COMMISSION DECISION

of 8 March 1995

fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used

(95/149/EC)

THE COMMISSION OF THE EUROPEAN COMMUNITIES,

Having regard to the Treaty establishing the European Community,

Having regard to Council Directive 91/493/EEC of 22 July 1991 laying down the health conditions for the production and the placing on the market of fishery products [1], and in particular Chapter V (II) (3) of the Annex thereto,

Whereas the checks provided for in Directive 91/493/EEC to prevent fishery products which are unfit for human consumption from being placed on the market may comprise certain chemical checks including checking total volatile basic nitrogen (TVB-N);

Whereas it is necessary to set levels of TVB-N which are not to be exceeded in the case of certain species categories and to specify the analysis methods to be used;

Whereas the analysis methods which are scientifically recognized for checking TVB-N must continue to be used as a matter of routine but it is advisable to specify a reference method which may be used in case of doubt regarding the results or in the event of dispute;

Whereas the measures provided for in this Decision are in accordance with the opinion of the Standing Veterinary Committee,

HAS ADOPTED THIS DECISION:

Article 1

Unprocessed fishery products belonging to the species categories listed in Annex I shall be regarded as unfit for human consumption where, organoleptic assessment having raised doubts as to their freshness, chemical checks reveal that the following TVB-N limits are exceeded:

1. 25 milligrams of nitrogen/100 grams of flesh for the species referred to in point A of Annex I;
2. 30 milligrams of nitrogen/100 grams of flesh for the species referred to in point B of Annex I;
3. 35 milligrams of nitrogen/100 grams of flesh for the species referred to in point C of Annex I.

Article 2

1. The reference method to be used for checking the TVB-N limit is the method involving distillation of an extract deproteinized by perchloric acid set out in Annex II.

2. Distillation as referred to in paragraph 1 must be performed using apparatus which complies with the principles of the diagram in Annex III.

3. The routine methods which may be used to check the TVB-N limit are as follows:

— microdiffusion method described by Conway and Byrne (1933),

— direct distillation method described by Antonacopoulos (1968),

— distillation of an extract deproteinized by trichloracetic acid (Codex Alimentarius Committee on Fish and Fishery Products (1968)).

4. The sample must consist of about one hundred grams of flesh, taken from at least three different points and mixed together by grinding.

Article 3

Member States shall recommend to official laboratories the use, as a matter of routine, of the reference method referred to in Article 2 (1). In case of doubt or in the event of dispute regarding the results of analysis performed by one of the routine methods only the reference method may be used to check the results.

Article 4

This Decision is addressed to the Member States.

Done at Brussels, 8 March 1995.

For the Commission

Franz FISCHLER

Member of the Commission

[1] OJ No L 268, 24. 9. 1991, p. 15.

ANNEX I

SPECIES CATEGORIES FOR WHICH A TVB-N LIMIT VALUE IS FIXED

A. *Sebastes* spp.
Helicolenus dactylopterus
Sebastichthys capensis

B. Species belonging to the Pleuronectidae family (with the exception of halibut: *Hippoglossus* spp.)

C. *Salmo salar*
Species belonging to the Merlucciidae family
Species belonging to the Gadidae family

ANNEX II

DETERMINATION OF THE CONCENTRATION OF VOLATILE NITROGENOUS BASES (TVB-N) IN FISH AND FISH PRODUCTS: A REFERENCE PROCEDURE

1. Purpose and area of application

This method describes a reference procedure for identifying the nitrogen concentration of volatile nitrogenous bases (Total-Volatile-Base-N: TVB-N) in fish and fish products. This procedure is applicable to TVB-N concentrations from 5 mg/100 g to at least 100 mg/100 g.

2. Definition

The TVB-N concentration is here understood to mean the nitrogen content of volatile nitrogenous bases determined by the procedure described. The concentration is stated in terms of mg/100 g.

3. Brief description

The volatile nitrogenous bases are extracted from a sample by a solution of 0,6 M perchloric acid. After alkalinization the extract is submitted to steam distillation and the volatile base components are absorbed by an acid receiver. The TVB-N concentration is determined by titration of the absorbed bases.

4. Chemicals

Unless otherwise indicated, reagent-grade chemicals should be used. The water used must be either distilled or demineralized and of at least the same purity. Unless indicated otherwise, a 'solution' is to be understood as an aqueous solution.

4.1. Perchloric acid solution = 6 g/100 ml.

4.2. Sodium hydroxide solution = 20 g/100 ml.

4.3. Hydrochloric acid standard solution 0,05 mol/l (0,05 N).

Note: When using an automatic distillation apparatus, titration should take place with a hydrochloric acid standard solution 0,01 mol/l (0,01 N).

4.4. Boric acid solution = 3 g/100 ml.

4.5. Silicone anti-foaming agent.

4.6. Phenolphtalein solution = 1 g/100 ml 95 % ethanol.

4.7. Indicator solution *(Tashiro Mixed Indicator)*

2 g Methyl — red and 1 g Methylene — blue are dissolved in 1 000 ml 95 % ethanol.

5. Instruments and accessories

5.1. A meat grinder to produce a sufficiently homogenous fish mince.

5.2. High-speed blender with revolutions between 8 000 min^{-1} and 45 000 min^{-1}.

5.3. Fluted filter, diameter 150 mm, quick-filtering.

5.4. Burette, 5 ml, graduated to 0,01 ml.

5.5. *Apparatus for steam distillation*

The apparatus must be able to regulate various amounts of steam and produce a constant amount of steam over a given period of time. It must ensure that during the addition of alkalizing substances the resulting free bases cannot escape.

6. **Execution**

Warning: When working with perchloric acid, which is strongly corrosive, necessary caution and preventive measures should be taken.

The samples should, if at all possible, be prepared according to paragraph 6.1 as soon as possible after their arrival.

6.1. *Preparation of the sample*

The sample to be analysed should be ground carefully by a meat grinder as described in secton 5.1. Exactly 10 g +/− 0,1 g of the ground sample are weighed in a suitable container, mixed with 90,0 ml perchloric acid solution as stated in section 4.1, homogenized for two minutes with a blender as described in section 5.2, and then filtered.

The extract thereby obtained can be kept for at least seven days at a temperature between approximately 2 °C and 6 °C.

6.2. *Steam distillation*

50,0 ml of the extract obtained according to section 6.1 are put in an apparatus for steam distillation as described in secton 5.5. For a later check on sufficient alkalinization of the extract, several drops of phenolphtalein as specified in section 4.6 are added. After adding a few drops silicone anti foaming agent, 6,5 ml of sodium hydroxide solution as specified in section 4.2 are added to the extract, and steam distillation begins immediately.

The steam distillation is regulated so that around 100 ml of distillate are produced within 10 minutes. The distillation outflow tube is submerged in a receiver with 100 ml boric acid solution as specified in section 4.4, to which three to five drops of the indicator solution as described in 4.7 have been added. After exactly 10 minutes the distillation is ended. The distillation outflow tube is removed from the receiver and washed out with water. The volatile bases contained in the receiver solution are determined by titration with standard hydrochloric solution as specified in secton 4.3.

The pH of the end point should be 5,0 +/− 0,1.

6.3. *Titration*

Duplicate analyses are required. The applied method is correct if the difference of the duplicates is not higher than 2 mg/100 g.

6.4. *Blank*

A blind test carried out as described in section 6.2.

Instead of the extract, 50,0 ml perchloric acid solution as specified in section 4.1 are used.

7. **Calculation of TVB-N**

By titration of the receiver solution with hydrochloric acid as in 4.3, the TVB-N concentration is calculated with the following equation:

$$\text{TVB-N (expressed in mg/100 g sample)} = \frac{(V_1 - V_0) \times 0{,}14 \times 2 \times 100}{M}$$

V_1 = Volume of 0,01 M hydrochloric acid solution in ml for sample;

V_0 = Volume of 0,01 M hydrochloric acid solution in ml for blanc;

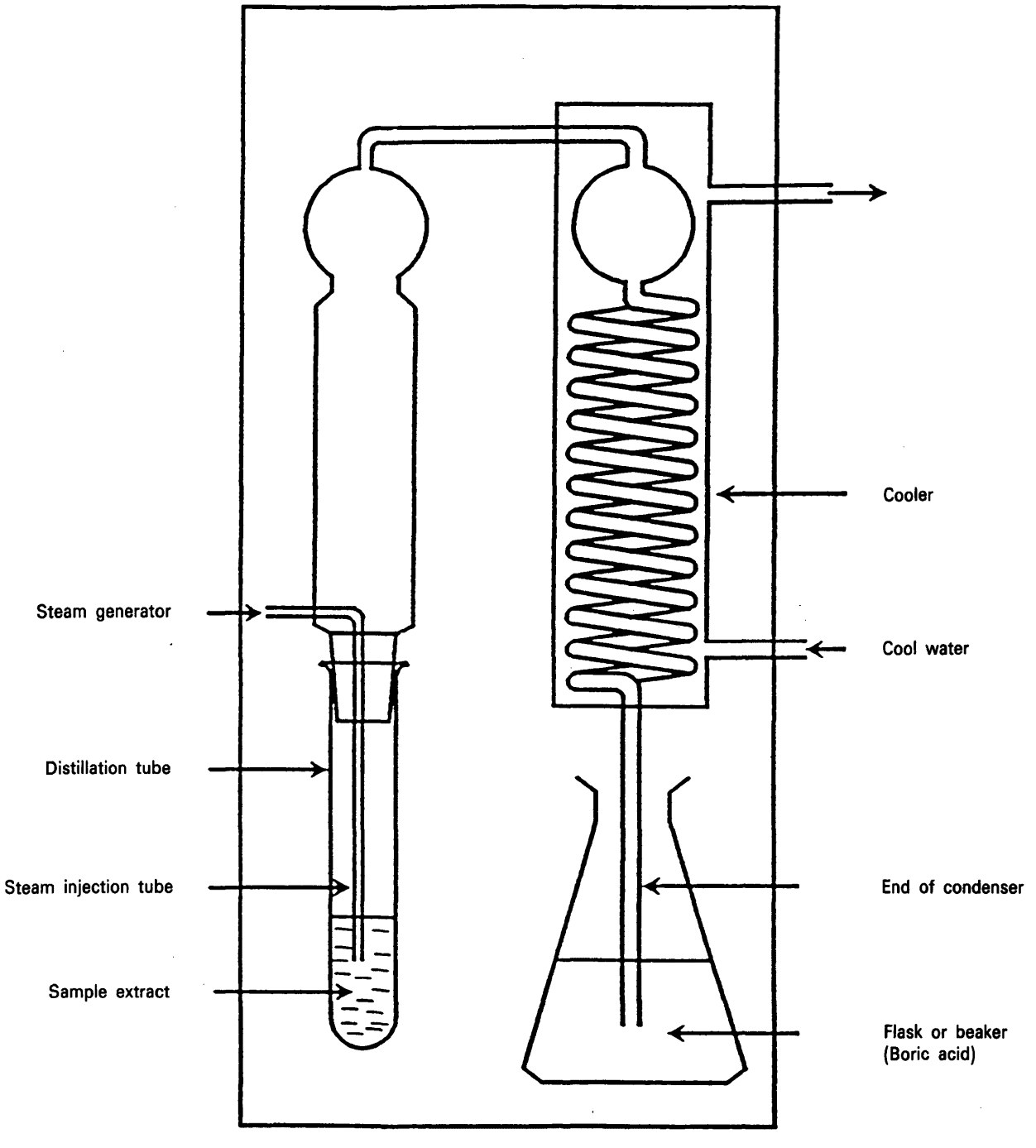
M = Weight of sample in g.

Remarks

1. Duplicate analyses are required. The applied method is correct if the difference between duplicates is not higher than 2 mg/100 g.
2. Check the equipment by distilling solutions of NH_4Cl equivalent to 50 mg TVB-/100 g.
3. Standard deviation of reproducibility S_r = 1,20 mg/100 g.

   Standard deviation of comparability S_R = 2,50mg/100 g.

ANNEX III



TVB-N steam distillation apparatus