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Standard Practice Guide for Evaluating Effects of Contaminants on Odor and Taste of Exposed Fish Suspected of Contamination 1

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INTRODUCTION

The flavor quality of fish and shellfish (hereinafter collectively termed "fish") can be related to their exposure to compounds that might be present in the food chain and the water in which they live. High-quality fresh fish have a low-intensity aroma and flavor impact. Certain compounds might cause deterioration of, or change to, the flavor of the fish's flesh. Examples of sources of contaminants include wood or other processing effluent, odorants of detergents, microbial genesis, and accidents involving petroleum products, industrial sewage, farm runoff, and feedstuffs. Although many known contaminant compounds can be detected by instrumental means, the presence of many unknown volatile contaminants is first detected through odor and flavor evaluation.

1. Scope

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- 1.1 The flavor quality of fish and shellfish (hereinafter collectively termed "fish") can be related to their exposure to compounds that might be present in the food chain and the water in which they live. High-quality fresh fish have a low-intensity aroma and flavor impact. Certain compounds might cause deterioration of, or change to, the flavor of the fish's flesh. Examples of sources of contaminants include wood or other processing effluent, odorants of detergents, microbial genesis, accidents involving petroleum products, industrial sewage, farm runoff, and feedstuffs. Although many known contaminant compounds can be detected by instrumental means, the presence of many unknown contaminants is first detected through odor and flavor evaluation. This practice describes methodology This guide covers procedures for determination of the effects of water-related contaminants on the odor and taste of exposed live fish, live fish or fishery products after alleged exposure where flavor impairment is a suspected issue. This practice supersedes the sensory evaluation procedures detailed in Practice D3696.
- 1.2 This guide addresses safety, harvested quality, sample preparation, assessor selection and training, testing procedures with assessor instructions, as well as test environment and parameters.
- 1.3 This guide is applicable to product categories from aquaculture and wild-caught sectors. The range of contaminated products could be from a small-scale water source, such as an estuary, or a limited river system, to a large-scale source, such as a bay, gulf or portion of an ocean. For details on how these methods compare to field- or laboratory-exposed fishery samples, see Ref (1).²
- 1.4 Also covered in this guide are fish species, harvest method (wild-caught versus aquaculture/farmed fish), post-harvest handling, processing methods, and storage.

¹ This <u>practiceguide</u> is under the jurisdiction of ASTM Committee E18 on Sensory Evaluation and is the direct responsibility of Subcommittee E18.06 on Food and Beverage Evaluation.

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² The boldface numbers in parentheses refer to the list of references at the end of this guide.

- 1.5 This guide provides suggested procedures and is not meant to exclude alternate procedures that may be effective. It also does not address all of the nuances of testing throughout the world. It is the responsibility of the user to be aware of their local guidelines and apply them as needed. Some useful resources are also cited in this guide.
- 1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.
- 1.7 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety safety, health, and health environmental practices and determine the applicability of regulatory limitations prior to use. Specific hazards statements are given in Section 7.
- 1.8 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 ASTM Standards:³

D3696 Practice for Evaluating an Effluent for Flavor Impairment to Fish Flesh (Withdrawn 2006)⁴

E253 Terminology Relating to Sensory Evaluation of Materials and Products

2.2 Federal Documents:⁵

21 CFR Part 50 Protection of Human Subjects

3. Terminology

- 3.1 Definitions:
- 3.1.1 For definitions of terms used in this guide, refer to Terminology E253. Definitions—See Terminology E253.

4. Summary of Practice

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4.1 Fish that are suspected of having been exposed to contamination are to be processed and maintained for sensory analysis in accordance with appropriate manufacturing practices. After cleaning and evisceration, fish are wrapped in protective covering such as aluminum foil (which will not impact flavor), placed in labeled plastic bags, and maintained at $4^{\circ}C_{4}$ or below, necessary for preservation of the product. Samples must be frozen if sensory testing cannot be conducted within 24 h. Immediately prior to sensory testing, the fish are thawed under refrigeration, if frozen, and homogeneous composite samples are prepared. Individually foil-wrapped aliquots of 20-g 20 g fish (sufficient to provide all panelists with nearly identical samples for testing) are steamed and presented to trained sensory panelists for odor or flavor evaluation, or both.

5. Significance and Use

- 5.1 This procedure is used to determine the effects of water-related contaminants on the odor and taste of exposed fish. This procedure may be used as evidence in showing compliance with regulatory procedures.
- 5.2 This <u>practiceguide</u> is designed for use by fish processors or research laboratories for evaluations by a trained and monitored sensory panel under the supervision of a sensory professional.

6. Apparatus

- 6.1 Aluminum Foil, heavy-duty, approximately 0.5-mm 0.5 mm thickness, or
 - 6.2 Polyethylene Bags, heat-sealable, as an alternative to aluminum foil.
 - 6.3 Steam Bath, with rack and lid.

³ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

⁴ The last approved version of this historical standard is referenced on www.astm.org.

⁵ Code of Federal Regulations, Available available from the-U.S. Government Printing Office, Washington, DC 20402: Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.



- 6.4 Thermometer, with a range from 20 to 100°C. 100 °C.
- 6.5 Electrical Warming Tray.

7. Precautions and Safety Hazards

- 7.1 Fish that are being prepared and eviscerated in the field should be visually evaluated to see if the outer coating on skin or shell has evidence of contamination. Determine if the coating should be disturbed or is significant to results. If the coating can be disposed of, wiping the skin or shell is preferable to washing. Use paper towels to wipe the fish clean. Do not use water containing the effluent or the dilution water (river, lake, and so forth). In the event that no clean water is available, the fish should be transported to a source of clean water for cleansing, eviscerating, and freezing.
- 7.2 Do not taste fish that have died or are suspected of having died as a result of exposure to contaminants, or that show any signs of toxic effects, because they might be toxic to the taster or possible tissue deterioration might influence the test results.
- 7.3 Where possible, if fish are to be frozen, they should have been eviscerated prior to freezing because the contents of the viscera may lead to subsequent flavor effects.
- 7.4 Minimize personal contact with the effluent or dilutions of the effluent because it is always possible that some hazardous material, bacterial, or viral pathogen might be present. Clean hands, clothing, and equipment after contact thoroughly.
- 7.5 Follow local water safety laws and practices in field studies. Check with local enforcement agencies because these laws vary from one area to another.
- 7.6 A current food handler's certificate might be required by local law for the cleaning, handling, and preparation of fish and shellfish samples.
- 7.7 Reasonable assurance of pertinent chemical and microbiological safety of the test samples should be assessed before sensory tests. If potential contaminants are known to be hazardous, then sensory assessment must be by odor evaluation only.
- 7.8 Panelists must read a statement that they are aware of the requirements of the test procedure. Prior to testing, all panelists must sign an informed consent form between themselves and the sponsoring organization (see 21 CFR Part 50).
- 7.9 Every attempt should be made to prevent further contamination of the samples. Panelists and sample preparers and servers must avoid introducing extraneous odors during preparation and testing from the use of products such as scented hand soap, hand creams, hairspray, perfume, odorous writing instruments or inks, etc.

8. Sampling Procedures

- 8.1 See Practice D3696 for conducting laboratory exposure of fishes.
- 8.2 See Ref (1).

9. Sample Preparation

- 9.1 The method of sample preparation should result in uniform samples for panelists. Preparation of homogeneous pooled samples is recommended because there might be flavor variation among fish, as well as within individual fish, such as differences between the anterior and posterior portions of a fish and dark versus light muscle areas of the fish.
- 9.1.1 For fresh fish, eviscerate and remove the head, tail, and large bones or shell crustaceans and molluscs. Thaw frozen fish in a refrigerator $(4^{\circ}C)$ for up to 18 h.
- 9.1.2 Observe if the outer coating on skin or shell has evidence of contamination. Determine if the coating should be disturbed



or is significant to results. If the coating can be disposed of, wiping the skin or shell is preferable to washing. Shred the fish flesh (with or without skin) or the entire flesh portion of shellfish (de-shelled) in a food processor for 4 s using chopping blades. Mix shredded fish muscle thoroughly to provide a homogeneous sample. Weigh out or portion $\frac{20-g-20}{g}$ samples of the composite mixture, the number of which is equivalent to the number of panelists factored by the number of replicates. Wrap each $\frac{20-g-20}{g}$ aliquot or sample in heavy-duty aluminum foil (see Appendix X1), and code foil packages with a three-digit code for identification in sensory analysis. Keep samples refrigerated at all times prior to cooking.

- 9.1.3 Preparation by steaming is preferable to other cooking methods such as frying or baking because it minimizes other flavor changes that would result from elevated temperatures and allows the preparation of individual, uniformly cooked samples. Pretested microwave oven procedures that do not overcook samples or expose samples to food-reactive equipment can be used.
- 9.1.4 Arrange foil-wrapped samples in a single layer on a rack in a steamer to allow adequate steam circulation. Do not puncture any of the packages. Cook the samples over steam for 7 min, and serve from electrical warming trays to maintain a constant sample temperature of 70°C 70 °C during panel sessions. No samples should be held longer than 15 min. The samples should be served to all panelists after an equivalent interval of time.
- 9.2 An alternative method of sample preparation is the use of heat-sealable polyethylene bags. The bags containing 20-g-20 g samples are boiled for 5 min in rapidly boiling water with this method. For all sample wrapping materials, the degree to which the material might impact the flavor of the fish must be known and addressed.
 - 10. Procedures for Training a-Sensory Panel for Flavor Impairment of Fish
 - 10.1 See Refs (2 and 3).
 - 10.1.1 Individuals who are selected to participate as sensory panelists should be selected for their experience and ability to detect and quantify the off-flavors from the suspected contaminant source; they should be trained in the evaluation procedure, and their performance should be validated before testing begins, as recommended in Ref (2). All training and test evaluations should be documented, and records should be retained.
 - 10.2 Determine the training needs based on the specific test objective. Tests may include attribute recognition, attribute intensity ratings, or difference tests, or some combination thereof (3). Panelists must be familiar with the test procedure and any rating scale(s) to be used for evaluating the samples.

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- 10.3 Terminology and Characteristics:
- 10.3.1 A limited lexicon specific for the descriptors and references for odors and flavors for fish and contaminants is included in Appendix X2 Appendix X2 and Appendix X3 and Appendix X3.
- 10.3.2 Odor recognition tests should include some of the contaminants if they are known to be present in the fish.
- 10.4 Prepare training samples that are characteristic of various odors and flavors and various intensity levels. Use Appendix X2, Appendix X3, and Ref (4) as guides. Evaluate a series of concentrations, starting with easily distinguished samples, and proceed to more difficult discriminations. Check the consistency of individual panelist's performance (2).

11. Sensory Testing Procedure

- 11.1 In these studies, there are a number of factors that should be considered in the design and execution of the testing procedure. There might not be an available control or reference sample against which to compare test samples in some situations. The number of available samples might be limited, thus restricting the number of replications in the test design. Samples might differ visually, and the difference might be difficult to disguise, thus leading to sample bias. An odor evaluation of the fish might be the only feasible test method due to hazards from contaminants. If flavor is evaluated, panelists *must be instructed to expectorate all samples*.
- 11.2 Experimental Designs With a Control Sample—Refer to Practice D3696.



12. Sensory Testing Facility

12.1 See Ref (5). All sensory testing procedures should be conducted in an appropriately designed facility. One important consideration for this facility is the requirement for a cooking area with its own ventilation system that is separated from the sensory panel evaluation area. There should also be an efficient air removal system in the sensory panel evaluation area.

13. Instructions to Panelists for Odor Evaluations

- 13.1 When testing samples with low intensity aromas, instruct panelists to smell an empty glass container to facilitate adaptation to extraneous odors and to allow for better discrimination between samples. For aroma evaluation, it is recommended that samples be placed in closed glass containers for an evaluation of the headspace odors. This is especially helpful for samples with weak aromas.
- 13.2 Present samples in random order and instruct panelists to evaluate them from left to right.
- 13.3 Instruct panelists to cut open the foil sample package or plastic sample bag containing the cooked fish sample, and sniff (use two to three short "bunny" sniffs). For all remaining samples the panelists should sniff in the same manner, keeping the distance from the nose, number of sniffs, and length of sniffing constant.
- 13.4 Instruct panelists to smell the back of their hand or a container of clean potable water before testing samples and between samples to help "zero" the nose and to prevent adaptation to the odors.

- 14.1 Instruct panelists to do the following:
- 14.1.1 Rinse the mouth well with warm $(50 \pm \frac{1^{\circ}\text{C}}{1})$ odor and flavor-free water before starting the flavor evaluation.
 - 14.1.2 Taste the samples in the order presented.
 - 14.1.3 Expectorate each sample; do not swallow the sample.
- 14.1.4 Rinse the mouth well with warm-temperature water $(50 \pm 1^{\circ}C)$ between samples for a predetermined amount of time to clear the mouth of residual flavors.
 - 14.1.5 Wait a predetermined amount of time before tasting subsequent samples to prevent taste fatigue; be consistent.
- 14.1.6 Additional methods to clear the mouth include unsalted soda crackers or a 50:50 blend of warm water (50°C) (50 °C) and room temperature sodium-free carbonated water.
 - 14.2 If residual flavors persist, repeat the procedure of rinsing and resting.

15. Procedure for Data Collection

- 15.1 Attribute Recognition—Use to describe, rank, and rate the overall intensity of specific odor and flavor attributes of a sample, including odors or flavors, or both, that contribute to off characteristics. If available, a highly trained sensory panel is the most sensitive method to use for data collection (see Ref (6)).
- 15.2 Difference Testing—An appropriate ASTM difference test is used to assess whether there is a difference between the contaminated sample and a known control, if available (see Ref-Ref (3)).

16. Data Handling

16.1 Statistical analysis of the data will depend on the type of test and test design. Reference Ref (3) contains statistical analyses appropriate for various sensory tests. Specific data handling methods for descriptive tests are presented in Ref (6).