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BUREAU OF INDIAN STANDARDS

DRAFT FOR COMMENTS ONLY

भारतीय मानक मसौदा

मांस और मांस उत्पाद — मांस में थिओबार्बिच्रिक अम्ल मान का निर्धारण — परीक्षण पद्धति

(आई एस 13401 का पहला पुनरीक्षण)

Draft Indian Standard

MEAT AND MEAT PRODUCTS- DETERMINATION OF THIOBARBITURIC ACID VALUE IN MEAT-TEST METHOD

(*First Revision of IS 13401*)

ICS 67.120.10

Slaughter House and Meat Industry Sectional Committee, FAD 18	Last date of comments: **16 April 2024**

FOREWORD

(*Formal clause will be added later*)

The long storage of meat and meat products may lead to considerable oxidative rancidity in the exposed fat. This oxidation may result in certain undesirable changes in the meat. The primary products of lipid oxidation are hydroperoxides and these are readily decomposed to produce various thiobarbituric acid reactive substances (TBARS) particularly carbonyl compounds. The 2-thiobarbituric acid reaction has been widely used as an objective measure of oxidative deterioration occurring in meat and meat products.

A specific micro method estimates TBA values in meat samples by direct spectrophotometry using reflux distillation to separate carbonyls. Incorporating the above-mentioned estimation method, this standard was originally published in the year 1992. In this first revision, scope of the standard has been modified for better representation and the standard has been brought out in the latest style and format of the Indian Standards.

For the purpose of deciding whether a particular requirement of this standard is complied with,

the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 2022 ‘Rules for rounding off numerical values (*second revision*)'. The number of significant places retained in the rounded-off value should be the same as that of the specified value in this standard.

1 SCOPE

This Indian Standard prescribes a test method for the determination of TBA value of meat and meat product.

2 PRINCIPLE

Animal tissue which has been incubated aerobically produces a colour with 2-thiobarbituric acid (TBA). This colour is due to the result of a complex formed from oxidation products of unsaturated fatty compounds and 2-thiobarbituric acid. The primary products of lipid oxidation are hydroperoxides and these are readily decomposed to produce various thiobarbituric acid reactive substances (TBARS) particularly carbonyl compounds. The 2-thiobarbituric acid reaction has been widely used as an objective measure of oxidative deterioration occurring in meat and meat products.

3 MATERIAL AND METHOD

3.1 Apparatus

3.1.1 *Distillation Assembly Unit*

3.1.2 *Blender*

3.1.3 *Water Bath*

3.1.4 Spectrophotometer

3.1.5 *Screw cap Test Tubes with Teflon Lined Caps* — 15 mm x 125 mm

3.1.6 *Magnetic Stirrer*

3.2 Reagents

3.2.1 *TBA Reagent*

Mix 1.44 g of 2-thiobarbiturie acid in 50 ml of distilled water in a 500 ml volumetric flask with vigorous stirring (magnetic stirrer). Add glacial acetic acid until the flask is two-third full. Vigorously stir the mixture for 10 minutes or until the 2-thiobarbituric is almost completely dissolved. Fill the flask to the mark with glacial acetic acid.

3.2.2 *TEP Standard Solution*

Accurately weigh 0.220 g Tetrethoxy propane (TEP) and transfer to a 100 ml volumetric flask. Dilute to volume with distilled water to produce a 1 x 10^5 M stock solution. Keep the solution under refrigeration. Prepare a 1 x10^5 M working solution by diluting 10 ml of the stock solution to 100 ml.

3.3 Preparation of Standard Curve

Accurately pipette aliquots of 0.4, 0.8, 1.2, 1.6 and 2.0 at working TEP standard solution into screw cap test tubes and add water to make a total volume of 5 ml. Add TBA reagent (5 ml) and tightly cap the tubes. The final concentration of TEP in the above 10 ml volumes correspond, to 0, 4.0, 8.0, 12.0, 16.0 and 20.0 x 10^{-7} mole per litre respectively. After thorough mixing, heat the test tubes in vigorously boiling water bath for 45 minutes and cool in tap water. Determine absorbance of the solutions at 538 nm within 30 min of cooling, setting the blank (0.0 ml TEP) to Zero.

The plot of TEP concentration (µm) against absorbance at 538 nm is linear up to 2.0 µm TEP under the conditions described here. The molar extinction coefficient of the colour developed (absorbance/ molarity) is 1.9 x 10^5 at 538 nm.

3.4 Procedure

Finely chop 100 g sample. Place 10 g sample in a small container and freeze immediately without thawing, transfer meat portion to the blender jar with 35 ml of distilled water and blend for 2 minutes or until the sample is finely divided. Transfer the sample homogenate to a tared 500 ml round bottom flask containing approximately 100 mg each of propylgallate and EDTA and add distilled water so that the total weight of the sample and water is 105 g. Flush the sample with nitrogen and add 95 ml of 4 M HCl. Keep the distillation rate at about one to two drops per second. The still, between samples, is rinsed with methanol and then with distilled water. Refrigerate TBARS distillate overnight, necessary.

3.5 Spectrophotometric Determination

Transfer 5 ml of each TBARS distillate and 5 ml of TBA reagent into screw-cap test lubes, cover tightly and treat as described in standard curve preparation. Simultaneously run a blank of 5 m of distilled water and 5 ml of TBA reagent. Sample solutions with absorbance greater than 0.5 shall be diluted with distilled water or alter natively the analysis shall be repeated using les TBARS distillate.

3.6 Calculation of TBARS Value

Express TBARS value as µmol malonaldehyde per kg meat. If a 5 ml aliquot of distillate obtained from 10 g meat, is used, then TBARS value may be calculated from the simplified formula:

$$c \times 10^7 = \text{TBARS}$$

where c (1.68 x 10^6) represents equivalent concentration in mol per litre of TEP determined from the standard curve.

For aliquot other than 5 ml the formula becomes,

$$\frac{5}{\text{aliquot size}} \times c \times 10^7 = \text{TBARS}$$