

**TVB-N IN FISH AND FISH PRODUCTS**

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**1. Principle**

When determining how fresh fish products are, the main emphasis is put on sensory criteria like e.g. looks, smell, taste or the texture of the filet. In addition however, also chemical parameters like the pH-value or the TVB-N content (total volatile base nitrogen content) as well as the bacterial count are determined.

In order to determine the TVB-N content, the volatile base nitrogen compounds - after making them alkaline - are expelled from a perchloric acid extract of the sample using water steam and received in an acid receiver. A titrimetric determination of the absorbed bases follows.

**2. Area of Usage**

Fish and fish products, crustaceans and mollusks - content of 5 to 100 mg per 100 g sample

**3. Method**

In accordance to BVL-Methode L10.00-3 Bestimmung des Gehaltes von flüchtigen stickstoffhaltigen Basen (TVB-N) in Fischen und Fischerzeugnissen – Referenzverfahren (reference method)

**4. Chemicals**

- 4.1. Perchloric acid solution  $c = 0.6 \text{ mol/l}$
- 4.2. Sodium hydroxide solution 200 g/l
- 4.3. Hydrochloric standard solution  $c = 0.1 \text{ mol/l}$
- 4.4. Boric acid solution 3 g/l
- 4.5. Silicone anti foam
- 4.6. Phenolphthalein solution 1 g/ 100 ml ethanol
- 4.7. Tashiro mixed indicator

**5. Instruments**

- 5.1. VAPODEST 10 to 50s
- 5.2. Moulinette or similar to make a homogenous paste
- 5.3. Mechanic mill like e.g. Ultratorax
- 5.4. Fluted filter (fast filtering)
- 5.5. Burette
- 5.6. Erlenmeyer flask with wide opening 300 ml

**6. Analysis****6.1. Sample Preparation**

A representative sample has to be crushed and homogenized e.g. in a Moulinette.

10 g (standard) of this sample are weighed into a recipient which can be used to work with the Ultratorax. With utmost care, 90 ml perchloric acid solution is added to this fish mash. Then, it has to be homogenized for about 1 to 2 minutes with the Ultratorax. This solution is then filtered using a fluted filter. The filter is then discarded; 50 ml of the extract are filled into a Kjeldahl flask e. g. type KTG 250 ml (cat. no. 12-0301). Should there be excessive foaming, it is advisable to use a Kjeldahl flask with wide neck opening, e. g. type KD 250, 500 or 750.

For later check of sufficient alkalization, a few drops of phenolphthalein indicator solution (4.6.) are added to the extract.

**6.2. Distillation and Programming of the VAPODEST**

The VAPODEST is started observing the instructions from the manual. At the beginning, an empty distillation is run in order to clean and to heat up the instrument. All chemicals must be available in sufficient quantities!

The Erlenmeyer flask containing 100 ml boric acid solution (4.4.) and a few drops of the Tashiro indicator (4.7.) is placed under the outlet tube of the distillate. Then, the sample flask is inserted into the VAPODEST.

Using the sodium lye pump, the sodium hydroxide solution (4.2.) is dosed into the sample flask. From this alkaline solution, the volatile bases are expelled and then determined titrimetrically.

The following table provides the program parameters for the various VAPODEST models.

The manual dosings have to be done with utmost care and having the potential dangers of the chemicals on



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mind.

	VAP10/s	VAP 20s	VAP 30s	VAP 45s	VAP 50s
H <sub>2</sub> O Addition	-	-	0 ml	0 ml	0 ml
NaOH Addition	~10 ml	~10 ml	~10 ml	~10 ml	~10 ml
Reaction Time	0 s	0 s	0 s	0 s	0 s
Distillation Time	600 s*	600 s*	600 s*	600 s*	600 s*
Steam Power	50 %	50 %	50 %	50 %	50 %
H <sub>3</sub> BO <sub>3</sub> Addition	70 - 80 ml <sup>1)</sup>	70 - 80 ml <sup>1)</sup>	70 - 80 ml <sup>1)</sup>	70 - 80 ml	70 - 80 ml
Suction Sample	<i>manually</i>	<i>manually</i>	0 s**	0 s**	0 s**
Suction Receiver	<i>manually</i>	<i>manually</i>	<i>manually</i>	30 s	30 s
Titration	<i>manually</i>	<i>manually</i>	<i>manually</i>	automatically <sup>2)</sup>	automatically
Calculation	<i>manually</i>	<i>manually</i>	<i>manually</i>	<i>manually</i>	automatically

\* The amount of distillate should be 100 ml

\*\* It is recommended not to suck the sample as there is a chance of blocking the suction tubings

<sup>1)</sup> All activities that have to be manually are written in italic print

<sup>2)</sup> external titrator

### 6.3. Determination of Blank Value

This is done identically to the previously described determination. However, instead of using the extract, 50 ml of perchloric acid (4.1.) are used.

### 7. Interpretation

The manual titration of the receiving solution is done with hydrochloric acid standard solution (4.3.) from green to grey (not necessary with automatic titration).

The TVB-N content in mg / 100 g sample is calculated by use of the following equation:

$$\text{TVB-N} = \frac{(V_1 - V_0) \cdot c \cdot F \cdot 2 \cdot 100 \cdot 14}{E}$$

where as:

V<sub>1</sub> - consumption of standard solution sample [ml]

V<sub>0</sub> - consumption of standard solution blank value [ml]

c - H<sup>+</sup> ion concentration of the standard solution [mol/l]

F - factor of the standard solution

E - initial sample weight [g]