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A Study on Salt-fermented Seahorse added with Proteolytic Enzyme (Protamex)*

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Abstract

We compared the fermentation of 0 to 4 weeks by manufacturing a rapid low salt-fermented seahorse with a commercial Protamex added to the functional food, *Hippocampus abdominalis*. We studied amino acid composition, content and major amino acids related to flavor during the fermentation process of salt-fermented seahorse. In the enzyme-free group, it showed little change in the content of non-protein nitrogenous compounds, the content of amino acids and degree of hydrolysis. The Protamex enzyme treatment group was rapidly hydrolyzed in one week of ripening, resulting in increased non-protein nitrogenous compounds content, amino acid content and degree of hydrolysis, and minimal changes in the four weeks. The total amino acid contents ratio showed the highest content of glutamic acid in the enzyme additive group, glycine, alanine, which indicates sweet taste, and serine, the content of glycine, alanine, serine, and lysine, indicating sweet taste, has increased significantly over the enzyme-free group. Twenty species of free amino acid in the four-week of salt-fermented seahorse were detected. It detected 43.0% (6 species) in the enzyme-free group and 63.96% (7 species) in the enzyme additive group.

Keywords: Sahorse, Hippocampus abdominalis, Salt-fermented, Protamex, Amino acid

Major classifications: Healthy Food

1. Introduction

The seahorse is used as a component of various drugs worldwide. Especially the *Hippocampus abdominalis* has a high medicinal value in the world (Craig, 2001). The *Hippocampus abdominalis* has antioxidant (Kim et al., 2016), anti-fatigue (Kang et al., 2017), inhibitory effect on tyrosinase (Qian, 2008), anti-cancer (Li et al., 2008), anti-septic arthritis (Ryu et al., 2010), physiological effects such as hepatoprotective (Son, 2016) were shown. This study sought to increase utilization by manufacturing a salt-fermented seahorse, which are not only nutritious but also health functional. As the protein breaks down during the fermentation process, the non-protein nitrogenous compounds

(N.P.N.) such as free amino acid, nucleic acid and related substances, amines, ammonia, and creatine increase, affecting the fineness and unique taste and aroma of salted fish (Dierick et al., 1974). Therefore, we compared the amino acid composition and content of protein hydrolysis by proteolytic enzymes during the fermenting process of fermenting process with the major amino acids that make up active peptide in process of the ferment with the ripening condition of the property *Hippocampus* using pulverized *Hippocampus abdominalis* and Protamex. The amino acids are an important ingredient that has various effects in the body, including regeneration and recovery, metabolism, and vitality. Also, we analyzed the free amino acid to analyze the flavor of salt-fermented seahorse and the ingredients of the unique taste after fermentation.

2. Research methods

2.1. Manufacture of a sample of salt-fermented seahorse

In this experiment we used after grinding *Hippocampus abdominalis* purchased in Australia and the frozen, adding 15% salts and 1% Protamex to 600g, and the ripening at room temperature(26±2°C) for 4 weeks.

2.2. Physicochemical properties analysis

We purchased the Protamex, a food hydrolysis enzyme used for the manufacture of salt-fermented seahorse adding proteolytic enzyme, from Novozymes A/S (Denmark). The samples for the measurement of TCA soluble nitrogen were prepared by adding 20% TCA solution to 5g of salt-fermented seahorse with a final TCA concentration of 10% and homogenizing and filtering. We purchased and analyzed the composition amino acid standard samples (Waco PH standard (Type H): 013-08391) and the free amino acid standard samples (Type B): 016-08641, (AN-II): 015-14461) from WaKo.

3. Results & discussion

3.1. Variation in the content of amino acid and non-protein nitrogenous compounds (N.P.N.)

In this study, it was changed in the content of the non-protein nitrogenous compounds (N.P.N.) and amino acids of salt-fermented seahorse in each fermented period were Table 1and Figure 1.

Non-protein nitrogenous compounds (N.P.N.) rapidly hydrolyzed to 0.23 mg/g in an enzyme-free group and 1.61 mg/g in salt-fermented seahorse with Protamex. It increased to 0.54 mg/g for the enzyme-free group and 1.68 mg/g for the enzyme additive group at 4 weeks of age. These results showed that the proteolytic enzyme greatly contributes to the increase in the initial speed of hydrolysis, which causes most hydrolysis per week of ripening, and that the content of non-protein nitrogenous compounds (N.P.N.) tends to increase over the fermented period.

Table 1: Change of amino acid content of salt-fermented seahorses and salt-fermented seahorses with Protamex during fermentation

Sample	Fermentation time(weeks)				
	0	**1**	**2**	**3**	**4**
Control (mM)	0	1.30	3.95	4.87	7.55
Protamex (mM)	0	315.61	462.70	668.73	954.86

Looking at the changes in amino acid content during the fermented period (Table 1), the salt-fermented seahorse with Protamex increased to 954.86 mM per 4 weeks of the fermentation, but the enzyme-free group showed little

change to 7.55 mM after 4 weeks of fermenting. The amino acid content of salt-fermented seahorse added with enzymes is higher than that of enzyme-free group, which appears to contain a large amount of low-molecular peptide as hydrolysis progresses rapidly due to the Protamex enzyme (Kim, 2016).

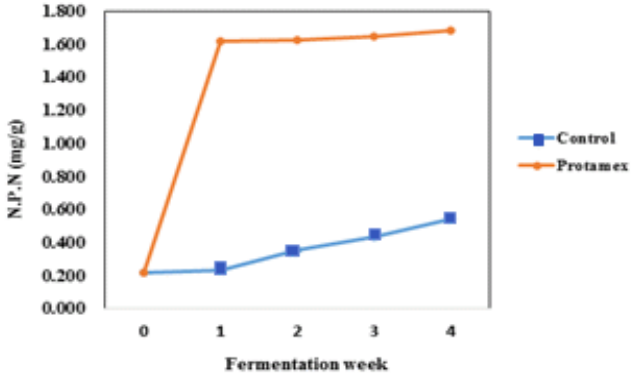

Figure 1: Change of non-protein nitrogenous compounds (N.P.N.) of salt-fermented seahorses and salt-fermented seahorses with Protamex

3.2. Degree of hydrolysis

The hydrolysis changed according to the fermentation period of the salt-fermented seahorse shown in Figure 2. Hydrolysis was found to be 1 percent in the enzyme-free group and 6.5 percent in the enzyme additive group in the first week of fermenting, and about 5 percent more in the enzyme additive group than in the enzyme-free group in the fourth week of fermenting. It is the same result that the hydrolysis rate of the enzyme-treated pearl shell salted seafood was higher than that of the enzyme-free group in Kim's study (Kim et al., 2006). However, the hydrolysis of each salted seafood group was less changed compared to a week of the fermentation. As a result of this experiment, the addition of Protamex, a proteolytic enzyme to it, increases the initial rate of hydrolysis, but after a certain period it maintains a nearly constant degree of decomposition. In this regard, hydrolysis products are believed to hinder hydrolysis speed after a certain period.

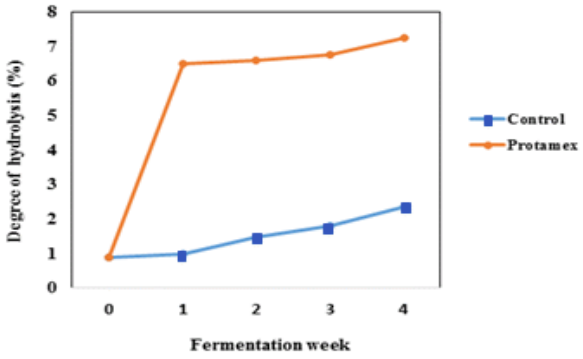

Figure 2: The degree of hydrolysis in salt-fermented seahorses and salt-fermented seahorses with Protamex during fermentation

3.3. Composition and content of total amino acid contents

Table 2 and Figure 3 was showed the results of analysis of composition total amino acid contents and content. As a result of the four-week fermentation of the enzyme-free salt-fermented seahorses, the total content of total amino acid contents was reduced. Each amino acid was low-grade carbonyl compound because of various enzyme interactions in the process of microorganism's fermentation (Pedraja, 1970). On the other hand, the total amino acid contents of the enzyme-added salt-fermented seahorses was 3.647 (g/100g), a doubling from the initial fermentation. In particular, the content of glutamic acid, the sweetening ingredients glycine, alanine, serine, and lysine were greatly increased compared to the enzyme-free group. Among them, the glutamic acid content, which is a savory taste, was the highest at 0.489 (g/100g).

Table 2: Total amino acid contents in salt-fermented seahorses and salt-fermented seahorses with Protamex

	Storage time(weeks)		
	0	4	
Amino acid	**Control (g/100g)**	**Control (g/100g)**	**Protamex (g/100g)**
Asp	0.154	0.128	0.333
Thr	0.074	0.065	0.170
Ser	0.078	0.068	0.199
Glu	0.205	0.169	0.489
Pro	0.066	0.054	0.238
Gly	0.068	0.067	0.417
Ala	0.107	0.105	0.300
Val	0.079	0.072	0.194
Ile	0.063	0.051	0.145
Leu	0.128	0.107	0.243
Tyr	0.055	0.041	0.060
Phe	0.071	0.058	0.139
Lys	0.127	0.108	0.270
His	0.044	0.035	0.089
Arg	0.091	0.074	0.257
Cys	0.032	0.022	0.050
Met	0.035	0.026	0.055
Total	**1.476**	**1.251**	**3.647**

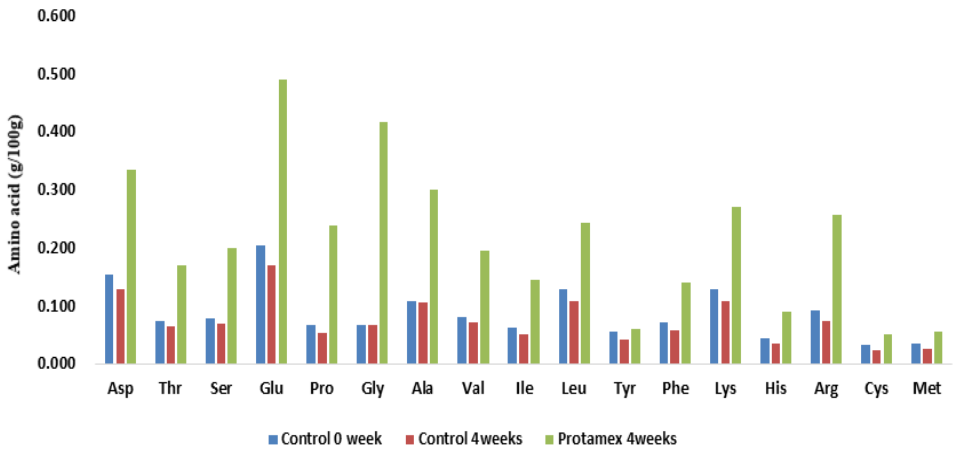

Figure 3: Amino acid contents in salt-fermented seahorses and salt-fermented seahorses with Protamex

3.4. Composition and content of free amino acid

A total of 20 kinds of free amino acid in salt-fermented seahorse were detected, and the composition was given in Figure 4, Figure 5, and Figure 6. When aged for four weeks, the major free amino acid in the enzyme-free group accounted for six species (43.0%) as a glutamic acid 197463.108(ug/L), glycine 134341.048(ug/L), alanine 374355.175 (ug/L), valine 159024.512(ug/L), lysine 235876.156(ug/L), leucine 197360.996(ug/L). And the enzyme additive group was leucine 1766559.064(ug/L) alanine 1439072.521(ug/L) arginine 1231572.93(ug/L), lysine 1188278.609(ug/L), valine 1105047.896(ug/L), isoleucine 1071589.655(ug/L) glutamic acid 1048277.482(ug/L), accounting for a total of seven types (63.96%). If you compare the content of free amino acid after the fermentation, glutamic acid, which tastes delicious in salted seafood with Protamex, was 5.3 times higher, aspartic acid was 5.6 times sweeter, leucine was 8.9 times higher, glycine was 3.6 times higher, threonine 5.6 times higher than in enzyme-free group.

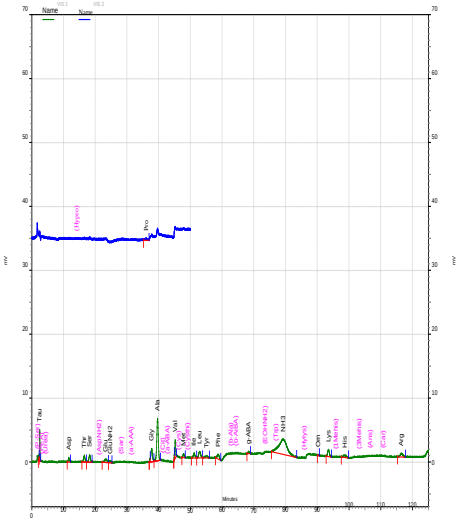

Figure 4: Free amino acid content of salt-fermented seahorses during 0 weeks fermentation

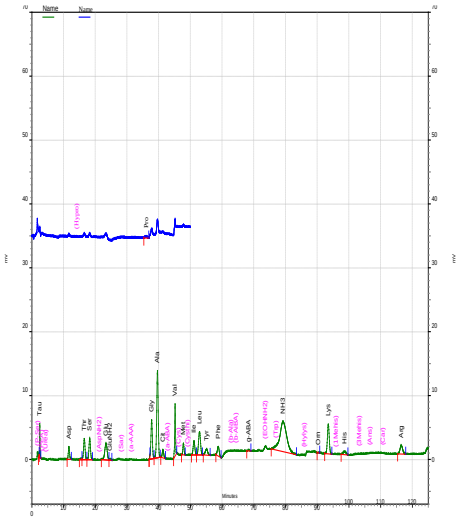

Figure 5: Free amino acid content of salt-fermented seahorses during 4 weeks fermentation

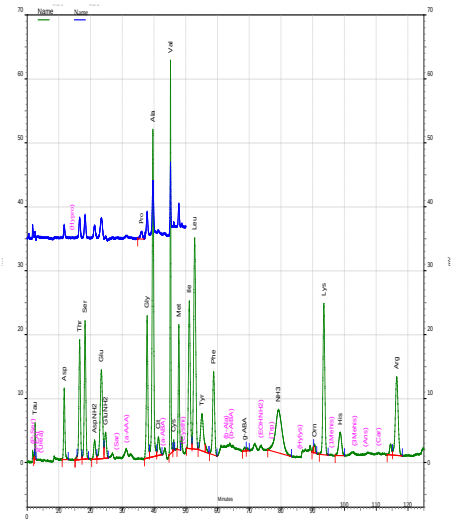

Figure 6: Free amino acid content of salt-fermented seahorses with Protamex during 4 weeks fermentation

4. Conclusion

This study was intended to increase the use as food by making seahorse, which currently has nutritional and health functional characteristics. We wanted to manufacture a rapid low salt-fermented seahorse using the seahorse and the commercially available enzyme and check the composition and content of amino acids involved in flavor in the process of fermentation by the proteolytic enzyme and the main amino acids that make up active peptide.

The changes in non-protein nitrogenous compounds (N.P.N.), content of amino acid content, and hydrolysis over the fermenting period showed little change in the enzyme-free group, and the enzyme additive group was rapidly hydrolyzed in one week of the ripening, showing an increase according to the fermentation period, but the changes were minimal in the four weeks of fermenting. The salt-fermented seahorse with Protamex, proteolytic enzyme is thought to contain a lot of low molecular weight peptides as most hydrolysis proceeds per week of fermenting.

As a result of examining the composition ratio of total amino acid contents, the content of the total amino acid contents of salt-fermented seahorse added in the enzyme-free group and enzyme additive group was increased from the early stages of the fermentation. The content of glutamic acid, which represent savory taste and glycine, alanine, serine, and lysine, which represent sweet taste in the enzyme additive group, was significantly increased than that of the enzyme-free group. In particular, the content of glutamic acid, which represent savory taste was the highest.

A total of 20 types of the free amino acid were detected in the analysis. After four weeks of fermenting, we identified six types (43.0%) of major free amino acid with glutamic acid, glycine, alanine, valine, lysine and leucine in the enzyme-free group and seven types (63.96%) of major free amino acid with leucine, alanine, arginine, lysine, valine, isoleucine, and glutamic acid in the enzyme additive group. In the salt-fermented seahorse added by Protamex, the content of free amino acid, which is savory taste (glutamic acid, aspartic acid) and sweet taste (alanine, leucine, glycine, lysine, threonine, proline), was three to eight times higher than that of the enzyme-free group.

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