Macroanalytical Procedures Manual (MPM)

V-8. Spices, Condiments, Flavors, and Crude Drugs

F. Supplemental Method for Whole Bay Leaves

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F. Supplemental Method for Whole Bay Leaves

(1) Scope

This method supplements Section 8.A. by describing procedures specific to whole bay leaves (*Laurus nobilis* L.) (Baldwin 1984). There are numerous plants often referred to informally as 'bay leaves'. These include California bay leaf, or as it currently is called, California laurel (*Umbellularia californica* (Hook. & Arn.) Nutt.); Indian bark (*Cinnamomum tamala* (Buch.-Ham.) T. Nees & C. H. Eberm.); Indonesian bay leaf (*Syzygium polyanthum* (Wight) Walp.); bay rum tree (*Pimenta racemosa* (Mill.) J. W. Moore); Mexican bay leaf (*Litsea glaucescens* Kunth) (Raman 2017) (Figure V-8-F-1). All of which can be examined under this method.

(2) Applicable Documents:

 <u>CPG Sec 525.150 Bay (Laurel) Leaves - Adulteration by Insect Filth; Mold;</u> <u>Mammalian Excreta | FDA</u>

(3) Defects:

Bay leaves are attacked by various field and storage pests and mold. Some of the common field pest include scale insects and mites. One type of field fungus that attacks bay leaves is Coryneum blight, or shot hole disease, *Stigmina carpophila* (Lév.) M. B. Ellis, which creates small holes in the leaves and resembles insect feeding damage.

(4) Procedure: Determination of Contamination in Whole Bay Caused by Arthropods, Animal Excreta, and Extraneous Material

Initial sample size should consist of six (6) subsamples, each with a minimum of 225 g. Examine entire contents of container.

b. Visual Examination-- Examine the product in small amounts with good light and against a white paper or other suitable contrasting background. One of the following techniques or combination of techniques can be used, but are not limited to: Examine a small portion of the product at a time, by placing a portion in a pile on white paper. Using a spatula or similar tool, move a small amount of product in a thin layer across the paper. Use a moving belt or other mechanical device if all the material can be seen easily.

Sifting may facilitate separation and concentration of certain types of objectionable matter. If sifting is performed, size of screens used, and method of use should be stated in the report of results.

Examine material visible to the naked eye up to 10x. After the initial examination, higher magnification may be used to confirm findings as necessary. If the magnification exceeds 10x in the initial analysis, then it should be stated in the report of the results.

Examine for rodent/bird excreta, manure, arthropods, and arthropod debris, mold clumps, miscellaneous objectionable matter, and other evidence of contamination. Note: if sample was received in plastic bags, filth elements may adhere to the bagging through static electricity. Examine the bagging for any adhering filth elements.

c. Classification of Contaminants -- Separate contaminants into suitable groupings relative to defect action levels, regulatory guidelines, or other applicable requirements. Add categories to tabulation of results depending on type(s) of contaminants found.

(i) *Arthropods and their fragments* -- Count the number of whole arthropods and equivalent visible to the naked eye (corrected as necessary for abnormal vision) with such magnification as may be necessary. If the magnification exceeds 10X, this should be stated in the report of results. After the initial examination, higher magnification can be used to identify arthropods, using appropriate arthropod identification keys. Classify arthropods as "Field" or "Storage", making a special notation when they are found alive. Note the size of any unidentified arthropods and larvae found.

(ii) *Rodent (Rat or Mouse) Excreta* -- Rodent excreta pellets are normally black or dark colored, roughly cylindrical, blunt at one end and pointed at the other. They range in length from 1.5 to 15 mm. They usually contain rat or mouse hairs, partially digested plant material, and sometimes insect parts. When wetted with water, rodent pellets form a characteristic gray mucous coating. Weigh suspect pellets and report as such *only* if rat or mouse hairs are present. Confirm identification by removing a hair from the pellet and identifying it microscopically. When none are present, proceed with AOAC Official Method 981.22 Mammalian Feces-Alkaline Phosphatase Test or AOAC Official Method 988.17 Mammalian Feces-Thin-Layer Chromatographic Method for Coprostanol. Record the size and weight of the excreta pellets before wetting with water.

(iii) *Animal Dung* -- Animal dung consists of an amorphous, usually dark colored material pressed into a matrix. Incorporated plant material usually consists of ligneous, fibrous material which is either pale-yellow or green. Parts of insects and small amounts of inorganic, earthy material may also be present. Weigh suspect material and report as animal dung or excreta, *only* when matricized plant material predominates. Confirm as excreta, using AOAC Official Method 981.22 Mammalian Feces-Alkaline Phosphatase Test or AOAC Official Method 988.17 Mammalian Feces-Thin-Layer Chromatographic Method for Coprostanol.

(iv) *Bird Excreta* -- Bird excreta will appear as rounded droppings, sometimes coiled with a white residue. Measure and weigh droppings and test a portion of the white, amorphous particles for uric acid AOAC Official Method 962.20 Excrement (Bird) on Food and Containers-Microchemical Test for Uric Acid or AOAC Official Method 986.29 Excrement (Bird and Insect) on Food and Containers-Thin-Layer Chromatographic Method for Uric.

(v) *Extraneous Material* – Any foreign material in a product associated with objectionable conditions and practices in production, storage, or distribution. In addition

to substances (i) to (iv) above, this includes but not limited to sand, glass, rust, plastic, sticks, paint chips etc.

d. Report -- Tabulate results as follows, adding additional categories as necessary (Table V-8-F-1). To determine excreta mg/lb use the following formula: Weigh excreta pellets (mg) x 454 g / Weight of product (g) x 1 lb = Excreta mg/lb

Table V-8-F-1

	Subsample	Subsample	Subsample	ata
	INO. I	INO. Z	INO. 3	eic.
Amount Examined (g)				
Whole and W/E Other				
Arthropods (count) ^a				
Whole Mites (count) ^a				
Arthropod Fragments				
(count) ^a				
Rat/Mouse Excreta				
Pellets (mg)				
Mammalian Excreta				
Pellets (mg)				
Bird Excreta Pellets (mg)				
Total Excreta (mg/kg) ^b				
Extraneous Material (g) ^c				
Other ^d				
Remarks:				

Notes: Identify and if whole state alive/dead. Excludes insect excreta pellets. Describe ((3)d) Substitute appropriate heading(s)

(5) Procedure: Determination of Arthropod Damaged, Moldy, and Otherwise Reject Material in Whole Bay Leaves

a. Sample Preparation – Weigh out 50 g of product from each of six (6) subsamples of sieved material remaining after completing procedure Section 8.F(4)a-d. Alternatively, draw a 50 g analytical unit directly from each subsample. State how analytical units are taken. Record weights in (Table V-8-F-2).

b. Visual Examination – Examine each bay leaf in the analytical unit for reject material visible to the naked eye up to 10x assisted magnification. Higher magnification may be

used for confirmation of findings after the initial examination. If the magnification exceeds 10x for the initial examination, this should be stated in the report of the results.

c. Classification of Reject Material

(i) Arthropod Damage – Any product material exhibiting definite evidence of arthropod feeding or containing one or more whole arthropod(s) or equivalent, webbing material, or excreta. Determine, if possible, whether infestation is "Field" or "Storage", making special notations for live arthropods. Field feeding damage will produce necrotic tissue where the arthropod fed. This necrotic tissue is caused by the death of the plant cells from the arthropod feeding damage. It will appear as a discoloration next to the feeding damage.

(Figure V-8-F-2) provide a guide to classify rejects due to arthropod damaged bay leaves. Examples of rejects include holes 1-5 mm in diameter with discolored necrotic tissue, or aggregate diameters \geq 1.0 cm (include necrotic tissue when determining longest diameter), leaf miner tunneling \geq 1.0 cm long, edge feeding with deep scalloping or notching \geq 3.0 mm (do not reject leaf if feeding damage is isolated to the edge of the leaf), hole > 5.0 mm with discolored necrotic tissue (include necrotic tissue when determining longest diameter). Do not reject holes without discolored necrotic tissue or leaf breaks showing mechanical damage.

(ii) *Moldy* -- Any product material bearing mold on more than 1/4 of its surface area or any material where the aggregate moldy area is greater than 1 cm² (Figure V-8-F-3 and Figure V-8-F-4). Confirm presence of mold with magnification as necessary, but determine the area affected without magnification. Describe general appearance of the moldy areas. Mold can be confirmed on a microscope slide with the use of lactophenol cotton blue.

(iii) *Animal-Contaminated* -- Any product material showing animal excreta, animal chewing, or gnawing. AOAC Official Method 945.88 Urine Stains on Foods and Containers has a flow chart on testing urine stains on product and containers.

(iv) *Otherwise Reject Material* -- Any product material that is not classified as above, but is otherwise decomposed, discolored, abnormal in appearance or otherwise unfit for food. This also includes product with adhering hair and or feather material but is not limited to these adhering materials. Describe rejects in remarks and document with photos.

d. Report -- Tabulate results as in (Table V-8-F-2).

Table V-8-F-2

	Subsample No. 1	Subsample No. 2	Subsample No. 3	etc.
Amount Examined (weight (g))				
Arthropod Damaged (weight (g)) ^a				
Moldy (weight (g)) ^b				
Animal Contamination ^c				
Otherwise Rejected Material (weight (g)) ^d				
Total weight (g) of rejects				
% by weight (g) of rejects				
Remarks:				

Notes:

- a. Describe ((5)c.(i)); report under Remarksb. Describe ((5)c.(ii)); report under Remarks
- c. Describe ((5)c.(iii)); report under Remarks
- d. Describe ((5)c.(iv)); report under Remarks

FIGURES



Figure V-8-F-1. A. Bay leaf, *Laurus nobilis* L. **B.** Indian bark, *Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm. **C.** California laurel, *Umbellularia californica* (Hook. & Arn.) Nutt.. (Source: Photos courtesy of H. Loechelt-Yoshioka, FDA).

CLASSIFICATION OF INSECT DAMAGE IN BAY LEAVES



Figure V-8-F-2. Drawing of bay leaf, *Laurus nobilis* L., showing classification of insect damaged leaves. (Source: Drawing courtesy of M. Zimmerman, FDA).



Figure V-8-F-3. A. and B. Insect feeding damage with arrows pointing to necrotic tissue (scale bar: 5mm). C. and D. Arrows pointing to examples of leaf mining damage. (scale bar: 5mm). E. Insect feeding damage with webbing material indicated by arrow. (scale bar: 5mm). F. Arrows pointing to scale insects. (scale bar: 5mm). G. Bracket indicates moldy area greater than 1 cm². Mold should be confirmed microscopically. Also present on the leaf, indicated by arrow, is a circular hole from insect feeding damage. (scale bar: 2mm). H. Mechanical damage, indicated by arrow is acceptable. (scale bar: 5mm) (Source: Photos courtesy of H. Loechelt-Yoshioka, FDA).



Figure V-8-F-4. Damage to Indian bark (*Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm.). A. and B. Show insect feeding damage. Arrows point to feeding damage in B. C. Leaves showing damage caused by mold. Mold should be confirmed microscopically. Arrows indicate damage on just a few of the leaves. (Source: Photos courtesy of I. Ali, FDA).

Acknowledgements

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References Cited in Section:

Baldwin, R. A. (1984) *Reject Criteria for Insect Damaged Bay Leaves.* U.S. Food and Drug Administration Memorandum, 3 pp.

Official Method 962.20 Excrement (Bird) on Food and Containers Microchemical Test for Uric Acid. *Official Methods of Analysis of AOAC INTERNATIONAL,* Chapter 16.

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Official Method 981.22 Mammalian Feces Alkaline Phosphatase Test. Official Methods of Analysis of AOAC INTERNATIONAL, Chapter 16.

Official Method 986.29 Excrement (Bird and Insect) on Food and Containers Thin-Layer Chromatographic Method for Uric Acid. *Official Methods of Analysis of AOAC INTERNATIONAL*, Chapter 16.

Official Method 988.17 Mammalian Feces: Thin-Layer Chromatographic Method for Coprostanol. *Official Methods of Analysis of AOAC INTERNATIONAL,* Chapter 16. Raman, V., Bussmann, R. W., Khan, I. A. (2017) Which Bay Leaf is in Your Spice Rack? – A Quality Control Study. *Planta Med* 83: 1058-1067.

Additional Information:

Informational articles not cited in the above section, but still useful:

American Spice Trade Association (2014). *ASTA Cleanliness Specifications for Spices, Seeds, and Herbs (Foreign and Domestically Produced).* ASTA,

Parry, J. W., (1962) *Spices - Their Morphology, Histology, and Chemistry*, Chemical Publishing Co., Inc., New York, NY.226 pp.

Revision History

Version No.	Purpose of change	Date
V0	New process	2025