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EAST AFRICAN STANDARD

Test methods for fish and fishery products — Part 1: Collection and storage of samples for analysis

EAST AFRICAN COMMUNITY

Foreword

Development of the East African Standards has been necessitated by the need for harmonizing requirements governing quality of products and services in East Africa. It is envisaged that through harmonized standardization, trade barriers which are encountered when goods and services are exchanged within the Community will be removed.

In order to meet the above objectives, the EAC Partner States have enacted an East African Standardization, Quality Assurance, Metrology and Test Act, 2006 (EAC SQMT Act, 2006) to make provisions for ensuring standardization, quality assurance, metrology and testing of products produced or originating in a third country and traded in the Community in order to facilitate industrial development and trade as well as helping to protect the health and safety of society and the environment in the Community.

East African Standards are formulated in accordance with the procedures established by the East African Standards Committee. The East African Standards Committee is established under the provisions of Article 4 of the EAC SQMT Act, 2006. The Committee is composed of representatives of the National Standards Bodies in Partner States, together with the representatives from the private sectors and consumer organizations. Draft East African Standards are circulated to stakeholders through the National Standards Bodies in the Partner States. The comments received are discussed and incorporated before finalization of standards, in accordance with the procedures of the Community.

Article 15(1) of the EAC SQMT Act, 2006 provides that "Within six months of the declaration of an East African Standard, the Partner States shall adopt, without deviation from the approved text of the standard, the East African Standard as a national standard and withdraw any existing national standard with similar scope and purpose".

East African Standards are subject to review, to keep pace with technological advances. Users of the East African Standards are therefore expected to ensure that they always have the latest versions of the standards they are implementing.

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Introduction

It is of utmost importance that all samples accurately reflect bacteriological conditions at the time that sampling is done. All sampling must be carried out aseptically so that no question as to the source of the bacteria present on a sample can arise. Samples must be processed as soon after collecting as is practicable. In the interim they must be held under conditions that will preserve the original bacterial flora as completely as possible, permitting neither die-off nor multiplication. Chilling the sample and holding it at the temperature of melting ice is usually the only feasible way in which samples can be stored without significantly changing the bacteriological picture. In some cases, samples must be frozen but it should be recognized that this may diminish bacterial numbers in the sample. Protracted frozen storage may further reduce the viability of bacterial in the samples. Do not freeze samples destined for *Vibrio parahaemolyticus* analysis. This standard is intended to offer guidance in minimizing errors due to mishandling of samples.

In the preparation of this East African Standard, the following sources were consulted extensively:

KS 1754-1:2003, *Test methods for fish and fishery products — Part 1: Collection and storage of samples for analysis*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

IS 4303-1:1975, *Code of hygienic conditions for fish industry — Part 1: Pre-processing stage*

IS 4303-2:1975, *Code of hygienic conditions for fish industry — Part 2: Canning stage*

Codex Alimentarius website: http://www.codexalimentarius.net/mrls/vetdrugs/jsp/vetd_q-e.jsp

USDA Foreign Agricultural Service website: <http://www.nrlidatabase.com>

USDA Agricultural Marketing Service website: <http://www.ams.usda.gov/AMSV1.0/Standards>

European Union: http://ec.europa.eu/enterprise/sectors/pharmaceuticals/veterinary-use/maximum-residue-limits/index_en.htm

Assistance derived from these sources is hereby acknowledged.

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Test methods for fish and fishery products — Part 1: Collection and storage of samples for analysis

1 Scope

This East African Standard applies to fish and products of fishery origin.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

CAC/GL 21, *Principles for the establishment and application of microbiological criteria for foods*

CAC/RCP 1, *Recommended international code of practice — General principles of food hygiene*

CAC/GL 30, *Principles and guidelines for the conduct of microbiological risk assessment*

CAC/GL 31, *Guidelines for the sensory evaluation of fish and shellfish in laboratories*

CAC/GL 48, *Model certificate for fish and fishery products*

CAC/RCP 52:2003(Rev. 4:2008), *Code of practice for fish and fishery products*

CAC/GL 53, *Guidelines on the judgement of equivalence of sanitary measures associated with food inspection and certification systems*

EAS 38, *Labelling of prepackaged foods — Specification*

3 Definitions

For the purpose of this standard the following definition shall apply:

3.1

lot

a quantity of food produced and handled under uniform conditions at a fixed location

3.2

sample

a representative portion of a lot

3.3

sample unit

one of a number of individual containers that make up a sample

3.4

analytical unit

the amount of product withdrawn from the sample unit for analysis. Unless otherwise specified, it will be 100 g.

4 Sampling plans

An attribute sampling plan or a variable sampling plan may be used to estimate compliance of product lots and water sources to established microbiological specifications. Attribute plans are based on the

presence or absence of a specific characteristic. They can be further subdivided into either two or three class plans. For example, Salmonella guidance are a two class plan whereas a three class plan introduces degrees of acceptability; for example, *Escherichia coli* guidelines show the product can be acceptable, marginally acceptable or unacceptable.

Variable sampling plans are based on the measurement of some continuous data and use of these plans requires knowledge of frequency and distribution. These plans are used in the evaluation of shellfish growing waters and the performance of depuration plants.

5 Sample size

Sampling should be representative of the lot. Five samples units per lot will be drawn randomly for analysis unless otherwise specified.

6 Laboratory apparatus

- (a) Sterile screw-cap or ground glass stopped bottles, or autoclavable, non-toxic plastic bottles, with or without added thiosulfate.
- (b) Felt pen.
- (c) Glass-marking crayon.
- (d) Sterile, wide mouth screw-cap jars — glass or autoclavable, non-toxic plastic.
- (e) Long forceps, stainless steel, or of noncorrodible, non-toxic material.
- (f) Sterile plastic bags.
- (g) Depth water sampler.
- (h) Insulated containers.
- (i) Ice or dry ice.

7 Media and reagents

Denatured Ethyl Alcohol 95 per cent for flaming instruments, or 70 per cent for general disinfection. Alternative disinfectants sterilization. The protective covering may be aluminium foil, rubberised cloth, heavy impermeable paper or bottle cover caps. Samples bottles to be used for collecting chlorinated water shall contain 0.1 ml of a 10 per cent solution of sodium thiosulfate prepared with distilled water. This is sufficient for a 100 ml water sample. For larger bottles, add a proportionately larger amount of the thiosulfate solution. Add the thiosulfate solution to the bottles before they are sterilized. Use sufficient thiosulfate solution to neutralize all chlorine present (5 parts thiosulfate will neutralize 1 part chlorine).

In order to obtain a representative sample from a tap, open the tap fully and allow to run for 2 or 3 minutes, or a sufficient time to permit clearing of the service line. Remove the stopper or cap of the sample bottle and hold by the protective covering. After the sample is drawn, replace the stopper or cap in such a way that the protective covering remains in place. When a still body of water is to be sampled, remove the cap from the bottle as already outlined, hold the bottle near the base and plunge neck downward below the surface to a depth of about 30 cm. Then tilt it with the neck pointed slightly upward and during filling, push the bottle horizontally forward in a direction away from the hand to avoid contamination. If any current exists, direct the mouth of the bottle against the current. When applicable, use a depth sampler. The more popular devices utilize the sample bottle and are designed so that, upon reaching the desired depth, the stopper may be raised to fill the bottle. Such devices are useful to depths of 10 to 20 metres. Beyond that depth, hydrostatic pressure makes it impossible to remove the stopper. When such samplers are not suitable, the capillary tube water sampler in general use for oceanographic work may be used.

The number of water samples to be taken from any one source may be left to the discretion of the laboratory concerned, but keep in mind that sampling must be sufficient to detect contamination under all conditions that might influence the quality of the water. These would include current, tides, wind action, precipitation, landwash, temperature and salinity gradients. Whenever possible, start the bacteriological examination of water samples immediately after collection. When this is not feasible, sample bottles must be held at temperatures below 5 °C until analysed. The holding time should not exceed 6 h for impure waters and for all sea water samples, and should not exceed 24 h in any case. Should this time limit be exceeded, record actual time between sampling and analysis.

During sampling, allow for sufficient headspace in the bottles to permit adequate mixing of the sample by shaking.

Analysis of water samples can be initiated in the field. Samples should be inoculated into screw cap fermentation tubes of Lauryl Tryptose Broth (LTB) or lactose broth. The screw cap tubes are required to prevent sample spills during transportation to the laboratory where they will be incubated at 35 °C. The portable membrane filtration kit, can also be used for on-site analysis. Samples should be analysed in the laboratory within 72 h of sampling.

8 Fishery products

Take samples at the end of the processing line, i.e., the point beyond which no further handling of the product takes place. Take samples as packaged by the processor or in new polyethylene bags. Samples may be transferred to the bags by the operators who normally handle the fish at the end of the line. Frozen samples may consist of factory produced packages, or of portions removed aseptically from such packages, and must be kept frozen. Fresh samples must be adequately refrigerated until analysed. Analysis of unfrozen fillets should take place within 24 h of sampling, otherwise report the time of sampling and the time of analysis. Reports must state whether or not the samples analysed have been frozen.

Inspections shall consist of 5 end-of-line samples spaced so as to be representative of the production of the plant for that particular run. When special sampling of a plant is being done, the point at which samples are to be taken and the numbers of samples to be taken will be left to the discretion of the laboratory. In principle, however, reported data should be based on a sampling schedule comparable in scope to that used in reporting results for end-of-line samples.

Store frozen samples at the laboratory at a temperature not higher than -20 °C. The samples may be defrosted at room temperature for a period of 3 h or overnight at 5 °C to simplify sample preparation.

9 Raw shellfish (molluscs)

Samples of shellstock and of shucked unfrozen shellfish should be examined within 24 h after collection. When analysis is unavoidably delayed beyond this point, actual time elapsed between collection and analysis must be reported.

Heavy plastic bags (6 mil gauge) are suitable for shellstock. Keep shellstock samples in refrigerated storage but avoid freezing. Do not permit shellstock to come into direct contact with ice.

In general, take 12-18 shellfish in order to obtain a representative sample and to allow for the selection of 10 sound animals suitable for shucking. For most species this sample size will yield approximately 200 g of meats and shell liquor.

A sterile, wide-mouth jar of suitable capacity with water-tight closure is an acceptable container for samples of shucked shellfish taken in shuckling houses. Transfer the shellfish to the samples jar with sterile forceps or spoon. Sampling of the final product may be taken in the packing cans or containers. Consumer packages are acceptable for examination. Refrigerate samples of shucked shellfish immediately after collection by packing in crushed ice and keep them in ice until examined. The shellfish must not come into direct contact with ice.

10 Breeding and batter

Transport dry ingredients in sterile wide-mouth screw-cap jars or in polyethylene bags. These need not be refrigerated. Transport batter in sterile jars and keep at 5 °C or lower until analysed.

11 Canned fish

Randomly select samples from lot(s) following appropriate directives for sample size. Transport the sample(s) to the laboratory at ambient temperature. Take special precautions when transporting cans that are obviously swollen or under pressure. Place swollen cans in a plastic bag and transport inside a box or a cooler. These cans should be examined immediately on arrival at the laboratory. **DO NOT INCUBATE SWOLLEN CANS.** Except for swollen cans, each sample unit must be judged by the laboratory as to whether or not preliminary incubation would be desirable. When cans are suspected of being non-sterile due to some apparent defect or because of loss of vacuum, such cans may also be opened without prior incubation are acceptable if they leave no toxic residues that may be transmitted to the sample. Sodium thiosulfate, 10 per cent solution.

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