CONTRIBUTION TO THE CHEMISTRY OF THE KING CRAB (Paralithodes camtschatica)

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ABSTRACT

Data are given on (1) yield of meat and waste from whole king crab; (2) proximate composition of meat and waste portions; (3) amount of protein and nonprotein nitrogen in the meat and the amount of sarcoplasmic, fibrillar, and stroma protein in the protein fraction; and (4) number, relative amounts, and electrophoretic mobilities of the protein components of the sarcoplasmic fraction.

BACKGROUND

The widespread acceptance of king crab products by the American consumer has been responsible for the recent and rapid expansion of an American fishery for these giant crab. Prior to 1940, imports of canned Japanese king crab formed the bulk of the domestic consumption of king crab meat. Since World War II, however,

the application of quick-freezing techniques to the preservation of king crab has stimulated the growth of the American industry. This growth has also been aided by investigations (Staff 1942) of the practical aspects of the king-crab fishery.

It has been established that techniques used in preparation and handling are of primary importance in maintaining the quality of the canned and frozen crab. In addition, it has been rec-



Fig. 1 - King crab (Paralithodes camtschatica).

ognized that such factors as habitat, age, moulting, and holding of the live crab all influence the quality of the final product.

Although king crab meat is more suitable for freezing than is the meat of several other species of crab, certain specific adverse changes in quality are occasionally encountered. Dominant among changes that might be mentioned are loss of the natural characteristic color and flavor during long holding periods; development of a yellowish or a bluish discoloration caused presumably by oxidation of tissue pigments; and undesirable texture changes and excessive drip, caused presumably by denaturation of muscle proteins and breakdown of the cellular structure of the tissue. Although studies (Dassow 1950) have been carried out to develop recommendations for processing procedures that would avoid such adverse changes in quality, little work has been done on the basic problems associated with the canning and freezing of king crab.

As part of a continuing investigation of fish proteins and associated technological studies carried out at this Laboratory, the comparative chemistry of the crude protein fraction of the meat of king crab has been studied. It was considered desirable to obtain such background information prior to investigating the specific technological problems that concern the industry. The present paper is a summary of the results of this preliminary study.

The object of this work was physically and chemically to characterize king crab and, in particular, to characterize the crude protein fraction of the edible meats. *Biochemist, Fishery Products Laboratory, Division of Industrial Research and Services, U. S. Bureau of Commercial Fisheries, Ketchikan, Alaska.

To achieve this purpose, the following determinations were carried out: (1) the raw crab was partitioned physically to obtain data on yield, (2) proximate analyses were made of the resulting meat portions and waste, (3) the major protein fractions of the various meat portions were partitioned chemically, and (4) low-ionic-strength extracts of the various meat portions were analyzed electrophoretically.

SAMPLES

Male king crab (<u>Paralithodes camtschatica</u>) obtained in September 1956 from Alitak Bay, Kodiak Island, Alaska, and in May 1957 from Pavilof Bay and Beaver Bay, Alaska, were used in the present study. The crab were placed, while alive, in a still-air freezer held at about -15° C. (5° F.) aboard the vessel. The frozen



Fig. 2 - Trawling for king crab.

crab then were individually wrapped in polyethylene bags transferred to the laborator y freezer at -30° C. $(-22^{\circ}$ F.), and held for subsequent analysis. The individual whole crab ranged in weight from 5.5 to 8.6 pounds with an average weight of 7.3 pounds

PHYSICAL PARTITION OF CRAB

PROCEDURE: To obtain data on yield, each one of four whole raw crabs was physically partitioned, while still frozen, into the following samples: (1) first three sections of all walking-legs,

(2) fourth and fifth sections of all walking-legs, (3) the four sections of both clawlegs, (4) shoulder-joint sections from all legs, (5) all shoulder (body) meat, (6) butc h ering waste, and (7) shell from samples 1 through 5.

RESULTS: The yield of meats from the four physically-partitioned whole king crabs (table 1) indicated that, on the average, 51 percent of the total weight of the

Sample	Decemintion	Yield <u>1</u> /						
Number	Description of Sample	From To	From Total Me					
Indilibei	or Sample	Range	Average	Range	Averag			
in the solution			(Per	cent)				
1	Meat from first three sections of all walking-legs)		18.2-18.9	18.6			
2	Meat from fourth and fifth sections of all walking-legs			35.5-41.1	37.6			
3	Meat from four sections of both claw-legs	\$47.6-53.0	50.9	12.7-13.7	13.2			
4	Meat from shoulder-joint sections from all legs			15.0-16.2	15.5			
5	Meat from shoulder (body))		12.9-16.8	15.2			
6	Butchering waste	26.4-36.0	31.3					
7	Shell from samples 1-5	11.0-26.0	17.8					

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crab was edible meat, consisting of approximately 85 percent total leg meat and 15 percent shoulder meat. Other data, not reported here, collected on a composite sample of six whole crab indicated a yield of 53 percent total meat, consisting of 84 percent total leg meat and 16 percent shoulder meat. The relative amount of meat for any one sample also appeared to be quite constant.

PROXIMATE ANALYSES

PROCEDURE: After the data on yield were obtained, each sample was made homogeneous by passing the frozen material through a prechilled electric grinder.

Proximate analyses (protein, oil, moisture, and ash) were carried out on portions of each sample by employing standard procedures of the <u>Association</u> of <u>Official Agricultural</u> <u>Chemists</u> (1955).

<u>RESULTS</u>: The proximate analyses of the raw king crab meat (table 2) averaged 10.8 percent protein, 0.67 percent oil, 86.2 percent moisture, and 1.95 percent ash. It was of interest to note that, starting at the extremities of the walking legs and progressing to

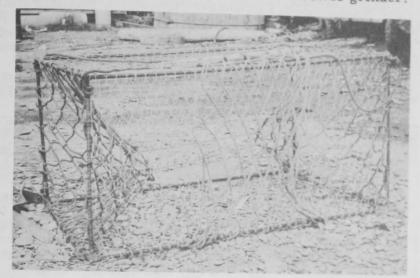


Fig. 3 - Typical crab pot used for king crab.

the shoulder, the protein content tended progressively to increase from 9.6 percent to 12.1 percent; the moisture content tended progressively to decrease from 87.4 percent to 84.3 percent; and the ash content tended progressively to increase from 1.80 percent to

ample	Description of Sample	Proximate Composition ^{1/}									
umber		Protein		Oil		Molsture		Ash			
		Range	Average	Range	Average	Range	Average	Range	Average		
1	First three sections	(Percent)									
	of all walking-legs	7.4-11.3	9.6	0.44-1.72	0.83	85.0-90.7	87.4	1.56-2.20	1.80		
2	Fourth and fifth sections of all walking-legs	8.0-12.9	10.8	0.43-1.04	0.63	83.9-90.2	86.3	1.56-2.16	1,76		
3	Four sections of both claw-legs	7.3-13.0	10.4	0.46-0.87	0.67	84.3-90.6	86.9	1.71-2.38	1.98		
4	Shoulder-joint sections from all legs	7.6-13.4	10.9	0.41-0.78	0.58	83.5-90.2	85.9	1.78-2.38	2,01		
5	All shoulder (body) meat	6.9-14.8	12.1	0.42-1.14	0.66	81.4-89.5	84.3	1.98-2.63	2.18		
-5	Total range and average	6.9-14.8	10.8	0.41-1.72	0.67	81.4-90.7	86.2	1.56-2.63	1.95		
6	Butchering waste	6.3- 9.4	7.9	0.74-2.46	1.54	81.7-85.8	83.4	4.7 -6.3	5.40		
om form i	Shell from samples 1-5 adividually analyzed crab as reported in		16.8	0.24-0.48	0.32	46.5-63.7	54.5	16.0 -22.4	18.5		

2.18 percent. The oil content remained fairly constant. The proximate composition of the claw meat appeared to be more like that of the shoulder-joint meat rather than to be like that of either the leg-section meat or shoulder meat.

The relatively high apparent protein content of the shell from the leg and shoulder sections (average, 16.8 percent; sample 7; table 2) and the low sum of proximate constituents for this material (total, 90 percent; sample 7; not totaled in the table) suggested that the normal proximate analysis scheme did not give an accurate picture of the crude constituents--particularly protein. One sample with a proximate composition of 20.1 percent protein, 0.24 percent oil, 46.5 percent moisture, and 22.5 percent ash (total, 89.3 percent) gave the following data when corrected for chitin nitrogen (Iverson 19561/) and reducing sugars (Hawk, Oser, and Summerson 1947): 18.9 percent protein, 0.24 percent oil, 46.5 percent moisture, 22.5 percent ash, 2.6 percent chitin, and 9.8 percent reducing sugars (less chitin, calculated as glucose), which totals 100.5 percent.

CHEMICAL PARTITION OF PROTEIN FRACTIONS

PROCEDURE: For the partition of the major protein fractions of the meats (samples 1 through 5), a scheme patterned after that of Robinson (1952) was employed. Duplicate 10-gram samples were accurately weighed and transferred to 100-ml. glass centrifuge tubes. Partition was effected as follows:

(1) Nonprotein Nitrogen and Sarcoplasmic Protein Nitrogen Fraction: To each sample were added 10 ml. of phosphate buffer (0.0156 molar potassium phosphate,

Sample	Nonprotein Nitrogen		Protein Nitrogen								
Number	-	U		lasmic		llar	Stroma				
	Mg. N Per g. Meat	% of Total N	Mg. N Per g. Meat	% of Total Protein N	Mg. N Per g. Meat	% of Total Protein N		% of Total Protein N			
1	3.69 (2.72-4.80)	22.7 (21.2-24.4)	4.38 (2.92-6.27)	34.6 (31.4-42.9)	7.74 (5.69-9.78)	61.9 (52.3-68.2)	0.45 (0.10-0.70)	3.6 (0.0-7.5)			
2	5.03 (3.06-6.30)	27.0 (22.0-32.6)	4.60 (3.29-5.45)	35.0 (32.4-42.6)	8.08 (6.45-10.43)	61.2 (53.0-65.6)	0.49 (0.32-0.69)	3.8 (2.0-5.0)			
3	4.43 (2.26-5.74)	21.9 (19.2-24.7)	4.73 (2.50-6.27)		10.20 (6.40-12.60)	66.2 (62.3-72.0)	0.48 (0.26-0.64)	2.8 (1.5-3.9)			
4	4.74 (2,88-6.30)	26.2 (22.8-28.0)	4.20 (2.99-6.86)	30.0 (20.4-41.1)	8.96 (5.93-11.53)	66.6 (57.9-74.3)	0.40 (0.16-0.64)	3.4 (1.0-5.5)			
5	5.13 (3.44-7.65)	24.2			9.82	61.3 (56.9-64.4)	0.28	1.9 (0.8-4.0)			

dibasic--0.0035 molar potassium phosphate, monobasic, pH 7.58, ionic strength 0.05) and a level teaspoon of Filter Cel washed in 0.1 normal hydrochloric acid.



Fig. 4 - Butchering king crab.

total nonprotein nitrogen and sarcoplasmic protein nitrogen. 1/Unpublished.

The samples were held overnight, with occasional stirring, at approximately 0° C. (32° F.). They then were centrifuged for 30 minutes at 0° C. (32°F) at 2,500 times gravity, and the supernatant fluids were filtered through coarse sinteredglass filters into 100-ml. volumetric flasks. The above ex-traction was repeated three times, allowing approximately 1 hour for each extraction. The extracts were diluted to volum = with the phosphate buffer. Duplicate 5-ml. aliquots were taken for each sample from the 100-ml. dilution for Kjeldahl-nitrogen determinations (Hiller, Plazin, and Van Slyke 1948), the results indicating

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In addition, duplicate 5-ml. aliquots were taken from each 100-ml. dilution and added to 5 ml. of 5-percent trichloroacetic acid in 15-ml. centrifuge tubes. The supernatant fluids, after being centrifuged, were filtered through paper and collected in Kjeldahl flasks. The trichloroacetic-acid precipitated protein was washed

twice with 1-percent trichloroacetic acid, the washing being added to the first supernatant fluid. Kjeldahl determinations of nitrogen on these filtrates gave the values for nonprotein nitrogen and sarcoplasmic protein nitrogen, was obtained by difference.

(2) Fibrillar Protein Nitrogen Fraction: The residue remaining from (1) was extracted three times with 25 ml. of 0.1 normal sodium hydroxide, allowing approximately 3 hours at room temperature for each extraction. The samples were centrifuged each time as indicated under (1) and filtered through

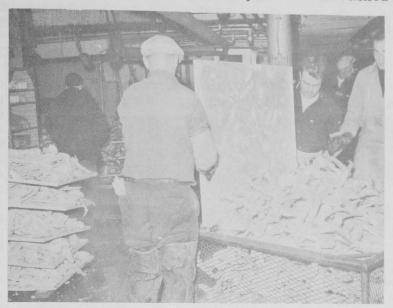


Fig. 5 - Frozen king crab legs being prepared for cold storage.

the original, coarse, sintered-glass filters into 200-ml. volumetric flasks and diluted to volume with 0.1 normal sodium hydroxide. Duplicate 10-ml. aliquots were taken from each sample for Kjeldahl-nitrogen determinations, the results giving total fibrillar protein nitrogen.

(3) Stroma Protein Nitrogen Fraction: The residue remaining from (2) was extracted for 2 hours with 10 ml. of 36 normal sulfuric acid over a bath of boiling water and then was centrifuged and filtered as before into 50-ml. volumetric flasks. The residue was washed twice briefly (10 minutes) with water. The combined supernatant fluids and washings were diluted to volume with water and duplicate 10ml. aliquots were taken for Kjeldahl-nitrogen determinations, the results giving total stroma protein nitrogen.

The sum of nonprotein nitrogen, sarcoplasmic protein nitrogen, fibrillar protein nitrogen, and stroma protein nitrogen indicated that 94 percent (average of 15 experiments) of the total nitrogen of the crab meat (determined separately) was recovered by the above procedure.

<u>RESULTS:</u> Regarding the total crude protein fraction of crab meat, it was determined (table 2) that, on the average, over 24 percent of the total nitrogen was composed of nonprotein nitrogen. This value is considerably larger than is that for most species of fish (Hamoir 1955), being exceeded only by the elasmobranchs, the muscles of which are known to contain appreciable amounts of trimethylamine oxide and urea.

It is of interest to note that considerable amounts of free amino acids (4.4 mg. mitrogen per gram of wet muscle) have been reported (Camien, Sarlet, Duchateau, and Florkin 1951) in North Sea lobster (Homarus vulgaris). In king crab, the average total nonprotein nitrogen was found to be approximately 4.6 mg. per gram of Wet meat.

As to the partition of the protein nitrogen, the average values (as percent of total protein) were 33.3 percent sarcoplasmic, 63.4 percent fibrillar, and 3.1 per-

percent stroma, which appear to be quite comparable with those of fish muscle (Hamoir 1955 and Bailey 1954). Although large differences in any one protein fraction from sample to sample were not noted, it appeared that the claw and shoulderjoint meats contained slightly more fibrillar but less sarcoplasmic protein than did either the walking-leg or shoulder meats. The shoulder meat was appreciably lower in stroma protein than was the other, more active, muscle.

ELECTROPHORETIC ANALYSES OF LOW-IONIC-STRENGTH EXTRACTS

PROCEDURE: For the electrophoretic analyses of low-ionic-strength extracts of crab meat, the following method was employed: A portion of a frozen sample



(samples 1 through 5) was added to an equal volume of the ice-cold, pH 7.58, ionic strength 0.05, phosphate buffer. The mixture was allowed to stand, with only occasional stirring, overnight at 0°-2°C. (32°-35.6° F.). The resulting mixture was centrifuged at 0° C. (32° F.) for 30 minutes at 4,000 times gravity. The supernatant fluid was dialyzed against three changes (1/10,v/v) of the pH 7.58 ionic strength 0.05 phosphate buffer over a 48-hour period at 0° to 2° C. The dialyzed protein was centrifuged at 0° C. for 30 minutes at 24,500 times gravity. The dialyzed, centrifuged protein was used for electrophoretic analyses, the final dialysate being used as

Fig. 6 - King crab meats being given a citric acid bath prior to canning.

supernatant fluid in the electrophoresis cell and to dilute the dialyzed protein solution to below 4 mg. of nitrogen per ml. Electrophoresis was conducted in a Perkin-Elmer Apparatus (Model 38A) at 1° C. (33.8° F.). The conductivity of the dialyzed protein was measured at 0° C.

<u>RESULTS</u>: Electrophoretic examination of low-ionic-strength extracts from the various meats of king crab indicated (fig. 7) the presence of 11 distinct components. Data on composition (table 4) for these components from the various sam-

			Exti	racts o	I Raw J	King-C	rab Me	eat			
Sample	Relative Amount of Component										
Number	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 1.
			. (Perc	ent of	Total A	rea of	Ascene	ding Di	agram)		
1	32.5	4.0	4.5	8.0	-11	.3-	8.3	8.1	6.2	11.7	5.8
2	16.5	5.5	3.9	8.8	6.0	7.8	17.0	13.9	7.3	9.0	7.2
3	25.9	5.2	4.7	8.1	4.6	9.5	12.7	7.1	8.8	9.0	4.9
4	18.4	7.8	5.3	6.4	6.4	8.7	17.3	10.1	5.3	8.7	7.8
5	14.2	11.0	4.7	6.3	-10	.1	18.9		-25.6-		9.2

ples of meat and their electrophoretic mobilities (table 5) were calculated from the ascending diagrams. Descending diagrams were not used for calculations because

considerable overlapping of adjacent boundaries occurred. Since the ascending mobilities have not been corrected, they should be considered as being only approximations.

An obvious difference between low-ionic-strength extracts obtained from kingcrab meat and miscellaneous species of fish (Connell 1953) is that the muscle of king crab contains a far greater proportion of proteins of high mobility (and by in-

ference, of lower molecular weight) than does fish muscle. It is also of interest to note that the mean electrophoretic mobility of kingcrab proteins extracted at low ionic strength is considerably higher than is the corresponding fraction from fish muscle. This observation is in agreement with the findings on lobster extracts (Dubuisson-Brouha 1953) and supports the theory (Hamoir 1955) that electrophoretic mobility increases progressively as the evolutionary ladder is descended.

Certain differences in the relative amounts of any

one protein component throughout the crab were also observed. Since the division of the diagrams into components is somewhat arbitrary, the significance of such diferences is necessarily debatable and will not be enlarged upon at the present time.

Sample		5 - Electrophoretic Mobilities of Components of Low-Ionic-Strength (0.05) Extracts of Raw King-Crab Meat Mobility of Component									
Numbers	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	[No. 11
1-5		11.2							1		2.7
		11.4	0.1	0.0	1.0	0.0	(5.1-5.7)	11 - 1 - 01	11 1 1 01	10 1 0 71	10 5 0 0

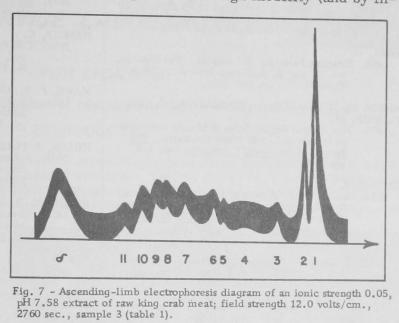
SUMMARY

1. King crab used in these studies ranged in weight from 2.5 to 3.9 kilograms and averaged 3.3 kilograms (7.3 pounds). The yield of meats from physically-partitioned raw whole king crab indicated that approximately 52 percent of the total weight of the crab is edible meat, consisting of approximately 85 percent leg meats and 15 percent shoulder meats.

2. Proximate analyses of the raw meats averaged 10.8 percent protein, 0.67 percent oil, 86.2 percent moisture, and 1.95 percent ash.

3. Of the total nitrogen fraction of the raw meats, over 24 percent proved to be nonprotein nitrogen. The total protein fraction of the meats consisted of 33.3 percent sarcoplasmic protein, 63.4 percent fibrillar protein, and 3.1 percent stroma protein.

4. Electrophoretic examination of low-ionic-strength extracts (sarcoplasmicprotein fraction) of the raw meats of king crab indicated the presence of 11 distinct



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components. Composition and mobility data calculated for these components indicated that the sarcoplasmic fraction from king crab muscle has a higher mean electrophoretic mobility and contains a much greater proportion of proteins of high electrophoretic mobility than does the corresponding fraction from fish muscle.

LITERATURE CITED

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1955. Official Methods of Analysis of the Association of Official Agricultural Chemists, Eight Edition, Washington, D. C., pp. 309-317.

BAILEY, K.

1954. Structure Proteins. II. Muscle. The Proteins, vol. 2, part B, Academic Press Inc., New York, N. Y., pp. 951-1055.

CAMIEN, M. N.; SARLET, H.; DUCHATEAU, G.; and FLORKIN, M.

1951. Non-Protein Amino Acids in Muscle and Blood of Marine and Fresh Water Crustacea. The Journal of Biological Chemistry, vol. 193, pp. 881-885.

CONNELL, J. J. 1953. Studies on the Protein of Fish Skeletal Muscle. 1. Electrophoretic Analysis of Codling Extracts of Low Ionic Strength. The Biochemi-cal Journal, vol. 54, pp. 119-126.

DASSOW, J. A. 1950. Freezing and Canning King Crab. U. S. Fish and Wildlife Service, Bureau of Commercial Fisheries, Fishery Leaflet 374, May, 9 pp.,

DUBUISSON-BROUHA, A. 1953. Bulletin de L'acad'emie Royale de Sciences de Belgique, vol. 39, pp. 121.

HAMOIR, C

- 1955. Fish Protein. Advances in Protein Chemistry, vol. 10, Academic Press Inc., New York, N. Y., pp. 227-288.
- HAWK, P. B.; OSER, B. L.; and SUMMERSON, W. H. 1947. Practical Physiological Chemistry. Twelfth Edition, the Blakiston Co., Philadelphia, Pa., pp. 523.
- HILLER, A; PLAZIN, J.; and VAN SLYKE, D. D. 1948. A Study of Conditions for Kjeldahl Determination of Nitrogen in Proteins. The Journal of Biological Chemistry, vol. 176, pp. 1401-1420.

ROBINSON, D. S.

1952. Changes in the Protein Composition of Chick Muscle During Development. <u>The Biochemi-</u> <u>cal Journal</u>, vol. 52, pp. 621-627.

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1942. The Alaskan King Crab. U.S. Fish and Wildlife Service, Fishery Market News, vol. 4, no. 5a (supplement), May, 107 pp., Washington, D. C.

Washington, D. C. NOTE: Acknowledgments: The author wishes to express his appreciation to King Crab Investigations, Bureau of Com-mercial Fisheries, U. S. Fish and Wildlife Service, for supplying the crab for this study, and to Russel L. Brown, John L. Iverson, and Lydianne Kyte, who assisted in certain parts of the work.



ANTIOXIDANT TO PROTECT SALTED SALMON FROM DETERIORATION

Experiments conducted in Japan have shown that out of several antioxidants examined, butylated hydroxyanisole (BHA) was found to be the most suitable for protecting salted salmon from deterioration due to the oxidation of its oil. The antioxidants were tested in the form of mixtures with common salt. Thawed samples of salmon, after removing the viscera and gill arches, were salted with the various salt mixtures, respectively, up to 40 percent of their weights. After keeping the salted samples for 10 days the adhering salts were removed, the fish being buried anew in batches of fresh salt that amounted to 10 percent of the initial fish weights. The fish treated with the salt mixture of appropriate BHA-content were recognized to be in a satisfactory condition even after 60 days' storage in respect of their organoleptic property as well as the character of their oil, while untreated ones deteriorated badly in 17 days. The optimum quantity of BHA to be used is in the range of 0.005 to 0.02 percent of the weight of raw fish (Bulletin of the Japanese Society of Scientific Fisheries, vol. 22, no. 3, 1956).