INTERNAL COOKING TEMPERATURE

Contents

DETERMINATIVE METHOD

A. INTRODUCTION

1. Theory

The enzyme (protein) acid phosphatase is denatured by heat. The activity of the phosphatase left after cooking is expressed as the amount of phenol formed when the sample is allowed to act upon the substrate disodium phenylphosphate for a constant time, at a constant temperature, and a fixed pH. The phenol produced is reacted with 2,6,-dibromoquinone chlorimide, to yield indophenol blue. The absorbance of the blue color formed is measured spectrophotometrically at 610 nm.

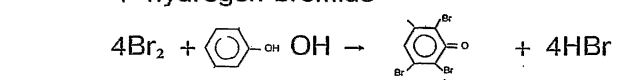
This method involves incubation of weighed samples with sodium phenyl phosphate in a constant temperature bath. Active phosphatase cleans this into phenol and sodium phosphate.

The mechanism for the standardization of the stock phenol solution is shown by the following equations:

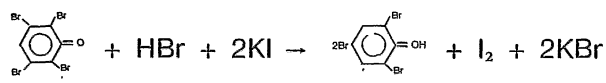
Potassium bromate + potassium bromide + H_2SO_4 → bromine + potassium chloride + water

$$KBrO_3 + 5KBr + 6H_2SO_4 \rightarrow 3Br_2 + 6KHSO_4 + 3H_2O$$

Phenol + bromine → tetrabromophenol hypobromite + hydrogen bromide



Tetrabromophenol hypobromite + hydrogen bromide + potassium iodide → tribromophenol + potassium bromide + iodine



The iodine is titrated with thiosulfate and the amount of phenol is calculated.

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

Each mL 0.1N potassium bromate = 0.001569 g phenol.

2. Applicability

The regulations require that all processed pork products be cooked to a temperature high enough to kill trichinae. In addition, APHIS Veterinary Services requires an internal cooking temperature of 156° F on imported pork products, from certain countries, to kill the foot-and-mouth virus and other exotic viruses. The ICT1 and ICT2 methods are used to determine the maximum internal cooking temperature reached in the processing of a meat product.

The coagulation test (method ICT2) is suitable as a screening method for use on all meat products for temperatures below 150° F . Above 150° F, the method is not accurate and this phosphatase procedure should be used, but only on canned picnics and canned hams, received either in the can or in a hard frozen condition.

DETERMINATIVE METHOD

B. EQUIPMENT

1. Apparatus	NOTE: Do not use plastic labware. a. 50 mL glass-stoppered centrifuge tubes (Pyrex 8424 or equivalent). b. 15 mL test tubes. c. Constant temperature waterbath (37° C). d. 500 mL Erlenmeyer flasks. e. pH meter.
2. Instrumentation	a. Spectrophotometer—suitable for reading at 610 nm. b. Pipettes—various sizes. c. Stopwatch. d. Syringe—100 μL.

DETERMINATIVE METHOD

C. REAGENTS AND SOLUTIONS

Reagent and Solution List

a. Citrate buffer, pH 6.5 ± 0.1: Dissolve 41.64 g trisodium citrate and 1.765 g citric acid in distilled water and dilute to 3 L. Adjust to pH 6.5 with pH meter. Preserve with 3 mL toluene and store in refrigerator.

b. 50% trichloroacetic acid: Dissolve 500 g TCA in distilled water and dilute to 1 L.

c. 20% trichloroacetic acid: Dilute 200 mL 50% TCA to 500 mL with distilled water.

d. 5% trichloroacetic acid: Dilute 100 mL 50% TCA to 1 L with distilled water.

e. Sodium carbonate 0.5M: Dissolve 53 g anhydrous sodium carbonate in distilled water and dilute to 1 L.

f. 2,6-dibromoquinone chlorimide (make fresh daily): Dissolve 40 mg 2,6-dibromoquinone chlorimide in 10 mL absolute alcohol. (Store reagent itself in a brown bottle in a desiccator).

g. Disodium phenyl phosphate 0.01M: Dissolve 0.436 g disodium phenyl phosphate in distilled water and dilute to 200 mL. (Prepare immediately before use.)

h. Stock phenol solution: Dissolve 1.000 g phenol in distilled water and dilute to 1 L.

i. Working phenol solution: Transfer 5 mL of stock phenol solution (item h above) to 1 L volumetric flask. Add 100 mL 50% TCA and dilute to volume with distilled water. Shake well.

The following reagents are for standardization of stock phenol solution:

j. Starch indicator (prepared solutions can be purchased): Mix 1 g soluble starch with 5 mL water. Add to 95 mL boiling water. Mix, cool, filter, and add 0.01 g HgI_2.

k. Sodium thiosulfate 0.1N: Dissolve 25 g sodium thiosulfate pentahydrate and 0.2 g sodium carbonate and dilute to 1 L with freshly boiled water.

l. Potassium iodide 10%: Dissolve 5 g KI and dilute to 50 mL with distilled water.

m. Sulfuric acid 2N: Dilute 5.6 mL concentrated H_2SO_4 to 100 mL with distilled water.

n. Hydrochloric acid 2N: Dilute 17.8 mL concentrated HCl to 100 mL with distilled water.

o. Potassium bromide: Reagent grade.

p. Potassium bromate 0.1N: Dissolve 2.783 g potassium bromate in distilled water and dilute to 1 L.

DETERMINATIVE METHOD

D. STANDARDS

1. Preparation of Standards	a. Pipet duplicate aliquots of 0.0 mL, 0.5 mL, 1.0 mL and 2.0 mL of working phenol solution into 15 mL test tubes (8 tubes total). b. Pipet 5.0 mL, 4.5 mL, 4.0, and 3.0 mL respectively, of 5% TCA, making each tube equal in volume (5 mL). c. Add 5.0 mL 0.5M sodium carbonate. d. Pipet 0.1 mL 2,6-dibromoquinone chlorimide into each tube. e. Swirl and develop color for at least 30 min in the dark. f. Measure the absorbance of each tube at 610 nm, using 1 cm cells and water as reference for setting spectrophotometer at 100% transmission.
2. Determination of Sodium Thiosulfate Factor	a. Add 2 g KI, 25 mL 0.1N potassium bromate and 20 mL 2N HCl in an 500 mL Erlenmeyer flask. b. Let stand 15 min in the dark. c. Slowly add 150 mL distilled water. d. With steady swirling or on magnetic stirrer, titrate with 0.1N sodium thiosulfate until the blue color disappears, using 1 mL of starch solution as indicator.
3. Determination of Phenol Concentration in Stock Phenol Solution	a. Pipet 50.0 mL stock phenol solution into glass-stoppered 500 mL Erlenmeyer flask. b. Pipet 50.0 mL 0.1N potassium bromate solution into the flask. c. Add 2.0 g potassium bromide. d. When the latter has dissolved, add 20 mL 2N H_2SO_4. Mix. e. Let stoppered solution sit for 15 min in the dark. f. Carefully pipet 10 mL 10% KI into mixture, removing stopper as little as possible. g. Shake well and let sit 15 min in the dark. h. Titrate with 0.1N sodium thiosulfate after rinsing stopper and sides of flask with distilled water, adding 1 mL starch indicator. End point is indicated by the absence of blue color from the gel particles. A bright light and white background may be necessary.

DETERMINATIVE METHOD

F. ANALYTICAL DETERMINATION

Determination

a. Weigh 2.50 g sample into each of four glass-stoppered 50 mL test tubes (A, B, C and D). Tubes A and B are to be used for duplicated determinations. Tubes C and D are duplicate control samples.

b. Pipet 10 mL citrate buffer into each tube.

c. Pipet 5 mL 20% TCA into control samples C and D only.

d. Stopper and shake well.

e. Place all tubes in a water bath at 37.0° C ± 0.5° C for 10 min before proceeding.

f. Pipet 5 mL disodium phenyl phosphate solution into each tube in turn at exactly 60-sec intervals using a stopwatch.

g. Shake all tubes at 10-min intervals.

h. After exactly 60 min, by stopwatch, pipet 5 mL 20% TCA to each tube in turn at 60 sec intervals *except tubes C and D.* (Tube A at 60 min, tube B at 61 min, etc.).

i. Remove each tube from water bath after addition of TCA, shake well and filter twice through Whatman 2V filter paper. (Filtrates may be stored at 4° C if needed.)

j. Pipet 3 mL clear filtrates into clean test tubes.

k. Pipet 3 mL sodium carbonate 0.5M, into each tube. Swirl to mix.

l. Add 100 µL 2,6-dibromoquinone chlorimide solution into each tube, using an 100 µL syringe. Mix well by swirling.

m. Develop color in the dark for at least 30 min (not overnight). If tube C or D is blue, contamination has occurred. Begin test again, using new 2.5 g samples and clean labware.

n. Read absorbance of each solution at 610 nm using 1 cm cells and water as reference for setting the spectrophotometer at 100% transmission. Spectrophotometer should be calibrated with a holmium oxide crystal.

DETERMINATIVE METHOD

G. CALCULATIONS

1. Procedure

a. A = 0.1N potassium bromate factor = $\frac{\text{g potassium bromate weighed}}{2.783}$

B = 0.1N sodium thiosulfate factor = $\frac{25.00 \times A}{\text{mL } C}$

C = mL thiosulfate titrated in section D.2, step d.

b. % Phenol = $\frac{[(50)(A) - (B)(D)]\ 0.1569}{50}$

D = mL thiosulfate titrated in section D.3, step h.

c. X = mg phenol/100 mL stock solution

d. Y = μmoles phenol/1000 mL working phenol solution = $\frac{(X)(5)(1)}{94.11}$

Molecular wt of phenol = 94.11

e. Extinction = $\frac{\text{(absorbance tube A + absorbance tube B)}}{2} - \frac{\text{(absorbance tube C + absorbance tube D)}}{2}$

Read absorbance to the nearest 0.001 absorbance unit.

After correcting for the blank (abs of the 0.0 mL standard substituted in the above formula in place of abs tubes C and D), the extinction values of the standard solutions are treated to determine the standard factor F.

f. F 0.5 standard = $\frac{0.5Y}{\text{extinction}}$ = μmol phenol/extinction unit

g. F 1.0 standard = $\frac{Y}{\text{extinction}}$ = μmol phenol/extinction unit

h. F 2.0 standard = $\frac{2Y}{\text{extinction}}$ = μmoles phenol/extinction unit

i. F = $\frac{\text{F 0.5 standard + F 1.0 standard + F 2.0 standard}}{3}$

DETERMINATIVE METHOD

G. CALCULATIONS (Continued)

To evaluate the phosphatase activity of the sample:

j. $EF' = \mu mol\ phenol/1000\ g\ sample = \frac{(F)(sample\ extinction)(1000)}{62.5}$

NOTE: 62.5 = the dilution factor used to convert g/mL to micro moles phenol, 1000 g sample.

$$\frac{2.5\ g}{20\ mL} \times \frac{3\ mL}{6\ mL} \times 1000$$

k. °C internal cooking temperature = 77.3985 − (5.7109)(Log EF')

l. $°F = \frac{9}{5}\,°C + 32$

The formula used to calculate the internal temperature was derived empirically. It may be necessary to redetermine the formula if processing procedures are changed.

NOTE: 1. Correction for products with salt content higher than 3.55%. Subtract 0.95° F for each 1% above 3.55%.

2. Repeat analysis if the following criteria are not met.

Abs of tubes A & B between	Acceptable abs difference between tubes A and B
0.35 to 0.50	0.035
0.20 to 0.34	0.028
0.10 to 0.19	0.020
Less than 0.10	0.016

2. Reference

Lind. J. Determination of Activity of Acid Phosphatase in Canned Hams, Danish Meat Products, Laboratory, The Royal Veterinary and Agriculture College, September 23, 1965.

DETERMINATIVE METHOD

H. HAZARD ANALYSIS

1. Method Title	Determination of Internal Cooking Temperature (Phosphatase).		
2. Required Protective Equipment	Safety glasses, plastic gloves, lab coat.		
3. Procedure Steps		*Hazards*	*Recommended Safe Procedures*
	Trichloroacetic acid	Eye, skin, and respiratory irritation.	Prepare and dispense in an efficient fume hood.
	2, 6-dibromoquinone chlorimide	Explosive at 120° C.	Store in refrigerator. Keep away from any heat source when using in lab.
	Phenol solutions	Highly toxic and suspected carcinogen. Rapidly absorbed through the skin.	Protective gear must be stressed. Work in cool, well-ventilated area.
4. Disposal Procedures	Colorimetric reaction solution	Mild irritant.	Flush into disposal sink with large quantities of water.
	Samples found to be undercooked	Spread of exotic animal diseases.	*Entire* sample must be autoclaved or incinerated.

DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN

1. Performance Standard

Procedure	*Analytical Range (°)*	*Repeatability % CV*	*Reproducibility % CV*
Maximum Internal Temperature	~145° F or ~62.83° C ~160° F or ~71.17° C	(±3° F)	(±5° F)

2. Critical Control Points and Specifications

Record	*Acceptable Control*
Labware	Do not use phenolic plastic labware.
Citrate buffer	6.5 ± 0.1 pH. Check with pH meter, calibrated at 6 or 7 pH, just prior to use.
Alcohol for 2,6-dibromoquinone chlorimide solution	Must be *absolute.*
Potassium bromate standardization	Standardize as in AOAC, 14 Ed., Sec 50.005, 50.006, 50.020, and 50.021.
Sample size	2.50 ± 0.02 g.
Water bath	37.0° C ± 0.5° C. Bring tubes to temperature before proceeding to next step.
Timed intervals	*Exactly* 60 sec, by stopwatch, for reagent additions from one tube to the next, and exactly 60 min, by stopwatch, for each tube in sequence, except C and D, for addition of TCA reagent.
Volume of BQC	Exactly 100 μL.
Color development	Must be in the dark for not less than 30 min. An hour is acceptable, but not overnight.
Spectrophotometer	Calibrated, capable of using 1 cm cells. Variable wavelength capability. Use distilled water as a reference for setting "0" absorbance.

DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN (Continued)

	Record	*Acceptable Control*
	Standards	Duplicate within ± 0.010 absorbance units.
	Sample tubes A & B	Duplicate within: 0.035 absorbance units for reading 0.35-0.50 0.028 absorbance units for reading 0.20-0.34 0.020 absorbance units for reading 0.10-0.19 0.016 absorbance units for reading < 0.10
	Calculations	Recheck.

3. Readiness To Perform

a. Familiarization.

i. Phase I: Standards—

(a) Prepare standards and measure absorbance.

(b) Determine sodium thiosulfate factor.

(c) Determine phenol concentration in stock phenol solution.

ii. Phase II: Fortified samples

iii. Phase III: Check samples for analyst accreditation.

b. Acceptability criteria.

See section J.1 above.

4. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: 1 per week not to exceed 20% of sample.

ii. Blind samples or random replicates chosen by supervisor after initial analysis.

iii. Records are to be maintained by analyst and reviewed by the supervisor and Laboratory QA Officer.

b. Acceptability criteria.

If unacceptable values are obtained, then:

i. Stop all official analyses for that analyst.

ii. Investigate and identify probable cause.

iii. Take corrective action.

iv. Repeat Phase III of section J.3 if cause was analyst-related.

DETERMINATIVE METHOD

J. QUALITY ASSURANCE PLAN

5. Sample Acceptablility and Stability

a. Matrix: Canned picnics and canned hams

b. Sample receipt size, minimum: 1 lb

c. Condition upon receipt: In can or hard frozen

d. Sample storage:

i. Time: One month

ii. Condition: Frozen