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The free amino acids as chemical indices of decomposition in fish

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Introduction

Fish are an important food supply for man. As an article of diet fish has a nutritional value similar to that of meat. Mean chemical composition of meat and fish is shown in Table 1 (1, 2).

Table 1

	Water %	Protein %	Fat %	Mineral elements %
Meat	35—74	10—20	4 —55	0,5—1
Fish	53—85	13—24	0,1—31	1 —2

Fish has always been a substantial part of the world's supply of high quality protein and from a nutritional standpoint fish protein is as useful for human

beings as protein from meat animals. Both the flesh and the fat of fish are rich in vitamins A and D. Fish is also a useful source of mineral elements.

The amount of Polish fish caught in 1966 was over 315 000 tons (3). On an average 5 kg of fish was eaten per head of population. Because of intensive fishing it is now necessary to face the problems associated with keeping the fish fresh and good for the consumption. The presentation of satisfactory fish and fish products to the public is an important and difficult question.

Spoilage of fish occurs from enzymes naturally present, from bacterial activity, and from oxydative changes, especially in fatty fish. Spoilage varies with differences in constituents of the fish body, muscle structure and bacterial load as well as with environmental factors. Nutritional status has also an important effect on the subsequent keeping quality of fish. Those containing more feed in some months spoil earlier than the same species of fish containing less.

The quality of fish is mostly determined by organoleptic means. Those experienced in the examination of fish have little difficulty in determining the presence of decomposition by appearance and odour. According to the new standards and regulations the freshness of fish should be determined besides organoleptic examination, by means of objective tests for decomposition, which should correlate well with the organoleptic grade assigned by experienced examiners.

Decomposition of fish is principally a progressive proteolysis of the muscle tissue brought about by the action of microorganisms and, to a lesser degree, by autolytic enzymes. Changes take place in the physical appearance of the fish as decomposition proceeds. It is generally recognized that the fish skin shows signs of spoilage earlier that the muscle. This was confirmed by *Ranke, Ranke* and *Bramstedt* (4).

From the results of numerous studies, it is evident that nonvolatile and volatile amines are produced by the action of bacteria during the decomposition of fish. In the initial development of decomposition volatile bases, amines, and organic acids as bacterial metabolites are formed by decarboxylation or by deamination of amino acids and organic bases.

When fish is stored the fat in the flesh undergoes deterioration and the degree of change in the quality increasing with the duration of storage. Rancidity in fatty fish becomes apparent in the advanced stages of the chemical deterioration.

According to *Stansby* and *Lemon* (5) fish decomposition can usually be divided into two main stages based on the types of products: primary changes, which lead to the formation of amino acids from protein or to certain types of intermediate products such as polypeptides and peptones; secondary changes including those which lead to the formation of such products as amines, indole, hydrogen sulphide and skatole. The secondary changes are caused by bacterial action.

There is general similarity between the bacterial flora responsible for the spoilage of fish and that existing in its environment. At first there is commonly a predominance of Micrococcus and Pseudomonas or Achromobacter species. Bacteria invading through gills and skin begin to grow by utilising nutritive substances including free amino acids, the vitamin B complex, trimethylamine oxide,

:c. In the early stage of bacterial spoilage amino acids content gradually decreases, and the corresponding amount of ammonia increases markedly. *Beatty* and *Collins* (6) found that later, there is an increase in both amino and ammonia nitrogen, caused by the increase of protein hydrolysis. One of the most characteristic biochemical changes in fish spoilage is the formation of reducing substances. Investigations have been made to compare the formation of these compounds and the degree of spoilage, as measured by other chemical tests and according to *Farber* (7) the method for determination of volatile reducing substances coincides with organoleptic judgments of raw and canned fish.

Difficulties met in assesment of suitability for consumption of fish and fish products stimulated searching for new indices of its quality. The need for a satisfactory objective test for the determination of spoilage in fish and fish products is evidenced by the large number of methods which have been published. Previous papers from this Department have described and evaluated the following methods used for the examination of fish fitness for consumption:

a) volatile bases (8, 9, 10);
b) trimethylamine nitrogen (11);
c) Walkiewicz test (12);
d) indol (13);
e) volatile acids (14, 15).

Recently we have reported — *Wierzchowski* and *Witkowski* (16) — a method to determine the freshness of fish meat by measuring the lower fatty acids content by paper chromatography procedures. Freshwater fish were analysed fresh and after storage for 24 and 48 hours at room temperature. The quality of different samples varied from good to poor. The contents of some lower fatty acids showed a marked increase on storage. It was assumed that the appearance of butyric acid in fish meat indicates its decomposition. The presence of 5 mg% of butyric acid in bream (Abramis brama), tench (Tinca vulgaris), pike (Esox lucius) and eel (Anguilla vulgaris) showed that the fish was unsuitable for consumption.

Bramstedt (17) reported that each species of fish has an amino acids pool which is rather specific and does not change fundamentally even under varying environmental conditions. The various factors like species of fish, age, sex, size, environment in which fish are stored, temperature of storage, and space of time of storage have influence upon some changes which take place in composition of free amino acids in fish. According to *Hughes* (18) among the free amino acids in herring histidine and taurine showed significant seasonal variation. In the author's opinion no correlation was found between sexual maturity and content of total or individual amino acids. In post mortem spoilage the amount of histidine after slight initial increase dropped to zero. Other essential amino acids like leucine, valine and threonine increased whereas lysine decreased.

The composition of free amino acids depends upon biochemical changes which take place in fish after capture, and which can readily be detected by variations

in the amount of individual amino acids established by paper chromatography. According to *Hodgkis* and *Jones* (19) during storage of fish in sterile conditions there are only minor changes of free amino acids.

To measure the degree of freshness of fish, a good scientific test is required not only from the technological point of view but also for sanitary purposes. The objective of this study was to determine if free amino acids estimation in fish would supply reproducible results in measuring the fittness of fish meat for consumption. The results reported here are part of a study to examine fish and fish products for compositional changes of some constituents during storage and relate these changes to loss of quality.

Experimental procedures

The fresh fish studied were pike (Esox lucius), bream (Abramis brama) and tench (Tinca vulgaris). Moreover frozen cod (Gadus morrhua) was analysed. The specimens of fish were obtained from the fish factory.

The quality of fish was checked on the basis of both organoleptic and laboratory tests. Organoleptic examination was carried out by a taste panel according to the obligatory standards:

a) for pike — RN-55-MŻ-09114;
b) for bream — RN-55-MŻ-09108;
c) for tench — RN-55-MŻ-09116;
d) for cod — PN-55/A-86757.

After removing bowels and bones the sample of fish was homogenised and subsequently homogenous specimens were analysed.

For the proper estimation of fitness of fish for consumption, the following laboratory tests were used:

a) determination of fats (20);
b) determination of acidity of fats (20);
c) determination of volatile fatty acids (14);
d) determination of acidity of water extract (20);
e) determination of ammonia (10, 21);
f) determination of moisture (PN- + 56/A-52110).

Paper chromatography was chosen as the best method of establishing the quantity of amino acids. Other investigators have also used this method with success.

The free amino acids were extracted by ethyl alcohol according to *Jones* (22) and *Hughes* (23). Further chromatographic procedures employed were the same as those previously reported by *Doboszyński* and *Wierzchowski* (24). We have used Partridges solvent modified by *Cramer* (25) and the amino acids were detected

with ninhydrin solution. The coloured parts of the paper (Whatman No. 1) chromatogram were cut out, cut into small pieces and eluted with methanol. The colour densities were determined on a Pulfrich Stepphotometer using S-53 filter and vessel F-4,992. Interpretation of density values was made from standard curves prepared in advance for each amino acids essayed.

The chemical data obtained for all laboratory analysis are presented in Tables 2 and 3.

Results and discussion

When fish is stored, the influence of the enzymes of the fish itself and of bacterial enzymes leads to deteriorating changes. There is a natural variation in concentration of enzymes between species as well as in substrate concentration in the flesh of fish and this can exert an influence on the end-product built up. The gross chemical and physical changes produced in fish as a result of the growth of spoilage bacteria are readily apparent and can be estimated organoleptically or chemically.

It has been found convenient to describe this progressive deterioration by dividing it into three classes of the fish. These designation run the gamut from perfectly fresh fish (first class) to fish in an advanced state of rottenness (third class). The second class of fish were samples kept in room temperature (ca. 20 ° C) during 24 hours — it was fish on the border of acceptability as food, approaching the condemnation level.

During this study opportunity was taken of investigating the correlation between taste panel scores and the concentration in the flesh of fish of certain substances in particular free amino acids.

The interesting point of the presented results is that amino acid number varies whit the different species of fish. The reason may be that the degradation processes of the free amino acids run much faster in some species or specimens of fish than in others. According to *Duchateau* and *Florkin* (26, 27) the constituents of free amino acids pool appear rather uniform but relative amounts of individual amino acids show characteristics specific for almost each fish species. The amino acids found in analysed fish were: histidine, glutamic acid, taurine, threonine, alanine, valine, leucine, lysine, arginine and methionine.

Pike. Data obtained indicate for pike eight free amino acids: histidine, glutamic acid, taurine, alanine, methionine, valine, threonine and leucine. All of these data have been summarized and determinations of individual amino acid for each organoleptic class are shown in Table 2. The results reported are the average of ten analysis of the homogenates carried out on ten specimens of fish.

Fresh fish contains relatively large amounts of alanine while fish found to be decomposed by organoleptic examination didn't prove histidine and alanine. Simultaneously with fish decomposition the amount of histidine and alanine decreased whereas glutamic acid, taurine, threonine, valine, methionine and leucine increased.

Table 2
The free amino acids in analysed specimens of fish mg%

	Pike			Bream			Tench			Cod defrosted on the air			Cod defrosted in the water		
	Organoleptic class			Organoleptic class			Organoleptic class			Organoleptic class			Organoleptic class		
	1-st	2-nd	3-rd	1-st	2-nd	3-rd	1-st	2-nd	3-rd	1-st	2-nd	3-rd	1-st	2-nd	3-rd
Histidine	33,1	18,0	—	29,6	1,3	—	29,9	10,4	1,5	44,3	18,8	—	44,5	20,3	—
Glutamic acid	47,9	84,6	122,0	157,9	163,2	175,5	141,0	143,5	158,5	14,5	97,5	161,7	14,2	97,9	163,4
Taurine	39,5	61,4	144,7	—	—	—	—	—	—	—	—	—	—	—	—
Threonine	12,8	41,2	140,1	49,4	84,7	134,1	127,0	138,7	138,2	13,2	76,9	121,5	14,2	77,9	120.5
Alanine	126,8	50,0	—	98,8	65,0	—	—	—	—	17,2	69,1	121,9	16,3	65,4	121,8
Metionine	59,7	127,3	129,9	17,9	50,5	65,5	—	—	—	6,1	83,0	130,0	4,9	82,4	131,7
Valine	17,5	50,6	93,2	—	—	—	—	—	—	—	—	—	—	—	—
Leucine	45,7	110,3	147,6	—	—	—	—	—	—	46,2	110,3	148,0	48,8	109,7	146,9
Lysine	—	—	—	15,9	15,4	—	15,4	15,2	15,5	17,6	20,1	45,7	16,7	18,9	47,2
Arginine	—	—	—	—	—	—	134,9	148,7	155,2	128,2	130,6	126,3	128,4	130,0	125,7
Total amino acids	383,0	543,4	777,5	369,5	380,1	375,1	448,2	456,5	468,9	287,3	606,3	855,1	288,0	602,5	857,2

Table 3
Organoleptic and chemical valuation of analysed specimens of fish

Species of fish	Pike			Bream			Tench			Cod defrosted on the air			Cod defrosted in the water		
	Organoleptic class			Organoleptic class			Organoleptic class			Organoleptic class			Organoleptic class		
	1-st	2-nd	3-rd	1-st	2-nd	3-rd	1-st	2-nd	3-rd	1-st	2-nd	3-rd	1-st	2-nd	3-rd
Moisture %	78,7	78,6	78,6	74,7	74,8	74,7	78,9	78,9	79,1	78,9	78,2	75,5	77,7	78,0	75,6
Fat %	0,56	0,48	0,53	1,03	1,04	1,07	1,55	1,73	1,72	0,62	0,64	0,59	0,71	0,71	0,68
Acidity of fats (grades)	4,8	6,4	13,6	12,7	18,8	23,1	9,6	13,6	19,8	4,6	5,2	11,2	4,6	5,2	12,2
Volatile fatty acids (ml 0,01 n Na OH/100 g)	49,4	61,7	85,1	50,6	59,3	64,1	55,3	71,0	78,0	45,1	47,6	54,4	42,6	49,9	53,2
Acidity of water extract (ml 0,01 n Na OH/100 g)	6,8	7,4	11,0	8,2	9,4	10,6	6,5	7,1	7,8	6,8	9,6	11,4	7,2	9,9	11,2
Ammonia mg %	57,6	62,1	79,4	11,4	16,7	24,0	34,5	58,8	58,3	12,7	19,5	25,9	13,3	21,0	27,2

Bream. The results reported in Table 2 indicate for bream six amino acids: histidine, glutamic acid, threonine, alanine, methionine and lysine. All of these data have been summarized in the same manner as those for pike.

In post mortem spoilage the amount of lysine remained constant whereas the amount of histidine and alanine decreased. The amount of glutamic acid, threonine and methionine increased. The significant differences between the amounts of glutamic acid and of other free amino acids were apparent especially in fresh fish.

Tench. In fresh fish the following amino acids were identified in order of increasing concentration: lysine, histidine, threonine, arginine and glutamic acid. In the progressive decomposition of fish the amount of lysine remained constant, whereas the amount of histidine decreased. Threonine and glutamic acid increased but not to such a degree as in pike and bream.

Cod. There were two kinds of specimens analysed — fish defrosted in air and in water. Among eight free amino acids identified the amount of histidine decreased, and in advanced state of decomposition dropped to zero like in other samples of analysed fish. Cod contains relatively large amounts of arginine which remained rather constant, whereas the amount of glutamic acid, threonine, alanine, methionine, leucine and lysine increased.

Conclusions

Relying on tabulated results a few particularly interesting points may be mentioned:

1. The high amount of free amino acids of fresh-water fish (pike, bream and tench) as compared with cod;
2. The changes in amount of free amino acids in the flesh of fish were followed during storage;
3. In samples of pike and cod the increase in amino acids quantity was more evident than in samples of bream and tench;
4. In these tests there was a noticeable decrease of histidine and increase of threonine in all specimens. From this it may be concluded, that two amino acids mentioned were found to be closely related as indexes of decomposition in flesh of fish.
5. We think that for sanitary purposes the content of histidine and threonine in the meat of fish is of interest and, relying on data received, we propose for fish good for consumption:
   a) for all four analysed species — at least 20 mg% of histidine;
   b) for pike, bream and cod — at the most 80 mg% of threonine.

Summary

The decomposition of fish is principally a progressive proteolysis of the muscle tissue, and it can lead to the formation of amino acids from protein.

The objective of this study was to determine if free amino acids estimation in fish would supply good results in measuring the fitness of fish meat for consumption.

The fresh fish studied were pike, bream and tench. Moreover frozen cod was analysed. Paper chromatography was chosen as the best method of establishing the quantity of amino acids. During this study opportunity was taken of investigating the correlation between taste panel scores and the concentration in the flesh of fish of amino acids and of certain other chemical indices.

Data obtained indicate that amino acid number varies with the different species of fish; the changes in amount of free amino acids in the flesh of fish were followed during storage. It has been found a noticeable decrease of histidine and an increase of threonine in all specimens.

The authors think that for sanitary purposes the content of histidine and threonine in fish meat is of interest, and relying on data received, they propose for fish good for consumption:

a) for all four analysed species — at least 20 mg% of histidine;
b) for pike, bream and cod — at the most 80 mg% of threonine.

Zusammenfassung

Die Verderbnis von Fisch ist hauptsächlich ein fortschreitender Eiweißabbau des Muskelgewebes und kann zur Bildung von Aminosäuren aus Eiweiß führen.

Das Ziel der vorliegenden Arbeit war, die freien Aminosäuren in Fisch zu bestimmen und festzustellen, ob diese schlüssige Resultate zur Abklärung der Eignung des Fisches für den Konsum ergeben.

Untersucht wurden folgende frische Fische: Hecht, Brassen und Schleie. Ueberdies wurde gefrorener Kabeljau untersucht. Zur Bestimmung der Aminosäure diente als bestgeeignete Methode die Papierchromatographie. Gleichzeitig wurde mit dieser Arbeit versucht, die Zusammenhänge zwischen den tabellarisch wiedergegebenen Ergebnissen über den Geschmack und der Aminosäuren-Konzentration und gewisser anderer chemischer Indikatoren zu ermitteln.

Die erhaltenen Daten zeigen, daß die Zahl der verschiedenen Aminosäuren bei den verschiedenen Fischarten variert; die Veränderung des Gehaltes an freien Aminosäuren wurde während der Lagerung beobachtet. Eine bemerkenswerte Abnahme an Histidin und eine Zunahme an Threonin konnte bei allen Arten gefunden werden.

Die Verfasser sind der Auffassung, daß der Gehalt an Histidin und Threonin in Fischfleisch aus gesundheitlichen Erwägungen von Interesse ist und gestützt auf die erhaltenen Ergebnisse, schlagen sie für die Feststellung der Konsumfähigkeit von Fischen vor:

a) für alle analysierten Arten (Hecht, Brassen, Schleie und Kabeljau, gefroren) — wenigstens 20 mg% Histidin;
b) für Hecht, Brassen und Kabeljau — höchstens 80 mg% Threonin.

Résumé

La décomposition du poisson est principalement une protéolyse du tissu musculaire qui peut conduire à la formation d'acides aminés. On a recherché et dosé les acides aminés ainsi libérés pour voir si l'on peut ainsi se rendre compte si le poisson est propre à la consommation. Diverses espèces de poissons ont été examinées et l'on a constaté par chromatographie sur papier une diminution de l'histidine et une augmentation de la thréonine, au cours du stockage. Les auteurs sont d'avis que les teneurs en ces deux acides

aminés permettent de se rendre compte si le poisson peut être consommé ou non; ils proposent les limites suivantes:

a) pour toutes les espèces examinées (brochet, brême, tanche et cabillaud congelé): au minimum 20 mg% d'histidine;
b) pour le brochet, la brème et le cabillaud: au maximum 80 mg% de thréonine.

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