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Microbiology of the food chain - Determination and use of cardinal values (ISO/DIS 23691:2024)

Mikrobiologie der Lebensmittelkette - Bestimmung und Verwendung von Kardinalwerten (ISO/DIS 23691:2024)

Microbiologie de la chaîne alimentaire - Détermination et utilisation des valeurs cardinales (ISO/DIS 23691:2024)

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Microbiology of the food chain — Determination and use of cardinal values

Microbiologie de la chaîne alimentaire — Détermination et utilisation des valeurs cardinales

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Contents

Page

Foreword ..... **v**
Introduction ..... **vi**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **2**
4 Principle **6**
4.1 General 6
4.2 Mathematical models 6
4.3 Process of cardinal values and food correction factor determination 9
4.4 Determination of the maximum specific growth rate 9
4.4.1 Binary dilution OD-based method 10
4.4.2 Direct plating method 11
4.5 Cardinal parameters determination 11
4.6 Correction factor determination 12
4.7 Validation 13
4.8 Use of cardinal values and food correction factor in predictions 14
5 Diluents, culture media and reagents **14**
6 Laboratory equipment and apparatus **14**
7 Experimental design and data collection **15**
7.1 General 15
7.2 Preparation of culture and medium 15
7.2.1 Choice and storage of studied strain 15
7.2.2 Preparation and inoculation of the microbial culture 16
7.2.3 Preparation of the modified nutrient broth 16
7.3 Levels per factor to estimate cardinal parameters 17
7.3.1 General 17
7.3.2 Temperature 17
7.3.3 pH 18
7.3.4 Water activity 19
7.3.5 Inhibitory compounds 20
7.4 Experimental design to estimate the maximum specific growth rate from the binary dilution OD-based method 21
7.5 Experimental design to estimate the maximum specific growth rate from the direct plating method 22
7.6 Determination of the food correction factor based on a challenge test 22
7.7 Validation 23
8 Expression of the results: Estimation of the growth parameters **23**
8.1 General 23
8.2 Assessment of maximum specific growth rate at each level of intrinsic or extrinsic factors (first step) 24
8.2.1 General 24
8.2.2 Assessment of maximum specific growth rates from direct plating data 24
8.2.3 Assessment of maximum specific growth rates by OD-based binary dilution method 24
8.3 Assessment of cardinal values and optimum growth rate in broth, μ_{Broth} (second step) 24
8.4 Assessment of C_f (third step) 25
8.5 Validation (fourth step) 26
9 Use of cardinal values to perform microbial growth predictions **26**
9.1 General 26

9.2 Prerequisites for growth predictions 27
9.3 Using cardinal values to simulate growth 28
9.3.1 Growth simulation at a static given temperature 28
9.3.2 Growth prediction with dynamic time-temperature scenario 29
9.3.3 Growth simulation at a static condition of temperature, pH and a_w 30
10 Test report **32**
11 Quality assurance **32**
Annex A (informative) **Indicative list of tools for primary and secondary fittings and simulations** **33**
Annex B (informative) **Guidance to obtain different aw values when using different humectants in broth** **35**
Annex C (informative) **Growth rate determination** **36**
Annex D (informative) **Plate design** **40**
Annex E (informative) **Example of the use of cardinal values for growth simulation and its variation** **41**
Bibliography **44**

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).

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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*.

Introduction

Under the general principles of the Codex Alimentarius on food hygiene, it is the responsibility of the Food Business Operators (FBO) to control microbiological hazards in foods and to manage microbial risks. Therefore, the FBO shall implement validated control measures, within the hazard analysis and critical control point (HACCP) system, and conduct 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. Predictive Microbiology is one of the recognized scientific 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, procedures and calculations to estimate the cardinal values of a microorganism of concern and use them in combination with challenge tests results to simulate and predict its growth in the raw materials, intermediate or end-products under reasonably foreseeable food processes, storage and use conditions.

To do so, different sections are developed:

— to identify the environmental factor(s) in scope (e.g. Temperature, pH, a_w, organic acids),

— to define the appropriate experimental design,

— to estimate the cardinal values of a microorganism in broth medium,

— to perform a challenge test in the matrix of interest and derive the food correction factor and the maximum microbial population density,

— to use the cardinal values and the food correction factor to predict the growth of the studied microorganism in different conditions of interest (e.g. changes in time and temperature throughout the chill chain, changes in formulation with addition of organic acids or preservatives).

Regulatory authorities may have specific recommendations, and these differences have been included as much as possible in this document. It is however possible that additional requirements need to be incorporated to get a regulatory approval of the study.

The use of the ISO 23691 involves expertise in relevant fields such as food microbiology, predictive microbiology and statistics. This 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 microbiology.

Microbiology of the food chain — Determination and use of cardinal values

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting *target microorganism(s)* are only undertaken in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

1 Scope

This document establishes basic principles and specifies requirements and methods to determine the cardinal values of bacteria and yeast strains and use them to predict microbial growth.

Four main steps are required: (1) Determination of the cardinal values in culture medium, (2) Determination of the correction factor in the target food, (3) Validation of the model and (4) Simulations.

Four environmental factors are considered: temperature, pH, a_w and inhibitors (e.g. organic acids).

NOTE Microbial competition is not considered as an inhibitor in this standard and can be addressed by proper modelling approaches.

The determination of cardinal values requires a two-step approach:

— the determination of maximum specific growth rates of the studied strain grown in broth under a defined range of values of the studied environmental factor(s), and

— the use of recognized predictive microbiology secondary models to fit the obtained experimental data to obtain the cardinal values.

The use of cardinal values in microbial growth simulation is based on predictive microbiology primary and secondary models. The cardinal values are combined with challenge test data to consider the matrix effect. Depending on the goal of the growth simulation, it is important to account for variation of cardinal values between strains within a bacterial or yeasts species.

Cardinal values are a good indicator of a strain growth ability for the studied environmental factors. They are therefore used as criteria to select strains, in addition to their origin and virulence, when performing growth challenge tests (standard ISO 20976-1) or in methods validation (ISO 16140 standards serie).

NOTE This document focuses on the determination of cardinal values for one strain. The same methodology can be used to characterize multiple strains independently to cover biological strain variability and include these results in the predictions.

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 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*

ISO 20976-1:2019, *Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products — Part 1: Challenge tests to study growth potential, lag time and maximum growth rate*

ISO 18787:2017, *Foodstuffs — Determination of water activity*

ISO 16140-2:2016, *Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*

ISO 5127:2017, *Information and documentation — Foundation and vocabulary*

3 Terms and definitions

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

ISO and IEC maintain terminological 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
binary dilution
method used to stepwise dilute a microbial suspension with a constant dilution factor of 2 in each step

3.2
biological independent replicate
refers to an experiment that has been performed using a newly prepared culture and a newly prepared medium

3.3
cardinal factor
extrinsic (Temperature) and intrinsic characteristics (pH, a_w, inhibitors) for which cardinal values are derived

3.4
cardinal parameter
cardinal value
estimated minimum, optimum or maximum values of extrinsic and intrinsic factors (e.g. temperature, pH, a_w, inhibitors) that characterize the growth of a given microbial strain

3.5
challenge test
study of the growth (or inactivation) of microorganism(s) artificially inoculated in food

3.6
coefficient of variation (CV)
ratio of the standard deviation to the mean

3.7
correction factor
dimensionless value used to link the broth and the food optimum growth rates. It is the ratio of the optimum growth rate estimated in the studied matrix ($\dot{\mu}_{Food}$) to the optimum growth rate value estimated in broth ($\dot{\mu}_{Broth}$)

3.8
detection time (td)
time at which the optical density (OD) reaches the pre-defined target during the exponential growth

3.9
exponential growth phase
phase during which the multiplication of the microbial population is the fastest. It is in this phase that the maximum specific growth rate is reached

3.10
extrinsic factor
factor in the surrounding environment of the food or the broth, such as temperature or packaging gaseous composition, which affects the growth kinetics of the microorganism

3.11
gamma concept
ɣ
the gamma concept establishes that intrinsic (e.g. pH, water activity, inhibitors) and extrinsic factors (e.g. temperature, packaging gaseous composition) affect the maximum specific growth rate independently, using linear and or non-linear functions e.g. ɣ(temperature), ɣ(pH), ɣ(water activity), ɣ(inhibitors), normalized between zero (no growth) and one (optimum condition for growth). When combined, the effect of the factors is multiplicative

3.12
gamma function
ɣ(X)
non linear, dimensionless function, normalized between zero (no growth) and one (optimum condition for growth) describing the relative effect of a studied factor (X) on the maximum specific growth rate (e.g. ɣ(temperature), ɣ(pH), ɣ(water activity), ɣ(inhibitors))

3.13
growth curve
graphic representation of the increasing number of living cells of a microbial population in any given intrinsic and extrinsic condition over a period of time

3.14
inoculum
microbial suspension used to contaminate the studied food or broth at a desired concentration

3.15
intrinsic factor
factor related to the food matrix itself or the broth, such as nutrients, water activity, organic acids or pH, and which affects the growth kinetics of the micro-organism

3.16
lag phase
phase, directly after inoculation, during which the microbial population is adapting to the environment, before it enters the *exponential growth phase* (3.9)

3.17
lag time
λ
kinetic parameter in time unit to characterize the duration of the *lag phase* (3.16)

3.18
maximum specific growth rate
μ_{max}
kinetic parameter (h^{-1}) to characterize the *exponential growth phase* (3.9), represented by the slope of the curve showing the evolution of the natural logarithm of the population as a function of time, under constant growth conditions When the maximum specific growth rate is estimated in food, this is noted $\mu_{max.food}$

3.19
Minimal Inhibitory Concentration
MIC
estimated parameter representing the lowest concentration of an inhibitor that gives a value of maximum specific growth rate of zero

3.20
modified broth
culture medium with specific composition (e.g. increased salt content) or characteristic (e.g. pH) to study intrinsic factors

3.21
Monte Carlo simulation
iterative random sampling method that propagates variability about model parameters to approximate the distribution of input variables. Monte Carlo simulations are extensively used in quantitative risk assessment and decision making

3.22
optimum growth rate
$\dot{\mu}$
highest value among the maximum specific growth rates, estimated at the optimum conditions for growth of the microorganism in a studied food or broth

3.23
optimum growth rate in broth
$\dot{\mu}_{Broth}$
highest value among the maximum specific growth rates in broth, estimated at the optimum conditions for growth of the microorganism

3.24
optimum growth rate in food
$\dot{\mu}_{Food}$
highest value among the maximum specific growth rates in the food, estimated at the optimum conditions for growth of the microorganism

Note 1 to entry: $\dot{\mu}_{Food}$ is a statistical parameter and is not measured in the food.

Note 2 to entry: $\dot{\mu}_{Food}$ is a mathematical result obtained when all studied factors are at their optimum values and the respective ɣ terms are equal to 1.

3.25
organizing laboratories
laboratories with responsibility for determining the *cardinal values* (3.4) and performing the simulations Data collection and data analysis (including fitting and simulation) are performed in a single or in multiple laboratories.

3.26
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 — Notes 1 and 2 to entry have been removed.]

3.27
pKa
quantitative measure (negative base-10 logarithm) of the acid dissociation constant or Ka value, which indicates the strength of an acid in solution (the lower the pKa value the stronger the acid)

3.28
primary model
mathematical model describing the changes of microbial concentration as a function of time under constant and known conditions of intrinsic and / or extrinsic factor(s)

3.29
relative standard error
r
standard error (se) (3.31) divided by the parameter estimate and expressed as a percentage

3.30
secondary model
mathematical model describing the effects of the intrinsic and / or extrinsic factor(s) (e.g. temperature, pH, a_w) on the parameters of the *primary model* (3.28) (e.g. maximum specific growth rate)

3.31
standard error
se
measure of the uncertainty associated with the estimated parameter or the overall model fit

3.32
stationary phase
phase in which the microbial population no longer increases (reaches and remains at its maximum concentration)

3.33
strong acid
is characterized by its negative pKa. It ionizes completely in an aqueous solution by losing one proton. Hydrochloric and sulfuric acids are examples of strong acids

3.34
uncertainty
refers to variation that originates from lack of or incomplete knowledge of some characteristics of a system. It originates from parameter uncertainty and model uncertainty. Sources of parameter uncertainty include lack of data, measurement errors, sampling errors and systematic errors. Sources of model uncertainty include model structure, excluded variables, model resolution, extrapolation. The standard error represents the uncertainty associated with the parameter

3.35
variability
refers to variation that is inherent to a given system, typically as a result of true heterogeneity of the studied population and is irreducible by additional measurement. Three variation sources are distinguished: between strain variability (intraspecies variability), within strain variability and analytical variability. The between strain variability is not included in this standard as it is designed to study only one strain at a time. The standard deviation represents the within strain biological variability associated with the parameter

3.36
water activity
a_w
ratio of the water-vapor pressure in the medium or foodstuff to the vapor pressure of pure water at the same temperature. It represents the water available for the microorganisms to use

[SOURCE: ISO 18787:2017, 3.1, modified — The definition has been condensed and the formula and Notes 1 and 2 to entry have been removed. The sentence “It represents the water available for the microorganisms to use” was added.]

3.37
weak acid
is characterized by its high pKa. It does not dissociate completely in aqueous solution. Acetic acid and citric acid are examples of weak acids

4 Principle

4.1 General

The general equation used to describe the effect of different independent intrinsic and extrinsic factors on the maximum specific growth rate of a microorganism is based on a modular approach called the "gamma concept"[28] and described in Equation (1).

$$\mu_{max} = \dot{\mu} \cdot \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \cdot \gamma\,(\text{inhibitors}) \quad (1)$$

where

μ_{max}	maximum specific growth rate (h^{-1}) of the studied strain in the matrix;
$\dot{\mu}$	optimum growth rate (h^{-1}) of the studied strain in the matrix;
$\gamma(T)$	dimensionless function describing the relative effect of the Temperature on microbial growth;
$\gamma(pH)$	dimensionless function describing the relative effect of the pH on microbial growth;
$\gamma(a_w)$	dimensionless function describing the relative effect of the a_w on microbial growth;
γ(inhibitors)	dimensionless functions describing the relative effect of different measurable inhibitors like the undissociated form of the weak (organic) acids (HA) or CO_2.

The γ terms all vary between 0 and 1, $\gamma = 0$ when growth is fully inhibited by the studied factor, and $\gamma = 1$ when growth is not at all inhibited by the studied factor.

There are various secondary models available in the literature to describe the mathematical expression of the gamma terms. In this standard, the cardinal models are used and presented in 4.2.

For the adequate use of the models and interpretation of data, knowledge of and experience in using predictive microbiology models is essential.

4.2 Mathematical models

Under the gamma concept, the different intrinsic and extrinsic factors (e.g. temperature, pH, water activity, inhibitors) have separate and independent effects on the maximum specific growth rate, which implies that the cardinal values associated with a factor are also estimated separately and independently.

Various mathematical models have been developed in the literature.

— For describing the effects of temperature, one of the two following models shall be used: the CTMI (Cardinal Temperature Model with Inflection) model (Equation 2) shall be used when optimal and super-optimal temperatures are required[25] while the restricted Ratkowsky (linear) model (Equation 3)[21] shall be used when the input temperature ranges from the minimum supporting growth up to a reference temperature that is below the optimal temperature.