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Under the term nitrogenous extractives is included a number of non-protein nitrogen constituents which occur to a small percentage in tissue and are extracted by water, alcohol or ether. Among other substances this group contains methylamine, creatine and creatinine, urea and several purine bases including xanthine, hypoxanthine and uric acid.

It is a matter of common knowledge that if muscle is boiled in water the greatest portion of the flavour of the fish or meat is extracted. It would appear likely therefore that the group of substances known as the non-protein nitrogen extractives were intimately concerned with the characteristic flavour of fish. Hence since the flavour of muscle largely determines its marketability it seemed highly desirable to undertake a study of the factors concerned, especially those pertaining to marine products. In addition to the above a second consideration must be admitted. When the nitrogen content of fish muscle is multiplied by the "protein factor", and the moisture, fat and ash content added, the total in certain species of fish adds to less than, in other species to greater than, 100%. This discrepancy must arise from the fact that different species of fish contain different amounts of non-protein nitrogen and hence the protein factor must vary from one species to another. Since only the protein and fat of fish muscle have any feeding value it is imperative that one should know the quantity of non-protein nitrogen and hence the correct "protein factor" for the muscle of each species in order to properly appraise and evaluate the biological values of different fish products.

Hence in an attempt to throw some light on these very interesting and highly important problems it seemed advisable to undertake a study of and eventually to attempt to account for the total non-protein nitrogen present in fish muscle. The study carried on consisted of a preliminary investigation and comparison of the quantity of ammonia, urea, creatine and uric acid present in different varieties of commercial fish. The investigation has not been extended sufficiently far as yet to enable one to draw any broad conclusions but has nevertheless shown some interesting results.

METHODS

Ammonia

The fish which were as fresh as could be procured, and which as a rule were not out of the water longer than about 3 hours at a maximum, were measured and the muscle to be analyzed was ground in a meat chopper as soon as the fish were received at the laboratory. Schmidt-Nielsen and Stine (1) have shown that the ammonia content of muscle chopped in air was somewhat higher than in muscle chopped in sodium borate solution owing to the decomposition in air of adenosine tri phosphate by muscular tissue. However, as our interest lay for the most part in the amount of ammonia present in the muscle under ordinary circumstances no such precaution as that followed by Schmidt-Nielsen was taken. Hunter (2) has pointed out that the creatine content of different muscles varies considerably, hence only the caudal muscle was used in this series of experiments. After mincing, samples of between 2 and 3 grams of the muscle were weighed into pyrex test tubes for ammonia determinations. These were carried out by aerating the minced muscle to which was added 5 cc. of saturated sodium carbonate, with air washed by passing through sulphuric acid. The muscle was aerated for 2 hours and the ammonia trapped in $\frac{N}{70}$ sulphuric acid and determined by back titration with $\frac{N}{70}$ standard alkali. The determination actually represents the total volatile base present, but since this can be almost quantitatively accounted for as ammonia and tri methylamine and as the amount of the latter is very low in fish muscle no correction was applied. Determinations were carried out in triplicate and checks made with an accuracy of 1%.

Urea determinations

Preparation of Urease

The urease used in these experiments was prepared by adding to 30 grams of jack bean meal 100 cc. of 30% ethyl alcohol and 2 grams of permutit previously washed with dilute acetic acid. This mixture was allowed to stand in an ice-box over night and was filtered next morning. Preliminary experiments with urea showed that 5 cc. of this preparation in the presence of small amounts of phosphate was sufficient to convert 5 mmg. of urea to ammonia in 30 minutes at room temperature.

Phosphate buffer

The phosphate buffer was prepared by making 39 grams of acid potassium phosphate and 87 grams of di potassium phosphate up to 500 cc. The pH of the solution was found to be 6.8.

Urea determinations were carried out by treating about 3 grams of minced muscle with 5 cc. of urease solution and 0.1 cc. of phosphate buffer and allowing the urease to react for 1 hour at room temperature. The tubes were then made alkaline with sodium carbonate and aerated for ammonia as described above. From the total ammonia value thus obtained was subtracted the value previously found and the difference calculated as urea. Determinations were carried out in triplicate with an accuracy of about 1%.

CREATINE DETERMINATIONS

Hunter (3) has shown that there is present little or no creatinine in fish muscle and hence no determination for this compound as such was carried out. Creatine was determined in the manner outlined by Hunter. Between 4 and 5 grams of muscle were weighed into tubes and hydrolyzed with 20 cc. of 5% sulphuric acid by heating on a water bath for 5 hours. The digest was then filtered and made up to a volume of 50 cc.; 10 cc. of this solution were then placed in a 250 cc. volumetric flask, about 150 cc. of distilled water, 25 cc. of saturated picric acid, and 10 cc. of 10% sodium hydroxide were added, and the flask made to volume with distilled water. After allowing the colour to develop for 20 minutes it was compared against a standard containing 5 mmg. of creatinine zinc chloride and prepared in a manner similar to the experimental flask except that in this case only 5 cc. of sodium hydroxide were added. Creatine determinations were carried out in duplicate with an accuracy of ½%.

URIC ACID DETERMINATIONS

For uric acid determinations samples of minced muscle of about 8 grams were weighed into tubes and hydrolyzed with 20 cc. of 5% sulphuric acid by heating on a boiling water bath for 5 hours. The tubes were then filtered and the filtrate treated with 15 cc. of 10% sodium tungstate. The precipitate was filtered off the filtrate, made slightly alkaline with lithium carbonate, and evaporated to a volume of 25 cc. The residue was then washed into a 50 cc. centrifuge tube and treated with 1 cc. of 3% silver lactate, 0.5 cc. of magnesia mixture, and 2 cc. of concentrated ammonia. The tube was allowed to stand overnight and the precipitate centrifuged off next morning. The clear liquid was discarded and the residue treated with 5 cc. of 10% sodium chloride in .1N hydrochloric acid. The tubes were then heated in boiling water and after cooling were again centrifuged; the extraction process was repeated and the combined liquids treated in a 50 cc. volumetric flask with 1 cc. of a 15% sodium cyanide solution and 0.5 cc. of Folin's uric acid reagent when the flask was made up to volume. After development of the blue colour it was compared against a standard prepared in a similar manner. When amounts of uric acid of the order of 0.02 mmg. were added to a sample of fish muscle the above procedure gave results which agreed to within 2%. However triplicate determinations on untreated muscle showed an accuracy of little greater than 10%.

Magnesia mixture

The magnesia mixture used in connection with the uric acid determinations was prepared by making 84 grams of magnesium chloride crystals, 100 grams of ammonium chloride and 200 cc. of concentrated ammonia up to 500 cc.

Folin's uric acid reagent

Folin's uric acid reagent was prepared by adding to 750 cc. of distilled water in a 2 litre Erlenmeyer 100 grams of sodium tungstate and 80 cc. of 85% phosphoric acid. The mixture was then boiled under a reflux condenser for 2 hours when 2 cc. of bromine water were added and the flask again boiled for 10 minutes.

EXPERIMENTAL

The experiments carried out consisted of an analysis for ammonia, urea, creatine and uric acid of a number of specimens of 5 different species of fish. The results obtained from that investigation are summarized in the following table.

TABLE I

The extractives from fish muscle

Species	Sex	Size cms.	Ammonia mmg./g.	Urea mmg./g.	Creatine mmg./g.	Uric Acid mmg./g.
Cod	Female	143	--	--	4.06	.0049
"	"	75	.164	.195	5.40	.0070
"	"	75	.177	.161	6.08	.0053
"	"	66	.162	.157	5.10	.0068
"	Male	65	.161	.244	5.62	.0073
"	"	81	.162	.212	6.11	.0117
"	Female	42	.189	.113	5.07	.0062
Haddock			.143	.0368	5.86	.0040
"			.185	.0189	6.12	.0047
"			.151	.0483	5.66	.0045
"			.147	.0159	6.01	.0044
"			--	--	6.52	--
"			.146	.0613	5.83	.0060
Hake	Female	75	.148	.065	3.49	0.0
"		80	.167	.055	3.77	.0075
"	"	60	.120	.063	3.66	.0030
"	"	65	.117	.119	3.11	.0020
"	"	54	--	.062	4.15	.0017
"	Male	46	.119	.040	4.26	.0029
"	Female	48	.108	.075	4.06	.0048
Mackerel		48	.225	.110	4.13	0.0
"	Male	43	.201	.189	5.85	.0084
"		36	.202	.192	5.71	.0149
"		36	.186	.185	4.87	.0137
Herring		--	.164	.120	6.68	--
"	Male	30	.232	.145	5.92	.0108

Although the number of mackerel and herring examined was hardly great enough to permit of any definite conclusion it appeared that the quantity of ammonia per gram of muscle was of the same order of magnitude in all species. The fast swimming surface fish, mackerel and herring, showed slightly higher values however, than the ground fish. In contrast to the relatively constant values for ammonia the quantity of other extractives studied varied rather widely from one species to another. Thus the urea value found for haddock was very much lower than that for its near relative the cod, which showed the highest value of any species investigated. Mackerel and herring occupied an intermediate position and hake showed values slightly lower. The lowest values for creatine were found on analysis of hake muscle. The highest were obtained on examination of haddock and herring, with intermediate values being shown by mackerel and cod. Prior to 1929 Hunter is authority for the statement that only 23 reliable determinations of creatine have been made on 15 different species within the class Pisces. Mellanby found only 0.28% in the flesh of skate, and 0.35% in that of cod. Much higher values were recorded, however, for cod by Hunter; thus he found for the caudal muscle of ling cod taken from the waters off the coast of British Columbia a value of around 0.6%, and for rock cod a value of about 0.51%. Although there is considerable variation in the figures reported in Table I Hunter's value for cod agrees very well with the average value found in our experiments provided that the creatine content of the large cod fish at the head of Table I, and which could not be regarded as normal since the water content of the muscle was over 90%, be neglected. On the other hand Hunter's value for Pacific herring of about 0.74% was much higher than that found in the case of the two herring examined in these experiments, where values of around 0.63% were observed. The lowest value of 0.48% recorded by Hunter was observed on analysis of skate muscle; this value was considerably higher than our lowest value of 0.38% for hake muscle. In spite of large individual differences Hunter was able to conclude that each species, and possibly all species of a single genus, showed fairly characteristic values. Observations on Table I appear to support this contention.

From the data appearing in Table I, one is struck by the large individual variations in uric acid content of the muscle of different fish. Because of this one is unable to conclude that any value is characteristic of any particular species. The table illustrates quite clearly, however, that the percentage of uric acid in fish muscle is of a much lower order of magnitude than that of human muscle being as it is of about 0.0006% in contrast to a figure of about 0.04% for human muscle recorded by Valenti.

SUMMARY

1. Examinations of a number of specimens of 5 different species of fish for ammonia, urea, creatine and uric acid have been carried out.

2. It was found that each species showed values for ammonia of about the same order of magnitude.

3. Each species, in spite of large individual variation, seemed to show a value for urea characteristic of that species.

4. Analysis for creatine showed highest values on examination of herring, lowest on analysis of hake, with haddock, cod and mackerel showing intermediate values.

5. A comparison of the quantity of uric acid in fish muscle with that of human muscle showed the former to be of a much lower order of magnitude.

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