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Sampling Plans to Determine the Minimum Core Temperature Reached during the Precooking of Tuna

ABSTRACT

This paper presents acceptance sampling plans that use existing statistical principles to ensure that the minimum core temperature can be used as a Critical Limit (CL) for a Critical Control Point (CCP) for precooking tuna. This strategy will ensure a 5 $\log_{10}$ reduction of the histamine-forming bacterium *Morganella morganii* and control the associated risk of histamine formation in tuna during the time needed for further processing and prior to retorting. Core temperatures of precooked tuna of different sized fish were gathered from industrial production for validation. The lower limit of the core temperature of the batch was calculated as: sample average – (3 times the standard deviation). For variable sampling plans, a sample size of 23 provided a 90% confidence level, with 99% percent acceptable, while a sample size of 35 provided a 95% confidence level, with 99% percent acceptable. Sample sizes are given for attribute sampling plans as well. The subsequent tables and sampling plans provided will allow tuna processors to develop HACCP plans to monitor CLs for a CCP for precooked tuna core temperature and allow for a significantly longer period of time in which to process tuna of any size after precooking.

INTRODUCTION

Purpose

The purpose of this paper is to: (a) develop risk-based sampling plans for monitoring tuna core temperature Critical Limits (CLs) for a commercial tuna precooking Critical Control Points (CCP) and (b) describe suitable practical monitoring practices for this processing step. This strategy involves sampling core temperatures of precooked tuna and measuring holding times to confirm that all the fish in the batch are heated sufficiently to control histamine formation during tuna processing. In this manuscript, the CL is defined as the *minimum* individual core temperature in a batch of precooked fish. The math and logic will be developed to predict the *lowest* temperature from a sample by calculating the [average core temperatures – (3 times the standard deviation)] of a normally distributed and representative sample of core temperatures. This strategy provides a robust

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estimate of the lowest temperature from a batch of precooked fish and is suitable for use in determining compliance with the CL.

Histamine and the tuna canning business

The canned tuna business is a multi-billion-dollar business spread throughout the world. Hamilton et al. *(23)* estimated that, in 2008, there were at least 144 tuna processing facilities around the world capable of producing either canned tuna or cooked, cleaned, and frozen tuna meat in loin bags destined for canning. The total capital investment in the global canned tuna industry was $15 billion U.S. dollars, the processing capacity was over 14,000 metric tons per day, and a round weight of 3 million metric tons was processed annually. The vast majority of tuna processing factories precook the tuna before further processing, with the average precooker capacity per cycle of about five metric tons. Based on these estimates, there are ca. 2,800 daily precooking cycles and ca. 600,000 precooking cycles performed annually.

The process and equipment used for precooking tuna and the factors that have the most impact on the heating rates and precooking time for commercially processed tuna have been described previously by DeBeer et al. *(8)*.

All seafood processed and/or sold in the United States must be processed under the U.S. Food and Drug Administration's (FDA) Seafood HACCP regulations *(18)*. The FDA has issued a series of recommendations for processing seafood in various HACCP guidance documents, with the latest issued in 2011 *(15)*. Histamine is one of the hazards that tuna processors are required to control. Histamine forms after the tuna dies, if the tuna is not chilled properly aboard the fishing vessel or is exposed to conditions conducive to histamine formation during processing at the factory *(26)*. These conditions allow histamine forming bacteria (HFB) to convert the free histidine found in certain fish to histamine by the inducible enzyme, histidine decarboxylase (HDC) *(41)*. What sets histamine apart from other seafood toxins is that its formation is 100% preventable, with timely and proper cold or heat treatments *(22, 26)*. The defect action level (DAL) for histamine in seafood in the United States is 50 ppm, with an action level of 500 ppm based on toxicity *(16)*. A 50 ppm DAL is associated with relatively small samples sizes and is most often viewed as evidence of mishandling and the possible presence of higher levels of histamine within the lot *(41)*.

The workflow for processing whole tuna, from receiving at the factory until shipping canned product, is shown in *Fig. 1.* Typically, there are 10 steps from thawing until retorting the canned product, usually processed in batches. The FDA's 2011 Seafood Hazard Analysis Critical Control Point (HACCP) guide *(15)* recommends a maximum exposure limit of 12 hours when temperatures are over 21°C for processing frozen tuna, in the absence of an approved intermediate heat (precook) step. However, 12 h may be not be sufficient for processing large tuna *(30)*, unless the fish are precooked to a minimum inhibitory core temperature (MICT) in order to pause histamine formation for an extended period of time. Under these circumstances, the MICT then becomes a Critical Limit (CL) in a HACCP plan. The FDA HACCP Guide *(15)* allows for a heating stage (precooking) for tuna but gives no guidance on the time and temperature of the heat treatment or the MICT. In the early 1970s, Peterson *(36)* recommended 57.3°C (135°F) as a minimum core temperature. Hence, that minimum core temperature has been the de facto standard for precooking tuna for almost 50 years.

Minimizing the risk of histamine formation during and after precooking

By the time the tuna is ready to be precooked, it will have passed the required initial HACCP histamine and organoleptic screening and is safe for processing into shelf-stable containers. The risk of histamine formation as a result of the precooking heating step is that a portion of some fish might not achieve the time and minimum fish core temperature necessary to reduce the HFB, which then might grow during the time the fish is cooled and cleaned, after precooking. The fish core temperature can be monitored in one of two ways: (a) the fish can be tested after precooking with thermometers at the backbone or geometric center or (b) the fish can be continuously monitored with temperature probes during precooking *(30)*.

Multiple factors can contribute to variability of the core temperatures of precooked tuna. These factors include fish size, thawing, initial core temperature before precooking, placement of the tuna on the trolleys, temperature distribution of the precookers, steam supply, and precooker maintenance. All of these factors need to be managed as much as possible. Some of the variation can be minimized by capital construction and simple maintenance, while other operational sources of variation can be minimized but not eliminated.

The canned tuna industry relies completely on the capture of free-swimming, wild tuna that weigh from 0.25 kg to 100 kg *(5)*. The fish is sorted by size when it arrives at the processing factory. The range of sizes within the size group has been reviewed elsewhere *(8)*. Even fish with the same weight can have different thicknesses, so there is always a thickness variation within a batch of fish from the same size category *(8)*, which can cause fish to thaw and heat differently. This size variation needs to be accounted for and dealt with accordingly, because it cannot be eliminated.

Several procedures can be implemented to minimize the temperature variation after precooking *(33)*. These include: (a) making certain there is a consistent, even temperature distribution throughout the precooker, (b) providing sufficient steam supply to maintain the targeted ambient steam temperatures, and (c) conducting proper maintenance

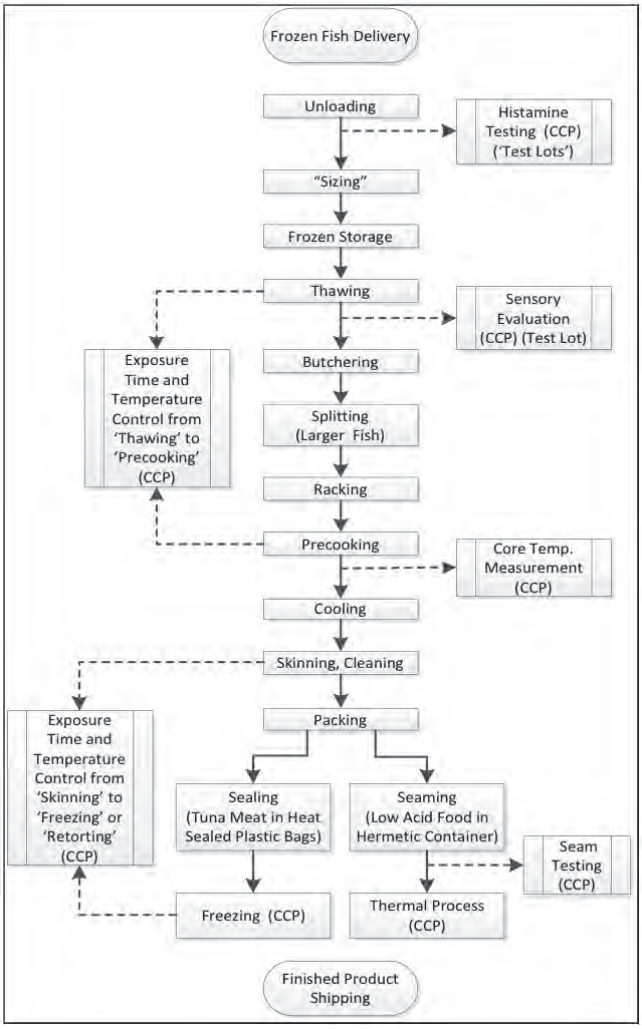

Figure 1. Flow chart of tuna processing: round fish to canned or frozen product

on the steam and air valves of the precookers to avoid leaks that could impact the temperature distribution.

Vogl et al. *(44)* conducted extensive testing and determined that precooking can halt histamine formation in deliberately spoiled tuna for up to 18 h, using 60°C as the *maximum* core temperature target. In another study, Enache et al. *(12)* evaluated five HFB, reported that *Morganella morganii* was the most prolific histamine former, and conducted thermal death time studies for *M. morganii* over the range of 50°C to 60°C. Nolte et al. *(35)* then proposed that if the core temperature of the tuna reached 60°C for 0.26 minutes during a precooking step, a 5 $\log_{10}$ reduction of *M. morganii* would be achieved. Furthermore, Kanki et al. *(27)* tested the HDC enzyme from four species of bacteria, including *M. morganii*, and observed no activity at 60°C. Given this information, it can be concluded that precooking provides the extra time and temperature needed to process even the largest tuna without the risk of histamine formation.

Since both the time and temperature are involved in reducing HDC activity, processors who use precooking as a CCP in their HACCP programs need to establish CLs and relevant holding times. Alternative CLs for the minimum core temperatures have been developed based on exposure times for the MICT *(9)*. In this paper, the target example utilizes a minimum core temperature of 60°C.

The fourth principle of HACCP is to establish monitoring procedures *(32)*. While the National Advisory Committee on the Microbiological Criteria for Foods (NACMCF) suggests that continuous monitoring is preferable, the expert committee acknowledges that "statistically designed data collection or sampling systems lend themselves to this purpose" and to reliable monitoring procedures *(32)*.

In this paper, various sampling plans will be proposed, reviewed, and recommended for use in control of histamine formation in tuna, thus allowing additional time to finish processing after the precooking step. A secondary goal

is to provide processors with simple and robust tools for precooking tuna.

The sampling plans proposed here are intended to be used within the context of tuna processed as described in FDA's HACCP Guidance *(15)* and the National Fisheries Institute (NFI) Tuna Council's HACCP for Canned Tuna Handbook *(33)*. This strategy should include: (a) an approved supplier program, (b) inspections for decomposition and histamine levels upon receiving, (c) controlling the time and temperature exposure prior to precooking within safe limits, and (d) precookers validated as per NFI Tuna HACCP Handbook *(33)*.

The authors plan a future manuscript that will provide processors with the prerequisites and tools to use the CLs and sampling plans developed herein. If a tuna processor chooses to monitor fish core temperature as a HACCP strategy to determine the adequacy of the heat treatment at the precooking CCP, they will need to develop a monitoring plan based on acceptance sampling to infer the minimum core temperature, since they cannot measure every single fish in each batch. DeBeer et al. *(8)* concluded that predicting precooking time cannot be an exact science because of the variability inherent in the fish.

The conditions that must be met to use core temperature as a monitoring technique are (a) collection and prompt analysis of the core temperature data after precooking, to determine if the CLs are met or if a Corrective Action is required, and (b) statistical confidence that the tuna flesh has been heat treated long enough to achieve a 5 $\log_{10}$ reduction in *M. morganii*. If these conditions are met, a statistically valid core temperature sampling plan could be employed to confirm that the tuna flesh has been heated sufficiently and thus that the formation of histamine can be controlled.

Acceptance sampling

The body of knowledge on acceptance sampling is very well developed *(11, 39, 42)*. There are two basic sampling procedures: (a) sampling by attribute (yes/no, or pass/no pass) and (b) sampling by variable (individual values). The two procedures have different sampling regimes and sample sizes for the same confidence levels.

Multiple sampling plans were reviewed for the development of the precooking core temperature sampling plan. The FDA Compliance Policy Guidance for decomposition and histamine in canned tuna *(13)* suggests a sample size of 24 cans. Regulatory action is recommended (i.e., the lot fails) if two cans show evidence of decomposition by having histamine levels exceeding 50 ppm. A sample size of 36 cans fails with three such cans. The FDA HACCP Guide *(15)* suggests that 18 fish be used for incoming histamine sampling, with the lot failing if any sample is over 50 ppm. The FDA's Seafood HACCP Guide *(15)* has recommended sample sizes for core (i.e., backbone) temperatures in at least three chapters: Chapter 7 (Control of Scombroid Toxin), Chapter 12 (Control of Pathogenic Bacteria toxins) and Chapter 13 (Control of *Clostridium botulinum* toxin). In each case, the recommended sample size for monitoring a temperature CL of 4°C was 12 fish. The Guide also recommended increased sample size if there was a high level of temperature variability or very small fish. Chapter 13 of the Guide also has a sample size of the three largest fish, each with a continuous temperature recording device for smoking fish to a core temperature of 62.8°C (145°F) for 30 minutes. The NFI Tuna HACCP Handbook *(33)* recommends measuring 24 fish for precooking core temperature control. The Inspection and Certification regulations for the National Marine Fisheries Service at 50CFR260.61 *(3)* has numerous examples of sample sizes for lot acceptance of fishery products, including decomposition or unwholesomeness. Sample size-based attributes with different levels of risk are detailed in the International Commission of Microbiological Specifications for Foods (ICMSF) *(1)*. A table of different biological sample sizes and their context is listed in *Appendix A*. None of these sampling plans or schemes cited is suitable for the task of lot acceptance on the basis of a minimum ending temperature of a sample of heated fish, except the example in Chapter 13 of the FDA HACCP Seafood Guide *(15)*.

A sampling plan should depend on the severity or risk of the hazard and associated risk management *(28, 29, 45)*. In this paper, the risks will be classified based on confidence levels and the percent of product required to be acceptable *(7)*, as shown in *Table 1*. For example, for Moderate Severity, processors need to be 95% confident that at least 95% of the precooked fish in the lot achieve a minimum core temperature to ensure the fish have been cooked sufficiently to inhibit further histamine formation. These statements are referred to as tolerance intervals. Additionally, there are three types of statistical intervals: confidence, prediction, and tolerance intervals *(34)*. Because processors need to be confident that a high percentage of fish have passed a certain minimum temperature, tolerance intervals, or limits, will be the focus of this paper.

A sampling plan in which the minimum core temperature of the fish is attained requires

1. knowledge of whether the shape of the distribution of the core temperature data is normally or near-normally distributed,
2. a decision on the confidence level and reliability or percent acceptable, based on the severity of the hazard,
3. a decision on the type of acceptance sampling plan to use (attribute or variable), and
4. the sample size (number of fish tested), which is determined by the aforementioned 3 items and are included in this paper.

TABLE 1. Risk classification

Severity	Confidence level	Minimum percent acceptable to be demonstrated	Comments
Critical	95%	99.5%	Likely to result in serious injury or death
High	95%	99%	Could cause serious injury or death
Moderate	95%	95%	Unlikely to cause serious injury or death
Low	95%	80%	No safety concerns, just cosmetic defects

Source: *(7)*.

TABLE 2. Selected values of d_2 used to calculate the standard deviation from the sample range

n	d_2	n	d_2
5	2.326	31	4.113
7	2.704	35	4.213
10	3.078	37	4.258
11	3.173	41	4.342
15	3.472	49	4.482
17	3.588	51	4.512
21	3.778	54	4.559
22	3.819	55	4.571
23	3.858	60	4.638
24	3.895	61	4.652
25	3.931	65	4.698
26	3.965	86	4.906
27	3.997	87	4.917
30	4.086	163	5.354

n = 1–25 *(21)*, n = > 25 *(7)*.

METHODS OF SAMPLING

Two methods of acceptance sampling are as follows:

1. Sampling for attributes: A simple pass/fail plan that makes no assumptions of normality
2. Sampling for variables: a random sampling plan that is based on a normal distribution of core temperatures and requires estimates of the mean (μ) and the standard deviation (σ)

The sample size for the two types of plan is quite different: the attribute or pass/fail plan requires a far larger sample size than a variable sampling plan. Duncan *(11)* indicates that there is almost a 7-fold difference in sample size between single sampling by attribute and single sampling by variables with a known standard deviation (σ).

For an attribute sampling plan, it is not necessary to know the exact maximum temperature achieved by each fish in the sample. Rather, processors need to ascertain only whether or not the core temperature has passed a certain predetermined minimum point, for example, 60°C. The operator can measure a fish, and, once the temperature measurement reaches or exceeds this minimum target, the thermometer can be removed, the data recorded (pass or fail) and another fish can be measured. HACCP regulations *(17)* do require that the

actual measurements or observations of CLs of times and/or temperatures need to be recorded. For the variable sampling plan, the actual core temperatures observed must be recorded for each fish in the sample to estimate the mean (μ) and standard deviation (σ) for all the fish in the precook batch.

Normal distribution parameter estimators

A computer can be used by the fish preparation personnel to estimate μ and σ from the sample mean ($\bar{x}$), as well as the sample standard deviation(s), thereby allowing for the determination of acceptance of the batch.

The use of the range/d_2 to estimate the standard deviation, σ, was first suggested by Tippett *(43)* in 1925. An excellent explanation of the mathematical derivation and the statistical validity is detailed by Luko *(31)*. A list of d_2 factors for n = 2 thru 100 was published in Grant and Leavenworth *(21)*, and selected values are listed in *Table 2 (7)*.

The median ($\tilde{x}$) can be used as an estimator of the mean in a normally-distributed population, with n > 25, which has been shown to be adequate by Hozo et al. *(25)*. With these two pieces of knowledge, median ($\tilde{x}$) and range/d_2, decisions can be made (e.g., determining whether the fish have been cooked sufficiently), using statistical methods when those techniques are needed. If the estimates are done manually, i.e., without using a calculator, the median and range/d_2 can be used to estimate μ and σ *(2)*. The median and range/d_2 is offered so that a factory without a computer could still use this technique.

The range of core temperatures will impact how close the lowest measured temperature will be to the value calculated to be three standard deviations (range/d_2) below the mean or median. For a fixed sample size, the standard deviation will increase with the size of the range. It is beneficial to the factory operators to minimize the range of the precooking core temperatures. The goal is to make the actual minimum core temperature as close as possible to the calculated lower limit of the core temperature.

Tests for normality

To use the variable sampling plan, processors will need to verify that a normal distribution provides a good model for the distribution of the actual core temperatures being measured. There are good discussions of testing for normality or near-normality in Geary *(20)* and Hart and Hart *(24)*. Two statistical tests for normality that work well for grouped data are the Ryan-Joiner test *(38)* and the Shapiro-Wilk test *(40)*; these are similar tests *(6)*. The data can also be easily analyzed and plotted in Minitab© or another statistical commercial software. The Shapiro-Wilk test is available online *(10)*. The Ryan-Joiner test is available in Minitab© and can be easily calculated with a computer spreadsheet with a statistical package installed. The spreadsheet application (to be discussed in a future paper) uses the Ryan-Joiner method for ascertaining normality.

METHODS AND MATERIALS

Sample size determination

The Minitab© statistical software system was used to develop the sample sizes for attribute and variable sampling plans, depending on confidence limits and reliability or percent acceptable. The sample size, with sampling by attribute, can change depending on the size of the population being sampled. If the approximate number of pieces in the lot is known, the attribute plan will have fewer samples than it would have if an infinite lot size was assumed. A detailed example of how the sampling sizes were developed for a variable acceptance plan using the sample median and standard deviation is presented in *Appendix B*.

Testing core temperature data for normality

To test if the core temperature data are normally distributed, two completely different data sets were collected from the same tuna processing factory and tested for normality. The first data set was collected from four different size groups of fish, but with different core temperature distributions (ranges) that were standardized and combined into one data set for analysis by use of a Minitab normality plot. The second data set was provided by one of the authors *(30)*, who collected precooking exit core temperatures from 24 fish, each from 296 precooking cycles, covering nine different fish size groups. Each 24-fish sample had a histogram charted for inspection and was tested for normality with a Minitab normality plot. The Ryan-Joiner statistic was calculated and recorded for all the data sets.

Testing attribute data for normality

No normality tests were required or conducted: the test used is a simple pass/fail test of whether or not the temperature passes the minimum target temperature.

Modeling how the lower limit of the tolerance interval changes based on a fixed minimum core temperature and varying ranges of core temperatures

The range in core temperatures will impact the lower end of the tolerance limit or percent acceptable. This approach was modeled by fixing the lowest core temperature and varying the range. The minimum measured core temperature of 60°C was chosen and the temperature ranges were varied in increments of 5°C, so Max/Min of 60°C/60°C, Max/ Min of 65°/60°C, Max/ Min of 70°/60°C and so forth were modeled. Using that information, we could estimate a median (midpoint of an ordered data values) and estimate the batch standard deviation, using range/d_2. We used an n of 60 with a d_2 value of 4.638 from *Table 2*.

RESULTS

Statistically valid sample sizes

Three distinct statistically valid sampling plans were developed with the same confidence levels and reliability.

1. Sampling by ATTRIBUTE:
   b. c = 0 (zero tolerance sampling, no non-conforming core temperatures allowed).
   c. Does not assume a normal distribution.
   d. Table 3 can be used by operators to determine the size of the population (n) of pieces in the precooker. Although *Table 3* shows a range of 16 to 8,400 pieces per precooker, the most common precooker batch sizes are between 2,000 and 3,000 pieces *(5)*.
   e. Requires counting the number of samples.
   f. Requires determining the number of samples based on different population sizes per precooker batch size:
      i. For 2400 pieces per precooker, the sample sizes are listed in *Table 4*.
      ii. For 4800 pieces per precooker, the sample sizes are listed in *Table 5*.
      iii. For 8400 pieces per precooker, the sample sizes are listed in *Table 6*.
      iv. For an infinite number of pieces, the sample sizes are listed in *Table 7*.
2. Sampling by VARIABLES, using the median $(\tilde{x})$ and range and d_2 from the sample data: sample sizes listed in *Table 8*.
   c. Verify normal or near-normal distribution of the core temperature data collected.
   d. Use sample median $(\tilde{x})$ and range/d_2 to accept or reject the lot:
      i. Lower limit is $y = [\tilde{x} - (3^*\text{range}/d_2)]$ and must be at least 60°C to pass. If n = 60 and the lot passes, we can be 95% confident at least 99% of the lot is good. If n = 10 and the lot passes, we can be 95% confident at least 95% of the lot is good.
   c. Requires counting, simple addition and division, and table look-ups.
   d. Very easy to use in that it involves only counting and simple arithmetic.
3. Sampling by VARIABLES using mean (μ) and standard deviation (σ) estimated from the sample data: sample sizes listed in *Table 9*.
   d. Verify normal or near-normal distribution of the core temperature data collected.
   e. Uses the sample mean $(\tilde{x})$ and sample standard deviation (s) to accept or reject the lot:
      i. Lower limit is $y = [\bar{x} - (3^* s)]$ and must be at least 60°C to pass. If n = 35 and the lot passes, we can be 95% confident that at least 99% of the lot is good. If n = 10 and the lot passes, we can be 95% confident that at least 95% of the lot is good.
   c. Requires multiple calculations and thus is more difficult to do in real time.
   d. Uses the smallest sample size.

TABLE 3. Lot sizes for a precooker load by fish size and precooker size

Precooker lot size — count of individual pieces/fish

		Fish per basket and average size								
Fish per basket		1	2	3	4	6	8	10	12	15
Size in kg > >		10 kg	8 kg	6 kg	4.2 kg	3 kg	2.25 kg	1.6 kg	1.2 kg	< 1 kg
Racks/ Cooker	Basket/ Rack									
1	16	16	32	48	64	96	128	160	192	240
5	16	80	160	240	320	480	640	800	960	1,200
10	16	160	320	480	640	960	1,280	1,600	1,920	2,400
15	16	240	480	720	960	1,440	1,920	2,400	2,880	3,600
20	16	320	640	960	1,280	1,920	2,560	3,200	3,840	4,800
25	16	400	800	1,200	1,600	2,400	3,200	4,000	4,800	6,000
30	16	480	960	1,440	1,920	2,880	3,840	4,800	5,760	7,200
35	16	560	1,120	1,680	2,240	3,360	4,480	5,600	6,720	8,400

TABLE 4. Sample sizes for c = 0 attribute acceptance sampling plans for lot size 2400

Confidence Level	Minimum percent acceptable to be demonstrated		
	95%	99%	99.5%
90%	45	219	419
95%	58	281	529
99%	89	418	764

Source: *(7)*.

TABLE 5. Sample sizes for c = 0 attribute acceptance sampling plans for lot size 4800

Confidence Level	Minimum percent acceptable to be demonstrated		
	95%	99%	99.5%
90%	45	224	439
95%	59	289	562
99%	89	437	837

Source: *(7)*.

TABLE 6. Sample sizes for c = 0 attribute acceptance sampling plans for lot size 8400

Confidence Level	Minimum percent acceptable to be demonstrated		
	95%	99%	99.5%
90%	45	227	448
95%	59	293	577
99%	90	446	871

Source *(7)*.

TABLE 7. Sample sizes for c = 0 attribute acceptance sampling plans for lot size infinite

Confidence Level	Minimum percent acceptable to be demonstrated		
	95%	99%	99.5%
90%	45	230	460
95%	59	299	598
99%	90	459	919

Source: *(7)*.

TABLE 8. Sample sizes for variable acceptance sampling plan using the median and range/d_2 as estimate of standard deviation

Confidence Level	Minimum percent acceptable to be demonstrated		
	95%	99%	99.5%
90%	7	30	100+
95%	10	60	100+
99%	20	100+	100+

Source: *(7)*.

TABLE 9. Sample sizes for variable acceptance sampling plan using the calculated mean and standard deviation

Confidence Level	Minimum percent acceptable to be demonstrated		
	95%	99%	99.5%
90%	7	23	54
95%	10	35	86
99%	17	65	100+

Source: *(7)*.

TABLE 10. Comparison of sample sizes for attribute and variable acceptance sampling plan using the calculated mean and standard deviation. Source: other tables

We are 95% confident of the percentage acceptable

Confidence Level		95%	99%	99.5%	Notes	Source
Attribute	95%	59	299	593	Infinite lot size	Table 7
Variable	95%	10	60	100+	$\tilde{x}$, range/d_2	Table 8
Variable	95%	10	35	86	$\tilde{x}$, s	Table 9

A comparison of the number of samples needed for testing by use of the attribute and variable sampling plans is shown in *Table 10*. The sample sizes listed have a 95% confidence and a reliability, or percent acceptable, of 95%, 99% and 99.5%. Using 99% reliability as an example, 3 different n's are displayed: 299 for attribute sampling, 60 from a variable plan based on the sample median and range/d_2, and 35 from a variable sampling plan based on the sample mean and standard deviation. At this confidence and reliability level, there is more than an 8-fold difference in sample size between the largest attribute and smallest variable sampling level.

Core temperature data normality test results

The grouped data from different-sized fish with different temperature distributions were standardized and combined for analysis. The data are plotted on a histogram (frequency distribution), as shown in *Fig. 2*, or normal probability plot, as shown in *Fig. 3*. With a Ryan-Joiner statistic of $P > 0.05$, the data distribution can be assumed to be normal or near-normal *(7)*.

The data from the 296 data sets of 24 core temperatures each were tested for normality. The Ryan-Joiner test rejected normality in six of the 296 precooks, when tested at the 95% confidence level. No significant departure was detect-

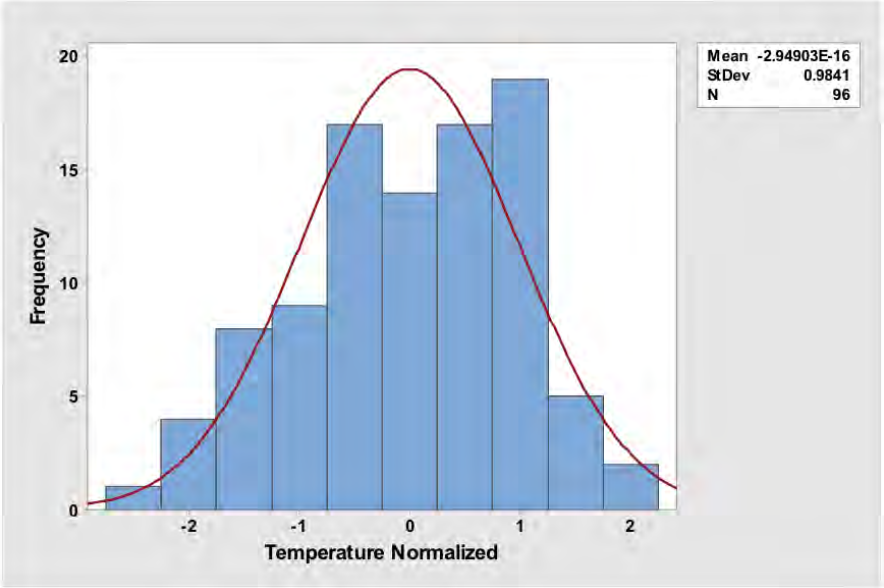

Figure 2. Histogram of normalized core temperatures: grouped data

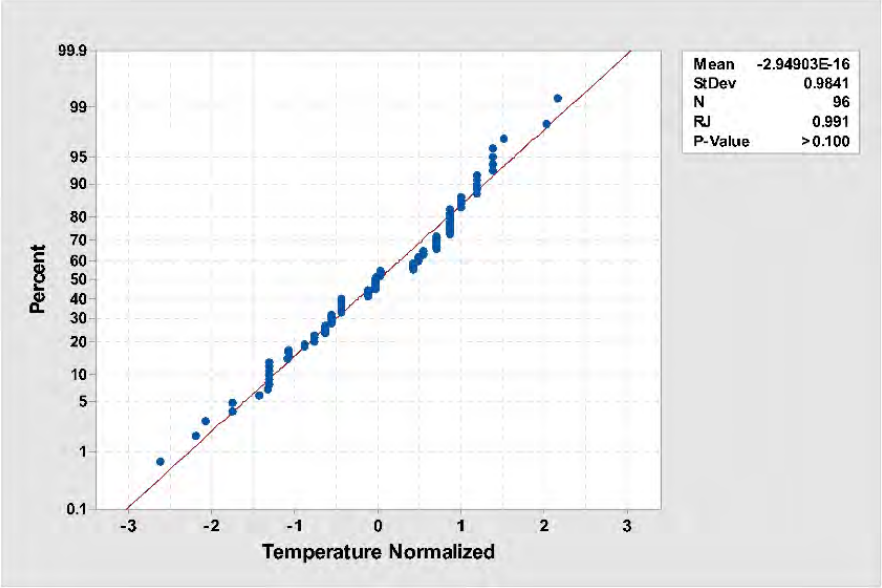

Figure 3. Normal probability plot of normalized core temperatures: grouped data

ed in the remaining 290 precooks. The histogram and normality plot for an example temperature data set with normal distribution is shown in *Fig. 4 and 5* (*P* value > 0.10). The histogram and normality plot for the data failing the normality plot are shown in *Fig. 6 and 7* (*P* value 0.025). These data sets indicate that precooking temperature data collected real time in a factory appear to be normally distributed in the range of interest. The outliers in the data sets that failed the test for normality primarily failed on the high side, with temperatures well over 70°C or 80°C.

Modeling the impact of the range of core temperature variation

In a hypothetical data set, the minimum recorded core temperatures were held constant at 60°C and the maximum core temperature was varied in value, increasing the range in 5°C increments. It is very evident how much the calculated

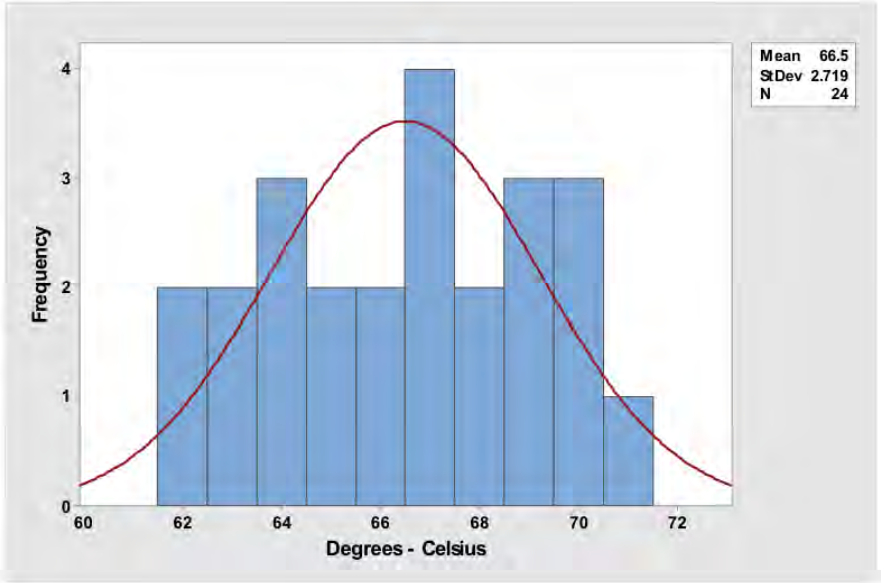

Figure 4. Histogram of normalized core temperatures: passed normality test

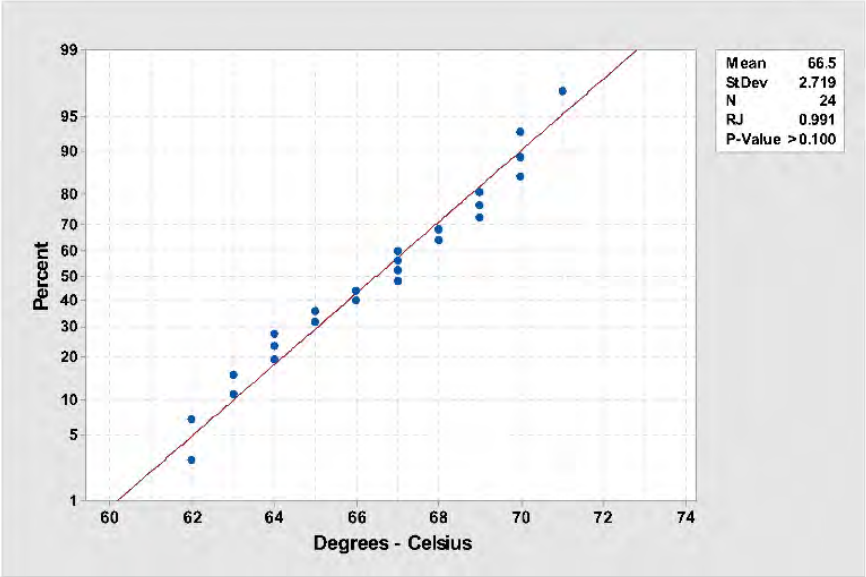

Figure 5. Normal probability plot of normalized core temperatures: passed normality test

three standard deviations (range/d_2) spread increased; see *Fig. 8*. As the range gets larger, the standard deviation increases, and tolerance limits widen. The net impact of this finding is that the factory needs to cook the fish longer to move the median higher and to make certain the lower tolerance limit at least meets the pre-set target of 60°C. This approach fully supports the argument that the factory needs to reduce the range and variance of the core temperatures to reduce overcooking of the fish.

DISCUSSION

Implementing core temperatures as a monitoring tool for a tuna processing CCP under HACCP

To use the core temperature monitoring procedure as a tool for a precooking CCP, the factory will need to integrate this procedure into their existing HACCP plan. The necessary prerequisites will be presented in a future publication.

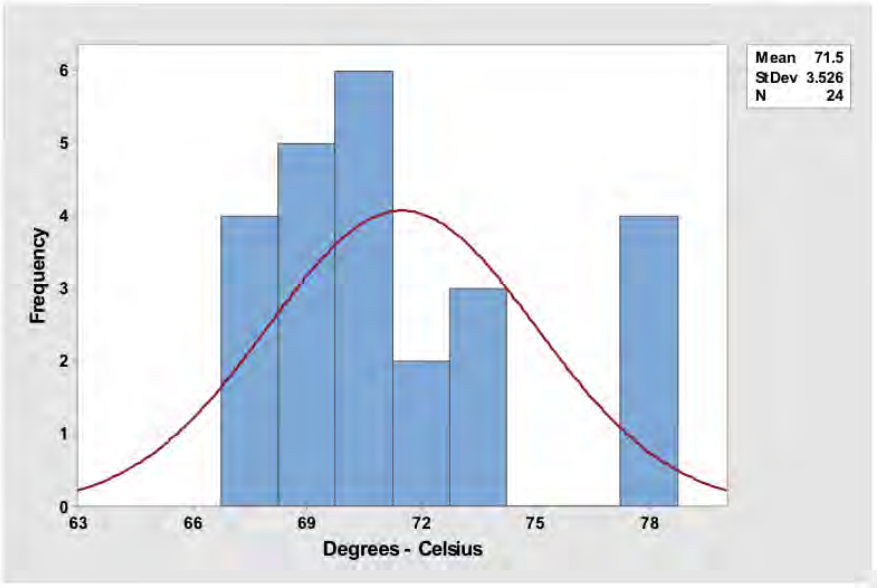

Figure 6. Histogram of normalized core temperatures: did not pass normality test

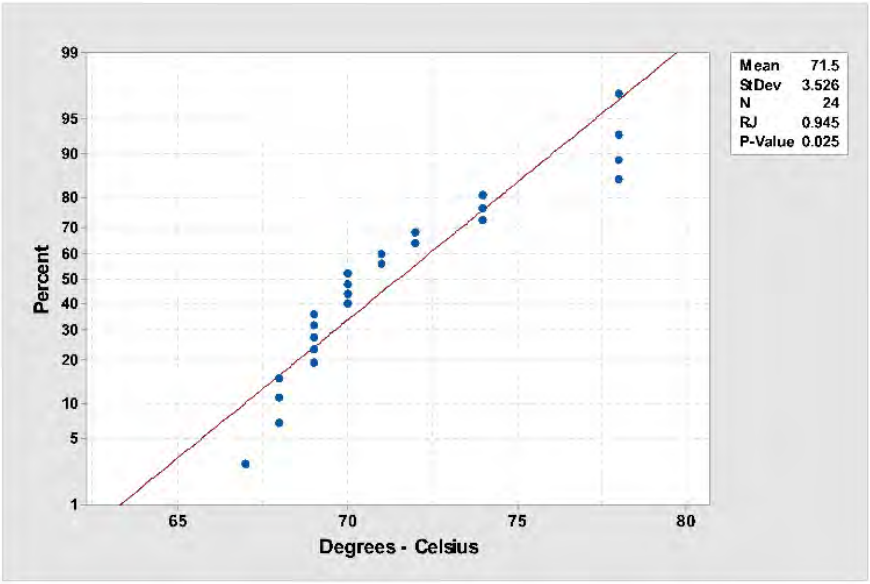

Figure 7. Normal probability plot of normalized core temperatures: did not pass normality test

Because it is very useful to quantify the use of the pre-cooking heating step for processing under HACCP, we have shown how this can be done by use of non-destructive testing of core temperatures of precooked fish. The advantage of being able to measure core temperatures quickly is that the precooker operators can wait and re-measure the core temperatures if the initial readings are too low to reach the minimum target, given that core temperatures continue to rise after the end of precooking. It is relatively simple to develop a computer spreadsheet that calculates the statistics relevant to the evaluation of the proposed CL using the data from a tally sheet if the factory management chooses to use the calculated average and standard deviation.

Normality testing is a fundamental requirement of using a variable sampling plan. When the core temperatures are grouped in a frequency chart or tally sheet, a histogram is naturally formed, and that histogram can be inspected by floor personnel for normality or near-normality. The histogram is effective only for moderate to large sample sizes ($n > = 50$) *(7)*. For smaller sample sizes ($n < 50$), grouped

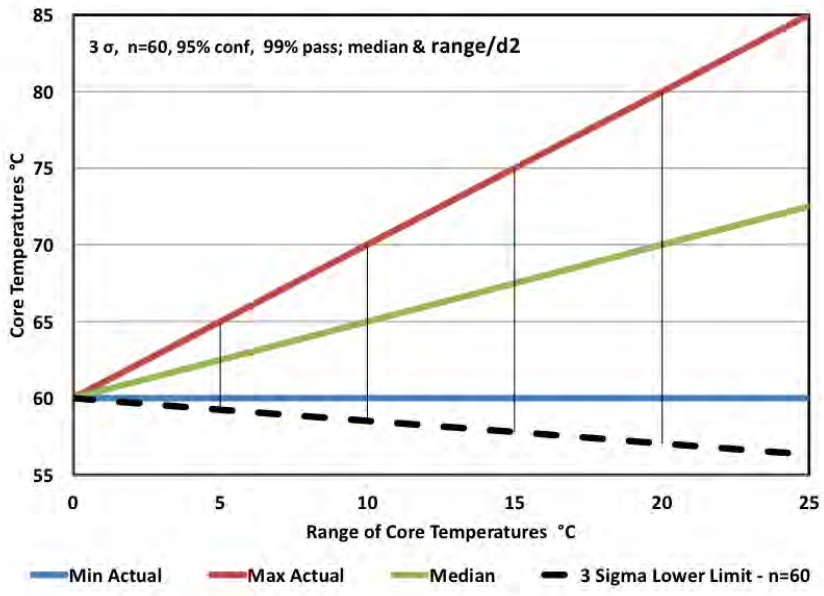

Figure 8. Temperature range chart – 3 sigma lower limit – using d_2 calculations

core temperature data can be graphed by floor personnel by calculating the adjusted cumulative frequency and plotting it against the individual core temperatures on normal probability paper *(2, 7)*. For an example of a variable tally sheet that displays a histogram of sample data, see *Appendix C-1*. This tally sheet also includes two sets of statistics: a) the average plus the standard deviation, and b) the median and the range/d_2. The normal probability plot of cumulative data from *Appendix C-1* is plotted in *Appendix C-2*; since the data are nearly linear, the distribution can be assumed to be normal or near-normal. Several authors use the term "fat pencil test" to test for normality or near normality. If a fat pencil covers the spots on a normal probability plot, the data can be assumed to be normal/near-normal *(4)*.

If there are questions on normality, plant personnel can plot the cumulative percentages on normal probability plot paper. Instructions on how to prepare a blank normal probability chart in an Excel spreadsheet can be found in *Appendix C-3*. The calculation and plotting can be done quickly by hand or with calculators in the precooking office. These normality plots with the precooking core temperature records are to be kept for HACCP verification during record review.

Variable sampling plans

Either variable sampling plan is a quick test if there are enough thermometers to use during the monitoring procedure. The tally sheet collection system is a simple yet very powerful technique. A tally sheet printed on waterproof paper *(37)* with 1-degree frequency bins will work very well. Waterproof paper is suggested because of the steamy environment around the precookers. The data can be quickly gathered, tallied, and analyzed with multiple fast-acting thermometers and a well-designed tally sheet.

The average and standard deviation method (i.e., the computer method) is preferred for sample size considerations. The calculations can be made with the grouped data tally sheet method and a spreadsheet, as is shown in *Appendix C-1*.

The median ($\tilde{x}$) and range/d_2 test for the minimum core temperature can be quickly and easily calculated from the collected and grouped data. The statistics can be calculated on the spot with simple counting techniques and a bit of division *(Appendix C-1)*. The [Median – (3 * Range/d_2)] will indicate the lower limit of the distribution of core temperatures, and a decision can be made whether to: (a) release, (b) measure again and release, or (c) rework with more precooking. With regard to the decision to wait and measure again, the decision can be based on whether the lower limit is below the lower specification (needs rework), between the lower specification and a predetermined marginal specification (wait) or above the marginal specification (release). For an example, see *Appendix C-1*. One can be X% confident that at least Y% of the core temperatures are above this lower limit. X and Y are indicated in *Table 8* based on the sample size. A visual inspection of the tally sheet can confirm a normal/near-normal distribution. If further verification of normality/near-normality is needed, the precooker operator can plot the cumulative relative frequency on normal probability plot paper *(Appendix C-3)*.

Attribute sampling plans

From an operational point of view, the use of attribute sampling is unlikely to be chosen because of the impact on

the optimal processing in a tuna canning factory. For an example of an attribute tally sheet, see *Appendix C-4*. The disadvantage of the attribute sampling plan in use with the precooked fish is the sheer volume of core temperature measurements that need to be collected quickly before the fish start cooling. From *Table 10*, for a confidence level and percent acceptable of (95%/99%), the sample size for attribute sampling plan is 299, versus a sample size of 35 for a variable sampling plan using the calculated mean and standard deviation. If the attribute sample size is 300 pieces and 299 pieces pass, but the 300th one fails, what is the corrective action? By the time the 300th fish has been measured the cooling has started, so a processor would have no proof that the fish attained the proper temperature, and the entire batch will have to be recooked as a corrective action, and all 300 pieces would have to be resampled.

CONCLUSIONS

Uniform initial core temperatures (ITs) at the start of precooking will shorten the precooking times in a conventional atmospheric precooker (CAP) because the fish was thawed properly outside of the precooker. A good thawing setup provides uniformly thawed fish to the butcher table and the precookers. The importance of properly thawing the fish is discussed in DeBeer et al. *(8)*. The initial core temperature will affect the speed of precooking and will need to be measured and verified for each precooking cycle.

After precooking, the fish must be cooled quickly. If the fish continues to heat up after being precooked to a safe core temperature, the fish will be overcooked, and usable fish recovery and yield will suffer. For this reason, the processing facilities should have cooling and chilling equipment with sufficient capacity to cool the fish as quickly as possible. If there are delays in the cooling area, the factory should delay the precooking process until the needed cooling capability is available.

The core temperature method of testing for the determination of whether an adequate precooking final core temperature has been attained is a very effective tool. The testing, which is non-destructive and relatively quick, is actually only a refinement of the widespread practice of collecting core (backbone) temperatures after precooking and therefore should be relatively simple to implement. This paper provides the scientific basis to support the historical practice. The technique gives the factory management and precooker operators sufficient time to optimally process fish of any size in a safe and effective manner.

The sampling plans(s) presented in this paper are conservative; they are based on the lower tolerance limit of three standard deviations below the average core temperatures of a normally distributed set of temperatures to determine whether a Critical Limit is met. In all cases, lethality is counted only while the temperatures are increasing or remaining the same, not during the cooling phase *(19)*. In a CAP, it actually takes longer to cool the fish than it does to heat it, so more log lethality accumulates during cooling. With this approach, the fish is safe for further processing past the original 12 hours recommended by the Seafood HACCP Guide *(15)*. Precooking is and has always been a good tool for preventing histamine formation in commercial tuna processing by precooking to the proper minimum backbone temperatures. A uniform core temperature range with very little variation is not only safe but also maximizes overall recovery and workability of the fish, making it economically feasible as well.

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APPENDIX A. SAMPLING PLANS FOR VARIOUS BIOLOGICAL HAZARDS

Reference	Hazard	Category	Sample Size and c	Critical Limit (s)	Attribute or Variable	Context
21CFR260.61 *(3)*	Grading and wholesomeness	Sensory	Depends on lot size		Attribute	Acceptance sampling for fishery products
BAM.A.1. *(14)*	*Salmonella*	Micro Testing	n = 15, c = 0	No positive samples	Attribute	Sampling for *Salmonella* for imported foods
FDA Compliance *(13)*	Histamine	Histamine	n = 24, c = 1 n = 36, c = 2	50 ppm	Attribute	FDA Compliance Policy Guidance in canned tuna
FHG 4th ed. C 7–p128 *(15)*	Histamine	Temperature	n = 12, c = 0	4.4°C (40°F)	Attribute	Sampling incoming fish
FHG 4th ed. C 7–p128 *(15)*	Histamine	Sensory	n = 118, c = 2		Attribute	Pass on 2 fail on 3, then corrective action
FHG 4th ed. C 7–p128 *(15)*	Histamine	Corrective Action	n = 60, c = 0	50 ppm	Attribute	Corrective action for temperature or decomposition CLs – sample for histamine
FHG 4th ed. C 7–p133 *(15)*	Histamine	Histamine	n = 18, c = 0	50 ppm	Attribute	Sampling incoming fish
FHG 4th ed. C12–p221 *(15)*	*S. aureus* or *B. cereus* toxins	Temperature	n = 12, c = 0	4.4°C (40°F)	Attribute	Transit Control: Transit Control for Refrigerated (Not Frozen) Cooked, Ready-to-Eat or Raw, Ready-to-Eat Fishery Products to be Stored or Processed without Further Cooking)
FHG 4th ed. C13–p265 *(15)*	*C. botulinum* or *B. cereus*	Temperature	n = 3, c = 0	Min 62.8°C for 30 min	Attribute	Smoking fish/3 largest fish in a smoker. Continuous recording. Minimum temperature 62.8°C (145°F) for at least 30 minutes
FHG 4th ed. C13-p268–p270 *(15)*	*C. botulinum* or *B. cereus*	Temperature	n = 12, c = 0	4.4°C (40°F)	Attribute,	Transit Control: Receipt of Products by Secondary Processor
ICMSF– C 5, p74 *(1)*	*S. aureus*	Micro Testing	n = 5, c = 1		Attribute, 3 class	From ICSMF sampling plan
NFI–Appendix 6–2 *(33)*	Histamine	Temperature	n = 24, c = 0	60°C	Attribute	core temperature – after precooking

APPENDIX B. DEVELOPMENT OF SAMPLE SIZES FOR MEDIAN AND RANGE/d_2 BY MINITAB SIMULATION

Sample Size Calculations

- Goal: determine the core temperature sample size for a variable sampling plan with a lower specification limit (LSL) and the following specifications:
- RQL = 1% and β = 0.05. Based on these values, we can be 95% confident that at least 99% of the population is good when we accept the lot.
- Core temperature follows a normal distribution.
- Instead of estimating μ with the *sample mean*, it is estimated with the *sample median*.
- Instead of estimating σ with the *sample standard deviation*, it is estimated with range/d_2.
- The k factor equals 3. In other words, the decision rule has the form

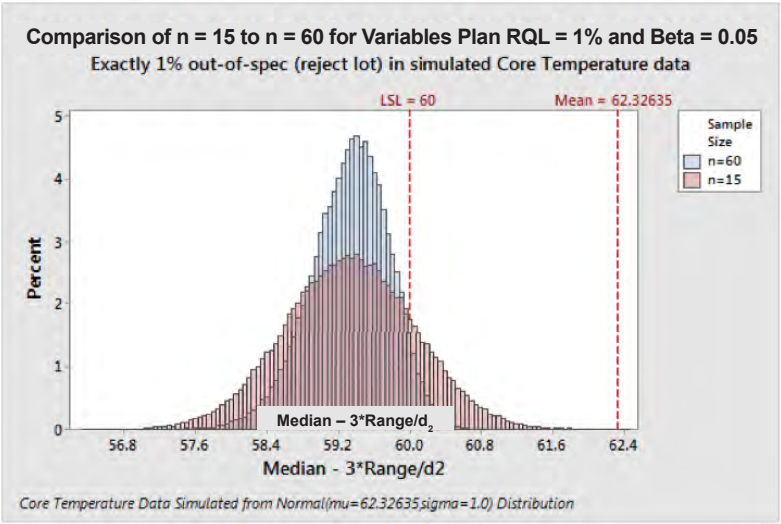
$$Accept\ the\ lot\ if\ median - 3 * \frac{range}{d_2} > LSL$$

Simulation Steps

1. Simulate 10,000 samples of size *n* from a normal distribution with a mean 2.32635 μ above the LSL. This results in exactly 1% of the population out-of-spec. When the true defect rate is equal to the RQL (1%) and β = 0.05, we should accept the lot 5% of the time and reject the lot 95% of the time.
2. Store the median and range for each of the 10,000 samples.
3. Estimate σ by dividing the range by d_2.
4. Calculate the percent of times the simulation accepts the lot and repeat steps 1–4 for different sample sizes *(n)* until the percent of times the lot is accepted $(median - 3 * \frac{range}{d_2} > LSL)$ equals 5%. In other words, when the true defect rate is equal to the RQL, as is the case here, we want to find the *n* so that we accept the lot 100 * β% of the time and reject the lot 100 * (1–β)% of the time.

To do this for an RQL = 5% and β = 0.05, replace step 1 with:

Simulate 10,000 samples of size n from a normal distribution with a mean 1.64485σ above the LSL. This results in exactly 5% of the population out-of-spec. When the true defect rate is equal to the RQL (5%) and β = 0.05, we should accept the lot 5% of the time and reject the lot 95% of the time.



Example of Simulation

The graph below shows a histogram of the simulated $median - 3 * \frac{range}{d_2}$ values for n = 15 and n = 60. The distribution for core temperatures in the simulation had a defect rate of 1%. When n = 60, the lot is accepted approximately 5% of the time and rejected approximately 95% of the time. Therefore, 60 is the correct sample size for an RQL = 1% and β = 0.05. When n = 15, the lot is incorrectly accepted much more than 5% of the time. This high β (or Type II error) indicates that a sample size of 15 is too small.

APPENDIX C-1. VARIABLE ACCEPTANCE SAMPLING PLAN — TALLY SHEET

	Sample size	
	Conf Level	99%
Using x-bar & s	95%	35
Using Median & Range/d_2	95%	60
Tools: Thermometers, pencil		

°C	Frequency distribution of temperatures	Freq	Cum Freq	Cum %
73		0	0	0
72		0	0	0
71		0	0	0
70		0	0	0
69		0	0	0
68		0	0	0
67	////	4	4	7
66	////\	6	10	16
65	////\////\	10	20	33
64	////\////\////\////\//	23	43	70
63	////\////\	10	53	87
62	///	3	56	92
61	///	3	59	97
60	/	1	60	98
59		0	60	98
58		0	60	98
57		0	60	98
56		0	60	98
55		0	60	98

Note: Median is the first bin where the cumulative probablity exceeds 50%

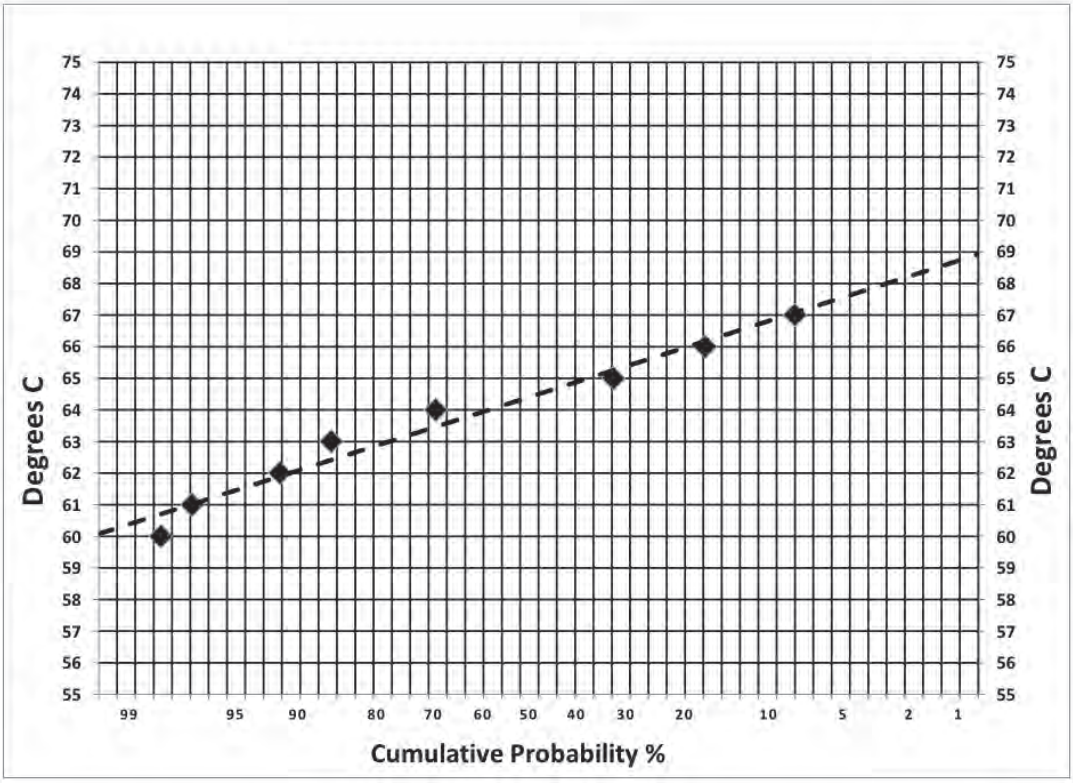
Average	64.1	Count	60	The cumulative percentage is adjusted by adding 1 to the divisor so as not to reach 100% probablity, used for the normal probablity plot
StdDev (s)	1.50	*Median*	64	
Max	68	*Range*	8	
Min	60	d_2 (60)	4.638	
Lower Limit	59.6	StdDev σ	1.72	
Based on (Avg - (3 * s))		Lower Limit - °C	58.7	

Action Plans

Measure temperatures from 60 fish
Determine median, maximum, and minimum temperatures
Calculate StdDev from Range and d_2 table - $R/(d_2)$
Calculate the Lower level - (Median -3σ)
Does this exceed the lower limit?
Pass, Wait, Re-test or Re-Cook

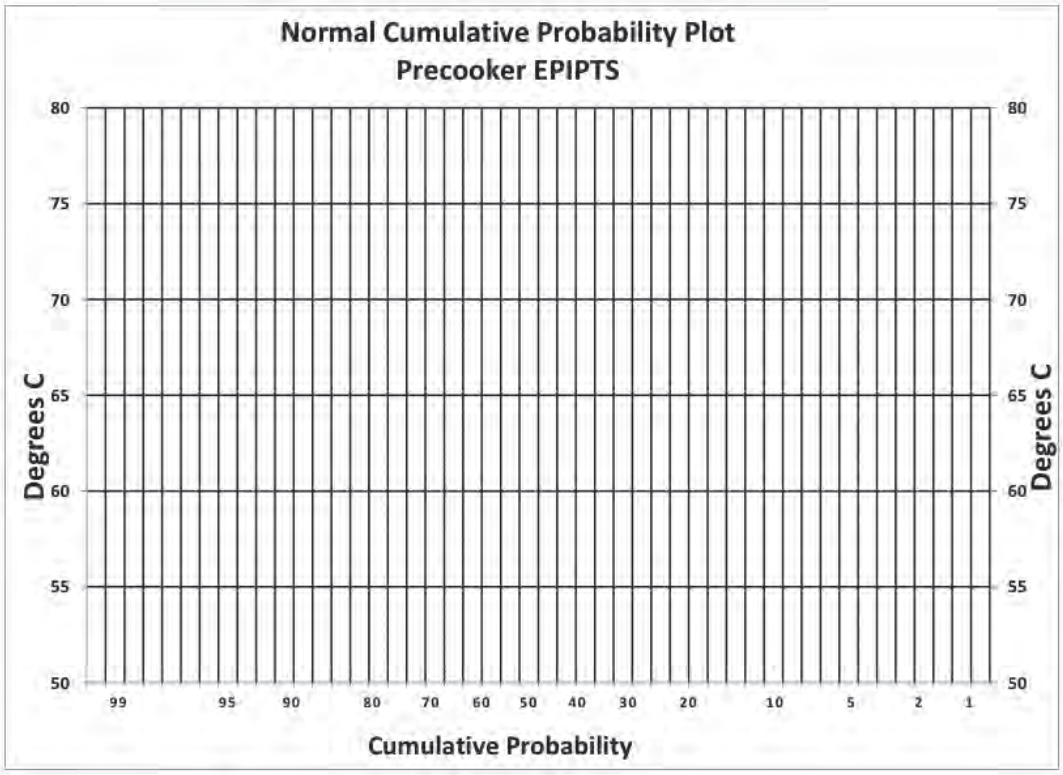
Corrective Actions Taken

APPENDIX C-2. NORMAL CUMULATIVE PROBABILITY PLOT OF CORE TEMPERATURES AT THE END OF PRECOOKING



APPENDIX C-3. BUILD A NORMAL PROBABILITY PLOT TEMPLATE

1. Open a fresh Exc]el spreadsheet
2. Starting in Col A, in cells A2 through A482 enter a list of values from +2.4 thru -2.40, in 0.01 increments
3. In Col B enter the formula "= (Normdist (A2,0,1, True)", copy down to B482
4. In Col C enter = text (100*Value(B2),"0") – copy down, converts Col B to text
5. Edit Col C so only the values 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99 show
6. Col D enter a series of values like D2 "= A2 + 55" – Fill the column
7. Col E enter a series of values like E2 "= A2 + 65" – Fill the column
8. Create a Line Chart with Col C = X-axis
9. Col D = Y1 axis
10. Col E = Y2 axis – Plot on 2nd axis
11. Format Y1 and Y2 axis as fix values from 50 to 80
12. Hi-Lite the data series for Y1, and Y2 and click on "no line"



APPENDIX C-4. ATTRIBUTE ACCEPTANCE SAMPLING PLAN — TALLY SHEET

	Sample Size		
	Reliability		
	Conf Level	95%	99%
	95%	59	299

Tools
Thermometers
Pen

Tally sheet – Acceptable - Yes or no

°C	Frequency distribution of temperatures	Freq
73		
72		
71		
70		
69	//// \/// \/// \/// \/// \///	29
68	//// \/// \/// \/// \/// \/// \	30
67	//// \/// \/// \/// \/// \/// \	30
66	//// \/// \/// \/// \/// \/// \	30
65	//// \/// \/// \/// \/// \/// \	30
64	//// \/// \/// \/// \/// \/// \	30
63	//// \/// \/// \/// \/// \/// \	30
62	//// \/// \/// \/// \/// \/// \	30
61	//// \/// \/// \/// \/// \/// \	30
60	//// \/// \/// \/// \/// \/// \	30
59		
58	/	1
57		
56		
55		

# of EPIPTs Passes	299
# of EPIPTs Fails	1

Action Plans

Measure temperatures from 299 fish
All Pass - OK
1 Fail - Recook
If 298 pass, and 299 fails, recook

Corrective Actions
Recook