Identification of Amino Acid Composition of Protein in Dulse (Laminaria Japonica Areschoung)

Jae Yong Lee

미역 蛋白質의 Amino acid 組成의 同定

李 載 容

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Introduction

Since Korean pennisula is surrounded by the ocean, the marine products have utmost importance on the Korean diet. Accordingly, in considerable amount of sea weeds such as dulse, sea-tangle and laver have been consumed as food stuff.

Above all, dulse is the one which is largely used as daily side dishes, and specially it was the traditional nutrient used for convalescence after child birth.

Though the origin or reason of this tradition are not definitly clarified however, through long experiences, dulse was known to increase secreting of milk and to promote health after child birth.

The chemical composition of sea weed is mainly of carbohydrate, protein and with little contents of fiber and fat. The carbohydrate is known as one of polysacchorides composed chiefly of galactose, and the protein belongs to glycoprotein which is complexes of protein and carbohydrate.

Since the true compounds of carbohydrate and protein in sea weeds are not known, the nutritional value was also not verified.

In spite of wide use of dulse in daily diet and nutriment for promoting health from child birth, the scientific evaluation of this food were almost completely neglected. Therefore, the writer made an investigation of dulse to determine its nutritional value.

Food's nutritional value is largely depend on contained protein and the nutritional value of protein is varied by kind and quantity of amino acid composition in the protein.

Because of the lack of information on amino acid composition of dulse protein[2], the result of study on amino acid was presented in this report.

Materials used and experiments

Dulse obtained from market without any previous treatment was used for this experiment. The protein contained in dulse was quantitatively analysed for soluble and insoluble crude proteins, and then the total nitrogen and proteineous nitrogen were determined by means of Kjeldahl method[3]To determine the amino acid composition of protein in dulse by means of hydrolysis products of water soluble and insoluble proteins were tested respectively and free amino acid was also examined for the reference purposes.

a). Preparation of sample for chromatography

(1) Hydrolysis of insoluble protein:

20 grams of sample(dulse) in water was put in an extractor. The container was heated to extract soluble protein. Discarded soluble protein.

The residue was dried in an oven at 100°C. 2 grams of sample added with 30 ml of 20% HCl was heated to 120-130°C on oil bath for 30 hours, cooled off, and then filtered.

The filtrate was evaporated to expel HCl and concentrated further on water bath then added 5 ml of distilled water to the concentrated solution which was to be used later for chromatographic test.

(2) Hydrolysis of soluble protein:

To this sample solution obtained through the previous process, 10% trichlor-acetic acid was added to create precipitation, filtered and dried in an oven at 80°C. Thus dried sample was hydrolyzed by acid as in the previous process to be used as sample for chromatography.

(3) Free amino acid:

To 10 grams of dulse powder in amortor, 200

ml of 80% ethanol was added, ground and was filtered in the next day. After filtrate was concentrated on a water bath, its residue was dissolved in water, again filtered and dried by evaporation, then extracted with isopropyl alcohol. After isopropyl alcohol was evaporated, water was again added to the residue and was separated by centrifuge. The seperated solution was used as sample.

b). Chromatography test

The filter paper used for this experiment was 23cm x 23cm size English made Whatman No. L.

The solvents used were phenal and 0.1% ammonia at the proportion of 4 to 1 and n-butanol, acetic acid and water at the proportion of 4 to 1 to 5 respectively for the first and secondary tinge development.

For the first development it took 10-11 hours at 25°C and for the secondary development it did approximately 6 hours.

Since the Brushes(4) reported that, when filter paper developed with phenol was to be dried,amino acid was abundantly decomposed by merely heating to 60°-105°C for 5 minutes, the writer attempted secondary development after phenol vapor was completely expelled at room temperature from the first filter paper developed.

After the secondary development, completely dried filter paper was tinted by using alcohol solution of ninhydrine. Consden[5] was the first one who used ninhydrine dissolved in butanol and later the Potten's[6] reported that ethanol instead of butanol was fast in tinging, reacted evenly on many amino acids and fading of amino acid's spot color was also even.

Based on this theory the writer used solution of ninhydrine dissolved in ethanol with which sprayed evenly on filter paper and heated 5 minutes at 100°C to develope coloring. Because the spots were faded in a few days, pink coloured spot was fixed immediately by spraying alcohol solution of 0.%8 cupper nitrate after tinging.

To identify amino acid in arginine, methionine, cystine, histidine, proline, tyrosine and phenyl alanine etc., the following special reagents were used.

1. Sakaguchi reagent[8]
2. Platinic iodide reagent[9]
3. Isatine reagent[10]
4. Sulfanilic reagent[11]

To determine amino acid in the samples, paper chromatogram for the standard amino acid was prepared for comparison.

C). Results and discussion

1. The total nitrogen contained in dulse was measured by Kjeldahl method and the result is shown in the following table.

Total Nitrogen (%)	Total nitrogen contained insoluble crude protein (%)
1.642	0.588
Proteineous nitrogen contained in soluble crude protein (%)	Total nitrogen contained in insoluble crude protein (%
0.492	1.089

The writer has identified 16 kinds of amino acid including alanine which is the component of insoluble protein, 14 kinds of insoluble protein and kinds of free amino acid excluding alanine were respectively acknowledged in dulse.

In determining amino acid the spots of valine and methionine were so closely appeared on the nearly same spot that its identification was difficult. But methionine was easily identified by platinic iodide reagents.

Phenyl alanine and leucine were not easily separated, but with ninhydrine solution phenyl alanine was appeared purple blue while leucine was tinged pink. Again with acetic acid containing isatine solution, phenyl alanine showed blue spot, and leucine did pink.

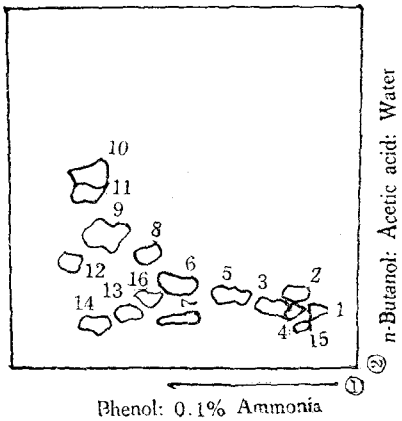
The spots of serine and glycine also appeared closely, however, they were identified as glycine in purple red and serine in red brown by ninhydrine solution, and with isatine solution glycine was appeared in pink and serine was coloured in light brown.

For identifying free amino acid, chlorophyll and other substances had to be eliminated in order to obtain better tinge development. In free amino acid, clear blue spots which were not observable in the products of hydrosis were seen. It was presumably known as methyl-histidine by location and color of the spot according to references.[12]

2. Amino acid composition of protein in dulse

Figure 1

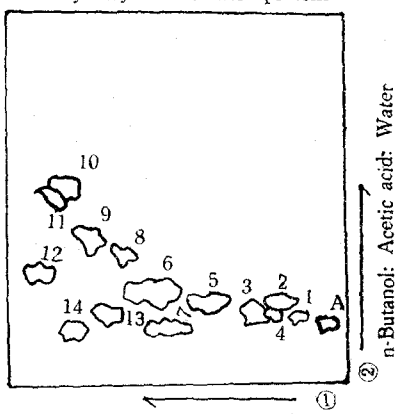
Standard amino acid Chromatogram



(1) Aspartic acid
(2) Glutamic acid
(3) Glycine
(4) Serine
(5) Threonine
(6) Alanine
(7) Lysine
(8) Tyrosine

Figure 3

Hydrolysis of soluble protein



A; Not identified

In assumption of spot size and shade of color appeared on chromatogram, the greater quantity of alanine, glutamic acid, glycine and serine were contained in decomposition products of insoluble protein while that of soluble protein contained more quantity of alanine, glutamieacid and glycine.

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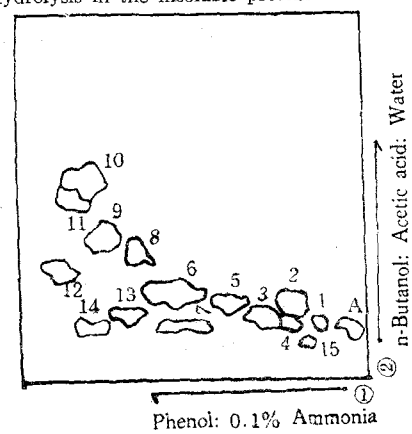
Figure 2

Hydrolysis in the insoluble protein

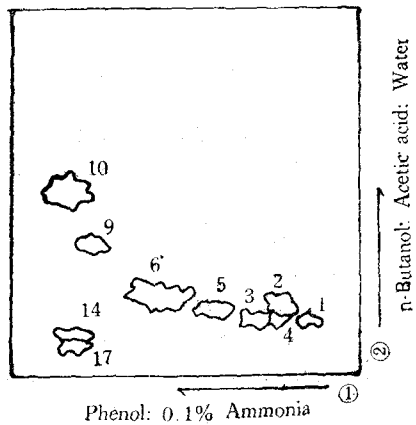


A: Not identified

(9) Valinemethionine
(10) Leucine
(11) Phenyl alanine
(12) Proline
(13) Histidine
(14) Arginine
(15) Cystine
(16) Oxyproline

Figure 4

Free amino acid



17: Methyl histidine?

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