

Microanalytical Analysis of Ground Capsicums (Excluding Paprika)

Purpose: To isolate extraneous material of insect, rodent, other animal and bird origin from ground capsicums, excluding 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) 32cm 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 H₂O aspirator or electric vacuum pump.
8. Sieve U.S. Standard No. 230, 8" or 12" diameter ("plain-not twill." weave).
9. Magnetic stirrer - hot plate.
10. Teflon covered stirring bars about 47mm x 9mm (egg-shaped, round or octagonal).
11. Beakers, glass - 1 liter, funnels, glass or metal, 6" diameter or greater.

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B. Reagents:

1. Isopropanol (IPA) - 99% and 40% by volume.
2. Ethanol 95% and 60% by volume.
3. Tween 80-ethanol-tetrasodium EDTA Premix: pour 420mL 60% ethanol in 1L graduate. Add 80mL Tween 80 to 250mL glass stoppered graduate. Invert 250mL graduate over 3L beaker and drain briefly. Rinse 250mL graduate with several portions of the 420mL 60% ethanol, pouring each into beaker. Add rest of 60% ethanol to beaker and start mag. stirring. Add 10g Tetrasodium EDTA to beaker while stirring rapidly. Add 500mL 60% ethanol and stir until uniform. Store in non-metal containers. Mixed reagent is stable several weeks.
4. 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).
5. Heptane - commercial heptane containing less than 8% toluene.
6. Flotation liquid - mineral oil and heptane (85+15).

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 g of capsicum and place in a filter paper cup formed by fitting a 32cm filter paper around a 400mL beaker. Place cup with ground capsicums 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 10 min.
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.

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5. Using a gentle stream of water, quantitatively transfer the sample to a prewashed No. 230 sieve. Avoid splashing and loss of sample.
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. (Note: Longer washing time than for paprika is needed.)
7. Add 600mL 60% ethanol 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 60% ethanol. Wash walls of flask and pour remainder of 600mL 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 cool to between 20 and 25° with cold water. Add 40mL flotation liquid down stirring rod.
10. Dilute to 800mL with 60% ethanol and stir mag. 5 minutes.
11. Set aside, add 100mL Tween 80-ethanol-tetrasodium EDTA premix, down stirring rod, and mix through liquid by gently swirling (to prevent foaming) stopper about 1 minute. Let stand 3 minutes. Wash sides of flask with 60% ethanol to keep solids down.
12. Slowly add 60% ethanol down trap rod, maintaining stopper above oil layer, until oil just reaches neck of flask.
13. Gently swirl stopper through lower portion of flask to suspend settling.
14. Add 60% ethanol down rod to bring bottom of oil layer to a level 1cm above raised stopper.
15. Clamp rod with stopper at mid point of flask. Let stand 15 minutes. Then gently swirl stopper through upper half of liquid to hasten rising of oil droplets.
16. Let stand 15 minutes undisturbed and trap into beaker, rinsing neck of flask with 60% ethanol. Filter onto ruled paper.
17. Add 30mL flotation liquid and stir manually 1 minute, with an up and down motion.

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18. Clamp rod at mid point and let stand 10 minutes. Swirl stopper gently through upper half of liquid and adjust oil level.
19. Let stand 15 minutes undisturbed and trap off.
20. Rinse neck of flask with 95% ethanol.
21. Filter onto ruled paper, rinsing beaker with 95% ethanol, and examine at 30X.

E. Calculation:

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

F. Statistics:

	<u>Repeatability</u>	<u>Reproducibility</u>
Rodent hairs	13.43%	14.66%
Elytra squares	8.10%	9.18%

G. Notes:

1. Periodically check the 32cm filter paper used under microscope 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.
4. Do not use any plastic equipment because fragments or hairs might adhere.

H. Reference:

AOAC Official Methods of Analysis 16.14.10 (978.22).
 JAOAC 61 900 (1978).