TBS/ AFDC 29 (2979) DTZS

DRAFT TANZANIA STANDARD

Determination of Cocoa shell in Cocoa and Cocoa products by spiral vessel Count

TANZANIA BUREAU OF STANDARDS

First Edition 2024

0. Foreword

This Tanzania standard prescribes determination of Cocoa Shell content in Cocoa mass or cocoa/chocolate Liquor and cocoa cake

In the preparation of this Tanzania standard assistance was derived from AOAC Official Method 968.10 (2023) Determination of Cocoa Shell and Germ % on fat free dry matter in Cocoa

In reporting the result 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 with TZS 4

1. Scope

This standard prescribes determination of Cocoa Shell content in Cocoa mass or cocoa/chocolate Liquor and cocoa cake

2. Principle

The method of counting the spiral vessels in a defatted, grinded and digested sample with the help of a microscope adjusted to mold counting examination and comparison with table

3. General Apparatus and Glassware

3.1. Sieve: - No. 230,5 inches (13cm) diameter, stainless steel.

3.2. Grinding equipment: - (1) Coarse grinding (cutting action) (2) Fine

grinding: - 13 cm (5 inches.) glass mortar and pestle or. electric mortar grinders

MG1 or MG2. Adjust MG2 so that pestle and shaft are not under tension by loosening top knob and lock nut by three turns and adjust closing spring control to ½ tension.

3.3. Aluminum Dish: - Diameter ca 77 mm, height ca 33 mm; with cover.

3.4. Brush: - No. 10, nylon, rubber set, oval sash paint brush with bristles cut to

4-4.5 Cm

4. Sample Preparation

4.1. (a) Chocolate liquor, chocolate: -

(1) Chill 200 g sweet or bitter chocolate until hard, and grate or shave to fine granular condition. Mix thoroughly and preserve in tightly stoppered bottle in cool place. Alternatively,

(2) Melt 200 g bitter, sweet, or milk chocolate by placing in suitable container and partly immersing container in bath ca 50°C. Stir frequently until test portion melts and reaches temperature of 45-50°C. Remove from bath, stir thoroughly, and while still liquid, remove test portion for analysis, using glass or metal tube, 4-10 mm diameter, provided with close- fitting plunger to expel test portion from tube, or disposable plastic syringe.

(b) Expeller cake: - Crush with mortar and pestle and grind to pass No. 30 sieve in mill, (b) (1), ca ½ teaspoonful at time. Mix well and store in tightly stoppered jar.

(c) Cocoa press cake: - Prepare and store as in (b) (Many test samples can be easily pulverized after drying 2-3 h at 60-70°C)

(d) Cocoa: - Use as is. Store as in (b).

4.2. Defatting and Grinding

4.2.1. Set up sieve in 15 cm (6 inches.) glass funnel with tip dipping 2 cm into 500 ml flat- bottom Pyrex centrifuge bottle.

4.2.2. Place 15 g cocoa, coarsely ground (30-40 mesh) cocoa press cake, or expeller cake, or 25-30 g chocolate or chocolate liquor in 250 ml centrifuge bottle. Add 100 ml ether, stopper, shake thoroughly to dissolve fat, and pour onto sieve. Wash material on sieve well with ether. Let material on sieve stand until dry.

4.2.3. Centrifuge mixture in 500 ml centrifuge bottle 10 min at 2000 rpm. Decant and discard supernate. Replace centrifuge bottle under funnel.

4.2.4. Place sieve with dried cocoa material in receiver (sieve bottom pan). Brush material through sieve with No. 10 sash paint brush. Transfer retains, using brush, to 12.7 cm glass mortar and grind 45 s with glass pestle, or grind 2 min in motor-driven mortar grinder. Transfer to sieve and rebrush.

4.2.5. Repeat grinding and brushing until virtually all material passes through sieve. Quantitatively transfer material, including small amount on sieve (< 20 mg), through funnel to the 500 ml centrifuge bottle.

4.2.6. Clean with brush, and clean brush against rim of sieve. Wash screen, receiver, mortar and pestle, and funnel (but not brush) with ether, letting washing run into centrifuge bottle.

4.2.7. Rub off coated material on funnel and other apparatus with policeman, rinsing with ether through funnel into centrifuge bottle. Stopper bottle and shake thoroughly.

4.2.8. Remove stopper and rinse with ether. Centrifuge 10 min at 2000 rpm. Decant and discard supernate. Add 100 ml ether and repeat extraction. Add 100 ml ether, Stopper, and shake. Immediately pour into fritted glass crucible (disk diameter 60 mm; medium porosity) under vacuum. Wash material from bottle

into crucible with ether. Wash twice with ca 35 ml ether and continue vacuum until dry (20 min).

4.2.9. Quantitatively transfer material from crucible to glass mortar and grind gently until fine. (Spoon may be used in transfer but use rubber policeman to scrape disc).

4.2.10. Quantitatively transfer ground material to Al dish. With cover in place, rotate dish until contents are well mixed. Dry on steam bath 10-15 min to remove traces of ether, and then in oven 1 h at 100 ^{o}C.

5. Method of analysis

5.1. Make duplicate determination. Accurately weigh 0.350 g extracted and dried material and transfer to 150 ml beaker. Gradually stir in 25 mL 4% NaOH solution (w/v) until smooth. Bring to initial boil, using electric hot plate.

5.2. Immediately reduce to low heat and boil gently 2 min with frequent stirring. Cool somewhat and transfer to 25 x 100 mm pyrex culture tube with small portions H2O.

5.3. Centrifuge until clear (3 min) at full speed of International clinical centrifuge, using No. 571curved rubber cushion in No. 320 shield, or equivalent. Decant carefully and discard supernate. Add H2O to tube until ¾ full, stopper, and shake until residue is well dispersed.

5.4. Centrifuge and decant as before. Add H2O to tube until ½ full, stopper, and shake until product is well dispersed. Transfer solution to 50 ml glass-stoppered graduate containing 25 ml glycerol.

5.5. Wash remaining material from tube to graduate with small portions H2O, stoppering and shaking tube to aid transfer. Dilute to 50 ml with H2O, and shake. Transfer to 100 ml beaker.

5.6. Stir well with vertical rotary motion. While stirring, withdraw small drop to Howard mold counting chamber, and make slide

5.7. Clean Howard cell and cover with cover glass so that Newton's rings are produced between slide and cover glass. Remove cover and with Knife blade or scalpel, place portion of well-mixed test sample on central disk; with same instrument, spread evenly over disk, and cover with glass so as to give uniform distribution. Discard any mount showing uneven distribution or absence of Newton's rings, or liquid that has been drawn across moat and between cover glass and shoulder.

5.8. With microscope adjusted for mold counting (field of view 1.382 mm at 100X), count fields positive for spiral vessel, at varying depths, at 200X in 25 fields of each of eight slides of each of the two determinations (total of 400 fields). Report as positive field one that contain any portion of section of spiral vessels, but none smaller than well developed "S" or "Z" either separate or attached to piece of shell. Average results and report as % positive field present. This is spiral vessel count.

6. Determination

% shell in chocolate component by comparison with standard curve prepared from spiral vessel count values listed in Table (A) plotted against % shell in chocolate component. Use column for counts listed under "≤ 15% shell

Table A: Standard spiral vessel count values

Spiral Vessel Count		
Shell in chocolate Component %	≤15% shell (0.350 g/ 50 ml)	≥15% shell (0.200 g/ 100 ml)
0	4.5	1.5
1	15	5.8
2	24.4	9.7
3	32.8	13.2
4	40	16.6
5	47	19.7
8	62.2	27.7
11	72.9	34.8
15	83.4	42.4
20	91.1	50.1
30	98.2	62.1
60		80.0
100		86.8

7. Calculation with units of expression

Spiral Vessel count Values

For 1-15 % shell (spiral vessel counts of 15-83.4), following formula gives values comparable to Table A

$$S = 538P-1777/ 7043-50P$$

Where

S= Shell in chocolate component and

P = spiral vessel count.

For test samples containing 15 % shell (spiral vessel count > 83.4) repeat determination throughout, but weigh 0.200 g test portion and dilute to 100 ml with H_2O in 100 ml glass stoppered graduate containing 50 ml glycerol. Count at 200X. Use column for counts listed under “>15% shell” for preparing standard curve.

Note

ca- approximately or around