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Formation of Chitin Oligosaccharides during Long Fermentation of Toha-jeot (Salt-Fermented Toha Shrimp)

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Abstract

Toha-jeot, salt-fermented Toha shrimp (*Caridina denticulata denticulata* De Haan) is a traditional fermented food in Korea. Samples of Toha-jeot used in the present study were a low-salt group of 15% sodium chloride (L), a high-salt group of 23% sodium chloride (H), a 50% conventional soybean sauce group (S), a low-salt group containing 2% wheat bran (W2%-L), a high-salt group containing 2% wheat bran (W2%-H), a low-salt group containing 4% wheat bran (W4%-L) and a high-salt group containing 4% wheat bran (W4%-H). These seven groups were refrigerated at 4±1°C and then taken out for analysis at three month intervals. We investigated the functional components of Toha-jeot during fermentation. Long fermentation of Toha-jeot lowered the viscosity of chitin and tended to reduce the distribution of molecular weight. The formation of chitin oligosaccharides on the other hand, increased significantly. After nine months of fermentation, chitin oligosaccharides (M.W. 823~1789) constituting 24.75% of Toha chitin were produced in the sample of W2%-H. During the same period, chitin oligosaccharides (M.W. 1436~1879) constituting 66.30% of Toha chitin were produced in the sample of S. However, chitin oligosaccharides were not produced in Jeotsaeu-jeot made of sea-water shrimp when fermented for six months.

Key words: Toha shrimp (*Caridina denticulata denticulata* De Haan), Toha-jeot, chitin oligosaccharides, Jeotsaeu-jeot

INTRODUCTION

Toha-jeot, salt-fermented Toha shrimp (*Caridina denticulata denticulata* De Haan), is a traditional fermented food which exists only in the area of Chonnam, Korea. Toha is found mainly in fresh lakes and unpolluted rice fields. Toha-jeot is made of live Toha, with its shell, preserved in salt. The chitin contained in the shell of Toha leads us to expect the functional effect of physiologic controls. Park et al (1). have reported that the benefits of chitin, the content of which in the natural Toha shells is 9.6% are aiding the recovery from abrasion wounds and assisting anti-tumor defenses.

Toha-jeot is superior in taste but there has been no scientific research into its food chemistry. Since 1990, there has been much interest in functional materials and functional foods. Several research projects have been performed on the chitin and chitosan found in Toha and Toha-jeot. One recent research discusses the characteristics of chitin and chitosan extracted and manufactured from Toha (1). Park and his colleagues have reported on the functionality and safety of chitin found in Toha-jeot (2) as well as the formation of chitin oligosaccharides (3) and the changes in nutritional components such as free amino acids, minerals and fatty acids (4) during fermentation. Kim (5) investigated the optimal fermenting conditions of Toha-jeot as well as the physicochemical properties of fermented Toha-jeot to improve the quality of Tohajeot. The antibacterial properties of Toha-jeot extracts were researched by Park et al. (6). There have also been reports about studies on the taste of Toha-jeot (7-9).

The present study is a part of researches (10,11) to study functional superiority and safety of Toha-jeot. Samples of Toha-jeot used in this study varied according to levels of salt concentration, the use of conventional soybean sauce instead of salt and the addition of different levels of wheat bran to the low and high salt concentrated samples. In order to enhance the fermentation process, wheat bran was added. These seven groups were fermented for nine months and taken out for analysis at three month intervals. It was observed that during fermentation there was a reduction in the viscosity and molecular weight of Toha chitin as well as the formation of chitin oligosacchrides.

MATERIALS AND METHODS

Preparation of materials

21.4 kg of experimental Toha were taken from a natural cultivated farm near Na-Ju City in Chonnam on January 13, 1996. The Toha were then prepared into seven different kinds of Toha-jeot (Table 1): a low-salt group of 15% sodium chloride (L), a high-salt group of 23% sodium chloride (H), a group preserved with 50% conventional soybean sauce

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Table 1. Ratio of materials of experimental Toha-jeot, abbreviations of Toha-jeot and fermentation conditions of Toha jeot

Ratio of materials of experimental Toha-jeot	Abbreviations of Toha-jeot type	Fermentation conditions of Toha-jeot
Raw Toha : Water : Table salt=1 : 1 : 0.35	L	Fermented with 15% NaCl concentration
Raw Toha : Water : Table salt=1 : 1 : 0.6	H	Fermented with 23% NaCl concentration
Raw Toha : Conventional soy sauce 1 : 1	S	Fermented with 50% conventional soy sauce
Raw Toha : Water : Table salt : Wheat bran=1 : 1 : 0.35 : 0.05	W2%-L	Fermented with 2% wheat bran at 15% NaCl concentration
Raw Toha : Water : Table salt : Wheat bran=1 : 1 : 0.6 : 0.05	W2%-H	Fermented with 2% wheat bran at 23% NaCl concentration
Raw Toha : Water : Table salt : Wheat bran=1 : 1 : 0.35 : 0.1	W4%-L	Fermented with 4% wheat bran at 15% NaCl concentration
Raw Toha : Water : Table salt : Wheat bran=1 : 1 : 0.6 : 0.1	W4%-H	Fermented with 4% wheat bran at 23% NaCl concentration

instead of salt (S), a low-salt group containing 2% wheat bran (W2%-L), a high-salt group containing 2% wheat bran (W2%-H), a low-salt group containing 4% wheat bran (W4%-L), and a high-salt group containing 4% wheat bran (W4%-H). Then each group was refrigerated at 4±1°C for nine months. Each was taken out at three month intervals to be analyzed. The control group was fixed with 95% ethanol immediately after being caught in order to evaluate the molecular weight of chitin prior to fermentation.

Extraction method of chitin

A modified Hackman method (12) was used to extrat chitin. To eliminate $CaCO_3$ and minerals, 1 L of 2 N HCl was added to 100 ml of ethyl alcohol. These were frozen to -30°C and then 200 g of dried Toha-jeot was poured to the mixture. To eliminate protein from demineralized Toha jeot, the mixture was shaken for three hours and then washed several times with distilled water. To suppress the decrease of molecular weight occurring in the chitin, inactivated nitrate gas was injected into 1 L of 5% NaOH solution and the temperature of the mixture was increased to 93°C. Toha-jeot intermediates in which mineral was eliminated were put to the mixture and shaken for three hours. This procedure was repeated several times for the elimination of protein. To eliminate the pigments in Toha-jeot, 500 ml of acetone was added to 100 g of demineralized and deproteined Toha jeot. It was then shaken for 24 hours at room temperature and filtered. After drying, white Toha chitin powder was obtained.

Viscosity measurement of Toha chitin

In order to evaluate the rate of reduction of molecular weight in Toha chitin during fermentation, the viscosity of chitin was measured using a viscometer (Brookfield viscometer LVDV II$^+$, USA). Using the method of Terbojevich et al. (13), a solution was made of 5% (w/w) lithium chloride-*N,N* dimethylacetamide (LiCl-DMAc). 0.05~0.5% of chitin dissolved in the solution absolutely.

Verifying conditions of Toha chitin molecular weight

Molecular verification was carried out using a gel-permeation chromatography (GPC; JASCO Model LC-900, JASCO Co., Japan) following the method of Hasegawa et al. (14). The analytical conditions for the verification consisted of a Pu-980 pump, AS-950 autosampler, CO-965 column oven and RI-930 detector. Pulluran (P-80 Shodex Co., Japan) was used as the standard materials. A SEC (size-exclusion chromatography) column filled with stylene divinyl benzene copolymer was used. The flow rate was 0.26 ml/min, the sample-injection volume was 20 μl and the column temperature was 40°C.

RESULTS AND DISCUSSION

The change in viscosity of extracted chitin during long fermentation of Toha-jeot

The change in viscosity of extracted chitin during long fermentation of Toha-jeot is shown on Table 2. The viscosity distribution of seven groups at 0-month fermentation ranged between 793 and 1020 cP. After three months of fermentation, the viscosity of the seven groups decreased to between 2.1% and 5.7% of 0-month fermentation ; L went down 4.6%, H to 2.5%, S to 2.1%, W2%-L to 4.8%, W2%-H to 3.1%, W4%-L to 3.4%, and W4%-H to 5.7%. Six months of fermentation showed that the viscosity of chitin decreased

Table 2. The change in viscosity of extracted chitin during long fermentation of Toha-jeot (unit : cP*)

Fermentation time (months) \ Sample	Control[1]	L	H	S	W2%-L	W2%-H	W4%-L	W4%-H
0	892	823.0 (100)[2]	1020.0 (100)	956.0 (100)	793.0 (100)	933.0 (100)	910.0 (100)	875.0 (100)
3	1120	38.0 (4.6)	25.0 (2.5)	20.0 (2.1)	38.0 (4.8)	29.0 (3.1)	31.0 (3.4)	50.0 (5.7)
6	1240	3.2 (0.4)	4.6 (0.5)	1.9 (0.2)	6.7 (0.9)	3.2 (0.3)	3.6 (0.4)	12.4 (1.4)
9	1060	2.4 (0.3)	3.2 (0.3)	1.5 (0.2)	3.1 (0.4)	2.5 (0.3)	1.8 (0.2)	4.5 (0.5)

[1]Fixed with 95% ethanol after sampling Toha jeot. [2]Percentage of viscosity to 0-month fermentation. cP*: centi Poise

Table 3. Changes in the average molecular weight (*Mw*), number average molecular weight (*Mn*) and top peak molecular (*Mp*) during long fermentation of Toha-jeot

Toha-jeot Months	Groups	*Mw*	*Mn*	$d^{1)}$=*Mw*/*Mn*	*Mp*
0	Control	1.022×10^6~1.638×10^6	2.540×10^5~4.599×10^5	3.02~4.11	1.15×10^6
3	L	81,874	51,388	1.59	94,141
	H	60,713	31,032	1.96	35,002
	S	11,582	923	8.22	150
	W2%-L	70,719	41,206	6.13	72,777
	W2%-H	156,458	128,795	1.21	174,563
	W4%-L	79,284	45,049	1.76	75,458
	W4%-H	85,023	58,766	1.45	73,839
6	L	59,779	44,431	1.35	48,695
	H	60,850	38,877	1.57	58,376
	S	9,083	1,222	7.43	187
	W2%-L	70,335	37,815	1.86	66,196
	W2%-H	8,270	5,551	1.49	7,617
	W4%-L	71,578	43,814	1.63	72,251
	W4%-H	80,623	72,408	1.11	61,039
9	L	7,576	3,422	2.21	6,193
	H	7,933	3,242	2.45	6,521
	S	1,114	1,795	1.01	1,828
	W2%-L	9,686	5,605	1.73	9,368
	W2%-H	4,234	1,854	2.28	4,311
	W4%-L	10,398	7,490	1.39	10,389
	W4%-H	8,865	4,960	1.79	9,613

[1)]Degree of dispersion

Table 4. The formation of chitin oligosaccharides (M.W. 800~1,800) during fermentation of Toha-jeot

Fermented month / M.W.[1)] / Toha-jeot samples	Raw Toha's chitin A.M.W.[2)]	3 months		6 months		9 months	
		A.M.W.	M.W. 800~1,800	A.M.W.	M.W. 800~1,800	A.M.W.	M.W. 800~1,800
L	1.33×10^6	8.19×10^4	0	5.98×10^4	0	7.58×10^3	16.78%
H	〃	6.07×10^4	0	6.09×10^4	0	7.93×10^3	15.49%
S	〃	7.58×10^3	11.73%	9.08×10^3	11.02%	1.81×10^3	66.30%
W2%-L	〃	7.07×10^4	1.65%	7.03×10^4	0	9.69×10^3	3.05%
W2%-H	〃	1.56×10^5	0	8.27×10^3	0.18%	4.23×10^3	24.75%
W4%-L	〃	7.92×10^4	0	7.16×10^4	0	1.04×10^4	?
W4%-H	〃	8.50×10^4	0	8.06×10^4	0	8.87×10^3	5.98%

[1)]Molecular weight, [2)]Average molecular weight

to between 0.2% and 1.45% of 0-month fermentation tremendously. At nine months of fermentation, the viscosity decreased to between 0.2% and 0.5% of 0-month fermentation ; L went down to 0.3%, H to 0.3%, S to 0.2%, W2%-L to 0.4%, W2%-H to 0.3%, W4%-L to 0.2%, and W4%-H to 0.5%. A considerable reduction of viscosity was observed as time progressed from three to nine months.

Molecular weight and distribution of Toha chitin during long fermentation

Table 3 shows the reduction of chitin molecular weight by GPC analysis. The average molecular weight of control ranged between 1.022×10^6 and 1.638×10^6. The average molecular weight of L dropped to 6.2% of control, H to 4.6%, S to 1.8%, W2%-L to 5.3%, W2%-H to 11.8%, W4%-L to 6.0% and W4%-H to 6.4% after three-month fermentation. At six-month fermentation, the average molecular weight of L dropped to 4.5% of the control, H to 4.6%, S to 0.7%, W2%-L to 5.3%, W2%-H to 0.6%, W4%-L to 5.4%, and W4%-H to 6.1%. At nine-month fermentation, it was observed that the average molecular weight of L dropped to 0.6% of the control, H to 0.6%, S to 0.1%, W2%-L to 0.7%, W2%-H to 0.3%, W4%-L to 0.8%, and W4%-H to 0.7%. These results were remarkably below the average molecular weight of the control. It seems that the hydrolysis by microorganism and chitinase in Toha-jeot is associated with molecular weight reduction during long fermentation. Table 4 shows the average molecular weights of Toha-jeot fermented for 3, 6 and 9 month periods repectively as well as the formation of chitin oligosaccharides of M.W. 800~1800. In three-month fermented Toha-jeot, chitin oligosaccharides were found in two groups, S and W2%-L. The average molecular weight of S was 7.58×10^3 and chitin oligosaccharides of M.W. 713 to 1546 made up 11.73% of Toha chitin. The average molecular weight of W2%-L was 7.07×10^3 and chitin oligosacchrides

of M.W. 1002 to 1572 made up 1.65% of Toha chitin. Other experimental groups, however, showed no formation of chitin oligosaccharides. In six-month fermented Toha-jeot, the average molecular weight of S was 9.08×10^4 and chitin oligosaccharides of M.W. 804 to 1650 made up 11.02% of Toha chitin and the average molecular weight of W2% H was 8.27×10^3 and chitin oligosaccharides of M.W. 1522 only made up 0.18% of Toha chitin. In nine-month fermented Toha-jeot, considerable amounts of chitin oligosaccharides were noticeable in six groups of except W4% L. The molecular weight and distribution of chitin oligosaccharides in each group were as follows; (L) M.W. 762 to 1655 constituting 16.78% of Toha chitin, (H) M.W. 714 to 1655 constituting 15.49%, (S) M.W. 1436 to 1879 constituting 66.30%, (W2%L) M.W. 782 to 1592 constituting 3.05%, (W2% H) M.W. 823 to 1789 constituting 24.75%, and (W4% H) M.W. 901 to 1721 constituting 5.98%. Among the chitin oligosaccharides of M.W. 823 to 1789, $(GlcNAc)_6$, M.W. 1236, also referred to as NACOS-6 and which has been found to possess anti-tumor properties (15), constituted 4.01-4.37% of Toha chitin. The distribution of molecular weight decreased and the formation of chitin oligosaccharides increased significantly as the fermentation process was prolonged.

In order to compare Toha-jeot with Jeotsaeu-jeot (*Actes japonicus* KISHINOUYE) which is made of sea-water shrimp, the latter was fermented for six months. After this period, the molecular weight of chitin in Jeotsaeu-jeot was between $n\times10^7$ and $n\times10^6$ and no reduction in molecular weight was observed. The molecular weight of Yuk-jeot, however, made of sea-water shrimp captured in June, was $n\times10^4$. The molecular weight of Yuk-jeot after six months of fermentation was similar to that of Toha-jeot after three months. Finally, the formation of chitin oligosaccharides was observed in Toha-jeot fermented over three months, but was not observed in Yuk-jeot or Jeotsaeu-jeot even after six months of fermentation. It would seem that Toha-jeot is superior to Yuk-jeot and Jeotsaeu-jeot in the functional effect of physiologic controls.

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