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Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products —

Part 2: **Challenge tests to study inactivation potential and kinetic parameters**

Microbiologie de la chaîne alimentaire — Exigences et lignes directrices pour la réalisation des tests d'épreuve microbiologiques —

Partie 2: Tests d'inactivation pour étudier le potentiel d'inactivation et les paramètres de la cinétique d'inactivation

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 20976 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Under the general principles of the Codex Alimentarius on food hygiene, it is the responsibility of the food business operators (FBOs) to control microbiological hazards in foods and to manage microbial risks. Therefore, FBOs implement validated control measures, within the hazard analysis and critical control point (HACCP) system, and conducts studies in order to investigate compliance with the food safety criteria throughout the food chain.

In the framework of microbial risk assessment (MRA), several complementary approaches are developed to estimate risks posed by pathogens or spoilage microorganisms in the food chain. MRA is adopted by regulators under the auspices of the international agency for setting food standards. Challenge testing is one of the recognized approaches used to validate control measures within the HACCP system, as well as to assess microbiological safety and quality of food, food production processes, food storage conditions, and food preparation recommendations dedicated to consumers.

Therefore, this document provides technical rules, calculations and approaches to investigate the ability of an inoculated microorganism of concern to grow, survive or be inactivated in the raw materials, intermediate or end products under reasonably foreseeable food processes, storage and use conditions. The objective and the scope of the study are to determine the experimental design and the selection of the study conditions, and to assess the extent of microbial inactivation. Regulatory authorities can have different recommendations, and these differences have been included as much as possible. It is, however, possible that specific requirements need to be incorporated to get a regulatory approval of the challenge test.

As the growth and inactivation studies are clearly different, the ISO 20976 series consists of two parts, under the general title *Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products*:

— *Part 1: Challenge tests to study the growth potential, lag time and the maximum growth rate*;

— *Part 2: Challenge tests to study inactivation potential and kinetic parameters*.

The use of the ISO 20976 series involves expertise in relevant areas such as food microbiology, food science, food processing and statistics. The statistical expertise encompasses an understanding of sampling theory and design of experiments, statistical analysis of microbiological data, and overview of scientifically recognized and available mathematical concepts used in predictive modelling.

For practical reasons, the term “food” includes feed.

Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products —

Part 2:
Challenge tests to study inactivation potential and kinetic parameters

1 Scope

This document specifies the protocols for conducting microbiological challenge tests for inactivation studies on vegetative bacteria and bacterial spores in the raw materials and ingredients, intermediate or end products.

The use of this document can be extended to yeasts which do not form mycelium.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
bacterial spore
resistant form of bacteria which is dormant until the *germination* (3.9) step

[SOURCE: ISO 20976-1:2019, 3.1]

3.2
batch
group or set of identifiable food obtained through a given process under practically identical circumstances and produced in a given place within one defined production period

Note 1 to entry: The batch is determined by parameters established beforehand by the organization and may be described by other terms, e.g. lot.

[SOURCE: Commission Regulation (EC) No 2073/2005, Article 2 (e)[10], modified — "food obtained through" has replaced "products obtained from" and Note 1 to entry has been added.]

3.3
bulk products
products that are not separated into individual items or units

[SOURCE: ISO/TS 17728:2015, 3.3.1]

3.4
challenge test
study of the growth or inactivation of microorganism(s) artificially inoculated in a food

[SOURCE: ISO 20976-1:2019, 3.5]

3.5
control unit
unit of food identical to the *test unit* (3.34) but not artificially inoculated (used as a blank)

[SOURCE: ISO 20976-1:2019, 3.4, modified — "inoculated" has replaced "contaminated".]

3.6
D value
decimal reduction
time or dose required to achieve reduction of 90 % of the tested microorganism under stated conditions (e.g. temperature, pH or chemical composition) in case of log linear *inactivation kinetics* (3.10)

3.7
δ value
first decimal reduction
time or dose required to achieve the first reduction of 90 % of the tested microorganism under stated conditions (e.g.: temperature, pH or chemical composition) in case of non-log linear *inactivation kinetics* (3.10)

3.8
experimental datapoint
result of analysis of a *test unit* (3.34) per unit mass, per unit volume, or per unit area

Note 1 to entry: The enumeration results may be expressed in $\log_{10}$ or most probable number (MPN).

[SOURCE: ISO 20976-1:2019, 3.6, modified — In the definition, "mass" has replaced "weight" and the units have been deleted. In Note 1 to entry, "for specific cases" has been deleted and "or most probable number (MPN)" has replaced "MPN".]

3.9
germination
mechanism in which a *bacterial spore* (3.1) initiates its transformation into a *vegetative cell* (3.36)

[SOURCE: ISO 20976-1:2019, 3.9, modified — "initiates its transformation into" has replaced "starts becoming".]

3.10
inactivation kinetics
change over time in the concentration of the target microorganism subjected to an inactivation process

3.11
inactivation parameter
mathematical estimate that describes the resistance/sensitivity of the target organism to the *treatment* (3.35), obtained by fitting *primary models* (3.18) and *secondary models* (3.24)

Note 1 to entry: Examples of these parameters are D, δ and p for the primary models and z for the secondary models.

3.12
inactivation potential
Δ value
log kill
log reduction
difference in the log concentration ($\log_{10}$ cfu/g or ml or cm^2) of the target microorganism between an earlier and a later time point expressed as $\log_{10}$

Note 1 to entry: In this document, the term "inactivation potential" refers to the type of inactivation study, and the terms "log kill" and "log reduction" refer to the result obtained.

3.13
inactivation treatment
process used to kill or inactivate the target microorganism

3.14
inoculum
microbial suspension at the desired concentration used to contaminate *test units* (3.34)

[SOURCE: ISO 20976-1:2019, 3.12]

3.15
k value
slope of the inactivation curve

3.16
p value
parameter describing the shape of the inactivation curve

3.17
pH value
measure of the concentration of acidity or alkalinity of a material in an aqueous solution

[SOURCE: ISO 5127:2017, 3.12.2.29, modified — The notes to entry have been deleted.]

3.18
primary model
mathematical model describing the changes of microbial counts as a function of time

[SOURCE: ISO 20976-1:2019, 3.16]

3.19
organizing laboratory
laboratory with responsibility for managing the *challenge tests* (3.4)

[SOURCE: ISO 20976-1:2019, 3.17]

3.20
pilot facility
manufacturing location used to run an experiment or test before introducing more widely

3.21
processing facility
location where products are made on a larger scale

3.22
sampling
selection of one or more units or portions of food such that the units or portions selected are representative of that food

[SOURCE: ISO 20976-1:2019, 3.18]

3.23
sampling point
time at which the *test units* (3.34) are taken for analyses

Note 1 to entry: When assessing *inactivation kinetics* (3.10), these are represented as *experimental datapoints* (3.8) on the inactivation graph.

[SOURCE: ISO 20976-1:2019, 3.19, modified — "taken for analyses" has replaced "analysed and which are represented as experimental datapoints on the kinetics graph" and Note 1 to entry has been added.]

3.24
secondary model
mathematical model describing the effects of the inactivation process factors (e.g. temperature, pH, a_w) on the parameters of the *primary model* (3.18) (e.g. D, δ)

[SOURCE: ISO 20976-1:2019, 3.20, modified — "inactivation process" has replaced "environmental" and "(e.g. D, δ)" has replaced "(e.g. growth rate)".]

3.25
sporulation
mechanism by which *vegetative cell* (3.36) forms spore

[SOURCE: ISO 20976-1:2019, 3.21]

3.26
surrogate
non-pathogenic microorganism that has similar or more robust *survival* (3.27) capability compared to the pathogen of concern both in the matrix and under the processing conditions being studied

3.27
survival
state of continuing to live or exist without significant increase or decrease in viability

3.28
target reduction level
target inactivation level expressed in $\log_{10}$

3.29
t_0
time at which the treatment starts

3.30
t_{end}
time at which the treatment is finished

3.31
t_{inoc}
time at which the microorganism is inoculated in the food

3.32
t_{xD}
time of treatment needed for x log reduction of the target microorganism

3.33
test portion
measured (volume or mass) representative sample taken from the *test unit* (3.34) for use in the analysis

[SOURCE: ISO 6887-1:2017, 3.5, modified — "test unit for use in the analysis" has replaced "laboratory sample for use in the preparation of the initial suspension" and Note 1 to entry has been deleted.]

3.34
test unit
measured (volume or mass) amount of the food used for inoculation, subsequent *treatment* (3.35) and analysis

[SOURCE: ISO 20976-1:2019, 3.24, modified — "subsequent treatment and analysis" has been added.]

3.35
treatment
any process, formulation or product characteristics, or a combination thereof, intended to inactivate the target microorganism

3.36
vegetative cell
state of microbial form that is capable of growing under favourable environmental conditions

[SOURCE: ISO 20976-1:2019, 3.25]

3.37
water activity
a_w
ratio of the water-vapour pressure in the foodstuff to the vapour pressure of pure water at the same temperature

[SOURCE: ISO 18787:2017, 3.1 modified — "water-vapour pressure in the foodstuff to the vapour pressure of pure water at" has replaced "partial water-vapour pressure in equilibrium with the product analysed to the water-vapour saturation pressure in equilibrium with", and the formula and the notes to entry has been deleted]

3.38
z value
change in *treatment* (3.35) (e.g. temperature, pH, a_w) that induces a 10-fold change in the *D value* (3.6)

Note 1 to entry: Temperature, pH and a_w can be indexed to the z value to denote the treatment being assessed.

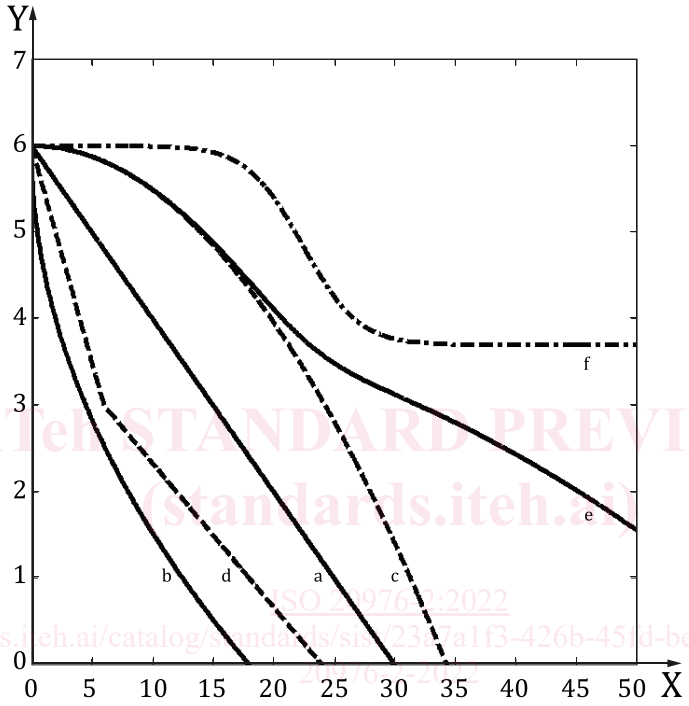
4 Principle

Inactivation studies are designed to determine the changes in the concentration of the target microorganism during the challenge test. These studies can be used to assess whether there is significant microbial inactivation in a foodstuff and to quantify the decrease in the target microorganism under a given set of processing conditions and/or formulation of the food product. The scope and the aim of the study shall be clearly specified (e.g. assessment/validation of the food process efficacy as a control measure, assessment of microbial stability and survival) including the target reduction level and the decision criteria. The experimental design shall be in accordance with that purpose and shall take into account the steps of the process and the food chain for which microbial inactivation is assessed. Knowledge from the FBO (e.g. on their products characteristics and production process) shall be combined with the expertise in food microbiology and analytical sciences to ensure the robustness of the study (see 14.3.2). Ideally, inactivation studies employ the target microorganism. In the challenge tests studies conducted within food production facilities, a validated surrogate shall be used in place of the target pathogen[26].

The organizing laboratory shall have knowledge and skills in food microbiology, food science and technology, and statistics to design and conduct the studies, interpret the results and draw the conclusions. The analyses shall be conducted under a quality assurance system (e.g. ISO/IEC 17025).

To conduct an inactivation challenge study, the inoculum should be prepared such that the microbial cells or spores have been adapted to the environmental conditions that mimic the food processing environment, thereby encouraging natural microbial response once inoculated into the food.

The same microbial strain could exhibit various shapes as a function of the treatment (see Figure 1)[14]. The heterogeneous shapes of microbial inactivation curves are the results of the microbial resistance or adaptive response or cell heterogeneity[16].



Key

X inactivation treatment (time or dose)

Y $\log_{10}(N)$

a Log-linear.

b Concave.

c Convex or with a shoulder.

d, e With a tail or biphasic.

f Sigmoïdal curve.

Figure 1 — Examples of microbial inactivation curve types

There are two types of inactivation study: the inactivation potential and the inactivation kinetics of a target microorganism.

The inactivation potential studies are most appropriate for process validation and/or product formulation. Inactivation potential results are specific to the conditions and matrix under study. To extrapolate to other conditions, inactivation kinetic parameters shall be used, or a new inactivation potential study conducted.

The inactivation kinetics studies are used to characterize the inactivation of the microorganism through the determination of inactivation parameters such as D, δ and z values. Those studies are more complex in terms of study design, execution, results interpretation and exploitation, particularly in the