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DRAFT TANZANIA STANDARD

Methods for determination of organic preservatives in foodstuffs – Part 2: propionic acid and its salts

TANZANIA BUREAU OF STANDARDS

0. Foreword

For protecting food from microbial deterioration, a number of methods as application of heat or cold, dehydration, fermentation, irradiation or addition of certain chemicals are employed. Besides extending the periods of use of food a chemical preservative should be safe for human consumption, should not impart undesirable organoleptic changes, be economical in use and be capable of being analyzed. While the use of preservative to be safe under conditions of use is governed by law, it is considered necessary to prescribe methods for their analysis. The use of these methods would not only ensure repeatable and reproducible results for their correct interpretation, but would also facilitate inter-laboratory comparisons

There are two classes of preservatives, class I and class II. Class I preservatives include common salt, sugar, dextrose, glucose (syrup), wood smoke, spices, vinegar honey, etc. class II preservatives include inorganic substances such as sulphurous acid including salts thereof, nitrates of sodium or potassium and organic substances like benzoic acid including salts thereof, sorbic acids and including its sodium, potassium and calcium salts and sodium and calcium propionate

This standard, covering the determination of organic preservatives, is being issued in three parts. This part (Part 2) covers the determination of propionic acid and its salts in foodstuffs. The part: 1 covers benzoic acid and its salts and part: 3 covers sorbic acid and its salts. In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance to with TZS 4

In the preparation of this standards assistance was drawn from IS 12014 (Part 2):1986, Methods for determination of organic preservatives in foodstuffs – Part 2: propionic acid and its salts published by Bureau of Indian Standards

1. Scope

This standard prescribes the methods for determination of propionic acid and its salts used as preservatives in foodstuffs

2. Normative References

The following standard, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies

TZS 59: Water for analytical laboratory use – Specification and test method

TZS 4: Rounding off numerical values

TZS 672: Automotive gasoline (premium motor spirit)

3. Quality of reagents

3.1 analytical grade chemicals and distilled water conforming to TZS 59 shall be employed in tests

4. General

4.1 This standard specifies three methods for determination of propionic acids and its salts, namely, paper and column chromatographic methods, titration and spectrophotometric methods. Paper chromatographic methods shall be used for qualitative detection and column chromatographic methods shall be used for quantitative estimation of propionic acid and its salts.

4.2 Principle

Propionic acid is soluble in water and organic solvents (alcohol) and being non-volatile can remains as residues when the solution is constantly dried. In solution propionic acid can be determined by quantitative analysis using sodium hydroxide solution

5. Paper chromatographic method

5.1 **Reagents**

5.1.1 Mobile Solvent

take two parts of acetone, one part of tertiary butyl alcohol, one part of n-butyl alcohol and one part of liquid ammonia and mix them. This solvent should always be prepared fresh

5.1.2 Chromogenic reagent

add 200mg each of methyl red and bromothymol blue to a mixture of 100mL formalin and 400mL absolute alcohol. Adjust to pH 6.2 with 0.1N sodium hydroxide

5.1.3 Sodium Hydroxide - 1N

5.1.4 Phosphotungstic acid - 20 percent solution in distilled water

5.1.6 Crystalline Magnesium Sulphate - $MgSO_4.7H_2O$

5.1.6 Sulphuric Acid - 1N.

5.1.7 cresol red indicator

5.1.8 propionic acid Standard solution

pipette 1mL of propionic acid into a 100mL volumetric flask and dilute to volume with distilled water. Pipette 1mL of this stock into a 25mL beaker and neutralize the acid with 0.1N sodium hydroxide using cresol red indicator avoiding excess alkali. Evaporate to 0.5mL in a water-bath

5.1.9. Congo Red Indicator Paper

5.2 Apparatus

5.2.1 Chromatographic tank

5.2.2 Pipette - with 0.1mL graduations

5.2.3 Chromatographic paper - Whatman No. 1, 20 x 20cm, sheet

5.2.4 Steam Distillation Apparatus

5.2.6 Beakers - 25mL capacity

5.3 Procedure

5.3.1 Sample Preparation

5.3.1.1 all types of bread not containing fruit

take one or half loaf of bread and cut it in slices of 2-3mm thickness. Spread the slices on the paper and let them dry in a room temperature until sufficiently crisp and brittle to grind well. Grind entire sample to pass through 860-micron sieve, mix well and keep in an air-tight container before proceeding for analysis

5.3.1.2 Bread containing raisins and fruits

proceed as in 5.3.1.1 except comminute by passing twice through food chopper instead of grinding and dry the air-dried sample in an uncovered dish for 16 hours at 70ºC under pressure of less 0.07999bars

5.3.2 Distillation

weigh accurately 10g of air-dried bread and transfer it to a 160mL distillation flask. Add 40mL of distilled water and 10 percent of 1N suphuric acid, mix thoroughly and add 10mL of 20 percent phosphotungstic acid solution. Mix the contents well and add 40g of magnesium sulphate. Swirl the contents well and make the solution acidic to Congo red indicator paper with 60 percent sulphuric acid. Connect the condenser and steam generator and distil 200mL in 36-40 minutes.

Immediately neutralize the distillate using cresol red and 0.1N sodium hydroxide. Evaporate the solution to 0.6mL or evaporate to just dryness and then take up in 0.6mL distilled water.

5.3.3 Paper Chromatography

5.3.3.1 Take a Whatman No. 1 (see 5.2.3) unwashed chromatographic paper and rule the starting line 2.6cm from the bottom edge with a hard pencil. Spot two 1µL spots with 1µl pipette on the paper 2.6cm apart from each other, leaving at least 2.6cm margin, the first spot being of propionic acid standard solution and the second of unknown sample. Let the paper dry and clip it to a glass rod and suspend it in the chromatographic tank with 60mL of mobile solvent in a trough. Do not saturate the tank with mobile solvent before inserting paper. Seal the glass cover with cellophanes or other suitable tape and let it develop until solvents reaches 2.6cm from top of paper. Remove the paper from the tank and let it air dry.

5.3.3.2 Spray chromogenic reagent on front side of the paper. Spraying should be uniform and rather heavy but not to the extent that chromogenic reagents runs or drips. Faint yellow spots indicate presence of propionic acid

5.3.3.3 To intensify the acid spots, place the paper in the atmosphere of ammonia fumes momentarily (by placing 60mL ammonium hydroxide in a 2litre beaker and exposing to fumes by placing each end in beaker momentarily), entire paper immediately turns green.

5.3.3.4 Remove paper from ammonia fumes, propionic acid gradually appears as red spots and presence of propionic acid in the sample may be determined by comparing its Rf value with that of standard propionic acid. Since colour of the acid is not stable, mark the spot with the pencil as soon as they are completely developed.

6. Column chromatographic method

6.1 Reagents

6.1.1 Sodium Hydroxide - 0.1 and 1N

6.1.2 Phosphotungstic acid solution – 20 percent in distilled water

6.1.3 Crystalline Magnesium Sulphate - $MgSO_4.7H_2O$

6.1.4 Sulphuric Acid – 1N.

6.1.5 Formic Acid - 0.01N

6.1.6 Alphamine Red R Indicator

6.1.7 Ammonium Hydroxide - 1N

6.1.8 Silicic Acid - 100 Mesh

6.1.9 Chloroform

6.1.10 butyl alcohol

6.1.11 Sulphuric Acid - 50 percent

6.1.12 Absolute ethanol

6.1.13 anhydrous sodium sulphate.

6.1.14 Butanol in Chloroform - 1 percent.

Remove alcohol in chloroform by washing three time with water. Add 10mL of n-butyl alcohol to 1litre of washed chloroform in separating funnel, shake vigorously, add 25mL of distilled water and shake again.

Let it stand until the lower layer clears. Drain and discard the upper aqueous layer. Store it in contact with granular sodium sulphate.

6.1.15 Cresol Red Indicator

dissolve 50mL of O – Cresol – Sulphonphthalein in 20mL of absolute alcohol, add 1.3mL of 0.1N sodium hydroxide and dilute to 50mL with distilled water. Use 2 drops for each 25mL of aqueous solution.

6.1.16 Barium Hydroxide Standard Solution - 0.01N

6.1.17 Sodium Acetate -sodium chloride solution

Dissolve 12g of sodium chloride and 25g of sodium acetate in distilled water and dilute to 500mL

6.1.18 standard propionic acid solution

6.2 Apparatus

6.2.1 Chromatographic Tube – approximately 15 x 250mm.

6.2.2 Rubber Bulb

6.2.3 Micro-funnel - 2mL capacity

6.2.4 Steam Distillation Apparatus

6.3 Procedure

6.3.1 **Sample Preparation**

6.3.1.1 **All type of bread not containing fruit**

take one or half loaf of bread and cut it in slices of 2-3mm thickness. Spread the slices on the paper and let them dry in a room temperature until sufficiently crisp and brittle to grind well. Grind entire sample to pass through 860-micron sieve, mix well and keep in an air-tight container before proceeding for analysis

6.3.1.2 Bread containing raisins and fruits

proceed as in 5.3.1.1 except comminute by passing twice through food chopper instead of grinding and dry the air-dried sample in an uncovered dish for 16 hours at 70 ºC under pressure of less than 0.07999 bars

6.3.2 Distillation

weigh accurately 10g of air-dried sample and transfer it to 150mL distilling flask. Add 40mL distilled water and 10mL of 1N sulphuric acid, mix thoroughly and add 10mL of 20 percent of phosphotungstic acid solution. Mix the content well and add 40g magnesium sulphate. Swirl the contents well and make the solution acidic to the Congo red paper with 50 percent sulphuric acid. Connect the condenser and steam

generator and distill 200mL in 35-40 minutes. Transfer the distillate to 400-600mL beaker, add 10mL 0.01N formic acid, make alkaline to phenolphthalein with 1N sodium hydroxide and evaporate to 5mL. transfer it into 225mL glass stoppered test tube rinsing the beaker with three portions of distilled water. If insoluble matter adheres to the beaker, rinse with 1N sulfuric acid. Make this solution alkaline to phenolphthalein and evaporate to dryness just by inserting the test tube in a steam bath.

6.3.3 Chromatographic Separation

6.3.3.1 Preparation of partition column

take 5g silicic acid in glazed porcelain evaporating dish and add 1mL of alpha mine red R indicator and just enough 1N ammonium hydroxide to alkaline colour of the indicator (1 drop is enough). Add maximum amount of distilled water that the silicic acid will hold without becoming sticky or agglomerating in the butyl alcohol-chloroform solution (this amount shall be determined for each of silicic acid and usually varies from 50 to 75 percent of the weight of the silicic acid). Homogenize the mixture thoroughly in a pestle. Add 25mL of 1 percent butyl alcohol in chloroform and mix to form a slurry that pours readily. Pour this slurry into a chromatographic tube containing a small cotton plug in the neck of the constricted end. To avoid air pocket, tilt the tube slightly while pouring. If air bubbles form while pouring, eliminate by stirring suspension in tube with long glass rod. Clamp the tube vertically in ring stand. In the tube insert a one-hole rubber stopper fitted with a glass tube bent to 90° angle and hold in place by a Bunsen clamp against the pressure to be exerted. Connect the bent glass tube to a pressure source. Adjust the pressure to 5-10 psi (0.3447-0.6895 bars) so that excess solvent is forced through the column dropwise.

During removal of excess solvent, gel packs down. As the column packs down, particles of gel adhere to the wall of the tube, but eventually the gel leaves the wall of the tube relatively clean. This is the point of optimum density of the column, and the column is ready to use. Apply pressure until solvent reaches the surface of the column. If solvent passes below surface causing drying or ‘cracking of column’ or if air pockets are present extrude packing from the tube, re-slurry with the solvent and repack the column.

6.3.3.2 Preparation of standard propionic acid solution

Prepare stock solution of propionic acid by diluting 5mL of propionic acid to 250mL with distilled water. Pipette 1mL of stock solution into a 125mL Erlenmeyer flask and titrate with 0.01N sodium hydroxide, using cresol red as indicator, to pink colour which persists for 45 seconds.mg acid/mL standard solution = titre value (mL) x normality of sodium hydroxide used x Factor (F)

F = 7.40 for propionic acid

6.3.3.3 Preparation of known samples – pipette out 50mL of standard solution into a 50mL beaker and just neutralize with 0.01N sodium hydroxide solution, using phenolphthalein and add 10 drops in excess. Evaporate it to dryness on a steam bath.

6.3.3.4 Column separation - to the dry residue add 2mL of 10 percent butyl alcohol in chloroform solution and while stirring with glass rod add 50 percent sulphuric acid dropwise until the sodium salt are converted to free acids (acid to congo red paper) and add 1g of anhydrous sodium sulphate. Place a 50mL graduated cylinder under the column as receiver. Decant the supernatant onto column, pouring it slowly down the side of the tube without disturbing level surface of the column. Apply pressure until

solvent reaches surface of the gel. Wash the beaker with 1mL solvent and pour into column. Apply pressure until the solvent just disappears into sodium sulphate layer. Fill the tube with the solvent and apply the pressure. Once the band reaches the point 2-5mm above the narrowest portion of constriction of tube, record the volume and remove the receiver.

Transfer the elute to a 125mL Erlenmeyer flask, rinsing the cylinder with three 5mL portion of distilled water. Add one drop of cresol red indicator and titrate with 0.1N barium hydroxide solution. As its end point approaches, stopper flask and shake vigorously to completely extract acid from solvent phase. Correct titration for blank as follows. Collect 25mL of butyl alcohol- chloroform mixture from column before the acid is transferred, add 15mL boiled and cooled distilled water and titrate as above with 0.01N barium hydroxide solution.

6.4 Calculation

Calculate the results for propionic acid as mg/100g sample

Propionic acid, mg/100g = 7.4mL x 0.01N barium hydroxide solution

Calcium propionate = propionic acid contents x 1.256

6.5 **Identification of propionic acid.**

Acid separated in butyl alcohol-chloroform solution may be further identified by paper chromatography as described in 5.3.3