Guideline for validation of thermal processes for reduction of *Salmonella* in cocoa processing

Working title: CocoaVal

First edition
Date: November 2024

Working group:
Validation experts by *ofi*, Cargill and BarryCallebaut

Reviewed by:
Relevant expert members of ECA and Caobisco, with acknowledgment to Mondelez, Nestle and Mars

Kindly supported and distributed by:
ECA and Caobisco

Table of Contents

1. Background

Within the food industry application of HACCP principles is required by EU and USA legislation (EC 178/2002; FSMA), as well as outlined in Codex Alimentarius guidelines. The application of HACCP principles put the focus on process controls. That includes the requirement of validations for control measures (process steps), where significant hazards are meant to be controlled. In that context validation is meant as "Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome" (Codex Alimentarius CAC/GL 69-2008). To support and harmonize industry approaches specifically in the validations of biological hazards, several guidelines for specific products have been published (e.g. ABC guideline, AIOE document 2012), and also a general guideline on how to approach that and what to consider is available (Ceylan et al., 2021).

In cocoa and cocoa-based products (e.g. chocolates) *Salmonella* has been recognized as the major pathogen of concern (ICMSF, FDA Appendix 1). Currently, there is no legal requirement for a specific log reduction of *Salmonella* in cocoa processing. However, general guidelines and approaches related to low moisture foods exist (GMA 2012, Schaffner et al, 2013) and can be used in an assessment to determine the appropriate target log reduction. Also, quantitative microbiological risk assessment has been recognized as a tool to define target performance criteria. For cocoa such an assessment has been recently presented at IAFP Europe (2024, Geneva[1]) providing support to determine the target log reduction in this commodity. Based on all the available information, for cocoa beans, nib and liquor a minimum 5-log reduction of *Salmonella* is currently considered adequate. Even when one thermal treatment step has been validated there are several other thermal treatment steps in the cocoa processing that might lead to significant log reduction of pathogens. However, evaluation of all those steps is not in the scope of this document.
So far there is no specific validation guideline available for cocoa processing which leads to ongoing discussions in industry towards the acceptance of validations, potentially resulting in double work / costs and in the worst case in incorrect outcomes and conclusions. To address that, this guidance was put together by a group of cocoa processors to align on validation approaches and thereby allow for comparison and increase acceptance of the validation studies performed.

Validation can be performed by a third party or by internal specialists. Both approaches have their advantages and disadvantages:
Advantages of conducting the validation through the use of external organizations.

- Evaluation is performed by unbiased, emotionally or economically not involved third party.
- Identification of issues that might escape the attention of the developer.
- Acceptance by the customers

Advantages of using internal experts (process authority):

- Detailed knowledge of the process
- Identification of issues that might escape the attention of the external party due to unfamiliarity with processing.

The validation process has several stages, generally these are:

[1] International Association for Food Protection, European Symposium

- Defining the validation approach
- Defining the team (including any external parties, where applicable) to coordinate and execute the validation
- Pre-validation work
- Validation work
- Interpretation of Results & documentation

The identified control step (usually a thermal process step including moisture increase due to water/steam additions) of each processing line should be validated. The validation approach will depend on the situation and technical aspects of the process line under validation. Due to the high complexity of processes in cocoa the currently preferred approach for validation in a processing environment is a validation using surrogate microorganisms for *Salmonella*. Another reason for validation by using surrogate is due to the inherent variability of these processes. To account for the process variability at least 3 runs are considered in the validation. The surrogate microorganism(s) should be carefully selected and show equivalent or slightly higher heat resistance in the process to be validated as the target pathogen.
In cases where using the surrogate is not feasible or limited by the process (e.g. liquor sterilization), laboratory (thermal death time (TDT) studies) or pilot plant studies may be used as a validation approach provided that all process parameters are considered.

The different validation stages are described below including the specific requirements per stage to enable a successful execution of a validation study.

2. Responsibilities

2.1. Responsibility of the validation site team

Site management should recognize that without their commitment, the validation team will not be provided with the resources required to ensure a successful validation outcome.
Each validation site team should:

- Include competent staff providing required expertise (e.g. HACCP team, engineering team, R&D team) to enable successful validation. By having in depth knowledge of:
    - The process and the equipment (e.g. process engineer, R&D, equipment suppliers),
    - Production flow and production process with respective parameters, also taking into account the product and process variability
    - Food safety plan / HACCP study at the plant (HACCP team)
    - Design of the study, including heat treatment studies and temperature distribution studies (process authority)
    - Microbiological methods (microbiologists)
    - (Microbiological) data analysis and knowledge about scientific experimental design
    - Conducting similar validation studies in the past
    - Human safety representative at the plant
    - Work in the laboratory to support study preparation

Once assembled the validation site team should:

- Be actively involved in the validation procedure of each line and should manage the timelines in the validation program and the deadline during the execution of validations.

- Each validation site team should have a team leader who coordinates the validation work including preparation of documents (e.g. validation protocol, validation report etc), laboratory analyses, data analysis.
- Be critically managing all external and internal stakeholders involved in the validation process and validation program.
- The validation site team should perform the validation independent of the site's operational priorities.
- Ensure that critical measurements in the line (temperature probes, load cells, water flow/dosing sensors etc.) are calibrated periodically (at least annually).
- Based on the outcome of the validation study establish critical process parameters and limits. Implement critical control point (CCP), monitoring and verification.

2.2. Responsibility of the process authority

Based on Institute for Thermal Processing Specialists (IFTPS) definition, the process authority (an individual, or group), should have expertise in the following (but note this list is by no means inclusive or exclusive):

- Knowledge of microbial risks
- Product characteristics
- Industrial food production and industrial thermal processes
- Knowledge and understanding of the appropriate design and methods of conducting heat treatment validation studies in low moisture food
- Applicable food safety and regulatory requirements
- Sample preparation, including sample homogeneity
- Microbiology and laboratory testing
- Ability to analyse data generated by scientific studies using statistical analysis, process calculations, analysis tools, and evaluation techniques related to thermal processing;
- Experience and ability to identify and evaluate process deviations
- Reporting the validation study outcomes, including requirements and recommendations

The process authority needs to be independent and unbiassed which would include consideration given to the reporting structure of the organisation.

Each process authority should define the expertise required in the validation site team.

Each process authority is responsible for the reliability of the execution and the results obtained during validation. The validation authority should be able to confirm the integrity of each validation and, when required, to demonstrate that no fraud took place.

3. Procedure for validation of thermal processes

Within cocoa processing there are several (heat-processing) steps which could be used as control measures for *Salmonella*. Typical examples of those are:

- Cocoa nibs or beans steam treatment (ambient pressure or vacuum).
- Cocoa alkalization treatments, often combined with roasting
- Cocoa liquor heat treatment
- Dry roasting processes (cocoa beans or nibs)
- Cocoa butter de-odorization.

The step(s) to be chosen as control measures at a particular line should be decided by the site HACCP team. To support their decisions the following considerations should be made:

1. Heat resistance of *Salmonella* (and other microorganisms) is significantly higher in dry heat. Therefore, preference should be given to processes where moisture is increased / added during processing (e.g. steam or water).
2. Dry processes show higher variability in their processing compared to wet processes. Therefore, more data may be needed in the validation of dry processes.
3. The choice of the control measure will also determine the segregation / zoning in a plant, i.e. separation between raw and processed areas. The consequences for infrastructure / movements / utilities need to be taken into account as well.
4. Critical parameters for the equipment / process steps can be determined based on the knowledge of the process.
5. Adequate monitoring options for critical parameters need to be in place for each process step. Depending on the equipment / process that can include (but is not limited to) temperatures during processing, pressure, time or throughput, moisture values / additions, ingoing product temperatures (especially for continuous processes), product bed depth, hot air dynamics and/or air velocity.
6. Worst case conditions are defined by the site team together with R&D / engineering / production and can be monitored.
7. Equipment design usually varies from one line to another, therefore, each line / step should be validated individually.
8. Validation of one step/line/equipment and then transferring the results to another **equivalent** line should be approached with caution and only enacted when carefully reviewed by the validation site team and approved by the process authority. It is important to keep in mind that the same equipment can be used with different recipes, linked to different utilities and run under different conditions at separate lines – and in those cases "grouping" of validations would not be adequate.

Minimum information for each validation are:

- Product characterization (product type: beans, nibs, liquor)
- Intrinsic product properties (moisture, water activity) and their variations
- Understanding equipment mode of action and thereby being able to determine the critical parameters for the process to control biological hazards
- Understanding of the variability and process capability of the process under validation, e.g. by using thermal mapping, variable in steam delivery, pressure etc. These variabilities are depending on the specific process type.
- Calibration of sensors
- Weak spots identification, e.g. cold spots in tanks, which will depend on the equipment used and the process
- Start up and end of the run procedure.

Validation approaches:

- Inoculation study with the appropriate surrogate microorganism(s) with known thermal resistance compared to *Salmonella*. That would require a pre-study (Thermal Death Time (TDT) study) to ensure the equivalent heat resistance of the chosen surrogate compared to *Salmonella* when undergoing the same process conditions.

- Validation process by mimicking the industrial process conditions in a laboratory (TDT study) or in the pilot plant. That is only possible, when those industrial process conditions can be mirrored in a laboratory/pilot plant, and the process allows for equivalent monitoring.

- Validation by using process data (potential future option, e.g. AI)- this validation strategy is only applicable if the line does not allow any other way for validation and a large amount of relevant historical and published data is available. Before starting the validation, the quality of the historical data should be validated for its suitability to be used for modeling purposes. The availability of advanced statistical and modeling expertise is a prerequisite for this type of validation as are:
    - Adequate predictive (mathematical) models that are able to mimic the process / product situation
    - A review of historical data available for the current process and
    - A review of methods and protocols to obtain the historical data and published literature on inactivation kinetics,
    - Identification of "weak" spots of the process
    - Careful analysis of results and data evaluation
    - Interpretation of the findings in the light of the validation procedure

The validation process has different phases: pre-validation, validation protocols, validation study, data evaluation, validation report and implementation.

3.1. Pre-validation

The pre-validation concerns evaluation of the routine and worst-case conditions at the lines that will be validated. During this phase information is collected and clustered to:

- Select lines and process step(s) for validation
- Define worst-case conditions to be validated
- Build validation site team and qualification of the validation lead
- Describe Product and Process, and their respective variabilities
- Ensure that sensors to measure defined critical parameters are installed and calibrated
- Describe all equipment with potential modifications
- Define success criteria

Validation site team analyzes the information and advises on the worst-case conditions for the validation. The theoretical log reduction can be calculated with various thermal conditions to investigate and predict the success of the validation with defined worst-case conditions.

3.2. Validation protocol

The validation site team should prepare the validation protocol to describe in detail the execution of the study. The validation protocol should include:

- Identified target pathogen to control
- Desired log reduction
- Surrogate selection (Hu and Gurtler, 2017)
    - surrogate selection should be the first step of the validation program, the results can be used for all following validations for the same product and process.

- The main criteria for the surrogate selected for process validation studies in food processing and pilot plant facilities are:
  - must be nonpathogenic to humans.
  - not causing spoilage (less relevant for low water activity products like cocoa)
  - have equivalent resistance when exposed to the same process conditions as the target organism (*Salmonella* spp. for cocoa)
  - possible to quantify using relatively simple methodology
  - easy to differentiate from other microbes
  - surrogate populations remain stable until utilized
- while there is no requirement towards usage of a specific surrogate in cocoa industry, the use of *Enterococcus faecium* ATCC 8459 (NRRL B-2354) as a surrogate organism for *Salmonella* during heat treatment of cocoa products is well documented (Potter et al., 2017).

- Procedure for surrogate qualification

  Surrogate qualification is a laboratory comparison of the thermal resistance of the surrogate microorganisms and pathogen (e.g. *Salmonella*) at the defined temperature, moisture, water activity relevant for the product and process (i.e. close to industrial process settings for worst case scenario)
  - TDT (thermal death time) study (comparison between heat resistance of surrogate and *Salmonella*) performed for product used in validation (e.g. cocoa beans, nibs, liquor)
  - Product relevant *Salmonella* strains and preferably highly thermoresistant *Salmonella* strains should be included in the surrogate qualification
  - *Salmonella* strains can be used as a "cocktail", i.e. mixture of strains
  - Inoculation method should be the same as that subsequently used in the validation study
  - Inoculation method should resemble as close as possible the natural contamination in the relevant cocoa matrix to provide a stable level of inoculum
  - TDT study should be available and referenced in validation report
  - It is recommended to perform surrogate qualification for every "batch" of inoculated material
  - The same laboratory requirements as outlined in chapter below should be applied

- Inoculation methodology and Product characteristics
  - Product should be described in detail including any variability in characteristics such as water activity (a_w), moisture, bulk density, background microbial flora etc.)
  - The inoculation procedure should be described and justified to achieve the same target level of studied microorganism in all test (homogeneity test) units without significantly modifying the product physico-chemical characteristics (pH, moisture and/or a_w included) (ISO 20976-2). The homogeneity of the inoculated material should be verified during the validation.

- Surrogate initial inoculation level and standard deviation should be described based on recommended practice in microbiological challenge tests (example: 0,3 – 0,5log) with a minimum of 3 inoculated samples tested.
- It is recommended to start with inoculation levels approximately 1 to 2 log higher than the target log reduction
- Instructions on chemical/physical parameters to be measured on inoculated and non-inoculated matrix/product.
- Stability of the inoculated material should be evaluated to make sure it meets the requirements for in plant validations
- Enumeration of inoculum must be done on the same medium that will be used for the validation study.

- Sample preparation
  - Instructions on how to handle the inoculated material
  - Instructions on how to prepare the initial and validation samples
  - Instructions on number of samples required per validation run, which will depend on process variability and capability (e.g. 3 - 5 samples may be sufficient for less variable processes, whereas 10 samples may be needed for others); also need to include samples for inoculation and non-inoculation controls / travel controls (when inoculated samples will be transported to a laboratory)
  - Instructions on chemical/physical parameters to be measured on inoculated and non-inoculated matrix/product.
  - (optional) Usage of external thermal data loggers.

- Execution of the validation runs:
  - Instruction on how to handle the validation sample before and during the validation process, including how to confine the samples (e.g. cages, "tea ball", bag), where to insert in equipment, how to retrieve etc.
  - Validation samples should be inserted and retrieved as close as possible to the process to be validated
  - Instruction on how to handle the validation samples after the process (i.e. chilling)
  - Instruction on labelling of samples
  - Instructions on chemical/physical parameters to be measured on inoculated and non-inoculated matrix/product.
  - Instructions on packaging of the samples for transportation, e.g. temperature, box, cooling, maximum transport time

- Instructions to the laboratory
  - Handling and storage of the samples upon receipt
  - Maximum storage time and temperature
  - Parameters to be measured, including microbiological, chemical and physical
  - Required replicates per sample and sample size to be analyzed
  - Reporting of the results

- Settings of the (worst-case) conditions to be validated, e.g.
  - Moisture content – adding moisture or steam
  - Homogeneity of the products – when to start the test

- Temperature interval (max-min allowed)
- Time interval (max-min allowed)
- Pressure interval (max-min allowed)
- Vacuum interval (max-min allowed)
- Capacity throughput
- Timeline: start and stop of the validation

3.3. Validation study

Each validation study should collect a complete set of results from at least 3 trials with the worst-case conditions performed during different runs. The thermal profile of each trial will be extracted and included in the validation report. Statistically representative sample sets should be collected for microbiological analysis.

3.3.1. Expertise requirements on-site

- During the validation study the site should provide adequate professionals and experts to understand and execute the validation protocol and ensure the safety of the employees involved.

- The validation study is executed by the professionals and experts from the site under the supervision of the process authority.

- Supervision can be physical, on-line or "on demand". When the supervision is "on demand" the validation study is prepared and executed by the professionals and experts from the site, the validation authority should have evidence for the integrity of the validation process, e.g. thermal profiles, pictures, movies, involvement of independent internal validation supervisor etc.

3.3.2. Laboratory requirements

- The validation samples should be analyzed by a competent laboratory.
- Competency can be shown either by accepted accreditations and/or internal Quality Assurances.
- The analytical methods (microbiological, chemical, physical) used for the validation should be accredited or at least validated and verified according to relevant ISO documents and for the relevant matrix.

  Example: if the process of cocoa powder sterilization is under validation, the laboratory should define the performance characteristics of the analytical methods on cocoa powder. For the relevant matrices, the analytical methods should have defined repeatability (*r*), intra-lab reproducibility ($R_{intra\text{-}lab}$), where applicable inter-lab reproducibility ($R_{inter\text{-}lab}$), accuracy and measurement uncertainty. When analyzing the validation sample the variability of the obtained results should comply with the limits set by the performance characteristics for the analytical methods.

- The performed analysis (microbiological, chemical, physical) should be adequate for the purpose of the validation and deliver unambiguous results.
  Example: If the log reduction is calculated from the counts of surrogate microorganisms (e.g. *Enterococcus faecium*), semi-specific medium can be used. Additionally, total plate count should be analyzed to evaluate the possibility for biased surrogate

microorganisms results by background microflora. If the results show high numbers on both media the accuracy of the semi-specific medium should be confirmed by additional colony identification.

3.3.3. Data evaluation

The evaluation and interpretation of the collected results should be performed by the validation site team.

The results of the critical parameters (e.g. time, temperature, pressure etc) collected from each trial should be available. The use of the worst-case conditions during each trial should be evaluated and confirmed. The critical parameter readings can be compared (when possible) with other data collected during the trials, e.g. external temperature loggers can be included in the validation.
If the worst-case conditions, in terms of time, temperature and moisture, were not applied the trial should be dismissed and a new trial should be conducted.

The log reduction of the surrogate should be calculated from the results collected during each validation trial. The log reduction is expressed as the difference in surrogate levels counted on non-processed (travel controls) and processed material (samples). The validity of the microbiological results should be verified by microbiological experts. Microbiological results can be excluded from the evaluation only if there is microbiological reason for that.
Any discrepancy in the obtained microbiological results should be justified.
The microbiological results can be linked to measured thermal treatment per sample, if available.

The calculated minimum log reduction is the acceptance limit for the validation.

3.3.4. Validation report

Validation results should be prepared by the validation experts and presented in a validation report. The validation report should include (see also Ceylan et al., 2021):

- Equipment under validation
- The conditions of the validation
- Validation protocol including surrogate qualifications
- Critical parameters settings and readings from at least 3 trials
- Microbiological and analytical methodology used in the validation
- Analysis of the results of all measured parameters relevant for the validation
- Analysis of microbiological results and their interpretation
- Log reduction calculations
- If applicable modelling methods and references, e.g. using TDT study or process data
- Conclusion on the validation
- Comparison of conditions used in reference validation or site recipe, with the establishment of operational settings accordingly
- Reference and supporting information

The validation authority should revise and approve the conclusions in the validation report.

4. Re-validation

There is no pre-defined time-based frequency for process re-validation. Re-validation should be considered in case of:

- New equipment (e.g. new roaster) installation or changes to the equipment that impact heat distribution e.g. installation of drum with different design.
- Equipment is moved to another location / line.
- Changes in the recipe/settings outside of the validated ranges - e.g. lower temp., shorter time, lower amount of water addition, line capacity/throughput
- Verification failure
- New scientific data or change in the performance criteria (towards more conservative) or target organisms

Validated processes should be reevaluated at a defined frequency (e. g. 24 months, or as dictated by an assessment) to evaluate performance against established critical parameters.

References

ABC (Almond Board of California Technical Expert Panel) (2014) Guidelines for using *Enterococcus faecium* NRRL B-2354 as a surrogate microorganism in almond process validation.

AIOE (GMA) (2012) Validating the reduction of *Salmonella* and other pathogens in heat processed low-moisture foods.

Ceylan, E., Amezquita, A., Anderson, N., Betts, R., Blayo, L., Garces-Vega, F., Gkogka, E., Harris, L. J., McClure, P., Winkler, A., den Besten, H. M. W. (2021) Guidance on Validation of Lethal Control Measures for Foodborne Pathogens in Foods. Comp. Reviews in Food Science and Food Safety. DOI: 10.1111/1541-4337.12746

Codex Alimentarius. GUIDELINES FOR THE VALIDATION OF FOOD SAFETY CONTROL MEASURES. CAC/GL 69 – 2008

Codex Alimentarius. GENERAL PRINCIPLES OF FOOD HYGIENE. CXC 1-1969 (update 2023)

EC 178/2002 https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002R0178-20220701 (accessed May 26, 2024)

FSMA (US FDA) Food Safety Modernization Act (FSMA) | FDA (accessed May 26, 2024)

FDA Appendix 1 (2024) Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventive Controls for Human Food [Draft Guidance for Industry: Hazard Analysis and Risk-Based Preventive Controls for Human Food | FDA]() (accessed May 26, 2024)

Hu, M. and Gurtler, J. B. (2017) Selection of Surrogate Bacteria for Use in Food Safety Challenge Studies: A Review. Journal of Food Protection, Vol. 80, No. 9, 2017, Pages 1506–1536 doi:10.4315/0362-028X.JFP-16-536

ISO 20976-2:2022 Microbiology of the food chain – Requirements and guidelines for conducting challenge test for food and feed products - Part 2: Challenge test to study inactivation potential and kinetic parameters.

ICMSF International Commission on Microbiological Specifications for Foods) Volume 8 (2011) Microorganisms in Foods: use of data for assessing process control and product acceptance. Springer, London

Potter, L., Limburn, R., Gaze, J., Ballard, C. and Leadley, C.E. (2017) Development of low Aw food and environmental decontamination: inactivation kinetics. Campden BRI R&D report.

Schaffner, D. W., Buchanan, R. L., Calhoun, S., Danyluk, M. D., Harris, L. J., Djordjevic, D. Whiting, R. C., Kottapalli, B., Wiedmann, M. (2013) Issues To Consider When Setting Intervention Targets with Limited Data for Low-Moisture Food Commodities: A Peanut Case Study. J. Food Prot. 76 (2): 360-369.