Studies on Flavor Components in Boiled Crabs—I Amino Acids and Related Compounds in the Extracts

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In order to elucidate the flavor components characteristic of boiled crabs, the suitable method of extraction was examined and extracts were prepared from different edible parts of five species of crab. The amino acid composition of the extracts was analyzed as the first step of a series of investigations on the flavor components. A common feature of the free amino acid composition of the leg meat extracts was a very high content of glycine and arginine, with somewhat lower amounts of proline and taurine; the former two comprised about 50% of the total of free amino acids. In the extracts of the hepatopancreas and ovary, the abundant amino acids were taurine, glycine, and arginine. After acid hydrolysis of the extracts, the total of free amino acids showed a 10–20% increase in the leg meat and the ovary and a 50–70% increase in the hepatopancreas. The main amino acids to increase were glutamic and aspartic acids.

A boiled crab is one of the most popular and tasty kinds of sea food in Japan, and it has a unique flavor somewhat different from those of boiled lobster or prawn. In spite of some sporadic data on free amino acids^{1,2)}, mineral constituents³⁾, lipid fraction⁴⁾, and nucleotides⁵⁾ of crab muscle, information on the flavor components of boiled crabs has been extremely limited apart from the report of Take *et al.*⁶⁾ who examined the meat extract of snow crab and concluded that some free amino acids played a leading role in the taste.

We have undertaken the present study in order to elucidate the contribution of each extractive component to the characteristic flavor of boiled crabs through detailed quantitative analysis of amino acids and related compounds, nucleotides, organic bases, organic acids, sugars, and minerals, followed by organoleptic testing of synthetic extracts prepared on the basis of the analytical data.

This paper deals with the method for preparation of extracts and with amino acids and related compounds in the extracts of meat, hepatopancreas, and ovary of boiled crabs. Proximate composition of the materials has also been determined.

Experimental and Results

Materials

Five species of live crab shown in Table 1 were used as materials. The crabs were cooked in a boiling water containing 3% sodium chloride for

15–25 min according to the commercial practice, and the meat of meri of the first walking legs (both sexes), the hepatopancreas (male), and the ovary were removed. They were packed in polyethylene bags, frozen immediately and brought back to the laboratory with dry ice.

Proximate Composition

Samples of the leg meat, hepatopancreas, and ovary were analyzed in duplicate for moisture, crude protein, crude fat, and crude ash according to the usual methods.

Results obtained are shown in Table 2. The meats, irrespective of species and sex, were similar in composition. Unlike the meat, the hepatopancreas was rich in crude fat, which ranged from 14.5% in Alaska king crab to 27.0% in horsehair crab. The level of crude protein was high in the ovary; 23.5% in Alaska king crab, 23.6% in snow crab, and 28.5% in king crab.

Preparation of Extract

For setting up an extraction method fit for the present purpose, the following eight kinds of extract were prepared from the meat of snow crab, and the contents of total and α -amino nitrogens in the extracts and their flavors were compared with each other. Total nitrogen was determined by the semi-micro Kjeldahl method. α -Amino nitrogen was measured before and after hydrolysis by the Van Slyke method. For hydrolysis 1 ml/portion of the extract was heated with 1 ml of

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Table 1. Characteristics of crab specimens

Species	Date of sampling Place of catch	Sex	Number of specimens	Body Weight (g)	Carapace Width (cm)
Snow crab "Zuwaigani"	Jan., 1976	M	11	346-1246 (739)*	9.5-14.0 (12.0)*
<i>Chionoecetes opilio</i>	Niigata	F	14	76- 234 (129)	6.5- 8.5 (7.2)
Blue crab "Gazami"	Sept., 1976	M	15	95- 290 (134)	12.0-18.0 (14.2)
<i>Portunus trituberculatus</i>	Hamamatsu	F	15	55- 170 (107)	11.5-14.5 (12.7)
Horsehair crab "Kegani"	Nov., 1976	M	11	530- 755 (615)	7.4- 8.6 (8.0)
<i>Erimacrus isenbeckii</i>	Kushiro	F	17	140- 247 (192)	6.0- 8.0 (7.0)
King crab "Hanasakigani"	Nov., 1976	M	6	572-1545 (860)	10.1-14.5 (11.7)
<i>Paralithodes brevipes</i>	Nemuro	F	9	432- 833 (588)	9.3-12.0 (10.0)
Alaska king crab "Tarabagani"	Nov., 1976	M	1	1612	17.3
<i>Paralithodes camtschaticus</i>	Nemuro	F	3	747-1340 (947)	12.6-14.1 (13.1)

* Numbers in parentheses indicate average values.

Table 2. Proximate composition of cooked crab tissues (%)

Part analyzed	Moisture	Crude protein	Crude fat	Crude ash
Snow crab				
leg meat (male)	78.6	18.5	1.2	1.4
leg meat (female)	81.6	16.6	0.7	0.6
hepatopancreas	64.9	9.1	23.3	1.1
ovary	60.0	23.6	11.4	1.3
Blue crab				
leg meat (male)	80.7	16.6	0.8	2.0
leg meat (female)	80.1	16.2	0.7	2.4
Horsehair crab				
leg meat (male)	76.9	20.8	0.6	1.8
leg meat (female)	76.4	20.1	0.7	1.9
hepatopancreas	56.2	11.2	27.0	1.7
King crab				
leg meat (male)	78.7	18.8	1.0	1.5
leg meat (female)	78.0	18.3	0.8	1.7
ovary	56.8	28.5	12.1	1.5
Alaska king crab				
leg meat (male)	80.0	16.4	0.5	1.7
leg meat (female)	81.9	15.1	0.7	2.0
hepatopancreas	71.5	11.1	14.5	1.7
ovary	61.5	23.5	11.0	1.4

conc. HCl at 110°C for 16 h in an evacuated sealed tube. The organoleptic tests were carried out by a panel consisting of four men and a woman of our laboratory.

Extracts I-III: Samples of 30 g each of the meat were homogenized with 34, 53, and 90 ml of ethanol, respectively, according to the method described in our previous paper⁷¹. After centrifugation, the precipitates were extracted twice with 60 ml portions of 60, 70, and 80% ethanol, respectively. The combined aqueous ethanolic extracts were concentrated to remove ethanol *in vacuo*. The resulting turbid solutions were treated five times with 30 ml portions of diethyl ether to remove lipids and made up to 60 ml with water. The extracts thus prepared were designated as E-I, II, and III, respectively.

Extracts IV-VI: The hot-water extract of 500 ml was prepared from 150 g of the meat after KOMATA *et al.*⁸¹ To each 100 ml of the extract were added 150, 233, and 400 ml of ethanol to make a final ethanol concentration of 60, 70, and 80%, respectively. The precipitates formed were centrifuged off and washed twice with 20 ml portions of 60, 70, and 80% ethanol. The com-

Table 3. Examination of the method for preparing the extract

Extract	(a)	Organoleptic test by five panelists				Total-N (mg/100 g)	α -Amino-N (mg/100 g)
		(b)	(c)	(d)	(e)		
E-I	+++	+++	+++	+	++* ¹	546	239 (66)* ²
E-II	+	+	+	++	+	496	224 (66)
E-III	++	+++	+	+	+	473	223 (82)
E-IV	+	+	++	++	+	639	250 (111)
E-V	+++	+++	++	+++	+++	579	258 (75)
E-VI	+	+++	+++	+++	++	571	264 (49)
E-VII	-	-	-	-	-	409	177 (60)
E-VIII	-	-	-	-	-	597	284 (11)

*¹ +++: Excellent, ++: Good, +: Fair, -: Poor.*² The amounts increased after hydrolysis are given in parentheses.

bined supernatants were evaporated under reduced pressure to remove ethanol and made up to 60 ml with water. The extract thus obtained were designated as E-IV, V, and VI, respectively.

Extract VII: The perchloric acid extract was prepared from 30 g of the meat after NAKAJIMA *et al.*⁹⁾ and made up to 60 ml with water. This extract was named E-VII.

Extract VIII: The trichloroacetic acid (TCA) extract prepared from 30 g of the meat as reported previously¹⁰⁾ was treated nine times with the same volume of diethyl ether to remove TCA and made up to 60 ml with water. It was designated as E-VIII.

The results of the organoleptic test as well as the amounts of total and α -amino nitrogens in the extracts are presented in Table 3. In the organoleptic test almost the same and high scores were given to E-I, V, and VI; most of the panelists answered that these extracts retained well the characteristic flavor of the boiled crab meat. The ratings of E-II, III, and IV were apparently lower than those of the above three, although some of the panelists give high scores to them. The flavors of both E-VII and VIII were unpleasant and quite different from that of the boiled crab meat, probably due to the remaining precipitants.

Among the three highly evaluated extracts, E-I, V, and VI, we preferred E-VI to the others on the basis of its higher values of total nitrogen and α -amino nitrogen before hydrolysis and its lower value of α -amino nitrogen increased after hydrolysis. Thus we adopted the following method for preparation of extract in a series of the present studies.

A 30 g portion of the material was homogenized with 50 ml of water by Ultra-Turrax (Janke & Kunkel KG). The homogenate was stirred in a boiling water bath for 15 min and centrifuged at 5,000 rpm for 15 min. The precipitate was extracted twice with 20 ml portions of water in the same way. The supernatants were combined and made up to 100 ml with water. To the solution was added 400 ml of absolute ethanol with stirring. The ethanolic solution was left for 15 min and centrifuged. The precipitate was washed twice with 20 ml portions of 80% ethanol in the same manner. The combined supernatants were evaporated under reduced pressure below 40°C to remove ethanol and made up to 30 ml with water. The extract obtained was stored at -20°C until analyzed.

Amino Acids in the Extract

Amino acids were measured with an automatic amino acid analyzer (Hitachi KLA-5) by the method for physiological fluid analysis. The results obtained are shown in Table 4. The increased amounts found after hydrolysis are given in parentheses.

A common striking feature of the free amino acid composition of the leg meat in 10 samples was the very high contents of glycine and arginine with somewhat lower amounts of proline and taurine, especially as the former two comprised about 50% of the total free amino acids. In comparison of the individual amino acid contents between both sexes of snow crab and Alaska king crab, the level of the major amino acids of female showed only half or two-thirds of those of male. Apart from proline, horsehair crab also showed a similar pattern in amino acid composition. On the other hand, there was little difference in amino acid composition of both sexes of blue crab and king crab.

In the extract of hepatopancreas analyzed, the abundant amino acids were taurine, glycine, and arginine. They comprised less than 50% of the total free amino acids, of which distribution was rather uniform in contrast to that of the leg meat. Proline, one of the major amino acids in the leg meat, was found to be rather poor in this tissue. It should also be noted that sarcosine in snow crab occurred in high concentration, next to arginine.

In the ovary, taurine, arginine, and glycine were also major amino acids like those in the hepatopancreas. Beside them, sarcosine and tryptophan in snow crab, and sarcosine in Alaska king crab, were present in greater quantities.

The contents of total free amino acids of the leg meat showed as high levels as 2,400–3,200 mg/100 g in horsehair crab, king crab, and Alaska king crab, being around 2,000 mg/100 g or less in snow crab and blue crab. Both in the hepatopancreas and in the ovary, less amounts of total free amino acids were determined than in the leg meat in most cases.

After acid hydrolysis, total free amino acids showed 10–20% increase in the leg meat and the ovary, and 50–70% in the hepatopancreas. It was suggested that hepatopancreas might be abundant in peptides which were soluble in 80% ethanol compared with other tissues. Glutamic and aspartic acids were major increasing amino acids when subjected to hydrolysis.

Table 4. Free amino acid

Compound	Snow crab				Blue crab	
	LmM* ¹	LmF	He	Ov	LmM	LmF
taurine	243	156	148 (17)	118 (1)	214 (27)	183 (2)
aspartic acid	10 (30)* ²	6 (34)	8 (51)	5 (38)	15 (23)	19 (26)
threonine	14 (5)	4 (9)	18 (48)	8 (15)	24 (8)	36 (6)
serine	17 (7)	3 (16)	19 (44)	8 (23)	23 (8)	37 (5)
asparagine* ³	+	10	44	32	147	133
sarcosine	77 (4)	39 (13)	201 (28)	107 (2)	— (+)	— (+)
proline	327 (1)	154 (9)	85 (72)	40 (20)	251 (8)	192 (14)
glutamic acid	19 (53)	11 (44)	22 (136)	16 (63)	43 (237)	47 (194)
citrulline	—	+	+	8	—	—
glycine	623 (33)	253 (9)	172 (59)	101 (33)	444 (33)	454 (13)
alanine	187 (6)	74 (8)	114 (28)	52 (12)	144 (4)	175
α -amino- <i>n</i> -butyric acid	2	+	1	+	4	6
valine	30 (5)	6 (9)	51 (24)	12 (10)	48 (3)	59 (1)
methionine	19 (2)	5 (1)	30	8 (6)	49 (6)	44 (2)
isoleucine	29 (2)	5 (5)	48 (28)	9 (6)	29 (2)	36 (1)
leucine	30 (9)	8 (14)	67 (41)	13 (9)	59 (6)	76 (3)
glucosamine	— (3)	— (2)	— (16)	— (12)	— (2)	— (3)
tyrosine	19 (4)	5 (6)	50 (15)	55 (17)	23	28
phenylalanine	17 (9)	5 (11)	50 (21)	17 (10)	20 (5)	27 (4)
β -alanine	— (+)	+	+	+	— (+)	— (+)
γ -amino- <i>n</i> -butyric acid	+	+	+	+	— (+)	1
ornithine	1 (6)	2 (1)	2 (2)	2 (1)	—	+
ethanolamine	— (8)	— (8)	— (19)	— (4)	— (4)	— (4)
lysine	25 (10)	8 (13)	80 (84)	53 (22)	44 (6)	52 (1)
1-methylhistidine	—	—	—	—	—	—
histidine	8 (3)	5 (5)	11 (17)	10 (11)	23 (6)	33
3-methylhistidine	3	+	4	2	7	+
tryptophan	10	+	12	105	6	9
carnosine	—	—	—	—	—	—
arginine	579 (1)	292 (26)	224 (16)	277 (36)	329 (133)	386 (14)
(ammonia)	4 (10)	— (10)	6 (21)	5 (19)	4 (22)	— (20)
total	2291	1055	1501	1063	1950	2033

*¹ LmM: first leg meat (male), LmF: first leg meat (female), He: hepatopancreas, Ov: ovary.*² The amounts increased after hydrolysis are given in parentheses.*³ asparagine + glutamine: calculated as asparagine.

Discussion

The crabs analyzed here are known to be palatable and commercially important species in Japan. In order to study the characteristic flavor of food, it is generally accepted that knowledge of composition of the extracts, especially that of free amino acids, is of primary consideration.

HUGGINS *et al.*¹¹⁾ stated in a review on the metabolism of crustacea that a striking feature of the free amino acid pool of crustaceans was the presence of large amounts of arginine, glycine, and proline along with lesser amounts of alanine, glutamic acid, and taurine.

One of the present authors¹²⁾ analyzed the amino acid composition of the muscle extracts

from a prawn, *Penaeus japonicus*, and found that glycine was most abundant and comprised about 50% of total free amino acids. Arginine, proline, serine, glutamic acid and alanine were relatively high in concentration. The recovery of amino acid-N, namely 70% showed that amino acids occupied a greater part in the extracted nitrogen.

CAMIEN *et al.*¹³⁾ assayed free amino acids in the muscle and blood serum of four species of marine and euryhaline crustacea including two species of crab, spider crab, *Maja squinado* and Chinese crab, *Eriocheir sinensis*. They showed that high concentrations of free amino acids, particularly glycine, proline, arginine, glutamic acid, and alanine, were found intracellularly in the muscle, and that their concentrations were

composition of crab extract (mg/100 g)

Horsehair crab			King crab			Alaska king crab			
LmM	LmF	He	LmM	LmF	Ov	LmM	LmF	He	Ov
550 (9)	487 (9)	328 (38)	363 (52)	356 (10)	245 (4)	372 (7)	264 (14)	513 (22)	283 (9)
10 (17)	19 (21)	18 (243)	7 (38)	8 (38)	7 (29)	10 (37)	3 (34)	20 (134)	5 (17)
12 (4)	13 (4)	44 (111)	31 (8)	47	13 (19)	35	52 (5)	35 (60)	12 (12)
23 (3)	18 (2)	50 (100)	46 (7)	66	20 (26)	17 (3)	58 (3)	30 (58)	17 (16)
34	113	118	143	136	49	255	204	109	34
—	+	+	(24)	—	(+)	13	37	+	(35)
383 (8)	529 (91)	102 (110)	319 (19)	370 (27)	32 (21)	502 (31)	226 (42)	75 (91)	72 (19)
22 (60)	31 (198)	90 (278)	75 (206)	80 (152)	38 (76)	72 (286)	55 (202)	38 (220)	30 (49)
—	+	6	+	+	—	—	—	+	+
894 (40)	575 (34)	411 (128)	741 (94)	700 (30)	293 (18)	611 (12)	412 (22)	136 (73)	190 (15)
158	141 (5)	152 (95)	176 (2)	187 (2)	74 (11)	186 (4)	180 (5)	128 (50)	72 (6)
+	+	—	5 (1)	9 (1)	3	3 (1)	+	1 (3)	+
14 (1)	12 (3)	89 (77)	56 (5)	62 (4)	16 (10)	54 (13)	64 (7)	60 (37)	16 (7)
13 (5)	17 (8)	57 (29)	43 (1)	52 (4)	8 (8)	38 (11)	43 (1)	30 (21)	11 (5)
5 (1)	5 (1)	69 (54)	48 (3)	65 (5)	14 (6)	31 (10)	54 (5)	44 (28)	14 (5)
9 (3)	9 (4)	36 (199)	72 (5)	105 (9)	25 (12)	63 (19)	86 (94)	86 (49)	26 (10)
—	—	(3)	—	(29)	—	(4)	—	(3)	—
10	19	88 (58)	50 (5)	49 (14)	23 (15)	56 (6)	37 (2)	56 (30)	17 (8)
7 (2)	6 (2)	115 (30)	42 (3)	53 (4)	20 (6)	54 (12)	49 (4)	60 (24)	19 (3)
—	+	—	2	2	1 (5)	5 (4)	+	6 (3)	2
+	1	—	3	4	1	3 (1)	+	15 (2)	2
+	1	3	1 (2)	2	+	3 (3)	5 (1)	2	2
— (5)	— (6)	— (21)	— (13)	— (8)	— (9)	— (9)	—	— (5)	— (5)
10 (3)	11 (3)	140 (61)	45 (6)	63 (8)	27 (16)	56 (17)	66 (9)	77 (60)	53 (9)
+	+	1	+	+	+	1	+	+	1
11 (2)	13 (2)	28 (35)	18 (4)	20 (3)	15 (10)	31 (9)	29 (2)	21 (19)	14 (4)
+	—	—	+	+	— (+)	+	+	— (+)	+
+	+	37	14	15	14	+	8	13	24
—	—	—	—	—	—	+	—	+	—
786 (5)	663 (5)	271 (12)	520 (7)	527 (8)	170 (16)	775 (137)	529 (36)	201 (41)	173 (11)
2 (11)	3 (16)	14 (39)	10 (18)	8 (17)	7 (18)	20 (24)	8 (33)	5 (26)	9 (14)
2953	2686	2267	2707	2836	1155	3252	2432	1826	1172

higher in marine species than in freshwater ones. They supposed that certain free amino acids are probably important for the regulation of osmotic pressure in crustacean muscle. The distribution of free amino acids found in crustacean muscle extracts, described in the foregoing papers, coincides well with the present results.

In spite of many investigations on free amino acids of crabs, most of them were carried out from the biochemical point of view, and food chemical studies of cooked crab are very limited. TAKE *et al.*⁶¹ carried out amino acid analyses on the extracts of boiled meat and viscera of snow crab, *Chionoecetes opilio*, by thin layer chromatography and microbiological analysis. The contents of eleven amino acids they found were very similar to our data, except that valine was present in greater quantity in their sample.

Most of the papers on the flavor of marine products up to this time appear to draw conclusions that certain amino acids contribute to the taste simply because they are present as the major components. In our case as well it is true that main amino acids such as glycine and proline could play marked roles in the taste of cooked crab but we have avoided jumping to such a conclusion until evidence is obtained through the omission test on an artificial extract.

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