Finished Product Sampling in Low-acid Canned Foods: Concepts and Discussion

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- Disclaimer: The information contained in this presentation is intended for general discussion purposes only and does not necessarily represent the opinions of or constitute recommendations from the presenter, contributors, their companies, or IFTPS.

Outline

-Introduction
-Purposes of Finished Product Sampling
-Sampling Plans
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-Pros and Cons of Finished Product Sampling

-Introduction

Finished product sampling (aka, incubation testing) has been identified as a subject of high interest during discussions within the IFTSP Aseptic Processing Committee meetings. The intent of this presentation is to share some basic concepts and prompt thoughtful consideration and fruitful discussions on the subject.

Due to differing business and regulatory perspectives, multiple potential applications, and various valid approaches, no particular recommendations are intended.

-Purposes of Finished Product Sampling

Finished product sampling may be in some instances **suggested**, or in other instances **required**, as a way to help verify compliance with internal and regulatory product commercial sterility requirements.

The sampling may be for process **validation**, for **release** or **monitoring** of ongoing production, or for **investigative** purposes. The purpose of the sampling will likely influence the sampling approach that is best suited.

M.H. Zwietering et al. / Food Control 60 (2016) 31–43

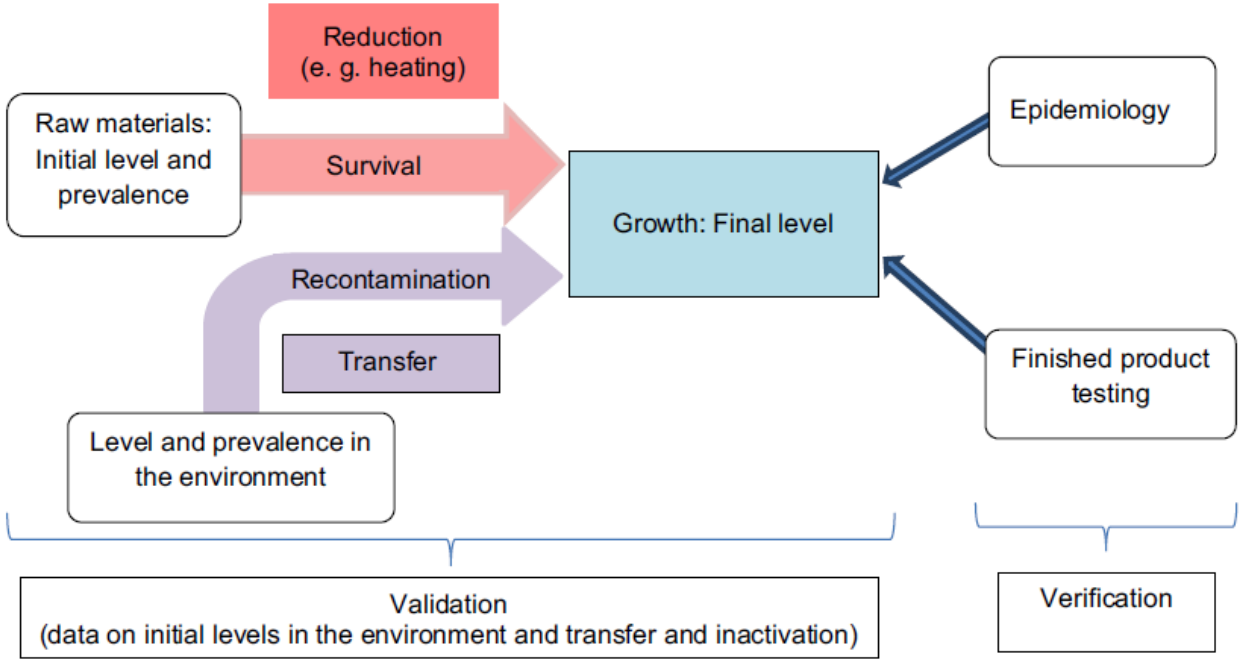

Fig. 1. Overview of the relevant phenomena in food safety control, indicating the position of validation and verification.

-Purposes of Finished Product Sampling (continued)

Some regulatory references to finished product testing.

-LACF Definitions: 21CFR113.3(k) "*Incubation* means the holding of a sample(s) at a specified temperature for a specified period of time for the purpose of permitting or stimulating the growth of microorganisms."
-Aseptic Processing & Packaging: 21CFR113.40(g)(3) "*Incubation.* Incubation tests **should** be conducted on a representative sample of containers of product from each code; records of the test results **should** be maintained."
-Meat/Poultry Thermal Processing: 9CFR431.3(b)(3) "Complete records concerning all aspects of the development or determination of a process schedule, including **any** associated incubation tests, **must be** made available by the establishment to the Program employee upon request"
–**Some*** Meat/Poultry products: 9CFR431.10(b)(1)(iii) & (iv): "*Product requiring incubation.* Shelf stable product requiring incubation includes: (A) Low acid products as defined in§ 431.1; and (B) Acidified low acid products as defined in § 431.1." Sampling: 1 per load (batch); 1 per 1,000 (continuous).

*Thermally processed commercially sterile meat/poultry products for which finished product inspections are not handled according to (1) An HACCP plan for canned product that addresses hazards associated with microbiological contamination; (2) An FSIS-approved total quality control system; (3) Alternative documented procedures that will ensure that only safe and stable product is shipped in commerce. See 9CFR431.10.

-Purposes of Finished Product Sampling (continued)

Isolated Lot-specific Evaluation:
For finished product sampling targeting evaluation of limited population, such as for **validation** or other **investigative** purposes, sampling a **larger quantity** of the produced population should be considered in order to provide a higher confidence level.

Ongoing production performance evaluation:
For finished product sampling targeting evaluation of ongoing production on an **established and well-performing process**, a **smaller sample quantity** from each lot produced may be considered. While the confidence level for each individual lot may be lower than applied in the case of an isolated lot evaluation, **accumulation of performance data** over time provides a higher confidence level.

Qualification Transitioning to Ongoing Production:
For a new or significantly modified process, it may be advisable to apply a sampling plan with a higher confidence level (more samples per lot) as part of the process qualification. This plan would be used until enough data is collected to **show acceptable performance**, allowing then a transition to a lower-level sampling plan for subsequent ongoing production.

-Sampling Plans

Isolated Lot-specific Evaluation:
Sampling plans for lots in isolation can be based on probability calculations. With the **assumed very low probability of insterilities expected** for food thermal processes, a **Poisson distribution** may be assumed, and related calculations used to estimate the distribution and confidence interval.

As with any sampling scheme, the **quantity of samples required will depend on the parameters set by the user**, in this case the required **acceptable performance** (maximum defect rate) and the **confidence level**.

The performance target and confidence level selected for sampling may be influenced by many factors, such as

- -Vulnerability of the end product consumer (e.g., medical foods, infant formula, baby food)
- -Sensitivity of the consumer or customer
- -Robustness of the process (e.g., retort vs. aseptic; low-acid vs. high-acid)
- -Reliability of the system (design, maintenance, age)
- -Experience with the technology (proven vs. novel)
- -Dependability of the employees (from operators to upper management)

-Sampling Plans (continued)

Isolated Lot-specific Evaluation (continued):

Some examples*:

-A validation to ensure a **< 1/10,000** defect rate at 95% confidence requires **~30,000** samples with 0 defects detected in all the samples. This may be required for a basic aseptic system validation.

-For a higher confidence level of 99%, ~46,000 samples with 0 defects.

-If an 80% confidence level is acceptable, ~16,000 samples, 0 defects.

-A validation to ensure a **< 1/30,000** defect rate at 95% confidence requires **~90,000** samples with 0 defects detected in all the samples. Such a high level of validation might be used for a new or complex system producing product for a highly sensitive consumer.

-Evaluation of a single lot (e.g., validation or precautionary sampling in the event of a minor deviation) to ensure a **< 1/1,000** defect rate at 95% confidence requires **3,000** samples with 0 defects.

-To ensure a **< 1/3,000** defect rate at 95% confidence requires **9,000** samples with 0 defects.

* Based on Poisson distribution calculation.

-Sampling Plans (continued)

Ongoing production performance evaluation / ANSI Sampling:

A common tool for ongoing product sampling is the standard **ANSI/ASQ Z1.4: Sampling Procedures and Table for Inspection by Attributes**. IS0 2859-1 is similar. The 'attribute' in this context would be commercial sterility. This standard includes many alternative **schemes** with the required sample size determined by **user-selected parameters** including lot-size ranges, inspection levels, and AQLs (Acceptance Quality Limits). The sample size will also depend on which inspection is in effect, i.e., Normal, Tightened, or Reduced, as determined by application of the switching rules which consider recent past performance of the process (number of consecutive lots accepted or not accepted).

Because of the potential severe risk that any sterility defect represents, **any single sterility defect should be considered as critical defect**, causing the affected lot to be initially "rejected" and put on hold (i.e., Acceptance number (Ac) for sterility defects = 0, Reject number (Re) for sterility defects = 1). However, lots which are "rejected" may not necessarily need to be scrapped. Such lots may be investigated and might be subsequently sorted, reworked, re-evaluated, etc.

It is important to **thoroughly understand this standard and all its requirements** to avoid improper or incomplete application.

Link: Attribute Sampling Plans - Inspection by Variables & Attributes Z1.4 & Z1.9 | ASQ

-Sampling Plans (continued)

Ongoing production performance evaluation / ANSI Sampling (continued):

Some important points to consider in applying this ANSI standard:
-The main sampling schemes are primarily used for and **applied to a series of lots** (vs. evaluation of isolated lots).
-Actual performance should be consistently better than the **selected AQL**.
-Samples should be selected at **random** from normal production.
  -If there are commonly occurring portions of production during which performance might tend to be less than average (e.g., Aseptic Filling Machine material splices, starts/re-starts, other line "Events"), a separate sampling and evaluation for these portions should be considered.
-The sampling scheme must consider the application of appropriate **switching rules** between Normal, Tightened, and Reduced inspection (and discontinuance of sampling inspection).
-**Any single sterility defect would usually be considered as critical defect**, causing the lot to be initially "rejected" and put on hold (i.e., Ac=0).
  -However, lots which are **"rejected" may not necessarily mean scrapped**. Such lots should be investigated and might be subsequently sorted, reworked, re-evaluated, etc.

Link to asq.org info: [Attribute Sampling Plans - Inspection by Variables & Attributes Z1.4 & Z1.9 | ASQ]

-Sampling Plans (continued)

Ongoing production performance evaluation / Examples using USDA sampling rates:

Using the Poisson distribution calculation for evaluation:

-Batch retort example: Incubation plan for thermally processed meat and poultry per 9CFR431.10(b)(1)(iv)(A): “From each load of product processed in a batch-type thermal processing system (still or agitation), the establishment must select at least one container for incubation.”

-Assume a batch-type retort that holds 5,000 containers per load and each lot has 30 retort loads:

-Lot size: 5,000 containers per load x 30 loads = 150,000 containers

-Sample size: 1 sample container per load x 30 loads = 30 sample containers

-Sampling rate: 30/150,000 = 1/5,000 = 0.02%

-30 sample with 0 defects with 95% confidence indicates a defect rate somewhere <1/10.

(-NB: Compare similar ANSI sample quantity (32) with General inspection level II (N), Multiple samplings, Reduced, AQL 10 (1/10).)

-After 10 lots: 300 samples with 0 defects with 95% confidence indicates a defect rate < 1/100.

-Continuous processing system: Incubation plan for thermally processed meat and poultry per 9CFR431.10(b)(1)(iv)(B): “For continuous rotary retorts, hydrostatic retorts, or other continuous type thermal processing systems, the establishment must select at least one container per 1,000 for incubation.” 1/1,000 = 0.1%

-Assume a continuous system runs 500 containers per minute (cpm) over a 12 hour shift:

-Lot size: 500cpm x 12hr x 60min/hr =360,000 containers.

-Sample quantity: 360,000 x 0.1% = 360

-360 sample with 0 defects with 95% confidence indicates a defect rate of <1/120.

(-NB: Compare similar ANSI sample quantity (315) with General inspection level II (P), Single sampling, Reduced, AQL 0.015 (1/6,667).)

-After 10 lots: 3,600 sample with 0 defects with 95% confidence indicates a defect rate <1/1,200.

-Clearly, the ANSI sampling approach depends on the accumulation of data over several lots, thus the importance of initial validation/verification.

-Sampling Plans (continued)

Validation/Qualification transitioning to ongoing production / VDMA Class V (aseptic) Example:

Example sampling plan for building confidence in commercial sterility after start-up of filling machines of VDMA class V[18]

Building Confidence in Commercial Sterility of UHT-products			
Confidence level	Sampling level	Results of microbiological investigation	Decision about future confidence level
Level 3: Performance trial 3 individual test runs	3 x 1,000 samples, aerobic investigation	0 failed packs in total	Go to level 2 (When former level was 1: return to level 1)
	3 x 100 samples for anaerobic investigation *	*No failed packs*	*Decision depends on aerobic counts (see above)*
Level 2: Intensified sampling during first 3 months of regular production In case of new process (equipment) or significantly different product	1%, but always ≥ 200 random samples per day/run, aerobic investigation At least 10,000 samples in total	≥ 10 failed packs in total, not resulting from incident(s)	Performance unacceptable, correct source and go back to level 3
		3 - 9 failed packs in total and not more than 1 in one single run	Investigate, record and adjust process Stay at level 2
		≤ 2 failed packs in total and not more than 1 in one single run	Go to level 1
Level 1: Regular production, stringent routine sampling	0.3%, but always ≥ 100 random samples per day/run, aerobic investigation; *incidental also anaerobic**	When confronted with 2 incident situations within 10 runs:	De-escalate to level 3; if source has been corrected, return to level 1
		When confronted with 1 incident situation:	Stay at level 1
		No failed packs in 10 successive runs:	go to level 0.
Level 0: Regular production, relaxed routine sampling	0.1%, but always ≥ 50 random samples per day/run, aerobic investigation; *incidental also anaerobic**	2 sporadic failures within 10 successive runs:	Go back to level 1
		1 confirmed sporadic failure in 10 successive runs:	Go back to level 1
		1 or none sporadic failures in 10 successive runs:	Continue at level 0

* Anaerobic investigation if appropriate, e.g. for soups. Anaerobic investigation gives an indication for the process rather than for the performance of the filling machines

[18] This example from practice was provided by a well known bottling and packaging company for publication in anonymized form. The performance test cited under Level 3 is a sterile test as defined in this VDMA document. The procedure is transferable to machines of class IV.

-Limitations of Sampling

Short of incubating and testing 100% of the product produced with a 100% foolproof testing method, there always remains a certain **possibility of accepting a defective lot** (consumer's risk, false negative, beta (β) risk, Type II Error). There is also the risk of rejecting a commercially sterile lot (manufacturer's risk, false positive, Type I Error, alpha (α) risk). It is important to understand the limitations of statistical sampling plans.

In most cases (given relatively large production lot sizes in food manufacturing) **only the number of samples determines the defect detection probability**, and there is little relation to lot size.

The following graph illustrates some of the limitations of finished product sampling, particularly when applied to **processes with very low failure rates**. Processes producing commercially sterile food products are expected to have extremely low sterility failure rates. So, **these limitations are important for our industry to recognize**.

For example, for a system with a defect rate of ~1 in 10,000, the probability of detecting a defect through sampling is quite low, and there is not much difference between 100, 200, 500, and even 1000 samples where, within an isolated lot with those sample quantities, the probability of detecting a defect is in the range of only 1 to 9%. So, the likelihood of NOT detecting a defect is 91 – 99%. This illustrates the importance of not relying only on finished product sampling, but to **use sampling only in combination with proper validation, operation and controls, and records review for each batch**, to ensure a decision for release is a valid one.

On the other hand, **a single defect detected from a small sampling quantity can indicate a potentially large issue**. For example, 1 defect in 500 samples could indicate an overall failure rate as high as 1 in 100. So, **each and every defect must be considered and investigated seriously**.

Sampling Plans (continued)

-Test Method Sensitivity

It is important to recognize and consider the sensitivity and limits of the method or methods that are being used to test the samples. **Some types of defects may not be identified by every method.**

The **sensitivity of the test method can vary** depending on may factors, including (but not limited to…)
-type of product (complexity, pH, ingredients, oxygen content, color, consistency)
-type of package (flexibility, transparency)
-product age
-incubation times and temperatures
-type of agar/growth media
-contaminating organisms (growth conditions, gas production, pH change, competition with other organisms)

Also, **multiple incubation and testing conditions may be needed** for the same product in order to identify different source of spoilage (e.g., aerobic vs. anaerobic; mesophilic vs. thermophilic)

-Test Method Sensitivity (continued)

Other general considerations on sterility testing:

-**A combination of test methods should be considered**.
For example, micro testing (e.g., direct streak plate, rapid methods) may be considered sensitive to identify organisms that do not produce gas or change the product pH. However, micro testing can give a false negative result, e.g., if the organism dies off before being tested. **Using micro and pH testing along with sensory examination (visual, odor) will help avoid the shortcomings of the individual methods**. Also, in the case of a true positive result, the **micro, pH and sensory exam information may provide useful information for the subsequent investigation**.

-**For less sensitive the test methods, a larger quantity of samples should be considered**.
A sampling approach may include a combination of sampling plans/test methods, e.g., many samples for container visual/dud detection only examination, several samples for pH only testing, and a few sample for micro + pH testing.

-Test Method Sensitivity (continued)

Other general considerations on sterility testing (continued):

-Consider including and documenting details that may be helpful in the case of a spoilage investigation.

- -pH of spoiled product
- -pH of normal product
- -visual observations of package
- -visual observations of product
- -specific odors

Spoilage manifestations in low-acid products

Group of organisms	Classification	Manifestations
Flat-sour	Package flat	Possible loss of vacuum on storage
	Product	Appearance not usually altered; pH markedly lowered, sour; may have slightly abnormal odor; sometimes cloudy liquor
Thermophilic anaerobe	Package swells	May burst
	Product	Fermented, sour, cheesy or butyric odor
Sulfide spoilage	Package flat	H_2S gas absorbed by product
	Product	Usually blackened; rotten egg odor
Putrefactive anaerobe	Package Swells	May burst; pH slightly above normal, odor
	Product	May be partially digested; pH slightly above normal; typical putrid odor
Aerobic sporeformers	Package flat/swollen	Usually no swelling; coagulated proteins; discolored vegetables

From FDA BAM, Warren L. Landry, Albert H. Schwab, and Gayle A. Lancette, Jan 2001. BAM Chapter 21A: Examination of Canned Foods | FDA

-Pros and Cons of Finished Product Sampling

Some ***potential*** advantages and disadvantages. *Actual* impacts will depend on many factors:

Pros:
-Added assurance, prior to product release, that no unidentified serious process or packaging failure has occurred.
-Added assurance of consumer safety
-Added brand protection
-Supporting information in the event of post-release failures or complaints.
-Accumulation of data to document and track line sterility performance trends.
-pH checks may identify other issues (e.g., pre-process spoilage, ingredient addition mistakes)

Cons:
-False sense of security leading to lowered vigilance in other, more important areas
-Improper reliance on sampling rather than on proper process validation, control, and records
-Added product release time
-Release delays and cost of dealing with 'false positive' results
-Added cost
  -unreleased inventory
  -unreleased product storage space
  -sample incubation space and operation
  -destructively tested samples
  -sample handling, storage, and testing

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…and the many, many other teachers and colleagues from over the years.

-Some hopefully helpful resources:

Mullan, W.M.A. (2020). Reliability of microbial sampling in assuring food safety and calculation of prevalence following negative tests. [On-line]. Available from: https://www.dairyscience.info/index.php/food-model/275-sampling.html

Attribute Sampling Plans - Inspection by Variables & Attributes Z1.4 & Z1.9 | ASQ
https://asq.org/quality-resources/sampling/attributes-variables-sampling

BAM Chapter 21A: Examination of Canned Foods | FDA
https://www.fda.gov/food/laboratory-methods-food/bam-chapter-21a-examination-canned-foods