Studies on Flavor Components in Boiled Crabs—III

Sugars, Organic Acids, and Minerals in the Extracts

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As a part of our studies on the flavor components of boiled crabs, we have analyzed the extracts from the leg meat, hepatopancreas, and ovary of five species of common edible crabs in Japan for sugars, organic acids, and minerals.

Glucose was found to be the most abundant monosaccharide in these tissues. Small amounts of ribose, fructose, and arabinose were also detected, together with inositol. The main organic acids were lactic and succinic acids. Very small amounts of acetic, propionic and oxalic acids were also detected. Most of the cations were Na^+ and K^+ . Cl^- was the major anion, with somewhat lower levels of PO_4^{3-} .

The sum of all the components analyzed for in the previous and present studies accounted for 92–96%, 83–89%, and 87–94% of the dry matter of the extracts of leg meat, hepatopancreas, and ovary, respectively.

In order to determine the origin of the characteristic flavor of boiled crabs, we have undertaken a series of studies involving a detailed quantitative analysis of extractive components, followed by organoleptic tests of synthetic extracts prepared on the basis of the analytical data. In the preceding papers,^{1,2)} we have reported the contents of nonprotein nitrogenous constituents, such as free and combined amino acids, nucleotides and related compounds, and organic bases, in the extracts from the leg meat, hepatopancreas, and ovary of five species of common edible crabs in Japan. As the final step of the analytical study, we have determined the contents of such nonnitrogenous constituents as sugars, organic acids, and minerals. The present paper deals with the distribution of these compounds and with the distribution of all the extractive components analyzed to date in this series of studies.

Experimental

Materials

Extracts prepared from the leg meat of both sexes, hepatopancreas of males, and ovary of the five species of boiled crabs in the previous study¹⁾ were used.

Sugars

The methods for the pretreatment of the extracts

with ion exchange resins and for the analysis of sugars by gas liquid chromatography (GLC) were essentially the same as described by JARBOE and MABROUK³⁾ and by ARAKAWA *et al.*,⁴⁾ respectively, and are outlined as follows.

The extracts (10 ml) were passed successively through columns of Amberlite CG-120 (H^+ form, 1×20 cm, 100–200 mesh) and Amberlite IR-45 (OH^- form, 1×10 cm, 20–50 mesh), and the columns were washed with water. The effluent and washings were combined and concentrated under reduced pressure. After addition of xylose as an internal standard, the sugars were converted to sugar alcohols with sodium borohydride and the sugar alcohols then to trifluoroacetates, an appropriate amount of which was subjected to GLC on a Shimadzu model GC 5A gas chromatograph with a hydrogen flame ionization detector and a gradient temperature programmer. The conditions of GLC were as follows: Column, glass ($2 \text{ m} \times 3.0 \text{ mm i.d.}$) packed with 2% OV-105 on 60–80 mesh Gas-Chrom P; injector temperature, 160°C ; column temperature, 130°C ; nitrogen carrier gas flow rate, 70 ml/min. Fructose, which is reduced by sodium borohydride to mannitol and sorbitol in equimolar amounts, was detected by thin layer chromatography (TLC).⁵⁾

Organic Acids

Organic acids, which had been adsorbed in the

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Table 1. Sugars and inositol in the extracts of boiled crabs (mg/100 g of tissue)

	Ribose	Arabinose	Fructose	Glucose	Inositol
Snow crab					
leg meat (male)	2	+	+	12	1
leg meat (female)	+	+	+	3	3
hepatopancreas	+	+	11	20	7
ovary	+	+	+	7	3
Blue crab					
leg meat (male)	1	+	+	26	+
leg meat (female)	+	+	+	20	+
Horsehair crab					
leg meat (male)	+	+	+	12	2
leg meat (female)	+	+	+	14	3
hepatopancreas	+	+	4	48	3
King crab					
leg meat (male)	+	+	12	49	1
leg meat (female)	2	+	+	86	+
ovary	+	+	10	22	+
Alaska king crab					
leg meat (male)	+	+	+	61	+
leg meat (female)	1	+	+	24	+
hepatopancreas	+	+	+	62	+
ovary	+	+	12	21	+

+, trace

Amberlite IR-45 column during the pretreatment of the extracts, were eluted with 1 N sodium hydroxide. The eluate was neutralized with 1 N sulfuric acid and was evaporated to dryness under reduced pressure. The residue was esterified with *n*-butanol after YAMASHITA *et al.*,⁶¹ and the resulting butyl esters were extracted with *n*-hexane. After *n*-dodecane was added as an internal standard, the solution was made up to 20 ml with *n*-hexane and a 4 μ l aliquot was injected into the above chromatograph. The conditions of GLC were as follows: Column, glass (2 m $\times$ 3.0 mm *i.d.*) packed with 20% DC 550 on 60–80 mesh Diasolid L; injector temperature, 240°C; column temperature, operated at 55°C for 5 min then programmed for 6°C/min to 215°C; nitrogen carrier gas flow rate, 25 ml/min. Since succinate, fumarate and maleate overlapped each other under the conditions applied here, TLC⁷¹ was also used for their identification.

Minerals

Cations were measured with an atomic absorption spectrophotometer (Nippon Jarrel Ash, Model AA-80) according to the usual method. Chloride and phosphate ions were analyzed by the titration⁸¹ and colorimetric⁹¹ methods, respectively.

Results

Sugars

The results of the sugar analyses are summarized in Table 1. Glucose was the most abundant monosaccharide in all the extracts. Ribose, which is distributed widely in marine products, was found at the level of 2 mg* at the highest in the leg meat and only in trace amounts in the hepatopancreas and ovary. Small amounts of fructose and arabinose were also detected in the extracts. The occurrence of inositol was also confirmed, and its content is shown in Table 1. Oligosaccharides, such as sucrose and maltose, were not detected.

Organic Acids

As shown in Table 2, the main organic acid in the extracts was lactic acid, which amounted to 30–200 mg in the leg meat and 4–70 mg in the viscera, except in the hepatopancreas of the horsehair crab. Succinic acid was also found, but its level was far lower than that of lactic acid. Traces of acetic, propionic, and oxalic acids were also recognized in all or most of the extracts.

Minerals

The contents of six inorganic ions are shown in

* Unless otherwise specified, the contents of extractive components are given in mg/100 g of wet tissues of boiled crabs in this paper.

Table 2. Organic acids in the extracts of boiled crabs (mg/100 g of tissue)

	Acetic	Propionic	Lactic	Oxalic	Succinic
Snow crab					
leg meat (male)	+	+	100	+	9
leg meat (female)	—	+	30	+	4
hepatopancreas	+	+	22	+	3
ovary	—	+	4	—	+
Blue crab					
leg meat (male)	—	+	76	+	3
leg meat (female)	—	+	150	—	5
Horsehair crab					
leg meat (male)	+	+	43	+	3
leg meat (female)	+	+	30	+	4
hepatopancreas	+	+	—	+	3
King crab					
leg meat (male)	+	+	120	+	6
leg meat (female)	—	+	140	—	6
ovary	—	+	14	+	4
Alaska king crab					
leg meat (male)	+	+	130	+	4
leg meat (female)	+	+	200	+	8
hepatopancreas	+	+	70	—	4
ovary	—	+	25	+	3

+, trace; —, not detected

Table 3. Minerals in the extracts of boiled crabs (mg/100 g of tissue)

	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Cl ⁻	PO ₄ ³⁻	Total
Snow crab							
leg meat (male)	191	197	+	+	336	217	941
leg meat (female)	119	82	1	+	195	98	495
hepatopancreas	213	135	1	4	317	21	691
ovary	153	72	1	2	211	16	455
Blue crab							
leg meat (male)	360	211	+	+	547	104	1222
leg meat (female)	432	211	1	+	597	26	1267
Horsehair crab							
leg meat (male)	269	296	+	+	540	236	1341
leg meat (female)	380	269	+	+	654	143	1446
hepatopancreas	318	179	1	4	468	35	1005
King crab							
leg meat (male)	382	277	+	+	625	134	1418
leg meat (female)	370	267	+	+	588	97	1322
ovary	239	105	1	5	396	28	717
Alaska king crab							
leg meat (male)	336	277	+	+	584	169	1366
leg meat (female)	422	217	+	1	600	53	1293
hepatopancreas	263	125	3	4	368	37	800
ovary	342	109	1	5	396	28	881

+, trace

Table 3. Sodium and potassium ions comprised the major part of the cations. The contents of calcium and magnesium ions were only 1 mg or less in the leg meat, and 1–5 mg in the viscera. As for the anions, the content of chloride ion was

around 600 mg in the leg meat of all species other than the snow crab, and was 200–500 mg in the hepatopancreas and ovary. Phosphate ion amounted to 100–200 mg in most of the leg meat samples, and 20–40 mg in the viscera. It is

Fig. 1. Distribution of the extractive components in the crab extracts.

noteworthy that the male leg meat contained a considerably larger amount of phosphate ion than the female leg meat in all the species analyzed.

Distribution of the Extractive Components in the Extracts

The contents of all the extractive components, *i.e.*, free and combined amino acids, nucleotides and related compounds, organic bases, sugars, organic acids, and inorganic components reported in the previous^{1,2)} and present papers were calculated as percentage of dry matter of the extracts and are shown in Fig. 1.

The sum of these six groups of compounds amounts to 92–96%, 83–89%, and 87–94% in the leg meat of both sexes, hepatopancreas, and ovary, respectively. These high recovery values indicate that most parts of the extractive components in our crab samples have been elucidated.

Discussion

Almost no detailed information on the free sugar composition of edible crabs has been available. NAGASAWA,¹⁰⁾ investigating the changes occurring in the amount of reducing sugars during the canning process of the Alaska king crab, analyzed the component sugars only on the juice in the can. TAKE *et al.*¹¹⁾ also determined the content of reducing sugars in the extracts of the

meat and viscera of the boiled snow crab, but they did not report the sugar composition. Thus, the present study may be the first to give in some detail the composition of the free sugars in edible crabs. It is shown that glucose is the most abundant free sugar in the meat as well as in the hepatopancreas and ovary. This feature seems to be in common with fish muscle.¹²⁾ In connection with the analysis of sugars, the glycogen content of the leg meat of the boiled male snow crab was determined, and was found as low as 178 mg.

With regard to the organic acids in edible crabs, TAKE *et al.*¹¹⁾ detected fairly large amounts of pyroglutamic, glycolic, malic, and citric acids in the extracts of meat and viscera of the boiled snow crab by silica gel column chromatography. However, none of these four acids were recognized in our samples, including the snow crab. Instead, lactic and succinic acids, which were not reported by TAKE *et al.*, were found to be the major organic acids. The reason for this discrepancy is not known.

LAUER *et al.*¹³⁾ reported the contents of Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and PO_4^{3-} in the ash of meats of the Atlantic queen crab, *Chionoecetes opilio*, Jonah crab, *Cancer borealis*, and red crab, *Geryon quinquedens*. Although the Na^+ , K^+ , and PO_4^{3-} levels were comparable to those of our meat samples, the Ca^{2+} and Mg^{2+} levels were con-

siderably higher than those of our samples. The difference may be ascribed to the fact that our analyses were on aqueous extracts rather than the ash.

As shown in Fig. 1, more than 92, 83, and 87% of the extractives from the meat, hepatopancreas, and ovary, respectively, were accounted for by some 60 components. These high recoveries indicate that the distribution of extractive components in the samples, especially in the meat, has largely been elucidated, and that the analytical data can be used for prescribing synthetic extracts, simulating natural crab extracts, for omission taste tests. These will follow the present analytical studies.

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