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Free Amino Acids in Hard Herring Roe

Variation during the Ripening Process

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Abstract:

The circlechromatografical separation method was found useful for both the qualitative and quantitative estimation of free amino acids in hard herring roe from Clupea Harengus. The following ten amino acids were detected: leucine, valine, alanine, serine, glutamic acid, lysine, taurine, proline, tyrosin and methionine, the 3 last ones in negletable amounts.

The amount of leucine, valine, alanine, serine, glutamic acid and lysine, increased during ripening untill the last stage before spawning, after which it showed a marked decrease. Taurin did not exhibit this property.

Introduction.

Hard herring roe taken from herring in the spawning state, will in contact with salt soon achieve a rubber-like consistence. This is not the case with hard roe in any of the other stages of maturity. The work reported in this paper had two main objects: first to develop a procedure for circlechromatographical separation of amino acids in fish and fish products, and second, as a preliminary investigation, to examine the free amino acids in the hard herring roe during the last weeks before spawning.

The roe to be investigated was taken from herring caught 18th—24th February 1961, near Kristiansund N. at the west coast of Norway. 8 different roe-samples were collected.

Method.

Two gonades, taken from two different fishes, visually classified to be in the same stage of maturity, were washed in fresh water and homogenized. Two samples of this mixture accurately weighed and of about 20.00 g were then shaken mechanically once for 5 minutes with 70 ml abs. ethanol and then three times with 50 ml 80 %ethanol. Between each shaking operation the suspension was filtered into a 250 ml graduated flask, which finally was filled to the mark with 80 % ethanol and stored at about — 20°C.

Experimental.

From each of the 16 alcohol extracts two samples of 25 and 50 ml respectively were extracted several times with chloroform[1]. The upper layers, containing the amino acids, were collected and evaporated to dryness at room temperature under reduced pressure and dissolved in 2 ml of water. To avoid bacterial decomposition of the amino acids during storage, minute amounts of thymol was added.

The amino acids were separated circlechromatographically on Whatman No 1, in a cabinet constructed by P. Juvik[2]). The following 5 solvent-systems were found necessary and sufficient to identify the amino acids present.

1. n-Butanol : acetic acid : water (4 : 1 : 5)
2. Methyl-ethyl-keton : pyridine : water (70 : 15 : 15)
3. Water-saturated phenol
4. Pyridine : acetic acid : water (70 : 15 : 15)
5. 3. 4.—Lutidine : ethanol : water (2 : 1 : 2)

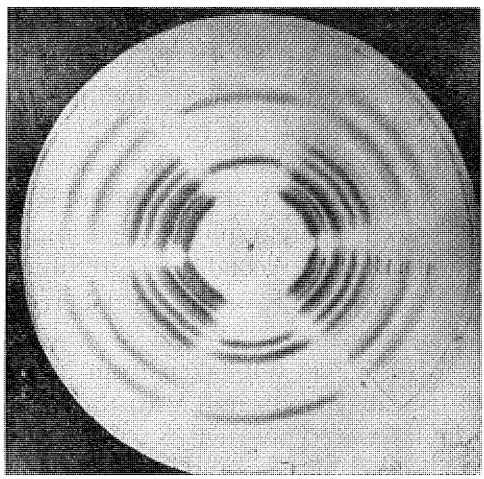

Fig. 1. Chromatogram of amino acids (Whatman № 1), BuOH:HAc:H_2O, (4 : 1 : 5)

Fig. 1 shows the chromatogram of 4 different roe extracts together with some of the standard amino acids. It was found possible with this solvent system (n—BuOH : HAc : H_2O) (4 : 1 : 5) to separate 13 different amino acids.

It was necessary, however, for an accurate identification, to confirm the results with 3 different solvent-systems.

The solvent-system 1. gave a sufficient separation for the quanti-

tative estimation of leucine, valine, alanine, glutamic acid and serine. For the determination of lysine and taurine the system 2. was found useful.

The amino acids separated circlechromatographically on Whatman No 1, were detected with ninhydrin. A 0.5 % ninhydrin solution in water-saturated n-buthanol was sprayed on the dried chromatogram and developed by heating at 80°C for 30 minutes.

The coloured spots were cut out and the colour extracted with 5.00 ml 70 % ethanol. The colour intensity of the extract was measured on a Beckman Colorimeter, Model C, green filter, (using 70 % ethanol as a reference). The amount of the acid was determined by comparing the transmission with a transmission-concentration-curve previously made by measuring the colour intensities of extracts from known amounts of chromatographically separated amino acids.

Results.

Desalting or any other pretreatment of the samples was not found necessary.

By the present method it proved to be possible to identify leucine, serine, glumatic acid, alanine, lysine, valanine, taurine, proline, tyrosine and methionine in each of the 8 roe samples. These are supposed to be the only free amino acids present in hard herring roe. The amount of proline, tyrosine and methionine were, however, so small compared with the other acids that any quantitative determination was found impossible.

The variation in the amount of leucine, serine, glumatic acid, alanine, lysine, valanine and taurine during the last weeks before spawning is illustrated in fig. 2 and fig. 3.

The point corresponding to the extreme left of the curves represents the free amino acid in unripened roe, whereas the point on the extreme right corresponds to the roe in the spawning state. The degree of maturity is supposed to increase from left to right.

Discussion.

Each point on the curves represents the mean value of at least 9 different chromatograms. As illustrated, the spread is rather moderate. The mean values, represented by the solid dots on the figure is considered to be near the correct ones, and the method is found acceptable for the quantitative estimation of free amino acids in fish. Applying the procedure described above, the analysis is rather quick

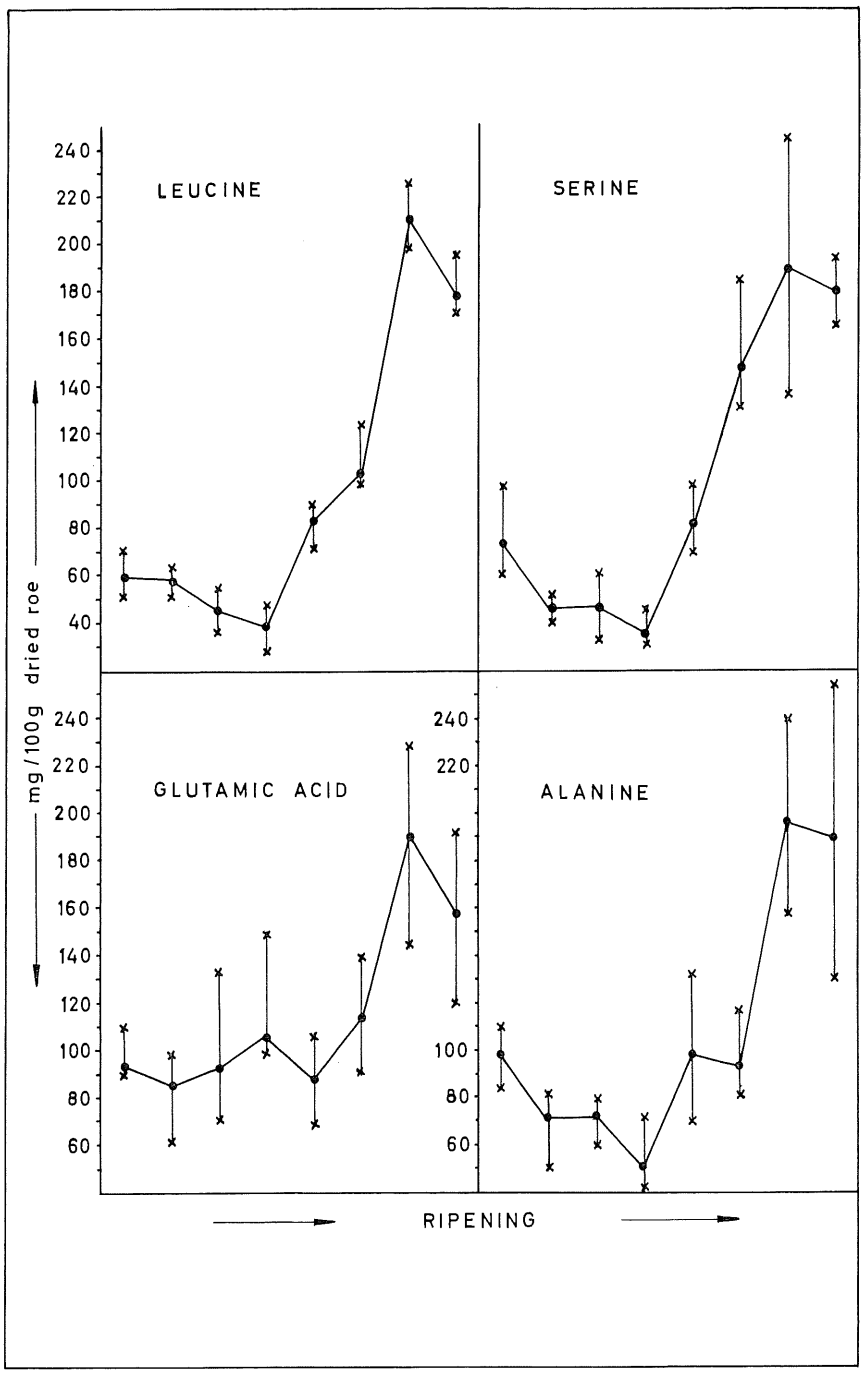

Fig. 2. The variation of amino acids in hard herring roe during the ripening process.

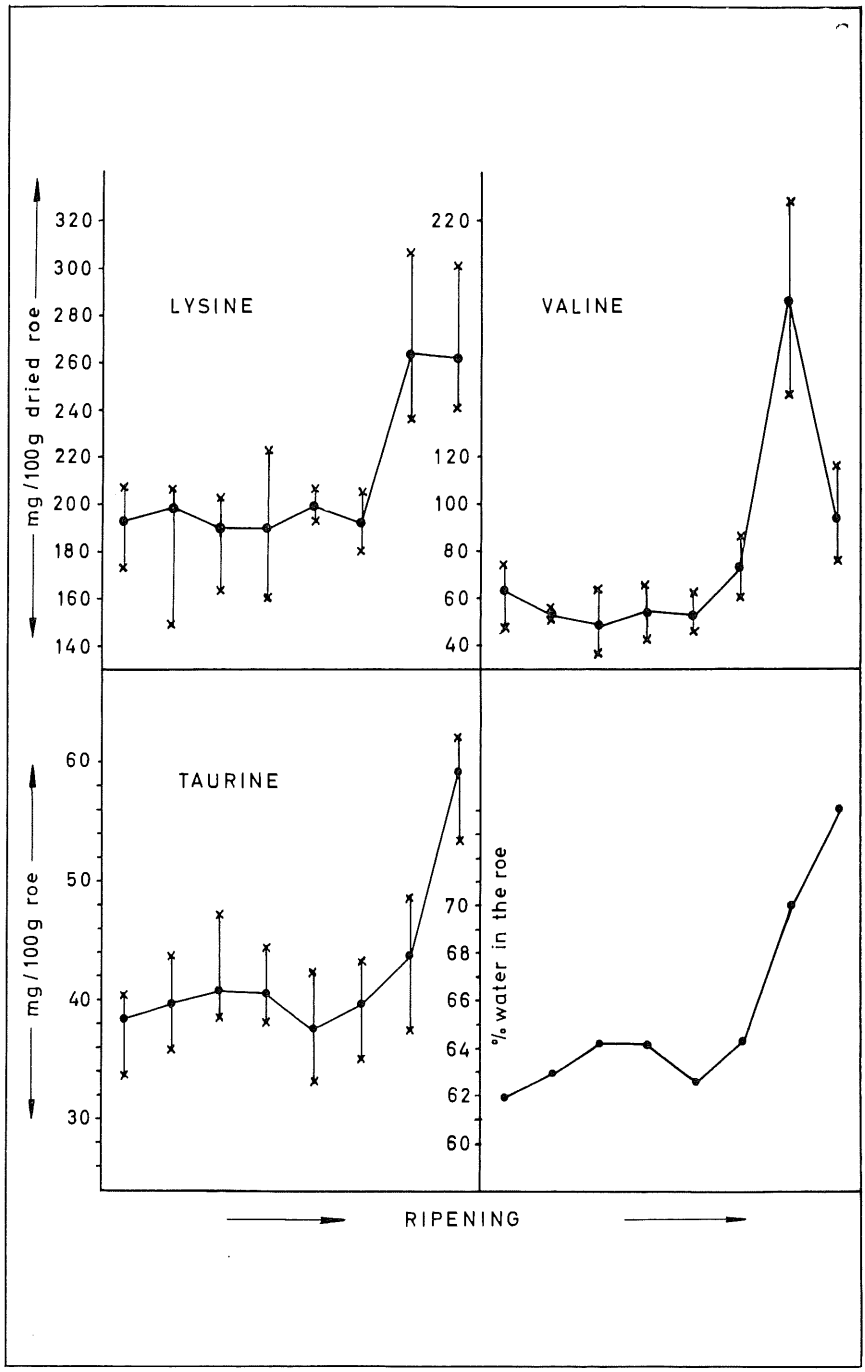

Fig. 3 The variation of amino acids and water content in hard herring roe during the ripening process.

to carry through, even if it is found necessary to confirm the results with several different chromatograms.

From fig. 3 it is seen that the water content of the hard herring roe has a pronounced increase during the last stages of the ripening process. A very similar curve illustrates the variation of taurine, the minimum is rather diffuse and there is no maximum. These minima are considered insignificant. The same is probably the case with regard to the curves illustrating the quantitative variation of leucine, serine, glutamic acid, alanine, lysine and valine. As regards the last six amino acids mentioned, the pronounced maximum just before full ripening, is, however, very interesting and should be subject to further investigation. The appearance of the roe corresponding to the maximum-point on the curves is very much like the full ripened roe; the roe-granules are hardly visible and they have a jellied soft consistence typical for the full ripened roe. The main difference appears when the roe is salted, which is believed to catalyse a coagulation process of the spawning roe.

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