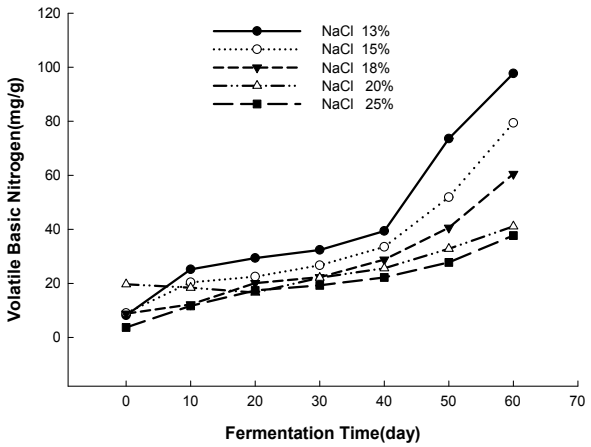

Fig. 2. Volatile basic nitrogen (VBN) variation during the abalone viscera *Jeotgal* fermentation.

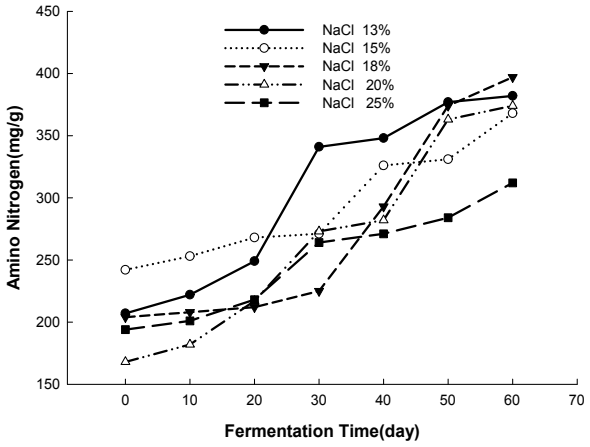

Fig. 3. Amino nitrogen (AN) variation during the abalone viscera Jeotgal fermentation.

Protease activity

During the fermentation, the proteases which would degrade the protein to peptides and amino acids were derived from two sources: the abalone viscera and the microorganisms. The protease activity (showed in Fig. 4) in this study indicated both the influence of the fermentation time and the salt concentration. As mentioned above, salt concentration may have impact on the growth of the microorganisms in the *Jeotgal* and such influence the protease produced by them indirectly. The results showed that higher salt concentration inhibited the protease activity compared with the *Jeotgal* added with lower salt concentration. We deduced that the lower number of the microorganisms and the changed conformation of the enzymes as the results of the higher salt concentration may lead to the lower protease activity. Moreover, the change of the protease activity suggested the influence of the fermentation time to the protease activity or the number of microorganisms in the fermentation. At the beginning of the fermentation, the microorganism number was much lower than the latter, at the 30th days for instance. At the 30th days the protease activity reached the highest. After that, the protease activity decreased accordingly. The change of the protease activity is related to the amino nitrogen and other flavor substance as well.

Free amino acid composition

As have been mentioned in the previous study (24), abalone viscera contained significant amount of free amino acids which conferred to abalone special flavor. We investigated and compared the free amino acids composition of abalone viscera and the abalone viscera *Jeotgal* at the end of the fermentation. The result was listed in the Table 2. Total free amino acids amount of abalone viscera was 30.37 mg/g which was less than half of that of the abalone viscera *Jeotgal*. Except for the taurine, β-alanine, DL-3-aminoisobutyric acid, γ-aminobutyric acid, DL-plus allo-δ-hydroxylysine, L-ornithine and L-anserine, all other free amino acids level in the abalone viscera *Jeotgal* was higher than the abalone viscera. Also in the abalone viscera *Jeotgal*, we detected L-3-methylhistidine which was not detected in the abalone viscera.

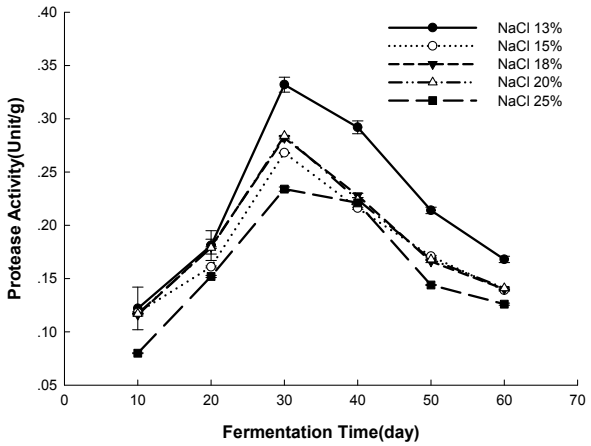

Fig. 4. Protease activity variation during the abalone viscera Jeotgal fermentation.

Sensory evaluation

In this study, the customer responses to the abalone viscera *Jeotgal* and three other *Jeotgal* used as control groups were investigated. Sensory evaluation result was listed in Table 3. The *changran Jeotgal* got the highest score in the color evaluation which was followed by abalone viscera *Jeotgal*, oyster *Jeotgal* and abalone meat *Jeotgal*. In this study, costumers showed similar response to the color of oyster *Jeotgal* and abalone viscera *Jeotgal* and significant bad perception for abalone meat *Jeotgal*. In the taste test the *changran Jeotgal* received the highest score which was followed by abalone meat *Jeotgal* and abalone viscera *Jeotgal* and the oyster *Jeotgal* got the lowest score. However, the scores for abalone viscera *Jeotgal* (2.85±0.93) and abalone meat *Jeotgal* (3.35±1.04) was not significant different. The abalone viscera *Jeotgal* presented inferior score compared with the abalone meat *Jeotgal* and *changran Jeotgal*. As to the overall acceptability score, *changran Jeotgal* got the highest score and followed by abalone viscera *Jeotgal*, abalone meat *Jeotgal* and oyster *Jeotgal*. *Changran Jeotgal* is generally considered as the superior than other *Jeotgal* in Korean cuisine (33) and this is also confirmed by the sensory evaluation conducted in this study. Abalone viscera *Jeotgal* showed higher score in the color and overall acceptability evaluation but lower score in the taste and flavor tests which indicated the possible likeness of the costumers.

Conclusion

Jeotgal is a fermented seafood and special dish in Korean cuisine. In this study we proposed *Jeotgal* prepared with the abalone viscera and investigated its physicochemical properties. The proximate composition of abalone viscera was analyzed and the results suggested that it is highly nutritious and suitable for fermentation. The pH change during the fermentation indicated that pH decline along with the fermentation but both the pH change and influence of salinity

Table 2. Comparison of free amino acids between abalone viscera and abalone viscera *Jeotgal* (mg/g)

Abalone viscera		Abalone viscera *Jeotgal*	
o-Phosphorserine	0.39	o-Phosphorserine	0.56
Taurine	13.92	Taurine	11.02
L-Aspartic Acid	1.33	L-Aspartic Acid	4.85
L-Threonine	0.72	L-Threonine	2.63
L-Serine	0.94	L-Serine	3.27
L-Glutamic acid	2.78	L-Glutamic acid	6.57
Sarcosine	0.06	Sarcosine	0.07
L-2-Aminoadipic Acid	0.12	L-2-Aminoadipic Acid	0.16
Glycine	1.72	Glycine	3.06
L-Alanine	1.21	L-Alanine	3.19
L-Citrulline	0.02	L-Citrulline	0.1
DL-2-Aminobutyric Acid	0.01	DL-2-Aminobutyric Acid	0.1
L-Valine	0.88	L-Valine	2.96
L(-)-Cystine	0.1	L(-)-Cystine	0.13
L-Methionine	0.04	L-Methionine	0.66
L-Isoleucine	0.58	L-Isoleucine	2.29
L-Leucine	0.15	L-Leucine	2.36
L-Tyrosine	0.53	L-Tyrosine	1.64
L-Phenylalanine	0.15	L-Phenylalanine	1.27
β-Alanine	0.05	β-Alanine	0.03
DL-3-Aminoisobutyric Acid	0.05	DL-3-Aminoisobutyric Acid	0.03
γ-Aminobutyric Acid	0.01	γ-Aminobutyric Acid	0.01
Ethanolamine	0.06	Ethanolamine	0.29
Ammonium Chloride	0.46	Ammonium Chloride	0.85
DL-plus allo-δ-Hydroxylysine	0.05	DL-plus allo-δ-Hydroxylysine	0.04
L-Ornithine	0.08	L-Ornithine	0.08
L-Lysine	1.2	L-Lysine	4.74
L-Histidine	0.25	L-Histidine	1.02
L-Anserine	0.29	L-Anserine	0.2
L-Carnosine	0.02	L-Carnosine	0.09
L-Arginine	2.16	L-Arginine	6.63
L(-)-Proline	0.55	L(-)-Proline	1.85
-	-	L-3-Methylhistidine	0.01
Total	30.37	Total	62.75

is not considered as significant. Furthermore, we also studied the change of VBN and AN which all reflect the influence of fermentation time. VBN result showed the influence of the different salinity as *Jeotgal* added with higher amount of salt released lower VBN than that of *Jeotgal* added with lower amount of salt. We see both the VBN and AN increased along with the fermentation. However, the influence of salinity on AN variation was not that clear. *Jeotgal* added with 25% of salt revealed lower AN at the end of the fermentation than other *Jeotgal* groups while *Jeotgal* added with 18% of salt presented the highest AN value. The protease activity change during the fermentation indicated both the salinity and fermentation process. In the middle of the fermentation (30 day) the protease activity of all *Jeotgal* groups reached highest. Also, *Jeotgal* fermented with the lowest salinity showed the highest protease activity while the *Jeotgal* with the highest salinity showed the lowest. We examined the free amino acids both in the abalone viscera and abalone viscera *Jeotgal* at the end of the fermentation. Seven kinds of free amino acids in the abalone viscera *Jeotgal* presented lower or the same quantity while the level of the rest 26 kinds of free amino acids in abalone viscera *Jeotgal* were higher. Moreover, the total amount of free amino acids in abalone viscera *Jeotgal* (62.75 mg/g) is more than twice of that of abalone viscera (30.37 mg/g). At last, the sensory evaluation was carried out. Results show that abalone viscera *Jeotgal* is more acceptable for the color and overall acceptability while need improvement on the taste and flavor. As a conclusion, we proposed the abalone viscera *Jeotgal* for the first time and researched its characteristics and hopefully it will provide the basis for future study.

Table 3. Sensory evaluation of abalone viscera *Jeotgal* in comparsion with the control groups

	Color	Taste	Flavor	Overall acceptability
A	$3.05\pm0.94^{ab1)}$	2.10 ± 0.64^{a}	2.35 ± 1.04^{a}	2.33 ± 0.69^{a}
B	2.70 ± 0.92^{a}	3.35 ± 1.04^{b}	3.30 ± 1.08^{b}	3.10 ± 0.97^{b}
C	3.70 ± 1.13^{b}	3.95 ± 0.83^{c}	3.65 ± 0.93^{b}	3.80 ± 0.77^{b}
D	3.15 ± 1.19^{ab}	2.85 ± 0.93^{b}	2.60 ± 1.31^{a}	3.15 ± 0.88^{c}

A, Oyster *Jeotgal*; B, Abalone meat *Jeotgal*; C, *Changran Jeotgal* (aged and seasoned intestine of *Alaska pollack*); D, Abalone viscera *Jeotgal*.
[1)]Mean±SD (n=3) in the same column with different superscripts are significantly different at $p<0.05$.

요 약

본 연구는 전복내장을 이용하여 젓갈을 제조과정 중 이

화학적 변화와 제조된 젓갈의 품질특성에 대하여 조사하였다. 전복내장의 영양학적 성분을 분석한 결과 탄수화물, 단백질 및 미네랄이 풍부하여 젓갈발효에 우수한 성분특성을 갖고 있었다. 젓갈 발효중 pH는 경시적으로 감소하는 경향을 보였으며 염도가 pH 변화에 영향을 미치지 않았다. 휘발성염기질소함량은 발효중 염농도에 따라 현저한 영향을 받았으며 고염발효의 경우 저염발효에 비하여 휘발성염기질소 함량이 현저하게 낮음을 알 수 있었다. 아미노태질소 함량은 발효중 꾸준히 증가하는 경향을 보였으며, 염농도가 낮을수록 높은 값을 보였으나 염농도에 따른 변화는 크지 않음을 알 수 있었다. 발효과정중 protease 활성 변화를 측정한 결과 고염에서 활성이 떨어지는 경향을 보였으며 발효 30 일경 최고 활성을 보였다. 전복내장과 발효 전복내장젓갈의 유리아미노산 함량을 분석한 결과 젓갈 중 7종의 아미노산이 내장에 비하여 낮게 나타났으며 26 종의 아미노산이 높게 나타났다. 전복내장 젓갈의 총유리아미노산 함량은 62.75 mg/g 으로 전복내장 30.37 mg/g에 비하여 약 2배 증가하였다. 관능평가 결과 색과 종합기호도에서 우수하였으며 상품화를 위해서는 맛과 향의 개선이 필요한 것으로 판단되었다. 전복내장젓갈에 대한 최초의 연구논문으로 향 후 타 연구에 기초자료를 제공할 수 있을 것으로 생각된다.

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