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Andelssild

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PRELIMINARY REPORT

AN EXAMINATION OF THE RELATIONSHIP BETWEEN THE CONTENT OF BIOGENIC AMINES IN FISHMEAL AND THE FRESHNESS OF THE RAW MATERIAL.

Summary.

Fishmeal, manufactured at Andelssild in Esbjerg, has been analysed for the content of certain biogenic amines. This has been related to the freshness of the used raw material measured in TVN (mg N/100g). Biogenic amines are the decomposition products of amino acids. It is known that some of the biogenic amines have a toxic effect in high concentrations, which, among other effects, may cause diarrhoea and diseases of the liver in the consuming organism. An exact limit of biogenic amines for toxic effect cannot be given from literature. A synergetic toxic effect is suggested between biogenic amines. It is also a possibility that not only biogenic amines cause the effects mentioned, but that biogenic amines rather are indicators of the degree of decomposition of amino acids.
This examination does not deal with toxic aspects, but only treat the problem whether the content of biogenic amines in a fishmeal indicate the freshness of the raw material used.
The examined raw materials are Norway pout, sandeel and sprat, all originating from the North Sea and the surrounding waters.
It appeared that as regards the raw materials of sandeel and sprat it is possible to see a relationship while the samples of Norway pout are too few for significance.

Background.

A fishmeal made from a fresh raw material or a fishmeal made from a raw material with a specified maximum TVN are part of the specifications which the customer often mention when special quality meal is discussed.

However, it has been impossible to prove the freshness of the used raw material. Agreements between buyer and seller, therefore, in this respect exclusively are based on confidence. This is hardly satisfactory to anybody and motivated Andelssild to solve the problem.

An interesting possibility turned up by the analysis of biogenic amines:

a) We know from laboratory experiments with ageing of fish that the content of biogenic amines grows when the decay of the raw material increases.

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b) None of the examined amines have boiling points below 130° C, and most of them have boiling points above 180° C. This means that they will not evaporate as TVN. The heating during the fishmeal process should not influence the content of biogenic amines.

Procedure.

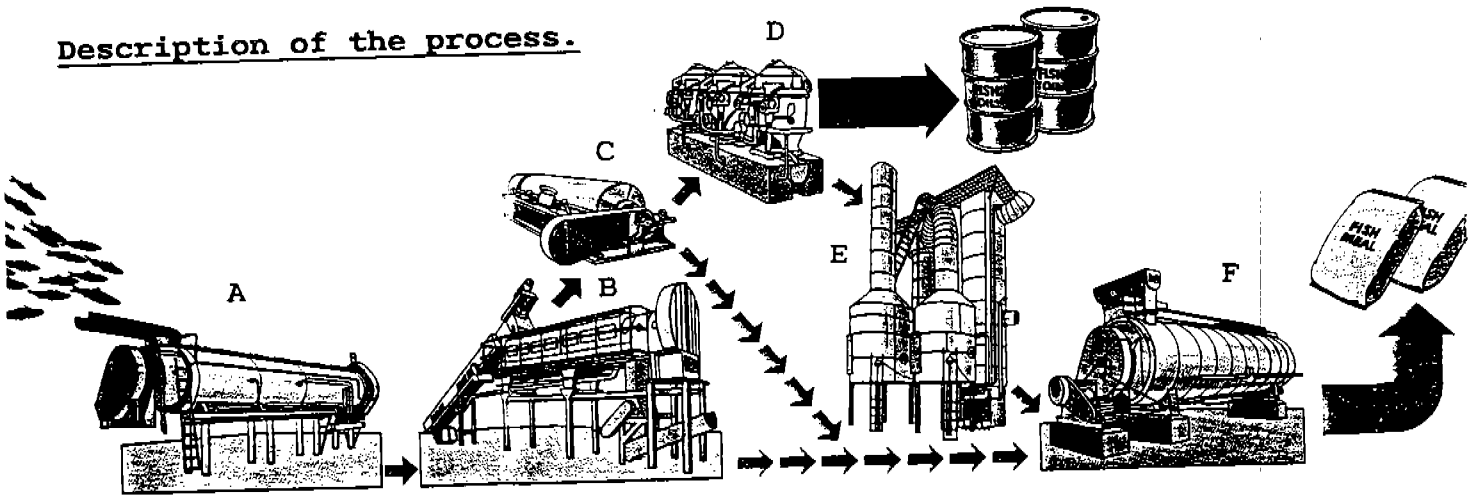
All samples were made at the fishmeal factory Andelssild, 999, in Esbjerg.

Average samples were drawn from 24 hours' meal production and analysed for: raw protein, humidity, fat, pH-10-value, amino acid composition and a number of biogenic amines.

Average samples were drawn from the unloading of the raw materials and analysed for: raw protein, humidity, fat and TVN. Species as well as the date of production were registered. In this way any seasonal variations were taken into consideration.

The analyses of the fishmeal were compared with the analyses of the raw material.

Description of the process.



A) The fish is heated in the cooker by indirect steam to app. 90°C. The boiling takes about 15 minutes.

B) The boiled fish mass is pressed and divided into two fractions: press cake and press water.

C) In the decanter the press water is divided into a solid phase and a fluid phase. The solid phase is mixed with the press cake.

D) The fluid phase from the decanter is lead into the centrifuge where the oil is separated. After the centrifuges the fluid mass is called stickwater.

E) The stickwater is lead into an evaporation plant where the solid phase in the stickwater is concentrated to about 45%. The product is called solubles, and it is mixed with press cake and the solid phase from the decanter.
If the solubles are not added to the press cake and solid phase from the decanter, the end product is called press cake meal.

The evaporation plant at Andelssild consists of an initial and a final evaporator.
The initial evaporator is a two-stage falling film evaporator. It uses waste heat as heating medium, and the highest temperature of the product is 80°C.
The final evaporator is a three- or a four-stage circulating evaporator, and the highest temperature of the product is 125°C.

F) In the drier the mixture of press cake, solid phase and solubles is dried to a minimum of 5% water. The driers at Andelssild are so-called plate driers with indirect steam heating. The temperature of the product rises gradually to about 90°C during the 2 hours' process.

The end product contains on the average:

crude protein	72%
moisture	8%
fat	8%

Results.

The results of the analyses are shown in the following 9 graphs. Methods of analysis are listed in the enclosures.

The samples were taken from 1 January 1987 to 15 December 1987 and so cover any seasonal variations.

Explanation of the graphs:

Graph 1:
Shows the pH-10-value versus water-soluble protein. There is a clear significance of a linear relationship. The pH-10 analysis is considerably easier and quicker to carry out than the analysis of water-soluble protein. In the following graphs the pH-10 analysis is used to indicate watersoluble protein (WATER-SOL.-PROT.).

pH-10 converted = -5.7 + 0.30*pH-10-value

PH-10-VALUE : ml 0.1 N sodium hydroxide/10 gram dry matter.

WATER-SOLUBLE PROTEIN: gram water-sol. prot./100 gram meal. The part of the protein which can be extracted by about 80°C hot water. The protein is determined by Kjeldahl analysis.

Graphs 2 and 3:
Show the relationship between the pH-10-value converted to water-soluble protein (WATER-SOL.PROT.) versus the sum of certain biogenic amines of the species sandeel and sprat. The sample material of Norway pout is yet inadequate.

There is a significant linear relationship meaning that added solubles to the meal increases the level of biogenic amines, and, conversely, an elimination of solubles reduces the level.

SUM BIO : the sum of biogenic amines tyramine, putrescine, cadaverine, histamine, agmatine, spermidine, phenethylamine and tryptamine. The result is shown as uMol/gram meal.

Graphs 4 and 5
Show the sum of certain biogenic amines in the meal (SUM BIO.) compared with TVN in the raw materials sprat and sandeel. The sample material for these graphs consists of meal with an admixture of the solubles belonging to the raw material. There is a reasonable relationship in the graph of sandeel which means that from the sum of certain biogenic amines in a whole meal it is possible to indicate the TVN of the raw material at the beginning of the process. Due to scarcity of samples it is impossible to verify relationship with sprat. However, there is an indication of a relationship. If the two graphs are compared, it can be seen that with a certain TVN the level of biogenic amines will be the same in the two species.

The weak point of determination of the biogenic amine level is that a decrease in the admixture of solubles will reduce the content of biogenic amines. The force is that an increase in the admixture of solubles will be revealed by a higher content

Graphs 6,7,8 and 9:
Show the relationship between TVN in the raw material versus the ratio of certain biogenic amines in the water-soluble protein (SUM BIO./WATER-SOL.PROT.) as regards the species sandeel (graph 6), sprat (graph 7) and Norway pout (graph 8). In graph 9 all the results mentioned above are gathered in one graph. From the graph of sandeel and sprat a clearly significant linear relationship can be seen. However, due to scarcity of samples the same clear relationship cannot be seen in graph 8.

If the three graphs are compared as it is done in graph 9, a clearly significant linear relationship appears. It can be seen that with a certain TVN in the raw material the level of the biogenic amines in the water-soluble protein will be the same for all three species. With a TVN of 75 mg N/100 g you will read about 200 uMol/g WSP.
This relationship shows the age of the raw material behind the water-soluble protein fraction, independent of the amounts of solubles added.

SUM BIO./WATER-SOL.PROT.	The sum of biogenic amines divided by calculated water-soluble protein. The calculation is made from the pH-10 analysis which as it can be seen from the enclosed graph 1 has a clear relationship with water-soluble protein. The unit is uMol/g water-soluble protein.

Please note that no results measured have been left out from the graph.

Comments.

The statistic analysis shows a clear significance of a linear relationship between TVN in the raw material and biogenic amines in the water-soluble part, (graph 9). The slope is probably correct whereas the absolute vertical position of the regression line is more uncertain according to the statistic analysis. This may be caused by problems in precisely determining the TVN of the raw material at the beginning of the process, as the samples are taken during unloading of the ship. It is expected that the calculated SEE of 13.6 TVN-units can be reduced considerably by analysing the raw material immediately before the cooker.

From the above it must be concluded that by combining the determination of the biogenic amines in the meal with the measurement of the pH-10 titration value i.e. the water-soluble protein, it is possible to indicate the TVN of the raw material at the beginning of the process. It all concerns the content of biogenic amines in the water-soluble phase.

Conversely it can be concluded that to a given TVN of the raw material there is a corresponding ratio of biogenic amines to the content of water-soluble protein in the meal. This circumstance is independent of the examined species, which are sandeel, Norway pout and sprat. (Graphs 6,7 and 8).

All measurements are made at one factory, and the conclusions can, therefore, only be related to this factory or to factories with the same process, type and equipment.

Example. (Graph 10)

A differentiation of all the mentioned meals without regard to species is made in graph 10. To define a meal made from a fresh raw material limits have been drawn as follows: SUM BIO. < 25 uMol/gram and SUM BIO./WATER-SOL.PROT. < 100 uMol/g. The chosen meals are indicated in the graph by a quadrangle. The meals which do not meet these criterions are indicated by a cross. From the graph it can be read that the meals which satisfy the requirements made are made from a raw material which has a TVN below 80. Some meals which are made from a fresher raw material cannot satisfy these requirements, but these meals can be explained by the circumstances of the process as addition of solubles from other fish.

In the example above SUM BIO. is used as well as SUM BIO./ WATER-SOL.PROT. The reason is that in the market exists fish-meal with widely varying contents of solubles or no solubles at all. It would be insufficient only to look at SUM BIO. as the biogenic amines follow the soluble phase. It is possible to provide a low content of SUM BIO. from a very decayed raw material just by leaving out the solubles phase. This will be disclosed, however, by measuring SUM BIO./WATER-SOL.PROT.
At present it is impossible to make significant statements about other raw materials than sandeel for the possible reasons:

a) most likely that for the time being there is not sufficiant material of the other species. The analyses continue to complete the material.

The biogenic amines without regard to raw material and season are:

	tyra-mine	putre-scine	cada-verine	hista-mine	agma-tine
% of sum	17	24	50	4	4

b) or that the biogenic amines develop differently in different species. If this is the case the analysis system will have to be extended to include more than biogenic amines. Sofar, however, there is no indication of this possibility.

At present, the influence of the process temperature on the retention of biogenic amines is being examined.

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Hans Otto Sørensen

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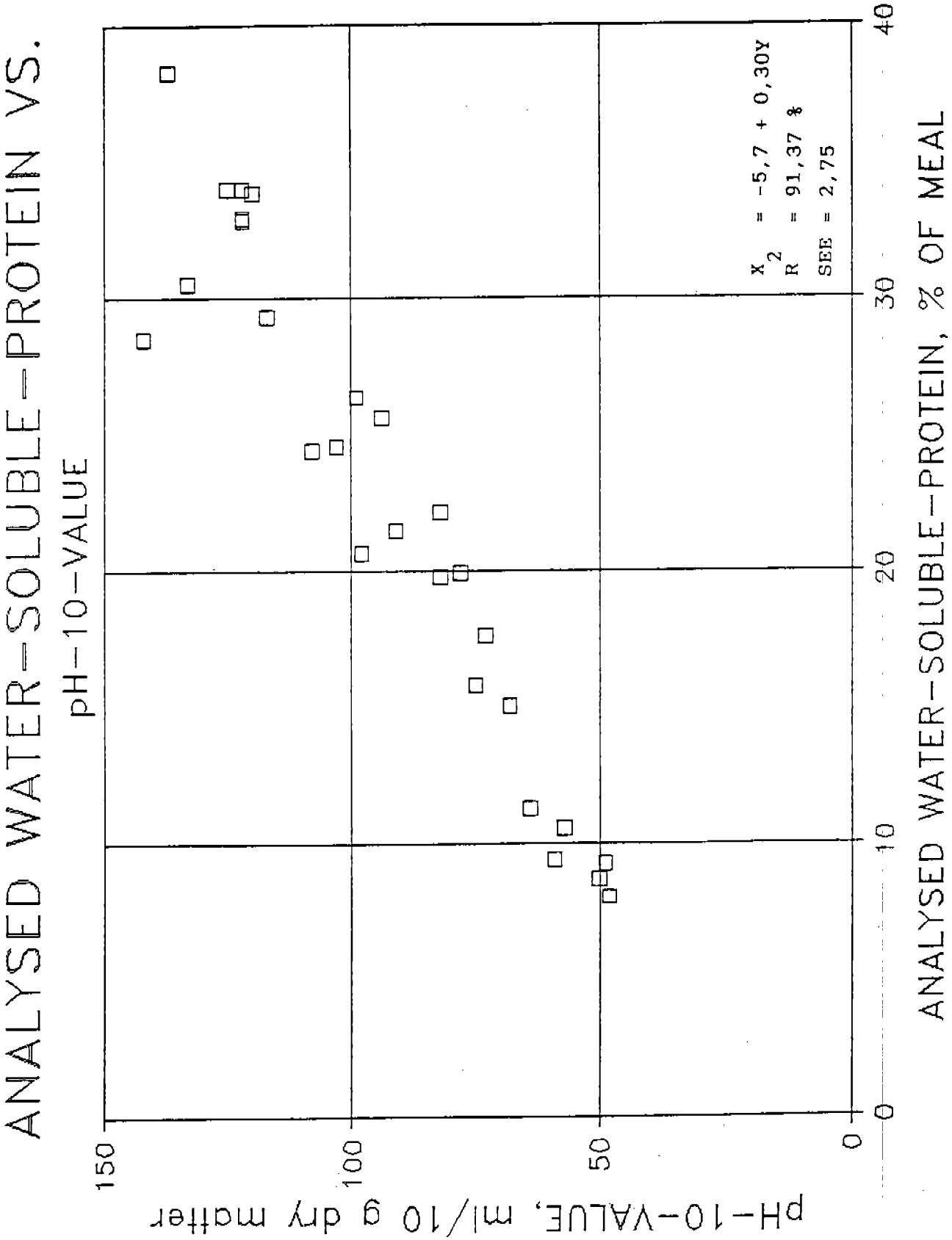
Graph 1



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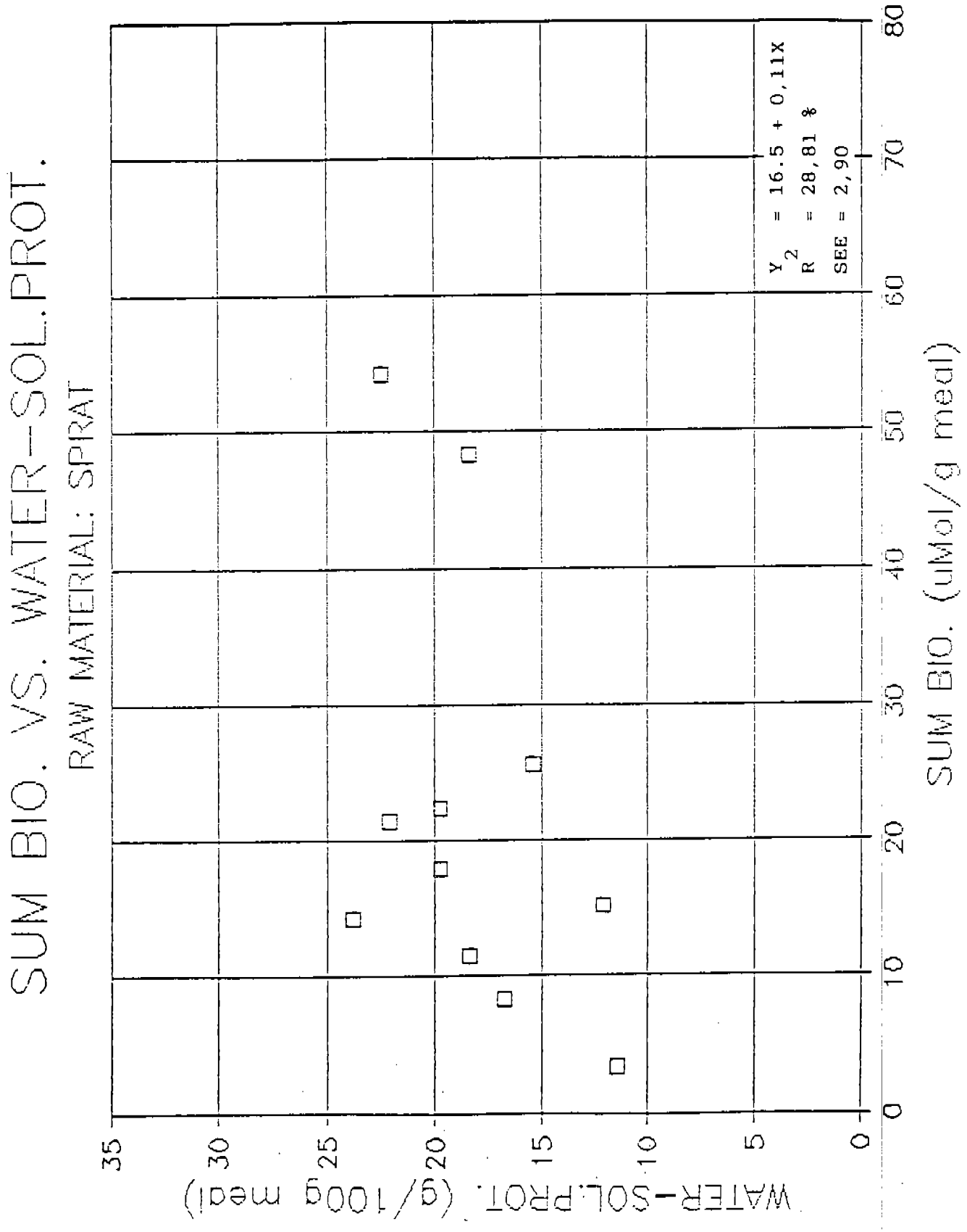
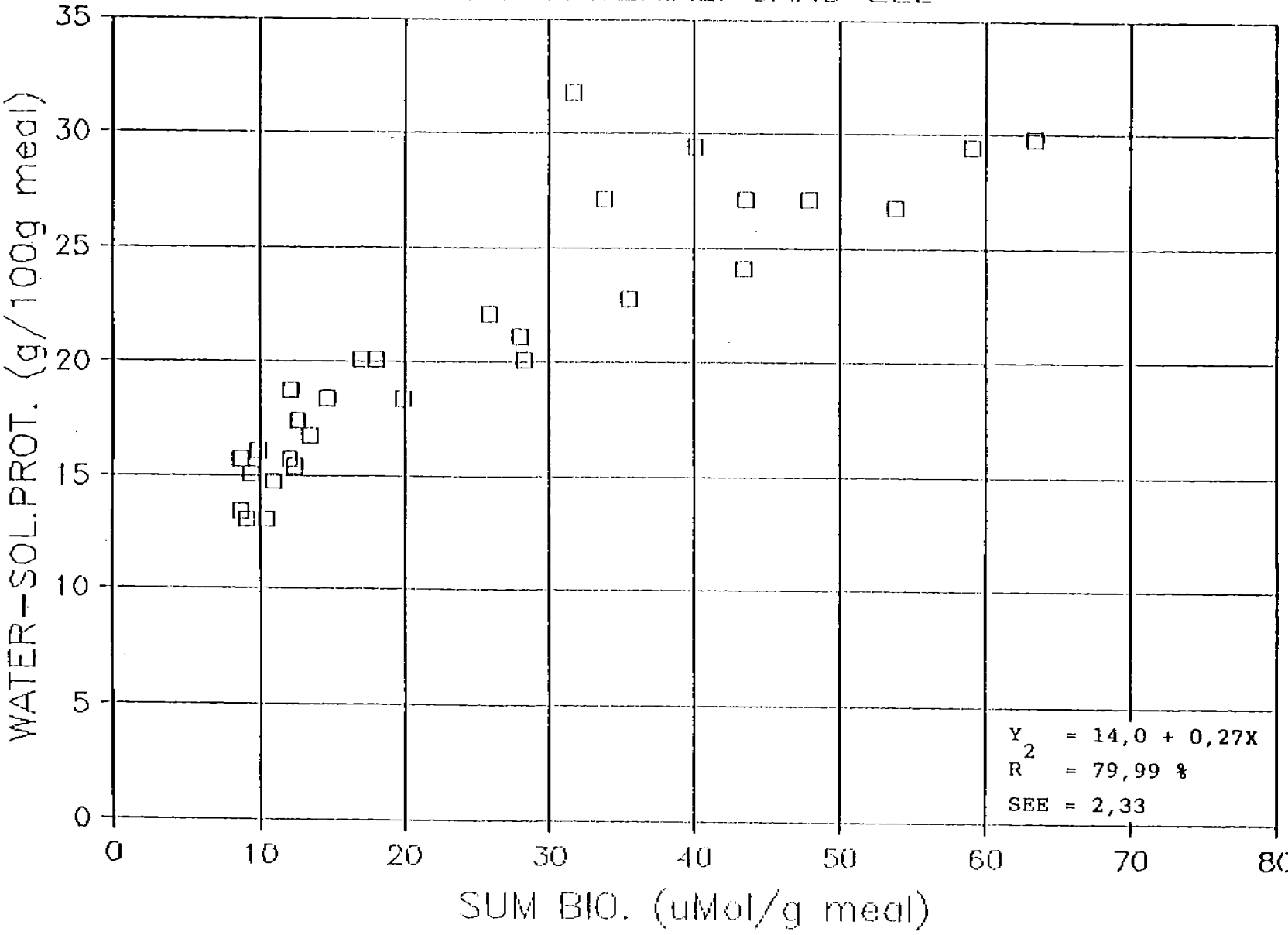
Graph 2



SUM BIO. VS. WATER-SOL.PROT.

RAW MATERIAL: SAND EEL



Graph 3

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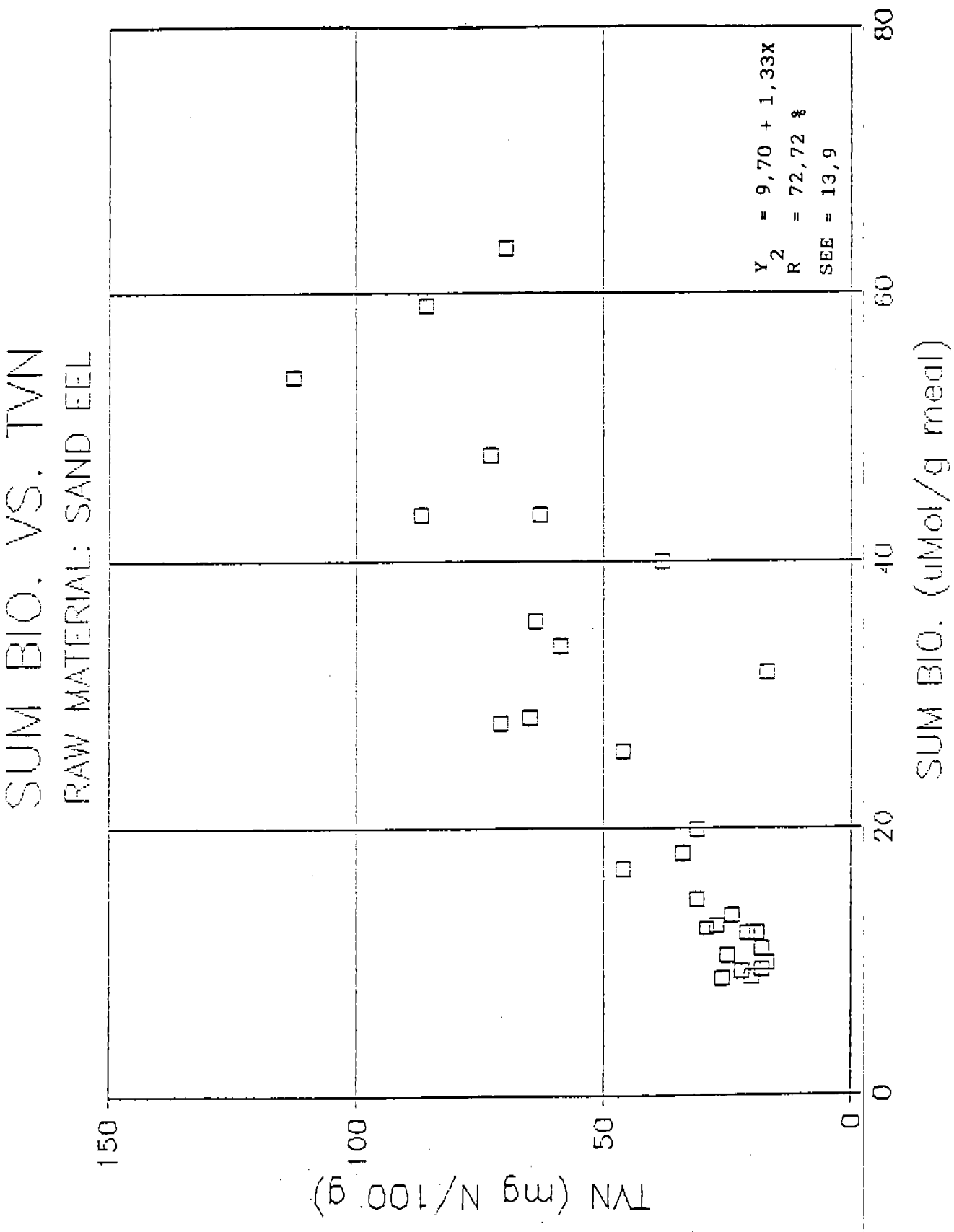
Graph 4



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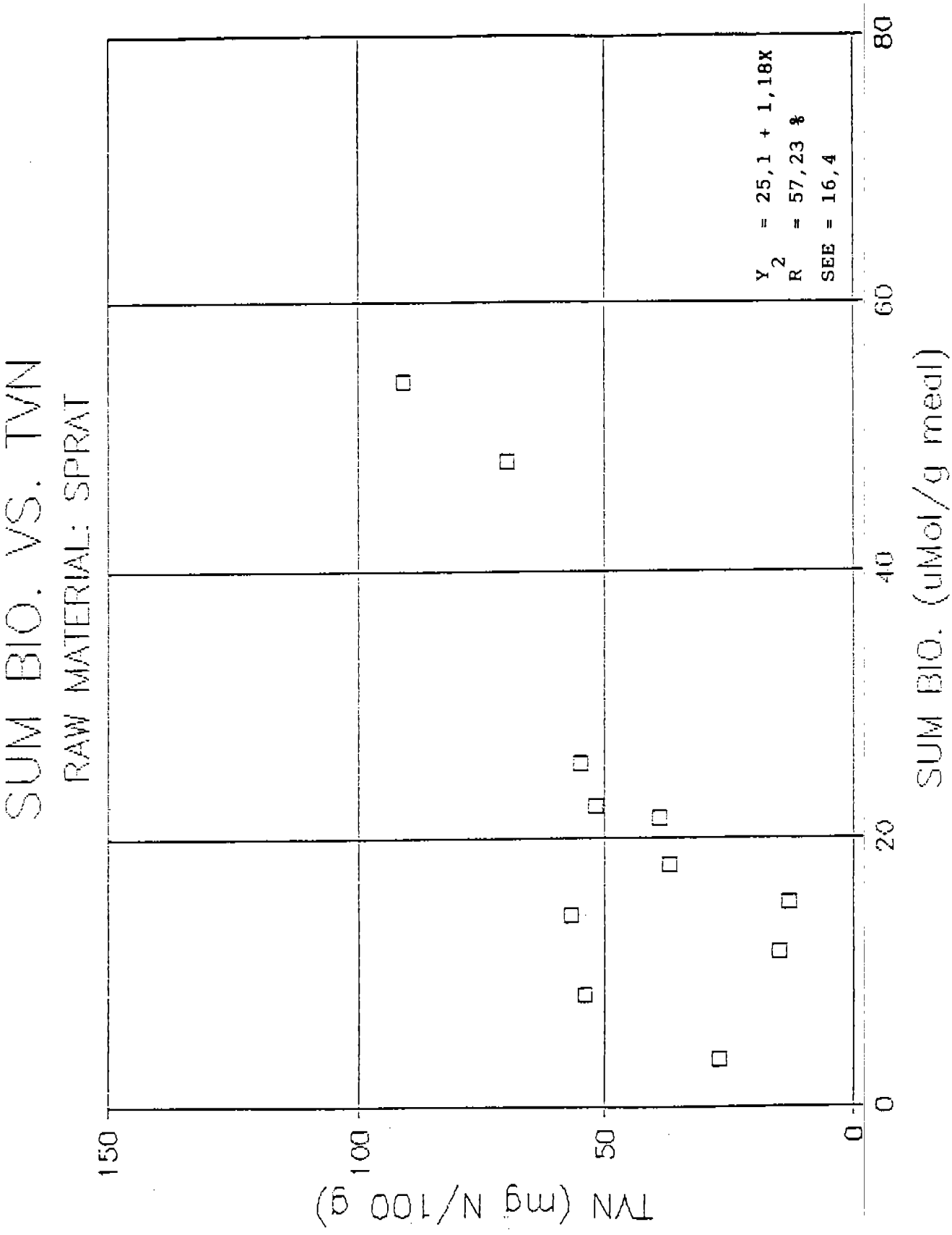
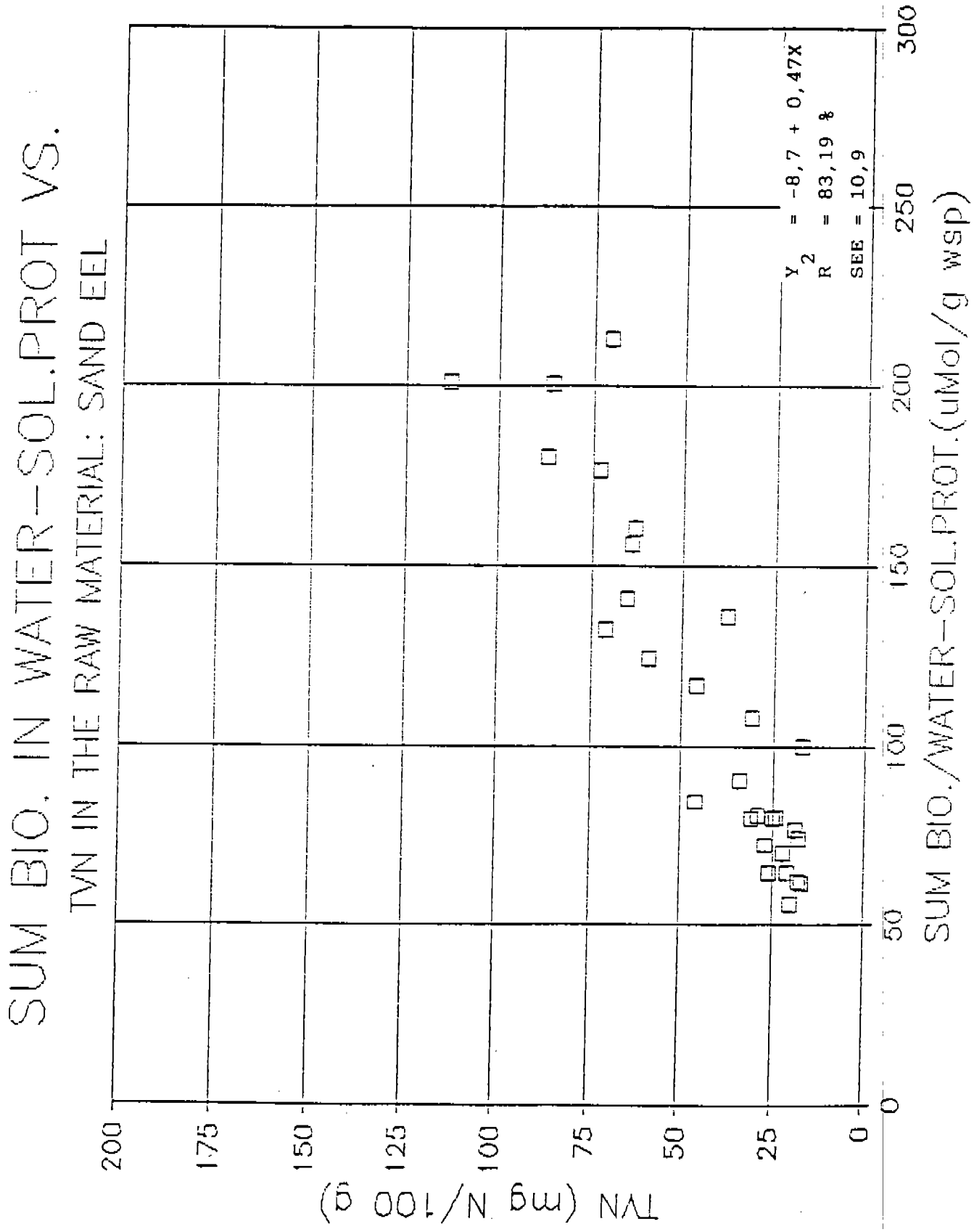
Graph 5

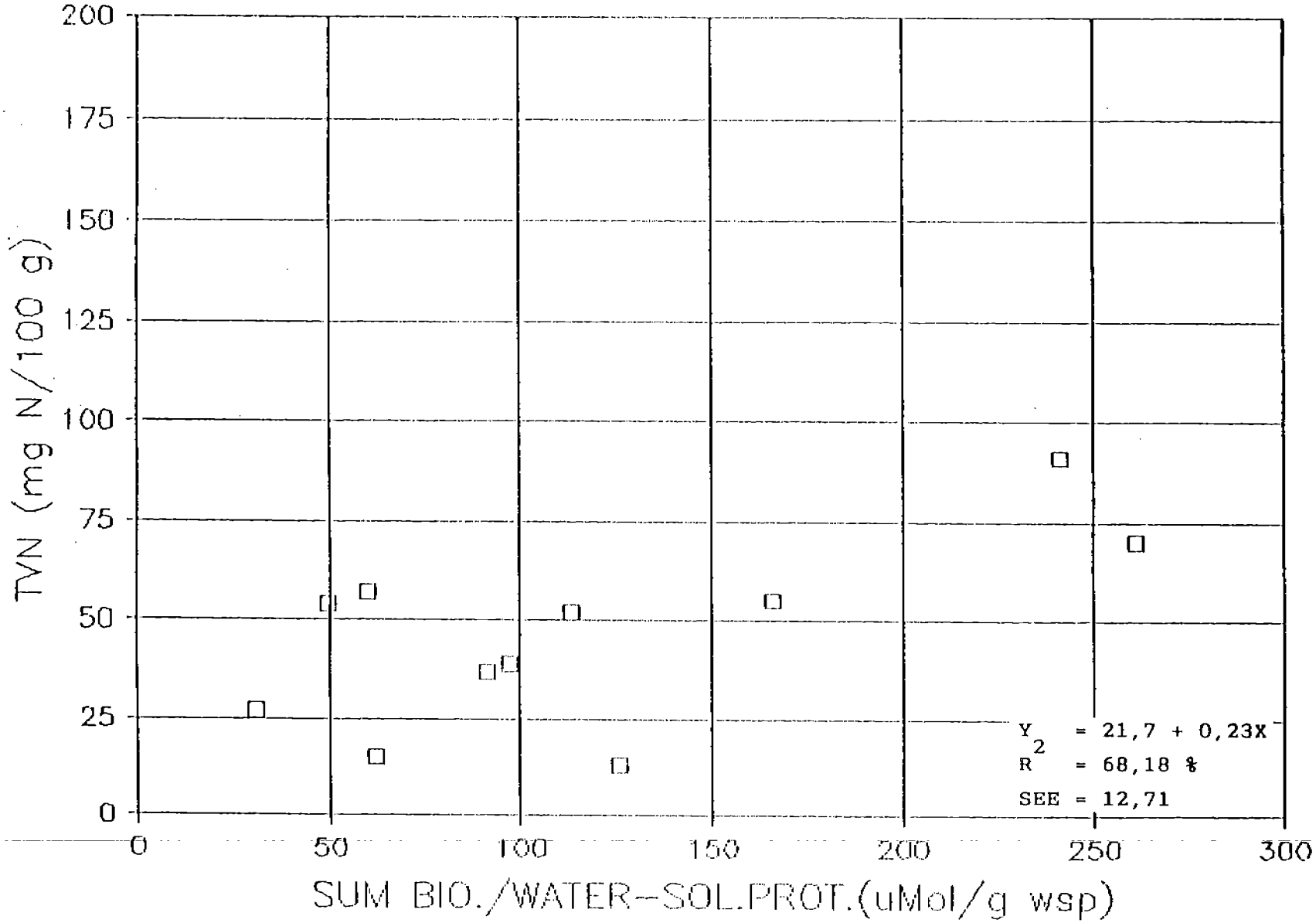


Graph 6


SUM BIO. IN WATER-SOL.PROT VS.
TVN IN THE RAW MATERIAL: SAND EEL
y = -8,7 + 0,47x
R2 = 83,19 %
SEE = 10,9
TVN (mg N/100 g)
SUM BIO./WATER-SOL.PROT.(uMol/g wsp)
0
25
50
75
100
125
150
175
200
0
50
100
150
200
250
300

SUM BIO. IN WATER-SOL.PROT VS.

TVN IN THE RAW MATERIAL: SPRAT



Graph 7

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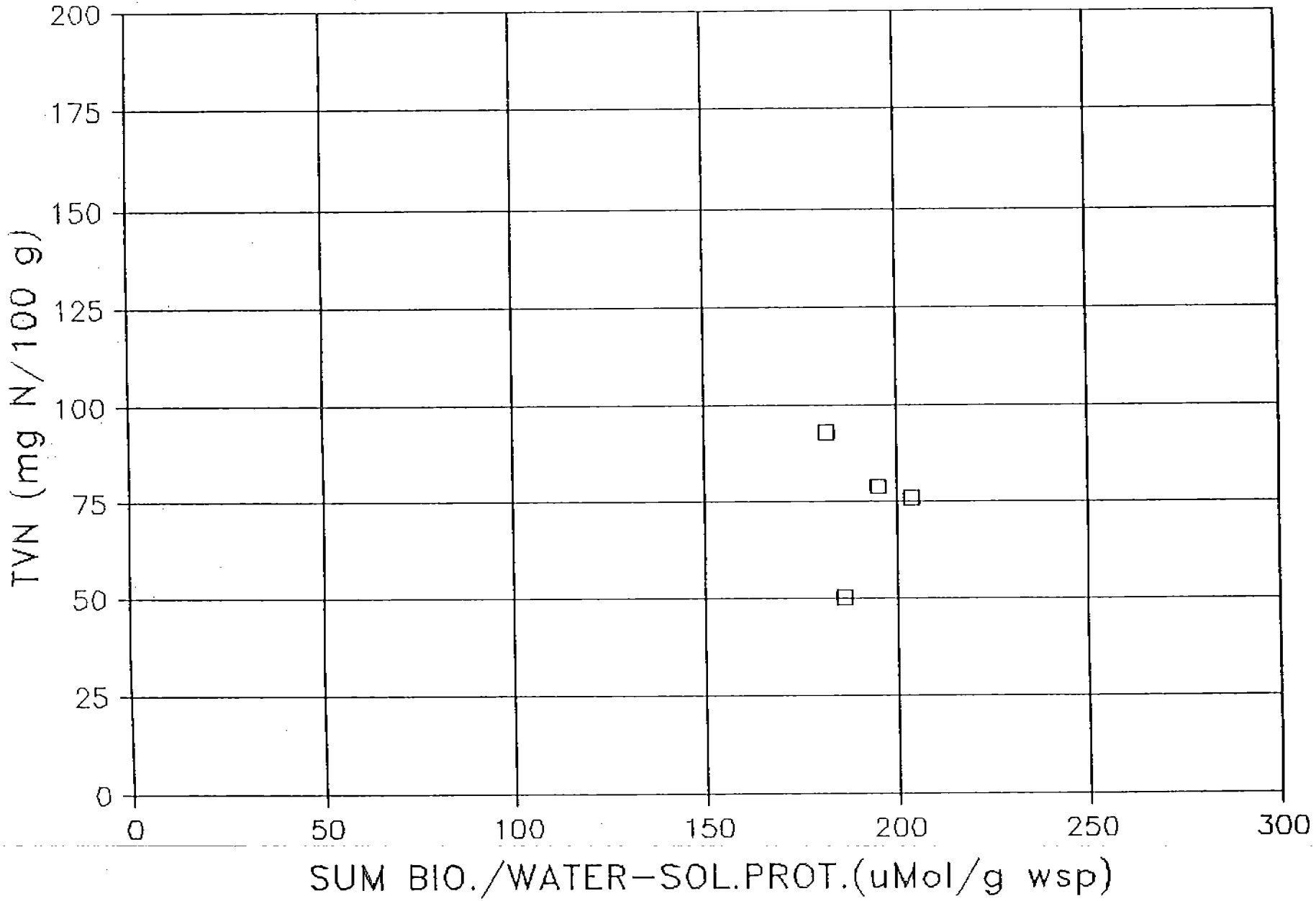
SUM BIO. IN WATER-SOL.PROT VS.

TVN IN THE RAW MATERIAL: NORWAY POUT



Graph 8

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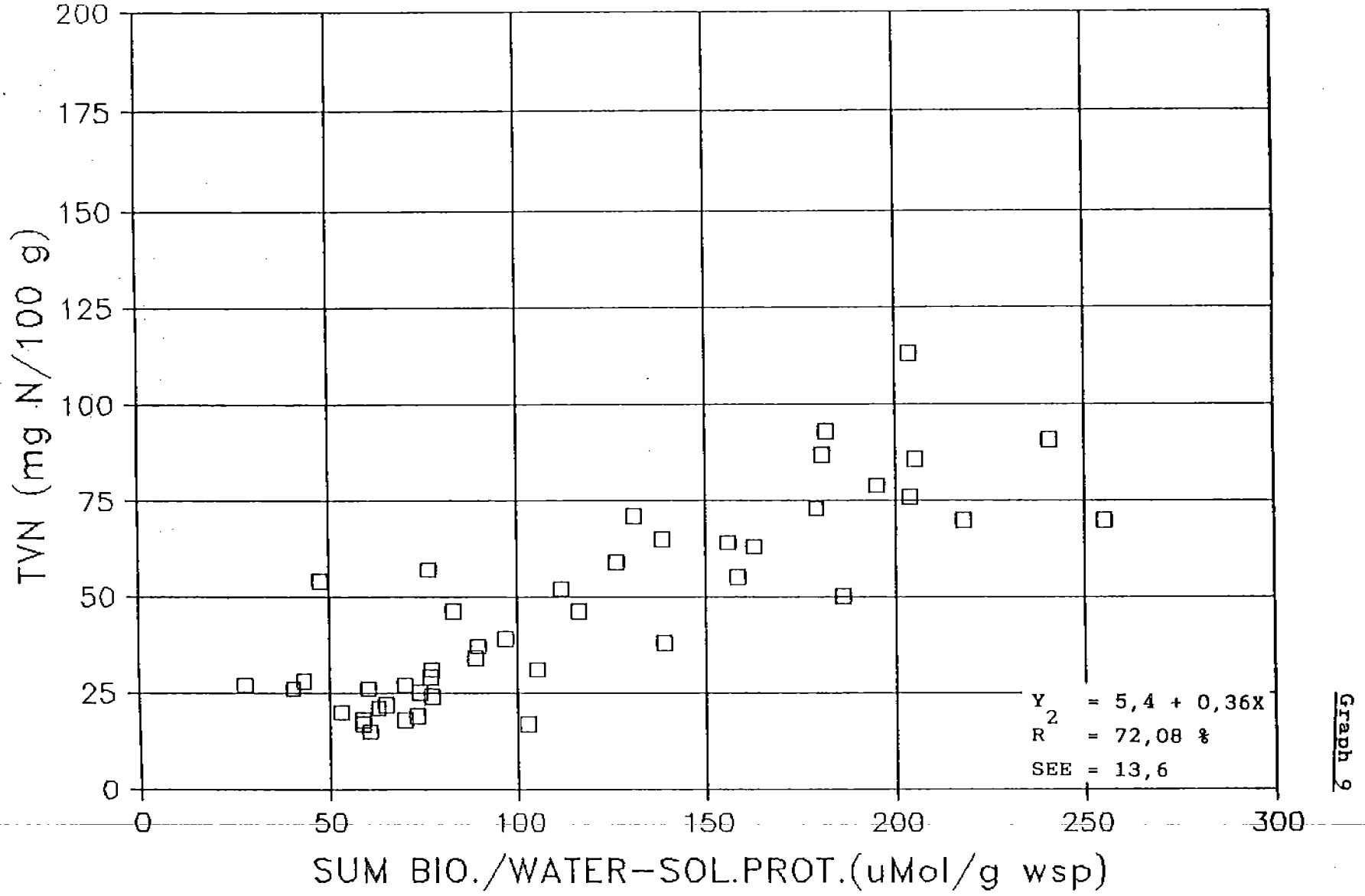
SUM BIO. IN WATER-SOL.PROT VS. TVN

RAW MATERIAL: SAND EEL, SPRAT, NORWAY POUT



Graph 9

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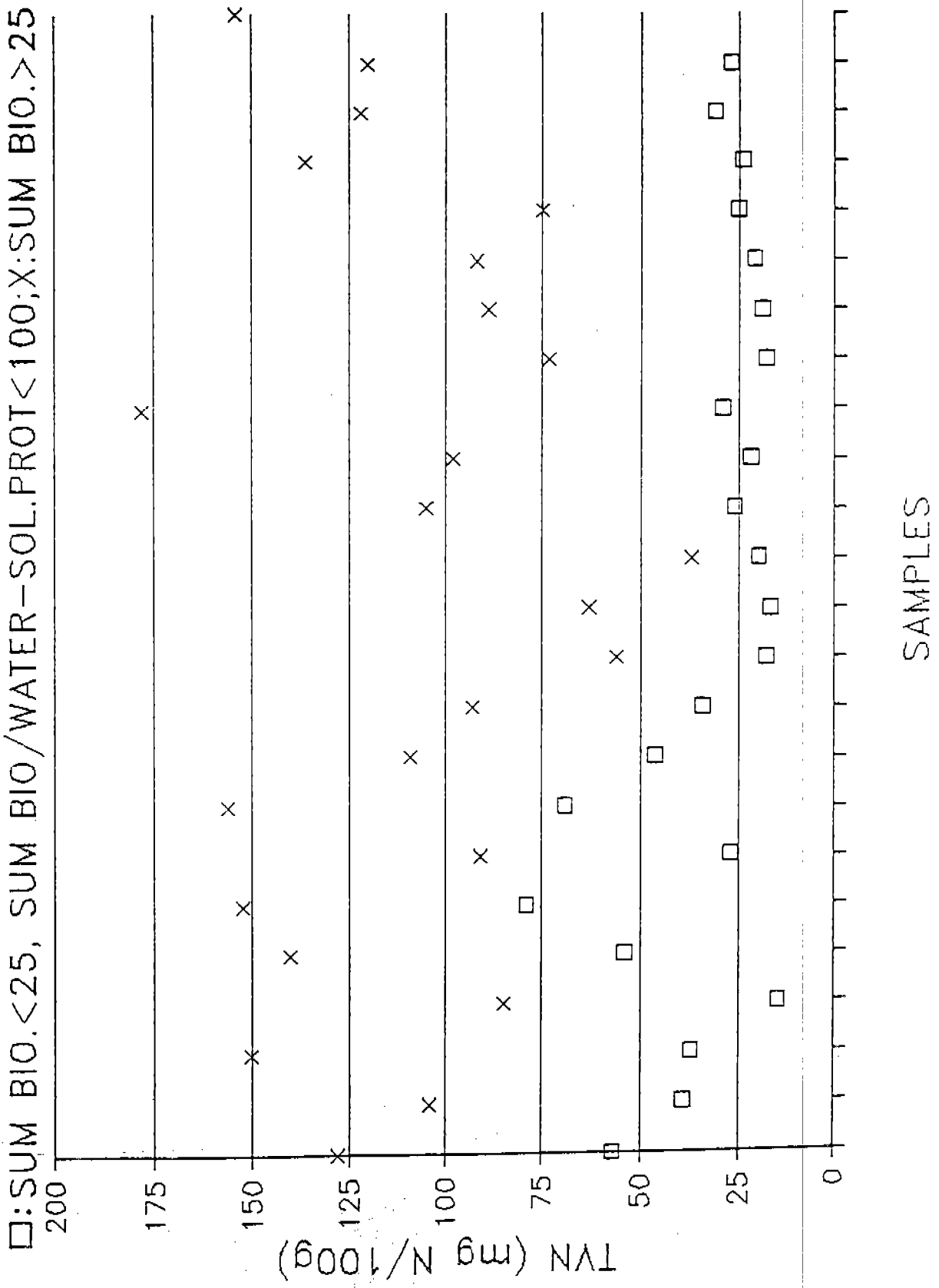
Graph 10


DIFFERENTIATION OF THE MEALS
□:SUM BIO.<25, SUM BIO/WATER-SOL.PROT<100;X:SUM BIO.>25
TVN (mg N/100g)
200
175
150
125
100
75
50
25
0
SAMPLES