Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls (Text with EEA relevance)

ANNEX VI

PRACTICAL ARRANGEMENTS FOR OFFICIAL CONTROLS ON FISHERY PRODUCTS IN ACCORDANCE WITH ARTICLE 70

CHAPTER II

CONTROLS ON TOTAL VOLATILE BASIC NITROGEN (TVB-N)

A. TVB-N limit values for certain categories of fishery products and analysis methods to be used

1. Unprocessed fishery products shall be regarded as unfit for human consumption where organoleptic assessment has raised doubts as to their freshness and chemical checks reveal that the following TVB-N limits are exceeded:

(a) 25 mg of nitrogen/100 g of flesh for the species referred to in point 1 of Part B of this Chapter;

(b) 30 mg of nitrogen/100 g of flesh for the species referred to in point 2 of Part B of this Chapter;

(c) 35 mg of nitrogen/100 g of flesh for the species referred to in point 3 of Part B of this Chapter;

(d) 60 mg of nitrogen/100 g of whole fishery product used directly for the preparation of fish oil for human consumption, as referred to in the second paragraph of point 1 of Chapter IV.B of Section VIII of Annex III to Regulation (EC) No 853/2004; however, where the raw material complies with points (a), (b) and (c) of the first paragraph of that point, Member States may set limits at a higher level for certain species pending the establishment of specific Union legislation.

The reference method to be used for checking the TVB-N limits involves distilling an extract deproteinised by perchloric acid as set out in Part C below.

2. Distillation as referred to in point 1 shall be performed using apparatus which complies with the diagram in Part D below.

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

(a) microdiffusion method described by Conway and Byrne (1933);

(b) direct distillation method described by Antonacopoulos (1968);

(c) distillation of an extract deproteinised by trichloracetic acid (Codex Alimentarius Committee on Fish and Fishery Products, 1968).

4. The sample shall consist of about 100 g of flesh, taken from at least three different points and mixed together by grinding.

Member States shall recommend that official laboratories use, as a matter of routine, the methods referred to above. Where the results are dubious 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.

B. Species categories for which TVB-N limit values are fixed

TVB-N limit values are fixed for the following species categories:

1. *Sebastes spp.*, *Helicolenus dactylopterus*, *Sebastichthys capensis*;

2. species belonging to the *Pleuronectidae* family (with the exception of halibut: *Hippoglossus spp.*);

3. *Salmo salar*, species belonging to the *Merlucciidae* family, species belonging to the *Gadidae* family.

C. **Reference procedure for determining the concentration of TVB-N in fish and fishery products**

1. Purpose and area of application

This method describes a reference procedure for identifying the nitrogen concentration of TVB-N in fish and fishery products. The procedure is applicable at TVB-N concentrations of 5 mg/100 g to at least 100 mg/100 g.

2. Definitions

'TVB-N concentration' means the nitrogen content of volatile nitrogenous bases as determined by the reference procedure described.

'Solution' means an aqueous solution as follows:

(a) perchloric acid solution = 6 g/100 ml;

(b) sodium hydroxide solution = 20 g/100 ml;

(c) hydrochloric acid standard solution 0,05 mol/l (0,05 N). When using an automatic distillation apparatus, titration must take place with a hydrochloric acid standard solution of 0,01 mol/l (0,01 N);

(d) boric acid solution = 3 g/100 ml;

(e) silicone anti-foaming agent;

(f) phenolphtalein solution = 1 g/100 ml 95 % ethanol;

(g) indicator solution (Tashiro mixed indicator) = 2 g methyl-red and 1 g methylene-blue dissolved in 1 000 ml 95 % ethanol.

3. Brief description

The volatile nitrogenous bases are extracted from a sample using a solution of 0,6 mol/l perchloric acid. After alkalinisation, the extract undergoes 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. The concentration is expressed in mg/100 g.

4. Chemicals

Unless otherwise indicated, reagent-grade chemicals shall be used. The water used shall be either distilled or demineralised and of at least the same purity.

5. The following instruments and accessories shall be used:

(a) a meat grinder to produce a sufficiently homogenous fish mince;

(b) high-speed blender with a speed of 8 000 to 45 000 revolutions/min;

(c) fluted filter, diameter 150 mm, quick-filtering;

(d) burette, 5 ml, graduated to 0,01 ml;

(e) 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 alkalising substances, the resulting free bases cannot escape.

6. Execution of the reference procedure

When working with perchloric acid, which is strongly corrosive, necessary caution and preventive measures shall be taken. The samples shall be prepared as soon as possible after their arrival, in accordance with the following instructions:

(a) Preparing the sample

The sample to be analysed is ground carefully using a meat grinder as described in point 5(a). An amount of 10 g ± 0,1 g of the ground sample is weighed out into a suitable container. This is mixed with 90,0 ml perchloric acid solution, homogenised for two minutes with a blender as described in point 5(b), and then filtered.

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

(b) Steam distillation

50,0 ml of the extract obtained in accordance with point (a) is put into an apparatus for steam distillation as described in point 5(e). For a later check on the extract's alkalinisation, several drops of phenolphtalein solution are added. After adding a few drops of silicone anti-foaming agent, 6,5 ml of sodium hydroxide solution is added to the extract and steam distillation begins immediately.

The steam distillation is regulated so that around 100 ml of distillate is produced in 10 minutes. The distillation outflow tube is submerged in a receiver with 100 ml boric acid solution, to which three to five drops of the indicator solution have been added. After exactly 10 minutes, 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 hydrochloric acid standard solution.

The pH of the end point must be 5,0 ± 0,1;

(c) Titration

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

(d) Blank

A blind test is carried out as described in point (b). Instead of the extract, 50,0 ml perchloric acid solution is used.

7. Calculation of TVB-N concentration

By titration of the receiver solution with hydrochloric acid standard solution, the TVB-N concentration is calculated using the following equation:

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

where:

V1 = volume of 0,01 mol hydrochloric acid standard solution in ml for sample;
V0 = volume of 0,01 mol hydrochloric acid standard solution in ml for blank;
M = mass of sample in g.

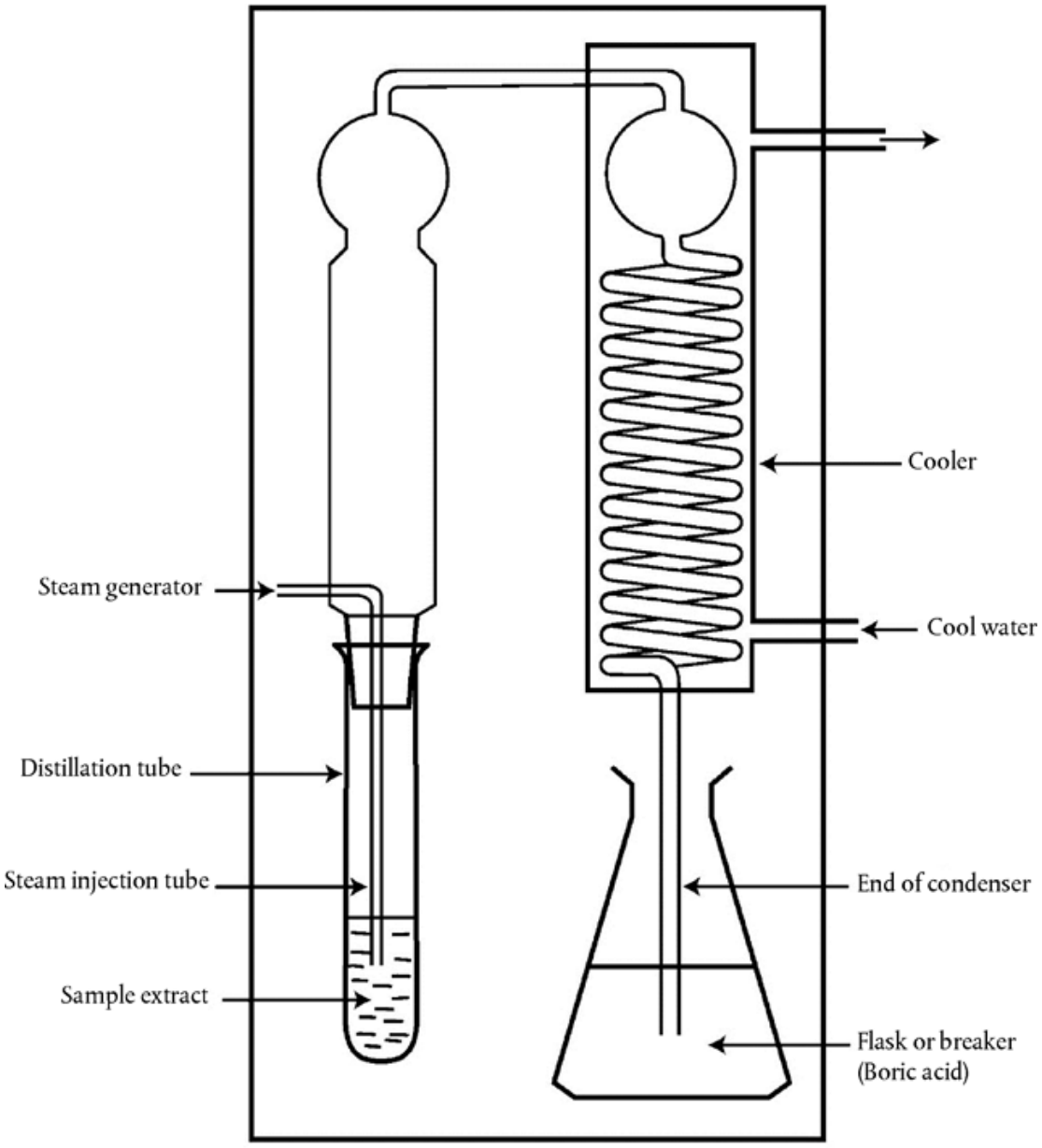
In addition, the following is required:

(a) duplicate analyses. The applied method is correct if the difference between duplicates is not greater than 2 mg/100 g;

(b) equipment check. The equipment is checked by distilling solutions of NH_4Cl equivalent to 50 mg TVB-N/100 g;

(c) standard deviations. The standard deviation for repeatability Sr = 1,20 mg/100 g and the standard deviation for reproducibility SR = 2,50 mg/100 g are calculated.

D. **TVB-N steam distillation apparatus**



Status:
Point in time view as at 31/01/2020.

Changes to legislation:
There are outstanding changes not yet made to Commission Implementing Regulation (EU) 2019/627. Any changes that have already been made to the legislation appear in the content and are referenced with annotations.