Amino Acid Composition and Pepsin Digestibility of Krill Meal

Krill meal (89.7% dry matter; 13.6% ash; 9.3% fat; 59.5% crude protein) was evaluated by determining the pepsin digestibility, the availability of lysine, and the amino acid composition. While the latter was similar to that of white fish meal, the pepsin digestibility of krill meal was poor, presumably resulting from the chitin content. The limitations of the digestibility method and the utilization of chitin by organisms are discussed.

In the last years considerable efforts were made to use the antarctic krill (*Euphausia superba* Dana) for human nutrition (Grantham, 1977; Sahrhage et al., 1978; Hempel et al., 1979). A number of products has been developed for direct human consumption; furthermore, krill meal was tested as feed for pigs (Schulz and Petersen, 1978), poultry (Vogt et al., 1980), and fish (Pfeffer and Becker, 1977; Jahn et al., 1978; Pfeffer and Meske, 1978; Vens-Capell and Horstmann, 1978; Beck et al., 1977; Hilge, 1979; Koops et al., 1979; von Lukowicz, 1979; Tiews et al., 1979). Especially in aquaculture it was demonstrated that krill meal can be utilized as well as fish meal. Beyond that no differences in the eating quality of trout, reared either with krill meal or fish meal, could be detected (Reinacher, 1979). Therefore, krill meal may have a potential market in the future if the production can be made profitable by increasing the catch rate (McElroy, 1980) and the yield of meal production (Reinacher, 1978). In this work the composition and chemical evaluation of krill meal produced during the II Antarctic Expedition of the Federal Republic of Germany, 1977–1978, are described.

MATERIALS AND METHODS

Krill was caught in the antarctic summer (Nov 1977 to March 1978) in the Scotia Sea. Meal has been produced on board with a commercial fish meal plant (Reinacher, 1978). Whereas the temperature of the dryer was set at 140 °C (resulting in a temperature of 70 °C of the meal leaving the dryer), the cooking temperature varied between 50 and 100 °C. Feeding krill (with green stomach) as well as starved krill were processed.

Dry matter was determined by vacuum drying at 70 °C overnight. For estimation of the ash content, samples were heated overnight at 550 °C in an electric muffle furance.

Fat evaluation was carried out by using the chloroform–methanol method in the modification of Winter (1963).

The crude protein (N × 6.25) content of meals was estimated by formaldehyde titration (Tegge and Bolling, 1959) of digests obtained by heating 200 mg of krill meal with 10 mL of concentrated sulfuric acid (95–97%; d = 1.84) and 5 mL of 30% hydrogen peroxide solution, until clear and colorless solutions resulted.

The amino acid composition of the krill meals was determined by ion-exchange chromatography (Spackman et al., 1958) using a two-column procedure (Multichrom B, program card no. 5 and 6, Beckman Instruments Co.). For hydrolysis of meal, samples of 50 mg were heated with 6 mL of 6 M hydrochloric acid (Suprapur, E. Merck, Darmstadt) under a nitrogen atmosphere for 24 h at 105 °C in test tubes sealed with ground-in stoppers.

Cysteine/cystine and methionine were determined as cysteic acid and methionine sulfone, respectively (Hirs, 1967). Tryptophan was determined with the *p*-(dimethylamino)benzaldehyde method (Spies and Chambers, 1948, 1949). For estimation of the availability of lysine, the method described by Roach et al. (1967) was used. Acid hydrolysis, however, was not performed under reflux but as described above.

Pepsin digestibility of krill meal was evaluated by the official West German procedure (Naumann and Bassler, 1976). For differentiation between true enzymatic digestion (d) and total solubilization (D) of the meals (Lovern et al., 1964), two values were calculated:

D = % total N solubilized by acid and pepsin

and

$$d = \frac{D - S}{100 - S} \times 100$$

d = true, corrected digestibility; S = percent total N solubilized by acid alone.

RESULTS AND DISCUSSION

Aliquots of krill meal from different hauls and at various temperatures of the cooker had been mixed to three final charges. From these charges samples were taken for analysis.

On an average, krill meal contained 89.7% dry matter, 13.6% ash, 9.3% fat, and 59.5% crude protein, thus corresponding to fish meal of medium quality.

The pepsin digestibility of krill meal (D = 84.1%; d = 78.9%), however, was rather low compared to that of fish meal (commercial fish meal, kindly supplied by the Nordsee Co., Bremerhaven) (62.5% crude protein; D = 93.3%; d = 88.9%). One explanation for the poor digestibility may be (heat?) damage of protein, which was also indicated by an availability of lysine of only 87.6%.

Another reason for the poor digestibility was given by the composition of krill meal: the meal contained ~10% crude fiber (Vogt et al., 1980); the chitin of the exoskeleton will not be digested by pepsin. The amount of chitin, determined as glucosamine in amino acid analysis, was 1.8%; ~1.5% of the total N content of krill meal was found in the glucosamine fraction.

As hydrolysis was not performed under optimal conditions for glucosamine determination [when pure chitin (Roth) was analyzed only 10% of the chitin N was found in glucosamine], it must be assumed that the actual contribution of chitin to the total N content was considerably higher.

By feeding krill shells to rats, it was shown that chitin N in part can be used in protein metabolism of the rat (Kühl et al., 1978). The utilization of *N*-acetylglucosamine in catabolic and anabolic pathways was demonstrated for several organs of the rat (Kikuchi and Tsuiki, 1979). Furthermore, in the digestive tract of many fishes, chitinases of bacterial and nonbacterial origin (gastric mucosa and pancreas) were found (Okutani, 1978; Fänge and Grove, 1979). Obviously, chitin must be regarded as a potential source of N (and carbohydrate) to many organisms (Jeuniaux and Cornelius, 1978); thus, by the pepsin digestibility method, the quality of krill meal will be underestimated.

The amino acid composition of krill meal and fish meals is given in Table I. Data from laboratories 1 and 2 can be compared directly, because these samples were taken from the final charges, whereas in the third laboratory meals produced at different temperatures had been

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Table I. Comparison of the Amino Acid Content of Krill Meals and Fish Meals[a]

	% crude protein									
	1,[b] krill meal		2,[c] krill meal		3,[d] krill meal		4,[e] krill meal		5,[f] fish meal	
amino acid	mean value	fluctuation	mean value	fluctuation	mean value	fluctuation	mean value	fluctuation	herring meal	white fish meal
aspartic acid	9.86	9.58-10.21	10.55	10.21-10.80	10.80	10.63-11.15	10.3	9.7-10.6	9.10	8.54
threonine	3.97	3.76-4.10	4.38	4.26-4.45	4.39	4.24-4.55	4.0	3.6-4.5	4.26	3.85
serine	4.08	3.97-4.24	4.11	4.03-4.16	3.74	3.48-4.01	3.2	3.1-3.5	3.82	4.75
glutamic acid	12.68	12.25-13.12	12.68	12.41-13.02	13.07	12.90-13.27	13.5	12.9-13.9	12.77	12.79
proline	3.76	3.66-3.89	3.98	3.71-4.23	3.89	3.64-4.11	4.1	2.2-5.2	4.15	5.34
glycine	4.86	4.56-5.13	4.86	4.75-4.95	5.45	5.18-5.65	5.5	5.1-5.9	5.97	9.92
alanine	5.16	4.97-5.37	5.43	5.30-5.55	5.54	5.31-5.67	5.8	5.6-5.9	6.25	6.31
½-cystine	1.21	1.13-1.27	1.26	1.16-1.42	1.17	1.10-1.20	ND		0.97	0.93
valine	4.64	4.53-4.78	5.32	4.86-5.45	5.29	5.05-5.51	5.5	5.2-5.9	5.41	4.47
methionine	2.66	2.61-2.76	2.54	2.44-2.60	2.66	2.41-2.76	2.4	2.0-2.9	2.86	2.60
isoleucine	4.44	4.30-4.56	4.87	4.71-4.08	5.56	5.19-5.69	5.3	4.9-6.0	4.49	3.70
leucine	7.35	7.11-7.56	7.47	7.27-7.60	8.03	7.63-8.23	7.7	7.3-8.0	7.50	6.48
tyrosine	3.45	3.29-3.55	4.02	3.69-4.19	3.71	3.41-3.98	2.9	2.6-3.1	3.13	2.60
phenylalanine	4.44	4.23-4.59	4.83	4.56-4.98	4.90	4.61-5.27	4.6	4.4-4.9	3.91	3.29
lysine	6.99	6.81-7.14	6.54	6.35-6.69	6.95	6.48-7.35	5.0	3.0-6.2	7.73	6.90
histidine	1.56	1.48-1.60	1.92	1.81-2.02	2.21	1.94-2.70	1.3	0.9-1.9	2.41	2.01
arginine	5.52	5.30-5.64	5.81	5.59-5.93	5.70	5.46-5.95	4.5	3.3-5.9	5.84	6.37
tryptophan	1.20	1.16-1.26	ND[g]		ND		1.5	1.5-1.5	1.15	0.94
crude protein	59.3	57.9-60.8	60.3	59.1-61.6	55.6	55.0-56.6	55.9	53.2-59.2	73.6	65.0
Σ amino acids	52.1	51.7-52.4	54.7	52.8-56.2	51.7	49.7-53.4	48.6	46.8-49.8	67.5	59.7
no. of meals analyzed	$n = 6$		$n = 9$		$n = 4$		$n = 3$			

[a] The data presented by the Bundesforschungsanstalt für Fischerei, Hamburg, the Degussa, Hanau (Spindler and Pohl, 1979), and the Institut für Kleintierzucht, Celle (Vogt et al., 1980), were obtained with krill meal produced on the II Antarctic Expedition of the Federal Republic of Germany, 1977-1978. The data from Egorova et al. (1970) were calculated from their krill meal sample no. 21, 23, and 53. [b] Bundesforschungsanstalt für Fischerei, Hamburg. [c] Degussa, Hanau. [d] Institut für Kleintierzucht, Celle. [e] Egorova et al. (1970). [f] Miller (1970). [g] ND, not determined.

Table II. Amino Acid Requirements[e] of Fish and Rat (Cowey and Sargent, 1979) Compared to the Amino Acid Content of Krill Meal and White Fish Mean Protein

	% crude protein					
amino acid	krill meal[a]	white fish meal[b]	Chinook salmon	Japanese eel	carp	rat
arginine	5.7	6.4	6.0	4.5	4.2	1.5
histidine	1.9	2.0	1.8	2.1	2.1	3.0
isoleucine	5.0	3.7	2.3	4.0	2.3	8.8
leucine	7.6	6.5	4.0	5.3	3.4	6.8
lysine	6.8	6.9	5.0	5.3	5.7	7.6
methionine + cystine	3.8	3.5	4.0	5.1	3.1[c]	4.5[c]
phenylalanine	4.7	3.3	5.3[d]	5.8[d]	6.5[d]	6.8[d]
threonine	4.2	3.9	2.3	4.0	3.9	3.8
tryptophan	1.2	0.9	0.5	1.1	0.8	1.5
valine	5.1	4.5	3.3	4.0	3.6	3.0

[a] Mean value of the data from laboratories 1-3. [b] For reference see Table I. [c] Methione requirement in the absence of cystine. [d] In the absence of tyrosine. [e] The values for amino acid requirements were measured at the optimal dietary protein intake for each species (in grams of protein per gram of dry diet): Chinook salmon, 40; Japanese eel, 37.7; carp, 38.5; rat, 13.19.

evaluated. In general, the data obtained by laboratories 1 and 2 are in good agreement: only for threonine, valine, and tyrosine were considerably higher values reported by laboratory 2, presumably because different conditions of hydrolysis had been applied [200 mg of meal was hydrolyzed with 800 mL of 6 M hydrochloric acid under a nitrogen atmosphere under reflux for 24 h (Beck et al., 1978)].

The amino acid composition of krill meal resembles that of white fish meal, especially if the essential amino acids (Table II) are compared. The amino acid composition of krill meal fulfills the requirements for optimal growth of fish and rat (Table II), explaining the good results obtained when krill meal was used in aquaculture. This had to be expected, because krill is a natural foodstuff of many species of antarctic fishes (*Champsocephalus gunnari*, *Chaenocephalus aceratus*, *Pseudochaenichthys georgianus*, and *Notothenia rossi marmorata*) (Kock, 1978).

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Thiazoles, Oxazoles, and Oxazolines Identified in the Volatile Flavor of Roasted Peanuts

Volatile flavor compounds were isolated from 70 kg of roasted peanuts by a specially designed apparatus. The isolated volatile flavor compounds were subjected to extensive gas chromatographic fractionation, and the pure fractions obtained were identified by infrared and mass spectrometry. Eight thiazoles, seven oxazoles, and three oxazolines were identified. All of them, except 4-methylthiazole, were new to the roasted peanut flavor.

The volatile flavor compounds of roasted peanuts have been studied by many researchers. Publications by Pattee et al. (1965), Mason et al. (1966, 1967), Brown et al. (1968), Walradt et al. (1971), and Johnson et al. (1971a,b) have described the isolation of an aroma complex from roasted peanuts and identification of many of its constituents. In a recent review, Van Straten (1977) stated that a total of 279 compounds had been identified.

Thiazoles, oxazoles, and oxazolines in food flavor have been receiving increased attention recently. Most thiazoles, oxazoles, and oxazolines possess some unique and potent sensory properties. For instance, 2-isobutylthiazole isolated from tomato flavor (Viani et al., 1969) was described as having a strong green odor resembling that of tomato leaf. 2-Isopropyl-4,5-diethyl-3-oxazoline, a synthetic flavor, possesses a typical cocoa aroma (Ohloff and Flament, 1979). However, only three thiazoles, namely, thiazole, 4-methylthiazole, and benzothiazole (Walradt et al., 1971), and no oxazoles and oxazolines have been reported in roasted peanut flavor.

The present paper reports the identification of eight thiazoles, seven oxazoles, and three oxazolines in the volatile flavor compounds of roasted peanuts.

EXPERIMENTAL SECTION

Material Used. Freshly roasted peanuts (kept at −20 °C) were used for isolation of the volatile compounds. Florunner peanuts were roasted in a commercial roaster to meet the Neotec color reflection of 25.0 ± 0.3 (green). Experienced tasters confirmed that the roasted peanuts had a typical roasted peanut aroma and flavor.

Isolation of Volatile Flavor Compounds (VFC). The VFC were isolated from 70 kg of roasted peanuts by using the apparatus described by Chang et al. (1977). The principle of the apparatus is the removal and subsequent condensation of the volatile compounds in the headspace of the roasted peanuts. Nitrogen gas was used to remove the VFC from roasted peanuts. Seven kilograms of roasted peanuts was used for each isolation which lasted 48 h. A total of 70 kg of roasted peanuts was used. The total condensate collected in the traps cooled with dry ice and acetone was treated in a manner similar to that described by Herz and Chang (1966). The ethyl ether extract of the condensate was dried with anhydrous sodium sulfate and then concentrated to a volume of 50 mL with the use of a 30-plate Oldershaw column. It was finally concentrated to a volume of 5 mL with a 200-plate spinning band still.

Fractionation of the Flavor Isolate. The initial preparative gas chromatography of the isolated volatile roasted peanut flavor compounds was performed on a Perkin-Elmer Sigma 3 gas chromatograph equipped with a flame ionization detector, fitted with a $^1/_8$ in. o.d. × 12 ft stainless steel column, packed with 10% OV-17 on 80–100-mesh Chromosorb W. The flow rate was 30 mL/min

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