Method 22.1

Microanalytical Analysis of Paprika

Purpose: To isolate extraneous material of insect, rodent, other animal and bird origin from ground paprika for microscopic detection and enumeration.

A. Apparatus:

1. Stereoscopic binocular microscope - wide field with following minimum specifications: 3 parfocal objectives - 1X, 3X and 6X or 7.5X; paired 10X wide field oculars, mounted on a base and capable of illumination by reflected light. Ordinarily 30X magnification is used for routine examination of filter papers. Confirmation of suspect material at higher magnification may be required.

2. Microscope illuminator - preferably with a transformer or rheostat to vary light intensity, a focusing adjustment to give uniformly lighted field of view, and blue-white color from a cool low-voltage source.

3. Wildman trap flask - consists of a 2L Erlenmeyer flask into which is inserted a close-fitting rubber stopper supported on a stiff metal rod, 3/16" diam., and about 4" longer than height of flask. Rod is threaded at lower end and furnished with nuts and washers to hold it in place on the stopper. Countersink lower nut and washer in the rubber stopper to prevent striking flask.

4. Filter paper:
   a) 32 cm folded rapid flow (S & S 588, or equivalent).
   b) 9 cm high wet strength, ruled, 5mm apart (S&S #8 or equivalent).

5. Hirsch funnel , porcelain, 56 mm plate diameter.

6. Büchner funnel, porcelain, 114 mm diameter.

7. Suction flask to provide suction by means of an H_2O aspirator or electric vacuum pump.

8. Sieve U.S. Standard No. 230, 8" or 12" diameter **(**plain-<u>not</u> twill weave)."

9. Magnetic stirrer - hot plate.

10. Teflon coated magnetic stirring bar, 1 3/4" - 2" x 3/8" (44.4 mm - 50.8 mm x 9.5 mm)**.**

11. Beakers, glass - 1 liter, funnels, glass or metal, 6" diameter or greater.

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B. Reagents

1. Isopropanol and 40% isopropanol in water.

2. Premix Tween 80-40% isopropanol-Tetrasodium EDTA: mix 250mL of (a) and 250mL of (b):
   a) Mix and filter 40mL Tween 80 and 210mL 40% isopropanol.
   b) Dissolve 5g Na_4 EDTA in 150mL H_2O add 100mL isopropanol, mix and filter. Mixed reagent is stable several weeks. Store in non-metal containers.

3. Mineral Oil - paraffin oil, white, light 125/135, saybolt viscosity (38°), specific gravity 0.84-0.86 (24°). Fisher Scientific Co. No. 0-119 or equivalent.

C. Preparation of Sample

1. The number of samples drawn should be six.

2. The sample size shall be 4 - 6 ounces (113 to 170 grams).

D. Procedure

1. Weigh 25.0g of paprika and place in a filter paper cup formed by fitting a 32cm filter paper around a 400mL beaker. Place cup with ground paprika in a 1L beaker.

2. Pour 400mL 99% isopropanol into the paper cup in the beaker. Place on a pre-heated hot plate, bring to a boil, then boil gently exactly 10 minutes (a "cold finger" should be used to condense vapors).

3. Remove cup from beaker without delay and place in a Büchner funnel and aspirate to slow drip. Discard liquid.

4. Replace cup in 1 liter beaker and repeat Step 2 and 3 twice using 400mL 99% isopropanol each time to remove oil and pigment.

5. Using a gentle stream of water, quantitatively transfer the sample to a prewashed No. 230 sieve. Avoid splashing and loss of sample.

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6. Wash the sample with a forceful stream of warm **(**55-70°C**)** water using a Fisher aerator until foam is gone and drainings are clear. Higher flow rates may cause breakage of fragments.

7. Add 400mL 40% isopropanol to wash bottle. Place 6" diameter funnel in trap flask. Wash sample to the edge of the sieve and quantitatively transfer to the trap flask with 40% isopropanol. Wash walls of flask and pour remainder of 400mL into flask.

8. Place on hot plate, bring to a boil, then boil gently 10 minutes, using gentle magnetic stirring to avoid splashing. Wash sides of flask every 2 minutes to prevent material from accumulating and drying on flask wall.

9. Remove from hot plate and immediately add 100mL premixed Tween 80-40% isopropanol - Na_4 EDTA solution down stirring rod.

10. Stir mag., gently, about 1 minute. Let stand 10 minutes.

11. Dilute to 800mL with 40% isopropanol added *slowly* down stirring rod, positioned with stopper just above liquid level.

12. Add 50mL mineral oil down stirring rod and stir mag. 3 minutes with stopper located above liquid level.

13. Add 40% isopropanol slowly down stirring rod to bring oil into neck of flask. Let stand about 10 minutes.

14. Raise stopper to middle of flask and swirl gently to hasten rising of oil droplets.

15. Rinse rod with 40% isopropanol and clamp so that stopper is at mid point of flask.

16. Add 40% isopropanol down rod to bring bottom of oil layer to level 1cm above raised stopper.

17. Let stand 10 minutes and swirl very gently again.

18. Let stand 10 minutes undisturbed and trap off into beaker, or onto ruled filter paper in Hirsch funnel.

19. Add 35mL mineral oil and hand stir 1 minute at speed to sufficiently keep oil moving through trap flask. Add about 20mL 40% isopropanol, stir gently at about 5 minute intervals for 20 - 25 minutes, then let stand undisturbed 5 - 10 minutes**.**

20. Trap off into second beaker or onto ruled filter paper in Hirsch funnel and rinse neck of flask with alcohol or undiluted isopropanol.

21. Filter onto ruled paper, rinsing beaker with isopropanol, and examine at 30X.

E. Calculation:

Report separately numbers of insect, rodent hair, animal hair and feather barbule fragments.

F. Statistics:

	Repeatability	Reproducibility
Rodent hairs	6.41%	8.59%
Elytra squares	5.89%	6.24%

G. Notes

1. Periodically check the 32cm filter paper used under microscope at 30X for completeness of transfer of fragments.

2. When 230 sieve draining slows, wash with detergent**,** then 50% sodium hydroxide (heated, if necessary).

3. Complete analysis without overnight interruption.

H. References

AOAC Official Methods of Analysis - 16.14.22 (977.25).
JAOAC 60 114 (1977).