The Neotia University

Laboratory Manual

Food Biotechnology

Course Code:

Semester: 8th Semester, 4th Year

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Index

Practical I: Estimation of ascorbic acid (vitamin C) from given food sample by titrimetric method

Aim: To estimate the ascorbic acid (vitamin C) from given food sample by titrimetric method

Principle: This method determines the vitamin C concentration in a solution by a redox titration using iodine. Vitamin C, more properly called ascorbic acid, is an essential antioxidant needed by the human body (see additional notes). As the iodine is added during the titration, the ascorbic acid is oxidised to dehydroascorbic acid, while the iodine is reduced to iodide ions. ascorbic acid + I2 → 2 I−+ dehydroascorbic acid Due to this reaction, the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidised, the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex. This is the endpoint of the titration. The method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables. NB: This method is more straight forward than the alternative method using potassium iodate, but as the potassium iodate solution is more stable than the iodine as a primary standard, the alternative method is more reliable.

Material Required:

- burette and stand
- 100 mL or 200 mL volumetric flask
- 20 mL pipette
- 10 mL and 100 mL measuring cylinders
- 250 mL conical flasks
- Solutions Needed Iodine solution: (0.005 mol L−1). Weigh 2 g of potassium iodide into a 100 mL beaker. Weigh 1.3 g of iodine and add it into the same beaker. Add a few mL of distilled water and swirl for a few minutes until iodine is dissolved. Transfer iodine solution to a 1 L volumetric flask, making sure to rinse all traces of solution into the volumetric flask using distilled water. Make the solution up to the 1 L mark with distilled water.
- Starch indicator solution: (0.5%). Weigh 0.25 g of soluble starch and add it to 50 mL of near boiling water in a 100 mL conical flask. Stir to dissolve and cool before using

Procedure:

Sample Preparation

For vitamin C tablets: Dissolve a single tablet in 200 mL of distilled water (in a volumetric flask if possible).

For fresh fruit juice: Strain the juice through cheesecloth to remove seeds and pulp which may block pipettes.

For packaged fruit juice: This may also need to be strained through cheesecloth if it contains a lot of pulp or seeds.

For fruits and vegetables: Cut a 100 g sample into small pieces and grind in a mortar and pestle. Add 10 mL portions of distilled water several times while grinding the sample, each time decanting off the liquid extract into a 100 mL volumetric flask. Finally, strain the ground fruit/vegetable pulp through cheesecloth, rinsing the pulp with a few 10 mL portions of water and collecting all filtrate and washings in the volumetric flask. Make the extracted solution up to 100 mL with distilled water. Alternatively the 100 g sample of fruit or vegetable may be blended in a food processor together with about 50 mL of distilled water. After blending, strain the pulp through cheesecloth, washing it with a few 10mL portions of distilled water, and make the extracted solution up to 100 mL in a volumetric flask.

Titration

1. Pipette a 20 mL aliquot of the sample solution into a 250 mL conical flask and add about 150 mL of distilled water and 1 mL of starch indicator solution.

2. Titrate the sample with 0.005 mol L^{-1} iodine solution. The endpoint of the titration is identified as the first permanent trace of a dark blue-black colour due to the starch-iodine complex.

3. Repeat the titration with further aliquots of sample solution until you obtain concordant results (titres agreeing within 0.1 mL).

Calculations

1. Calculate the average volume of iodine solution used from your concordant titres.

2. Calculate the moles of iodine reacting.

3. Using the equation of the titration (below) determine

the number of moles of ascorbic acid reacting.

ascorbic acid + I_2 $\rightarrow$ 2 I^- + dehydroascorbic acid

4. Calculate the concentration in mol L^{-1} of ascorbic acid in the solution obtained from fruit/vegetable/ juice. Also, calculate the concentration, in mg/100mL

or mg/100g of ascorbic acid, in the sample of fruit/ vegetable/juice.

Result:

Discussion:

Conclusion:

Practical II: Determination of microbial count in milk by standard plate count (SPC) method

Aim: Determination of microbial count in milk by standard plate count (SPC) method

Principle: The standard plate count (SPC) is suitable for estimating bacterial populations in most types of dairy products, and it is a reference method specified in the Grade A Pasteurized Milk Ordinance to be used to examine raw and pasteurized milk. This procedure is also recommended for application in detecting sources of contamination by testing line-samples taken at successive stages in the processing.

The test employs aserial dilution technique for easy quantification of the micro-organisms. The appropriate dilutions of the milk sample are mixed with a sterile nutrient medium that can support the growth of the micro-organisms, when incubated at a suitable temperature. Each bacterial colony that develops on the plate is presumed to have grown from one bacterium or clump of bacteria in the inoculums. The total number of colonies counted on the plates multiplied by the dilution factor to represent the number of viable micro-organisms present in the sample tested.

Materials Required:

- Nutrient agar/ Tryptone dextrose agar
- Dilution water: Use only phosphate water for dilution
- Dilution water blanks: Fill dilution bottles with phosphate water so that each bottle will contain 99 or 9 ml (test tube) andautoclave at 121 °C for 15 to 30 min

Procedure:

Samples

Process the samples as soon as possible, but if necessary store the samples at 0 to 4.4C until tested. Samples must be tested within 36 hours after the initial collection, and the time of plating must be recorded. Fluid milk samples that have been frozen should not be tested microbiologically, because freezing causes a significant change in the viable bacterial count in milk and hence, may give enormous results.

18.3.5 Sample preparation

Mark each plate with sample number, dilution, and other desired information before making dilutions.

Before opening a sample container, remove from the closure all obvious materials that may contaminate the sample. If desired, wipe the tops of unopened sample containers with a sterile cloth or paper towel saturated with 70% ethyl alcohol. Invert filled retail containers that contain air space 25 times, or until the contents are homogeneous. To ensure a homogeneous sample, where no air space is present, aseptically open the container and pour the product from the filled carton into a sterile container. The interval between mixing and removing the test aliquot must not exceed 3 min. Immediately before transferring the test portions of milk and of dilutions thereof, shake the container, making around 20 complete up-and-down (or back-and-forth) movements of about 30 cm in 7 s. Mechanical shaker may be used, if desired, to shake the dilution blanks uniformly for 15 s.

18.3.6 Dilution of samples

For SPCs, select dilution(s) so that the total number of colonies on a plate is between 30 and 300. For example, where an SPC is expected to reach a number 5000, prepare plates containing 10^{-2} dilutions.

Use a sterile pipette for initial and subsequent transfers from the same container, if the pipette is not contaminated. If the pipette becomes contaminated before transfers are completed, replace it with another sterile pipette. Do not flame to decontaminate. Use a separate sterile pipette for transfers from each different dilution.

18.3.7 Precautions to be taken

Do not prepare or dispense dilutions or pour plates in direct sunlight. When removing a sterile pipette from the pipette container, do not drag the pipette tip over the exposed exteriors of the pipettes remaining in case because the exposed ends of such pipettes are subject to contamination. Do not wipe or drag the pipetteacross the lips and necks of vials or dilution bottles. Do not insert the pipette more than 2.5 cm below the surface of the sample or dilution. Draw test portions above the pipette graduation; then raise the pipette tip above the liquid level and adjust to the desired mark by allowing the lower side of the pipette tip to touch the inside of the container so that drainage is complete and excess liquid does not adhere when the pipette is removed from the sample or dilution bottle. Do not flame sterile pipettes.

When delivering a diluted sample of a dairy product, hold the pipette at an angle of about 45 while the tip is touching the inside bottom of a Petri dish or the inside neck of a dilution bottle. Lift the cover of the Petri dish just high enough to insert the pipette. Deposit the sample away from the center of the dish to aid in mixing the sample with medium. Allow 2 to 4 s for the diluted milk or cream to drain from the graduation mark to the rest point in the pipette tip; then, holding the pipette in a vertical position, touch its tip once against a dry spot on Petri dish or on inside of the dilution bottle neck. Do not blowout. When 0.1 ml quantities are measured, hold the pipette as directed and let the diluted sample drain to the proper graduation point but do not retouch the pipette to the plate. After depositing the test portions in each series of plates, pour the medium.

18.3.8 Plating

Melt the required amount of medium quickly in boiling water, in a microwave oven, or by exposing it to flowing steam in a partially closed container, but avoid prolonged exposure to unnecessarily high temperatures during and after melting. Discard melted nutrient agar or tryptone dextrose agar that develops a precipitate. Do not melt more medium than will be used in 3 hours. Do not re-sterilize the medium.Cool the melted medium promptly to approximately 45C and hold it in a water bath between 44 and 46C. Place the bulb of a thermometer in the medium or an aqueous solution of 1.5% agar in a separate container identical to that used for the medium; this temperaturecontrolled solution must have been exposed to the same heating and cooling as the medium. Do not depend on the sense of touch to indicate the proper temperature of the medium when pouring agar.

Select the number of samples to be plated in anyone series so that not more than 20 minelapse between diluting the first sample and pouring the last plate in the Petri dish.

Wipe water from the outside of the medium bottles before pouring. Introduce 12 to 15 ml of liquefied medium at 44 to 46C into each plate by gently lifting the cover of the Petri dish high enough to pour the medium. Carefully avoid spilling the medium on the outside of the container or on the inside of the plate lid when pouring. As each plate is poured, thoroughly mix the medium with the test portions in the Petri dish by rotating the dish first in one direction and then in the opposite direction, rotating and tilting the dish by hand or using mechanical rotators. Take care not to splash the mixture over the edge. Having thus spread the mixture evenly over the bottom of the plate, allow it to solidify on a plain surface. After solidification, invert the

plates to prevent spreading colonies from developing because of accumulated moisture, and place the plates in the incubator.

18.3.9 Sterility controls of medium, dilutions, and lab wares

Check sterility of dilution water, medium, pipette, and Petri dishes by pouring control plates for each sterilization lot.

18.3.10 Incubating

Incubate plates at 321C or 37C for 48 3 h for SPC. Plates must reach the temperature of incubation within 2 h. Avoid excessive humidity in incubator to reduce the tendency toward spreader formation, but prevent excessive drying of the medium by controlling ventilation and air circulation. Agar in plates should not lose more than 15% of its weight during 48 h of incubation.

18.3.11 Counting of colonies on agar plates

Count the plates after the desired incubation period. Record the dilutions used and number of colonies counted on each plate. If it is impossible to count at once, after the required incubation store the plates at 0 to 4.4C for not more than 24 h. For each lot of samples, record the results of sterility tests on materials used when pouring plates and the incubation temperature used.

Count colonies with the aid of magnification under uniform and properly controlled artificial illumination. Plates should be examined in subdued light. Routinely use a colony counter equipped with a guide plate ruled in square centimeters. Avoid mistaking particles of un-dissolved medium or sample, or precipitated matter in plates, for pinpoint colonies. Examine doubtful objects carefully, using higher magnification where required, to distinguish colonies from foreign matter.

a. Count all colonies, including those of pinpoint size, on selected plate. Record the dilution used and the total number of colonies counted.

b. Do not record counts on crowded plates from the highest dilution as too numerous to count. If the number of colonies per plate exceeds 300, count colonies in those portions of the plate that are representa tive of colony distribution, and calculate an estimated SPC from these counts. If there are fewer than 10 colonies per square centimeter, count colonies in 12 squares; select, if representative, 6 consecutive squares horizontally across the plate and 6 consecutive squares at right angles, being careful not to count a square more than once. When there are more than 10 colonies per square centimeter,

count colonies in four such representative portions. In both instances, multiply the average number of colonies found per square centimeter by the area of the plate used to determine the estimated number of colonies per plate. Each laboratory must determine the area in square centimeters of the plates in use.

c. Spreading colonies are usually of three distinct types. The first type is a chain of colonies, not too distinctly separated, that appears to be caused by the disintegration of a bacterial clump. The second type develops in film of water between the agar and bottom of the dish. The third type forms in the film of water at the edge or on the surface of agar.

Automated colony counters, when determined in individual laboratories to yield counts that 90% of the time are within 10% of those obtained manually may be used for counting plates. When using colony-counters take the following precautions:

a. Align the Petri dish carefully on colony-counter stage.
b. Avoid counting the stacking ribs or legs of plastic Petri dishes.
c. Do not count plates having unsmooth agar surfaces.
d. Avoid plates having food particles or air bubbles in the agar.
e. Do not count plates having spreaders.
f. Avoid scratched plates.
g. Wipe fingerprints and films off the Petri dish bottom before counting.

Result:

Discussion:

Conclusion:

Practical III: Determination of lactose content in milk by LaneEynon method

Aim: Determination of lactose content in milk by LaneEynon method

Principle: There are many methods, both chemical and physical, for determining lactose in milk, but they do not always give concordant results Gas-liquid chromatography (g.l.c.) of trimethylsilyl ethers (TMS) has recently been shown to be a convenient, sensitive, and specific technique for the analysis of lactose. Generally speaking, equilibrium is established between the anomers of a reducing sugar in aqueous solution. The anomers give rise to separate TMS derivatives so that more than one chromatographic peak is obtained from each reducing sugar. Analyses based on the TMS-lactose method, therefore, have 2 main disadvantages: it is necessary to sum the area of 2 peaks, corresponding to a- and /^-lactose, and the possibility of peaks overlapping increases when the determination is carried out in the presence of other sugars. Reducing the number of peaks may be achieved by converting sugars to their alditols or oximes before forming the TMS. Although the former method was used to determine lactose in human colostrum, we were unable to obtain accurate results from cow's milk with the method. In this paper, the successful use of the lactose oximeTMS procedure for determining lactose in milk by g.l.c. and a comparison of this procedure with the Lane & Eynon method are described.

Materials Required:

The sugars used as standards. It was confirmed from moisture determinations that the lactose and trehalose were the monohydrate and dihydrate respectively. A dual flame-ionization detector was used, the injection port being heated to 280 °C. The carrier gas was N2 at a flow-rate of 40 ml/min. A stainless steel column (2m x 3 mm i.d.) was packed with 1-5% SE-52 on Chromosorb W(AW-DMCS, 60-80 mesh) and held at 215 CC

Procedure:

Approximately 5 g milk was weighed accurately into a 100 ml volumetric flask and diluted to volume with water. A 0-6 ml portion of the diluted sample was transferred with 0-6 ml of 0-5 % trehalose solution to a small glass vial equipped with a ground glass stopper and evaporated to dryness below 50 °C in vacuo. To this residue, 1 ml of 2-5% hydroxylamine hydrochloride in pyridine was added, the mixture heated at 70-80 °C for 30 min, and then evaporated to

dryness below 50 °C. Removal of the last trace of water as an azeotrope by adding a drop of benzene to the dried oximated sample was not absolutely essential. The residue was dissolved in 0-25 ml dry pyridine (stored on KOH pellets) and 0-225 ml hexamethyldisilazane added, followed by 0-025 ml trifluoroacetic acid. The mixture was shaken vigorously for 30 s. This solution was allowed to stand for 30 min, then centrifuged for 2 min at 3000 rev/min. A 0-5 fil aliquot of the clear supernatant was injected into the gas chromatograph within 24 h of the trimethylsilylation. Following this oxime-TMS treatment, a single peak for lactose was obtained instead of the double peaks of the a- and /ff-anomers. The area of a peak was calculated from the product of peak height and width at half height. As a preliminary test of the method 0-5 % lactose solutions were made up and subjected to the treatment described above. The method of Lane & Eynon (1923) was used for comparison. The lactose content obtained was given as a percentage of lactose monohydrate in the sample.

Result:

Discussion:

Conclusion:

Practical IV: Methylene blue reduction test for assessing Raw Milk

Aim: Methylene blue reduction test for assessing Raw Milk

Principle: Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk. This test is based on the fact that the blue colour of the dye solution added to the milk get decolourized when the oxygen present in the milk get exhausted due to microbial activity. The sooner the decolourization, more inferior is the bacteriological quality of milk assumed to be. This test is widely used at the dairy reception dock, processing units and milk chilling centres where it is followed as acceptance/rejection criteria for the raw and processed milk

Grading of raw milk based on MBRT:

MBRT test may be utilized for grading of milk which may be useful for the milk processor to take a decision on further processing of milk. As per BIS 1479 (Part 3): 1977 criterion for grading of raw milk based on MBRT is as below:

5 hrs and above	Very good
3 to 4 hrs	Good
1 to 2 hrs	Fair
Less than ½ hrs	Poor

Materials Required:

- Pasteurized milk
- Non-pasteurized milk
- Test tubes
- Micro pipette
- Methylene blue
- Test tube rack

Procedure:

- The test has to be done under sterile conditions. Take 10 ml milk sample in sterile MBRT test tube.
- Add 1 ml MBRT dye solution (dye concentration 0.005%).
- Stopper the tubes with sterilized rubber stopper and carefully place them in a test tube stand dipped in a serological water bath maintained at 37±1ºC.
- Record this time as the beginning of the incubation period. Decolorization is considered complete when only a faint blue ring (about 5mm) persists at the top.

Result:

Discussion:

Conclusion: