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Research Paper

Characterization of dried bonito extract residue using acid hydrolysis

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ABSTRACT

The common method to extract useful components of dried bonito shaving dust result in the formation of residue (dregs from the extraction process) that contains over 80% of high-quality protein, and the currently available options are only to sell it as low-cost animal feed or fertilizer. In this study, we processed the extraction residue using an acid hydrolysis method with the aim of developing a functional food ingredient and analyzed the protein recovery rate, flavor component ratio, anti-oxidative properties and anti-mutagenicity. Our results revealed a protein recovery rate of 5 to 92%, an amino acid content of 49 to 844 mg/100 ml, anti-oxidative properties of 5 to 27 units/100 ml and anti-mutagenicity against the direct-acting mutagen furylfuramide (AF-2) of 9 to 40%. These findings provide new insight into the hydrolysate obtained in this study as a novel and effective seasoning material.

Keywords: Dried bonito, extraction residue, amino acids, anti-oxidative properties, anti-mutagenicity.

INTRODUCTION

In recent years, although the volume of marine products consumed worldwide has tended to increase, it is feared that marine resources as a food source will one day be exhausted due to increases in fish catches, the deterioration of marine habitats and increases in the global population. Therefore, the advanced utilization of by-products from marine product processing and the creation of value-added products using waste from the processing of such marine products are anticipated.

One type of waste from the processing of marine products is dried bonito residue, which is produced in large quantities during the various stages of producing *katsuobushi* (dried bonito), a traditional ingredient in Japanese cooking; this residue accounts for 82 to 84% of the weight of the fish prior to processing (Wada, 1999). This residue, which is produced during the process of turning boiled and smoked bonito into fermented and dried bonito, is the dust that results from shaving off the surface of the fermenting bonito. Calculated on the basis of the annual dried bonito production volume, approximately 4,000 tons of shaving dust is produced each year (Wada, 1999).

The main methods for extracting useful components from this shaving dust are alcohol extraction and hot water extraction. However, flavor components are mainly extracted using the former method and only some soluble substances are extracted using the latter method; during this secondary processing, residue that contains high-quality protein (dregs from the extraction process referred to as "dregs") is produced, but at present, the only available options are to sell it as low-cost animal feed or fertilizer. Although dregs hardly have any taste, they still contain large volumes of protein. If this protein can be broken down into amino acids or low-molecular-weight peptides that have taste/function, they can be reutilized as a seafood seasoning material with taste and function, which in turn will improve their value.

An enzyme or hydrochloric acid (HCl) is often used for protein hydrolysis. The action of an enzyme is insufficient to completely break down the entire protein into free amino acids and as such this approach result in more peptides than free amino acids in the enzymatic decomposition product. Moreover, it has been reported that a seasoning material made with an enzymatic

decomposition product has certain downsides, such as the relatively high cost of the enzymes considering the low protein recovery rate, in addition to problems associated with its taste, such as it being weak and sometimes accompanied by bitterness (Suzuki and Tamura, 2014; Ren, 2014). However, components with diverse physiological properties, including anti-oxidative and anti-hypertensive properties, have been differentiated and identified from the enzymatic decomposition product of fish-derived proteins such as dried bonito (Yokoyama et al., 1992; Terasawa et al., 2010; Suetsuna, 1999).

Hydrochloric acid is more effective than enzymes in breaking down proteins, and when hydrolysis is performed using HCl (6 mol/L) at 110°C for 24 h, the protein is almost completely decomposed into free amino acids. However, by adjusting the hydrolysis time and temperature, as well as, the HCl concentration, it is possible to achieve a high rate of protein recovery and to produce an excellent protein hydrolysate containing free amino acids and low-molecular-weight peptides with functions such as anti-oxidative properties and anti-mutagenicity.

Although it has been reported that decomposition products obtained from the dregs of bonito flakes used for making broth that were decomposed using enzymes or Koji mold (*Aspergillus oryzae*) have nutritional value and functionality, the scope of such studies has mostly been limited to protein decomposition methods such as enzymatic hydrolysis and Koji mold treatment and there was hardly any study that focused on dregs produced when reutilizing dried bonito shaving dust.

In this study, with the aim of developing a functional food ingredient from high-protein dregs produced when reutilizing shaving dust produced during the production of dried bonito, we used HCl to perform acid hydrolysis of dregs under various conditions. Samples taken from the hydrolysate were then evaluated for taste/nutritional value/functional amino acid content and correlations among tested variables, such as dreg protein recovery rate, anti-oxidative properties, anti-mutagenicity and acid hydrolysis conditions were investigated.

MATERIALS AND METHODS

Test samples

Dregs provided by a dried bonito processor in Makurazaki City, Kagoshima Prefecture, Japan were used as test samples. The total nitrogen content of the dregs was 7.36%.

Acid hydrolysis sample preparation

Although acid hydrolysis of proteins is normally performed using HCl (6 mol/L) at 110°C for 24 h; in this study, to study the influence of acid hydrolysis conditions on not only free amino acids but also other functional changes, such as anti-oxidative properties and anti-mutagenicity, decomposition temperatures of 100 and 110°C were used with decomposition times of 5, 10 and 20 h at HCl concentrations of 1, 2, 3, 4, 5, and 6 mol/L, respectively. Dregs were weighed in 0.1 g samples and then mixed with 5 ml of HCl solution (of varying concentrations). The acid hydrolysate was filtered, repeatedly vacuum-concentrated/evaporated for complete removal of all traces of HCl filtrate, replenished with distilled water to make a volume of 5 ml, and then frozen as acid hydrolysis samples until testing could be performed.

Amino acid analysis

Processing of amino acid analysis test samples

The acid hydrolysis samples were diluted with a sodium citrate buffer solution (pH 2) and filtered through a 0.2 µm membrane filter prior to being used as measurement samples.

Amino acid analysis conditions and amino acid content calculation

The amino acid content was measured using high performance liquid chromatography (HPLC, Prominence LC-20, Shimadzu Corporation). ISC-30Na/S0504 columns filled with an ion-exchange resin were used; the mobile phase used a sodium-type mobile phase kit, while the reaction solution was an OPA reaction solution. The column temperature was 60°C, reaction temperature was 65°C, while flow rate was 0.6 ml/min and spectrofluorimetry performed with an excitation wavelength of 348 nm and an emission wavelength of 450 nm. Although the qualitative analysis of amino acids was performed as permitted by the retention time of the separated substances, the amino acid content per 100 ml of the acid hydrolysis sample being tested and the amino acid content per 100 g of dregs were calculated in quantitative analyses using absolute calibration against an internal reference solution. Amino acid analysis was performed only once.

Antioxidant properties

Acid hydrolysates were used as test samples to test their anti-oxidative properties. The XYZ-dish method of ultra weak chemiluminescence reported by Iwai et al. (2000) was used to evaluate the anti-oxidative properties. Atto

Luminisensor PSN (model AB-2200CP) was used to measure hydrogen peroxide (a reactive oxygen species). Gallic acid, which is widely used as a food additive (as an antioxidant), was used as a positive control for antioxidant activity. The antioxidant effect was evaluated by first defining the antioxidant effect of 0.1 mmol gallic acid as one unit (integral value), then measuring the sample under the same conditions and dividing the resulting integral value by the unit corresponding to the antioxidant effect of 0.1 mmol gallic acid, and finally calculating unit numbers for each 100-ml sample of the acid hydrolysate. The antioxidant activities of all samples were measured thrice, while average values and standard errors were calculated.

Mutagenicity and anti-mutagenicity

The hydrolysate was used as a sample. Although there are several methods for detecting mutagenicity and antimutagenicity; in light of the properties of the sample, which contained free histidine, a simple method of the UMU assay, which is not influenced by the presence of histidine, was used (Muraoka, 2007). An Umulac AT kit (Jimuro Co., Ltd., Gunma, Japan) was used for the UMU assay. A direct mutagen [furylfuramide (AF-2)] and an indirect mutagen [2-aminoanthracene (2-AA)], which shows mutagenicity in the presence of the rat liver S9 fraction (9,000 g supernatant fraction) were used as the known mutagens. Tests were performed in accordance with the instructions on the package insert, and 0.1 μg/ml AF-2 and 0.03 μg/ml 2-AA used to evaluate the antimutagenicity of samples, with a diluent used as the control.

Anti-mutagenicity evaluation

The intensity of antimutagenicity in a sample was evaluated using the formula:

Antimutagenic performance (%) = $[(A - B)/A] \times 100\%$

Where:
A = Mean light absorption in the mutagen (0.1 μg/ml AF-2 or 0.03 μg/ml 2-AA) and in the diluents;
B = Mean light absorption in the mutagen (0.1 μg/ml AF-2 or 0.03 μg/ml 2-AA) and in the sample.

Measurements were taken thrice with each sample, and the mean and standard deviation calculated.

Statistical analysis

Mean value ± standard deviation (SD) was denoted as the presented form and was evaluated by one-way analysis of variance (ANOVA). Among three independent conditions: decomposition temperature, reaction duration and HCl concentration, respectively two of them were kept unchanged and the anti-oxidative and anti-mutagenic activity were compared under single variable by Dunnett's test to calculate the significance of differences at the level of $p < 0.05$.

RESULTS

Effect of acid hydrolysis as seen from amino acid content and protein recovery rate

Samples were hydrolyzed using 1 to 6 mol/L HCl, and the amino acid content converted on a per 100 g of dregs basis (Tables 1 and 2). When the total amount of amino acids was compared according to the decomposition time (5, 10, and 20 h) and HCL concentration (1 to 6 mol/L) for each sample hydrolyzed at 100°C, 2.5 to 27.1 g, 6.4 to 30.8 g, and 10.7 to 36.8 g, respectively, of amino acids was detected per 100 g of dregs. On the other hand, from samples hydrolyzed at 110°C, 15.5 to 33.8 g, 23.7 to 37.9 g and 27.7 to 42.2 g of amino acids were detected per 100 g of dregs at the corresponding decomposition times. Although the amino acid content tended to increase with an increase in the hydrolysis time and HCl concentration at both temperatures, the highest amino acid content was found in the sample processed at 110°C in 6 mol/L HCl for 20 h. The protein recovery rate calculated using the residual protein content in the dregs was 46 g/100 g and the amino acid content in each acid hydrolysis sample was 5 to 80% when the temperature was 100°C, while it was 34 to 92% when the temperature was 110°C (Table 2). At either temperature, the protein recovery rate remarkably increased with an increase in the processing time and HCl concentration. The highest recovery rate (92%) was observed in the sample processed at 110°C in 6 mol/L HCl for 20 h.

Effect of acid hydrolysis as seen from anti-oxidative performance

Table 3 shows the anti-oxidative performance on hydrogen peroxide for each acid hydrolysis sample. A comparison of the anti-oxidative performance per 100 ml hydrolysate according to the decomposition time (5, 10, and 20 h) and HCl concentration (1 to 6 mol/L for samples hydrolyzed at 100°C revealed an anti-oxidative performance of 27 to 14 units, 22 to 11 units, and 20 to 9 units, respectively. On the other hand, for samples hydrolyzed at 110°C, the anti-oxidative performance was 21 to 8 units, 20 to 6, units and 13 to 5 units, respectively. At either temperature, a tendency for the anti-oxidative performance to decline was observed as acid hydrolysis

Table 1: Effect of acid hydrolysis conditions (100°C 5・10・20 h) on amino acid content (g/100 g).

18AA	1 M	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M
	100°C-5 h						100°C -10 h						100°C -20 h					
Tau	0.03	0.04	0.04	0.04	0.04	0.03	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.03
Asp	0.14	0.80	1.02	1.30	1.51	2.35	1.03	1.82	2.02	2.13	2.14	2.76	1.81	2.30	2.23	2.16	2.87	2.91
Thr	0.03	0.16	0.17	0.25	0.54	0.95	0.07	0.18	0.36	0.67	0.88	1.19	0.23	0.66	1.09	1.11	1.44	1.82
Ser	0.05	0.30	0.33	0.47	0.80	1.18	0.12	0.32	0.55	0.95	1.02	1.26	0.32	0.86	1.24	1.24	1.28	1.66
Glu	0.24	1.05	1.00	1.44	2.46	3.77	0.40	1.16	1.93	3.09	3.27	4.22	1.34	1.64	2.60	4.04	4.43	5.70
Pro	0.07	0.43	0.47	0.65	1.16	1.74	0.17	0.45	0.86	1.38	1.64	2.06	0.34	0.92	1.74	1.41	2.12	1.89
Gly	0.13	0.98	1.02	1.25	1.67	2.06	0.49	1.02	1.36	1.83	1.82	2.15	0.88	1.60	2.09	1.82	2.03	2.14
Ala	0.13	0.74	0.72	0.97	1.50	2.11	0.31	0.77	1.18	1.84	1.85	2.29	0.75	1.64	2.35	2.01	2.21	2.53
Cys	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Val	0.01	0.09	0.09	0.14	0.31	0.57	0.03	0.09	0.20	0.36	0.51	0.73	0.14	0.39	0.58	0.67	0.95	1.41
Met	0.03	0.15	0.14	0.21	0.35	0.55	0.07	0.16	0.24	0.41	0.48	0.63	0.14	0.40	0.55	0.61	0.65	0.91
Ile	0.01	0.03	0.03	0.07	0.23	0.51	0.02	0.04	0.13	0.30	0.44	0.67	0.07	0.29	0.53	0.54	0.92	1.33
Leu	0.03	0.42	0.45	0.68	1.32	2.16	0.13	0.46	0.93	1.57	1.92	2.53	0.53	1.46	2.18	2.18	2.72	3.44
Tyr	0.17	0.82	0.79	0.77	0.97	1.17	0.88	0.72	0.69	0.84	1.04	1.23	0.65	0.80	0.93	1.01	1.16	1.31
Phe	0.28	1.00	1.02	1.09	1.17	1.40	0.74	0.96	0.97	1.40	1.22	1.62	0.76	1.05	1.27	1.12	1.51	1.44
His	0.77	1.29	1.43	1.49	1.45	1.50	1.12	1.26	1.27	1.40	1.44	1.55	1.14	1.22	1.62	1.41	1.14	1.10
Lys	0.29	1.10	1.32	1.64	2.61	3.71	0.57	1.11	1.98	2.65	3.53	4.29	1.16	2.25	3.12	3.47	4.50	5.11
Arg	0.03	0.39	0.36	0.51	0.91	1.36	0.20	0.42	0.62	1.17	1.21	1.56	0.38	1.14	1.50	1.41	1.63	2.09
Total	2.5	9.8	10.4	13.0	19.0	27.1	6.4	11.0	15.3	22.0	24.4	30.8	10.7	18.7	25.7	26.2	31.6	36.8

18AA	1 M	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M
	110°C-5 h						110°C-10 h						110°C-20 h					
Tau	0.03	0.03	0.03	0.03	0.03	0.02	0.03	0.03	0.04	0.03	0.04	0.03	0.04	0.03	0.03	0.04	0.03	0.03
Asp	4.22	4.12	3.47	3.11	3.34	2.97	4.53	4.32	4.00	3.75	3.44	2.84	6.21	5.74	4.82	3.92	3.33	3.18
Thr	0.19	0.54	0.82	1.13	1.46	1.49	0.74	1.35	2.21	1.88	1.78	1.74	0.71	1.33	1.58	1.41	2.05	2.09
Ser	0.33	0.79	0.96	1.45	1.38	1.33	0.83	1.32	1.44	1.58	1.39	1.64	0.89	1.45	1.46	1.28	1.53	1.73
Glu	1.16	3.86	3.94	4.75	4.89	5.34	3.67	5.60	5.77	5.68	5.83	6.61	5.66	6.14	6.18	6.30	6.60	6.97
Pro	0.50	0.99	1.43	1.16	2.30	2.17	0.93	1.53	1.86	1.84	2.93	2.78	1.00	1.39	1.97	2.72	2.63	2.99
Gly	1.19	1.57	1.73	1.98	2.25	2.04	1.60	1.95	2.00	2.13	2.21	2.58	1.82	2.02	2.11	2.24	2.45	2.32
Ala	0.88	1.69	1.88	2.38	2.45	2.56	1.62	2.31	2.37	2.46	2.41	2.52	2.07	2.20	2.62	2.69	2.69	2.80
Cys	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Val	0.12	0.34	0.49	0.78	0.96	1.06	0.48	0.86	1.12	1.32	1.55	1.74	0.45	0.76	0.96	1.55	1.69	1.86
Met	0.17	0.33	0.45	0.65	0.71	0.68	0.39	0.70	0.95	0.85	0.74	0.87	0.37	0.69	0.70	0.79	0.83	0.99
Ile	0.03	0.28	0.44	0.75	0.92	1.05	0.39	0.79	1.52	1.78	1.59	1.78	0.37	0.67	0.93	1.78	1.77	1.93
Leu	0.46	1.29	1.71	2.43	2.82	2.83	1.52	2.51	2.58	3.56	2.78	2.76	1.54	2.70	2.71	2.70	3.06	3.27
Tyr	1.33	0.68	0.76	0.95	1.21	1.18	0.76	0.99	1.26	1.28	1.31	1.30	0.78	0.93	0.97	1.20	1.38	1.41
Phe	1.39	1.00	0.99	1.36	1.61	1.55	0.96	1.19	1.34	1.46	1.61	1.65	1.13	1.37	1.38	1.68	1.79	1.64
His	1.47	1.43	1.41	1.32	1.18	1.16	1.76	1.44	1.34	1.31	1.20	1.11	1.81	1.67	1.50	1.50	1.22	1.20
Lys	1.51	1.82	2.42	3.21	4.56	4.60	2.36	3.17	4.06	5.11	5.03	3.74	1.91	2.32	3.37	3.43	4.18	5.50
Arg	0.50	0.86	1.15	1.42	1.71	1.70	1.13	1.70	2.40	2.15	1.93	2.24	0.95	1.50	1.71	2.32	2.05	2.29
Total	15.5	21.6	24.1	28.9	33.8	33.7	23.7	31.8	36.3	38.1	37.8	37.9	27.7	32.9	35.0	37.6	39.3	42.2

Table 2: Effect of acid hydrolysis conditions on protein recovery rate (%).

HCl concentration	100°C			110°C		
	5 h	10 h	20 h	5 h	10 h	20 h
1 M	5	14	23	34	52	60
2 M	21	24	41	47	69	72
3 M	23	33	56	52	79	76
4 M	28	48	57	63	83	82
5 M	41	53	69	73	82	85
6 M	59	67	80	73	82	92

Table 3: Effect of acid hydrolysis conditions on anti-oxidative activity (units/100 g).

HCl concentration	100°C			110°C		
	5 h	10 h	20 h	5 h	10 h	20 h
1 M	26.5±0.4	22.2±0.2	20.1±0.2	21.1±0.1	20.1±0.1	13.1±0.2
2 M	20.1±0.3	18.1±0.2	15.1±0.2	17.1±0.2	14.2±0.1	10.0±0.1
3 M	18.3±0.5	17.1±0.2	13.1±0.1	15.0±0.2	13.0±0.2	8.1±0.1
4 M	16.0±0.2	15.1±0.2	12.0±0.1	12.0±0.2	10.1±0.2	6.2±0.1
5 M	15.8±0.2	13.0±0.1	10.1±0.2	10.0±0.2	8.1±0.2	5.1±0.1
6 M	14.0±0.1	11.0±0.1	9.0±0.1	8.2±0.2	6.0±0.2	5.0±0.2

Table 4: Effect of acid hydrolysis conditions on anti-mutagenicity (AF-2 0.1 µg/ml)).

HCl concentration	100°C			110°C		
	5 h	10 h	20 h	5 h	10 h	20 h
1 M	9.0±0.1	16.0±0.1	19.0±0.1	17.0±0.1	21.2±0.3	15.0±0.0
2 M	16.0±0.1	27.0±0.1	32.0±0.1	25.0±0.0	30.0±0.1	23.0±0.1
3 M	23.0±0.1	29.9±0.1	34.0±0.1	30.0±0.1	33.9±0.1	28.0±0.1
4 M	27.0±0.1	36.0±0.1	40.0±0.1	34.0±0.1	38.9±0.1	24.1±0.1
5 M	30.0±0.1	39.0±0.1	33.2±0.2	38.0±0.1	31.9±0.2	20.0±0.1
6 M	36.0±0.1	31.2±0.1	24.9±0.2	33.1±0.2	26.0±0.1	9.8±0.1

conditions became more intense. Except for between 4 and 5 mol/L at 100°C for 5 h and between 5 and 6 mol/L at 110°C for 20 h, respectively, this tendency was statistically significant.

Effect of acid hydrolysis as seen from anti-mutagenicity

Although a suppressive effect on the direct mutagen AF-2 was detected from the hydrolysate at a rate of 9 to 40% (Table 4), no such effect was detected for the indirect mutagen 2-AA. The suppressive effect on AF-2 was compared according to the hydrolysis temperature, decomposition time and HCl concentration, and excluding three samples (6 mol/L HCl at 110°C for 5 h and 5 and 6 mol/L HCl at 110°C for 10 h), the suppressive effect on AF-2 in the samples processed at 110°C for 5 or 10 h was 17 to 39%, resulting in a higher performance than that observed in samples processed at 100°C for 5 or 10 h. In contrast, the suppressive effect on AF-2 was 19 to 40% in samples processed at 100°C for 20 h, resulting in a higher performance than that observed in samples processed at 110°C for 20 h. Both differences were significant.

DISCUSSION

Wide-ranging migratory fish, such as bonito, contain large amounts of lean muscles rich in hemoglobin. The dry content of *katsuobushi*, which is manufactured in a complicated process from only the muscle of bonito, contains at least 85% protein. As the protein is denatured when it undergoes vitrification, among other processes, during roasting and drying, *katsuobushi* is known to be the hardest food worldwide. As a consequence, when extracted with hot water or ethanol solutions, only about 10 to 15% of the nitrogen content is extracted as essence,

Table 5: Effect of acid hydrolysis conditions (100℃ 5・10・20 h) on amino acid content (mg/100 ml acid hydrolysate).

18AA	1 M	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M
	100℃-5 h						100℃ -10 h						100℃ -20 h					
Tau	0.6	0.9	0.8	0.8	0.7	0.7	0.8	0.8	0.7	0.7	0.6	0.6	0.6	0.7	0.7	0.6	0.5	0.5
Asp	2.8	16.0	20.5	26.0	30.2	46.9	20.7	36.4	40.4	42.6	42.8	55.1	36.1	45.9	44.6	43.2	57.4	58.2
Thr	0.5	3.1	3.4	5.0	10.8	19.0	1.3	3.5	7.2	13.3	17.6	23.9	4.6	13.1	21.9	22.1	28.8	36.4
Ser	1.1	6.0	6.6	9.4	15.9	23.5	2.4	6.5	11.0	19.0	20.4	25.2	6.3	17.1	24.9	24.8	25.6	33.3
Glu	4.8	20.9	20.0	28.8	49.1	75.4	8.0	23.1	38.6	61.7	65.4	84.4	26.8	32.9	52.0	80.8	88.6	113.9
Pro	1.4	8.6	9.3	13.0	23.3	34.8	3.3	9.0	17.2	27.5	32.7	41.1	6.9	18.3	34.9	28.3	42.4	37.8
Gly	2.5	19.7	20.4	25.0	33.3	41.2	9.7	20.4	27.2	36.7	36.4	43.0	17.6	32.1	41.8	36.4	40.6	42.9
Ala	2.7	14.8	14.5	19.5	29.9	42.1	6.2	15.3	23.7	36.8	37.0	45.8	15.0	32.8	47.0	40.2	44.2	50.6
Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Val	0.3	1.8	1.8	2.7	6.3	11.5	0.7	1.8	4.0	7.2	10.2	14.6	2.8	7.8	11.6	13.3	18.9	28.3
Met	0.5	3.1	2.9	4.2	6.9	10.9	1.3	3.1	4.8	8.2	9.6	12.5	2.8	8.0	11.0	12.1	13.0	18.2
Ile	0.2	0.7	0.6	1.5	4.5	10.2	0.4	0.8	2.7	5.9	8.8	13.4	1.4	5.9	10.6	10.9	18.3	26.6
Leu	0.7	8.5	9.1	13.7	26.3	43.2	2.5	9.2	18.6	31.5	38.4	50.7	10.7	29.1	43.6	43.7	54.3	68.8
Tyr	3.4	16.3	15.8	15.4	19.4	23.4	17.6	14.3	13.7	16.9	20.8	24.5	13.1	16.1	18.6	20.1	23.2	26.2
Phe	5.7	20.0	20.4	21.8	23.3	28.0	14.8	19.2	19.5	27.9	24.3	32.4	15.3	21.0	25.4	22.4	30.3	28.9
His	15.5	25.9	28.6	29.8	28.9	30.0	22.5	25.2	25.4	28.0	28.8	31.0	22.8	24.5	32.3	28.1	22.8	22.0
Lys	5.8	22.0	26.3	32.9	52.1	74.2	11.4	22.2	39.5	53.1	70.7	85.8	23.2	45.0	62.4	69.4	90.0	102.2
Arg	0.5	7.7	7.3	10.1	18.2	27.2	3.9	8.3	12.3	23.3	24.2	31.1	7.6	22.9	30.0	28.3	32.6	41.8
Total	49.1	195.9	208.1	259.5	379.4	542.1	127.6	219.3	306.6	440.3	488.8	615.2	213.5	373.1	513.1	524.6	631.7	736.4

18AA (mg/100 ml acid hydrolysate)	1 M	2 M	3 M	4 M	5 M	6 M	1M-	2 M	3 M	4 M	5 M	6 M	1 M	2 M	3 M	4 M	5 M	6 M
	110℃-5 h						110℃-10 h						110℃-20 h					
Tau	0.7	0.6	0.6	0.6	0.6	0.4	0.6	0.6	0.7	0.6	0.8	0.6	0.7	0.6	0.6	0.8	0.6	0.6
Asp	84.4	82.3	69.5	62.2	66.7	59.4	90.5	86.4	80.1	75.0	68.9	56.8	124.1	114.8	96.4	78.5	66.7	63.6
Thr	3.7	10.7	16.5	22.6	29.3	29.8	14.9	27.1	44.3	37.6	35.7	34.8	14.3	26.6	31.5	28.3	40.9	41.7
Ser	6.5	15.7	19.1	29.0	27.6	26.6	16.7	26.4	28.9	31.6	27.7	32.7	17.8	28.9	29.1	25.6	30.5	34.6
Glu	23.3	77.2	78.9	95.0	97.8	106.8	73.4	112.0	115.4	113.6	116.5	132.3	113.2	122.8	123.6	126.1	132.0	139.5
Pro	10.0	19.8	28.5	23.2	45.9	43.4	18.6	30.6	37.2	36.8	58.6	55.6	20.1	27.7	39.3	54.5	52.6	59.8
Gly	23.9	31.5	34.5	39.6	45.0	40.9	32.0	39.1	40.0	42.6	44.3	51.7	36.4	40.5	42.3	44.8	49.0	46.3
Ala	17.5	33.9	37.6	47.6	49.0	51.3	32.4	46.2	47.4	49.1	48.1	50.4	41.5	43.9	52.4	53.8	53.7	56.0
Cys	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Val	2.4	6.8	9.8	15.7	19.1	21.3	9.6	17.2	22.5	26.4	30.9	34.7	9.1	15.2	19.2	30.9	33.8	37.1
Met	3.5	6.6	8.9	13.0	14.1	13.6	7.8	13.9	19.0	16.9	14.7	17.5	7.4	13.7	14.1	15.8	16.5	19.9
Ile	0.7	5.5	8.9	15.0	18.4	21.0	7.8	15.8	30.4	35.5	31.9	35.5	7.5	13.4	18.6	35.6	35.4	38.7
Leu	9.3	25.8	34.2	48.6	56.3	56.6	30.5	50.3	51.6	71.1	55.6	55.2	30.8	54.1	54.2	54.1	61.2	65.4
Tyr	26.6	13.5	15.2	19.0	24.2	23.6	15.1	19.7	25.1	25.5	26.1	26.0	15.6	18.6	19.4	24.1	27.6	28.2
Phe	27.8	20.0	19.9	27.2	32.2	30.9	19.2	23.8	26.8	29.1	32.2	33.1	22.5	27.4	27.6	33.5	35.8	32.8

Table 5: Conts. Effect of acid hydrolysis conditions (100°C 5・10・20 h) on amino acid content (mg/100 ml acid hydrolysate).

His	29.5	28.6	28.2	26.4	23.7	23.2	35.3	28.8	26.7	26.3	24.0	22.2	36.3	33.5	30.0	30.0	24.5	24.0
Lys	30.2	36.3	48.4	64.2	91.3	92.0	47.2	63.5	81.2	102.2	100.6	74.7	38.1	46.4	67.5	68.6	83.6	110.1
Arg	10.0	17.2	23.1	28.4	34.1	34.1	22.7	34.1	48.0	42.9	38.6	44.8	18.9	30.0	34.2	46.5	41.1	45.9
Total	309.9	432.2	481.7	577.5	675.3	675.0	474.2	635.6	725.3	762.8	755.3	758.6	554.2	658.2	699.9	751.3	785.7	844.1

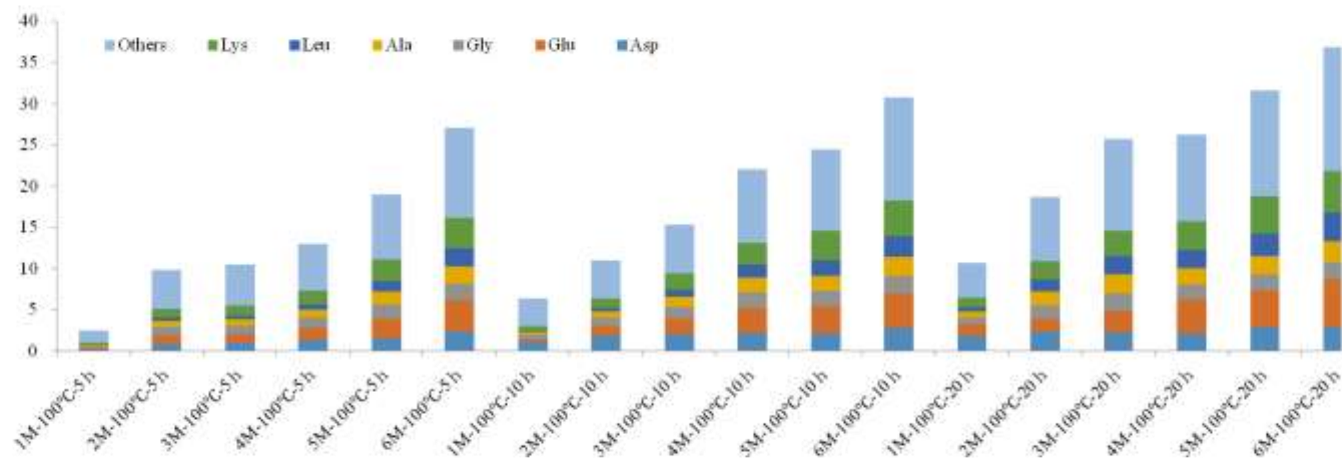

Figure 1: Effect of acid hydrolysis conditions (100°C 5・10・20 h) on flavorful and functional amino acids (g/100 g).

and the remnant contains not less than 80% of the protein of the dry content of *katsuobushi* (Yokoyamas et al., 2002). Accordingly, large quantities of dregs are created when making broth from *katsuobushi*, most of these are used as poultry feeds (Yokoyama et al., 2011).

In recent years, broth dregs were studied as food ingredients. Although bonito broth dregs made from dried bonito treated with *A. oryzae* have been observed to improve lipid metabolism to a certain degree when fed to rats on a high-fat diet (Tanaka et al., 2006); to have anti-hypertensive properties and to be very useful as a seasoning material methods of degrading the protein are limited to enzymatic degradation and *A. oryzae* treatment (Yokoyamas et al., 2002). However, enzymatic decomposition is difficult because the protein undergoes changes such as vitrification and we believe that the low rate of protein recovery from bonito broth residue (despite treatment with enzymes and *A. oryzae*) is a problem. In this study, we therefore used strong acid hydrolysis to recover as much high-quality protein from the dregs as possible, with the aim of using it as a flavoring and functional food ingredient.

First, as a result of acid hydrolysis, 2.5 to 42.2 g of amino acids was recovered from 100 g of dregs. Table 5 shows the quantity of each amino acid recovered (converted into quantity per 100 ml of acid hydrolysate).

Although the main components of *umami* in seafood, such as dried bonito, are nucleic acids such as IMP, amino acids such as glutamic acid, glycine, alanine, histidine, arginine and proline are also important factors that constitute flavor (Yamazaki, 1994). In this study, samples degraded at 100 and 110°C with 6 mol/L HCl for 20 h contained more amino acids, with the exception of histidine, than broth derived from dried bonito itself, as reported by Yamazaki (1994) and Maekawa et al. (2005, 2007). Furthermore, Figures 1 and 2 show that there were large quantities of flavorful and functional aspartic acid, glutamic acid, glycine, alanine, leucine and lysine among the detected amino acids, comprising 40 to 61% of the total

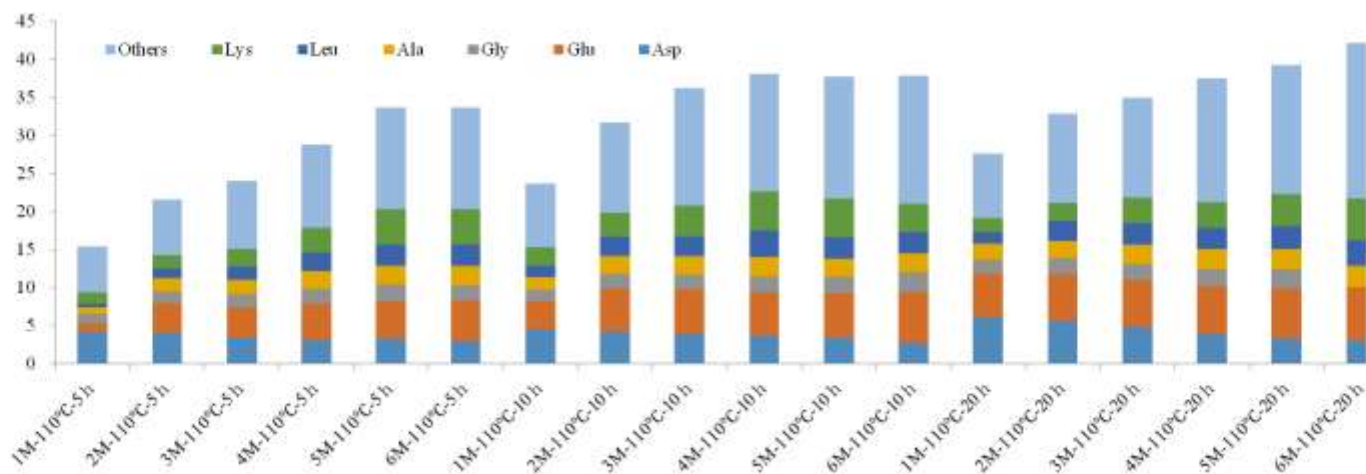

Figure 2: Effect of acid hydrolysis conditions (110°C 5・10・20 h) on flavorful and functional amino acids (g/100 g).

amino acid content when samples were degraded at 100°C and 51 to 69% when they were degraded at 110°C.

Dried bonito and the broth derived from it not only contain excellent flavor components but also are reported to have functional properties, such as anti-oxidative and anti-hypertensive properties and help control reduced stress response (Yokoyama et al., 1992; Suzuki and Motosugi, 1996; Yamada et al., 2008; Nashimoto et al., 2008; Yamada and Matsuda, 2007). Suzuki and Motosugi (1991) reported that a study of the anti-oxidative properties of water-soluble constituents, such as peptides, amino acids, and browning substances, revealed that the anti-oxidative effect of bonito extract was due to the effects of amino acids (serine, proline, glycine, isoleucine, asparagine, aspartic acid, and glutamic acid), peptides, and the browning substances and phenols formed during roasting and drying.

In this study, when acid hydrolysis of the shaving dust in the supplied dregs was performed, each acid hydrolysate displayed an inhibition ratio (5 to 27 units/100 ml) against hydrogen peroxide and against the direct mutagen AF-2 (9 to 40%). Furthermore, Fukunaga and Kubomura (2007) reported that peptides with anti-cancer properties were extracted from the so-called dregs remaining after the essential constituents had been extracted from dried bonito (used as an ingredient in the manufacture of flavorings and processed food) by treatment with organic solvents such as methanol and ethanol. In this study, although we did not measure the peptide content in the samples tested, the free amino acid and protein recovery rate remarkably increased along with increases in time, temperature and HCl concentration used for degradation. However, a tendency for anti-oxidative properties and anti-mutagenicity to decrease with these increases was observed; there is the possibility that samples treated with a low level of acid hydrolysis possess functional benefits.

On the basis of the analyses performed in this study, we can degrade the thermally denatured protein in the dregs into peptides and free amino acids with taste and functional properties by adjusting the conditions under which acid hydrolysis is performed.

The dregs tested in this study mostly contained nearly a tasteless, thermally denatured protein, a quality it shares with bonito broth dregs. In the future, we plan to process bonito broth dregs and also bonito shaving dust under analytical conditions and to study not only amino acids, peptides, and antihypertensive properties but also polycyclic aromatic hydrocarbons (due to concerns from the standpoint of safety in recent years about their formation during the smoking process, hence, we can clarify methods that can guarantee the safe reutilization of bonito dregs (Kasane et al., 2015; Commission, 2013).

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