Predictive tools and strategies for establishing risk-based Microbiological Criteria in Foods

Valero A.

in

Sanchís V. (ed.), Líebana E. (ed.), Romagosa I. (ed.), López-Francos A. (ed.).
Food safety challenges for mediterranean products

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 111

2015
pages 67-74

Predictive tools and strategies for establishing risk-based Microbiological Criteria in Foods

A. Valero

Department of Food Science and Technology, Faculty of Veterinary, University of Cordoba, Campus de Rabanales s/n, Edif. Darwin-Anexo (Spain)

Abstract. One of the fundamental objectives of food legislation is the assurance of an appropriate level of health protection, as already stated in the EC Regulation No. 178/2002 concerning the food safety and hazard analysis policies. However, the increasing food exports between countries to a large number of consumers give rise to the need of a further harmonization of the control procedures leading to increase food safety. To date, due to the lack of homogeneity in the development of scientific risk assessments for different pathogens in foods, a sufficiently cohesive and integrated food safety policy has not been yet developed. To make feasible the implementation of food safety management schemes, the routine and successful use of software applications by the food industry, governments or educational agencies, should be promoted. One useful way is to create decision-support tools assessing the behaviour of potential microbial hazards along the food chain and their impact on public health. Their use might depend on the availability of user-friendly software, which encompass predictive modelling tools and risk assessment modules to allow different users to retrieve information from them in a rapid and convenient way. The performance of risk-based metrics and the establishment of microbiological criteria could help to identify critical steps along the food chain that influence on the final risk associated to a specific pathogen. Throughout this paper, some examples on how to elucidate microbiological criteria basing on established risk-based metrics (namely, Performance Objectives and/or Food Safety Objectives) set as (i) numerical limit of pathogen concentration; (ii) frequency or proportion terms; and (iii) in qualitative to non detectable values.

Keywords. Microbiological Criteria – Performance Objectives – Food Safety Objectives – Sampling plans – Predictive modelling.

Instruments de prédiction et stratégies visant à établir des critères microbiologiques fondés sur le risque pour les denrées alimentaires

Résumé. *Un des objectifs fondamentaux de la législation relative aux aliments est l'assurance d'un niveau approprié de protection de la santé, comme le manifestait déjà le Règlement CE Nº 178/2002 concernant les politiques de sécurité des denrées alimentaires et d'analyse des risques. Néanmoins, l'augmentation des exportations alimentaires entre pays vers un grand nombre de consommateurs, rend nécessaire une harmonisation plus poussée des procédures de contrôle pour une meilleure sécurité des aliments. Actuellement, en raison du manque d'homogénéité en matière de développement de l'évaluation scientifique des risques pour différents pathogènes d'origine alimentaire, on n'est pas encore parvenu à une politique de sécurité des aliments qui soit suffisamment cohésive et intégrée. Pour permettre la mise en place de démarches de gestion de la sécurité des aliments, il conviendrait de promouvoir l'utilisation routinière et performante de logiciels par l'industrie alimentaire, les gouvernements ou les instituts de formation. Une façon d'aller dans ce sens consisteriat à créer des outils d'aide à la décision évaluant le comportement des dangers microbiens potentiels sur toute la chaîne alimentaire ainsi que leur impact sur la santé publique. Leur exploitation pourrait dépendre de la disponibilité de software convivial, englobant les outils de modélisation prédictive et les modules d'évaluation des risques pour permettre aux différents usagers d'en extraire des informations de façon rapide et appropriée. Les performances de métrique basées sur les risques et la définition de critères microbiologiques pourraient contribuer à identifier les étapes critiques sur toute la chaîne alimentaire ayant une influence sur le risque final lié à un pathogène spécifique. Dans cet article, quelques exemples sont présentés sur la façon d'élucider les critères microbiologiques en se basant sur la métrique établie concernant les risques (à savoir, Objectifs de Performance et/ou Objectifs de Sécurité des Aliments) en tant que (i) limite numérique de la concentration de pathogènes ; (ii) termes de fréquence ou de proportion ; et (iii) valeurs allant de qualitatives à non détectables.*

Mots-clés. *Critères microbiologiques – Objectifs de performance – Objectifs de sécurité des aliments – Plans d'échantillonnage – Modélisation prédictive.*

I – Introduction

Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs has established specific guidelines for different food commodities regarding the compliance with microbiological limits. This regulation introduced two different types of criteria: Food Safety Criteria (FSC) and Process Hygiene Criteria (PHC).

Regarding the establishment of FSC for pathogenic microorganisms harmonized standards on the acceptability of food are provided for both authorities and industry within the EU and for products imported from third countries. FSC will impact the entire food chain, as they are set for products placed on the market.

Implementation of FSC may be achieved through the establishment of risk-based metrics, namely Performance Objectives (PO) or Food Safety Objectives (FSO). A PO is a risk-based metric that allows government risk managers and food operators to quantify the stringency of a food safety management system in a particular point in the food chain. An FSO is defined as the maximum frequency and/or concentration of a hazard in a food at the moment of consumption that provides or contributes to reach an Appropriate Level of Protection (ALOP) for human health. These metrics are usually proposed by the competent authority although they can also be set by the food business operators as a part of their management systems. In any case, actions are taken throughout the food process in order to meet with such objectives. The International Commission on Microbiological Specifications for Foods (ICMSF, 2002) established the link between a public health measures and food safety management concepts throughout the food chain.

Microbiological Criteria (MC) constitute tools for lot acceptance or rejection under specific targets implemented by food operators. To evaluate if the PO is accomplished for a specific food/risk combination the establishment of MC can be set at different stages of the food chain. However, they should not be considered without other aspects of EU food legislation, in particular Hazard Analysis of Critical Control Points (HACCP) principles and official controls to audit food business operators' compliance. Microbiological food safety targets are international theoretical concepts already included in several documents (Codex Alimentarius Commission, 1997; ICMSF, 2002; EFSA, 2007). However, microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans, particularly in the cases where the hazard presents an unacceptable risk at low concentrations and/or low and variable prevalence.

To articulate a MC coming from a PO, several decisions must be taken:

(i) Assumption of the distribution of the pathogen in the lot of food.

(ii) Definition of the 'maximum frequency/concentration' of the hazard that will be used to specify the PO/FSO. Regarding this, the risk manager can set different targets to know the most probable concentration limits that must satisfy the PO.

(iii) Specification of the level of confidence needed to ensure that a non-conforming lot is detected and rejected by the specific number and size of samples taken (generally, the default value is set at 95%).

(iv) Finally, the analytical procedure used is specified in case of qualitative tests, enrichment, and enumeration techniques.

The sampling plan appropriate to assess an MC depends on the specific situation for which the PO is established. Note that the PO can be translated into frequency and/or concentration terms. At low concentration values, prevalence and concentration are not independent so that qualitative tests or enrichment techniques are applied. On the contrary, when dealing with high contaminated samples, PO limits are established on concentration terms.

The stringency of an MC is defined by the values of n (number of samples taken from a food lot), c (maximum allowable number of samples exceeding a certain limit), m (lower microbiological limit) and M (upper microbiological limit). Overall, when more samples are needed with a smaller number of acceptable positive units (c) and/or lower limits are chosen; or sample unit is larger, the sampling plan becomes more stringent.

Throughout this paper three generic examples applicable to different microbial food/risk combinations are presented to provide guidance on how to derive an MC from a PO. The examples were elaborated in accordance with the established principles stated by the Codex Alimentarius Commission (CAC, 2004), as well as other relevant published papers about setting Food Safety Criteria and sampling procedures (Stringer, 2005; Whiting *et al*., 2006; Van Schothorst *et al*., 2009; Zwietering *et al*., 2010).

II – Establishment of a MC from a PO that is set in concentration terms

For the purpose of this scenario, we assumed that the competent authority has established a PO for the concentration of a microbial foodborne pathogen in a specific matrix.

The PO can be established at different points in the food chain. For illustration purposes, a PO could be stated as a pathogen level lower than 4 log cfu/g for 99.75% of the samples comprising the lot. This can be understood as 'no more than 0.25% of the sampling units in the lot will have a concentration higher than 4 log cfu/g'.

Following the steps above described, we must have an approximate knowledge of the distribution of the microbial concentration in the lot. Where such data are not available, it is a good choice to assume a log normal distribution of concentrations. Furthermore, we know that the standard deviation (σ) is 0.8 (taken as a reference value for solid foods, as shown in van Schothorst *et al.,* 2009).

The 99.75 quantile ($x_{99.75}$) corresponding to a PO (≤ 4 log cfu/g) belongs to a log normal density distribution with σ =0.8 with a specific unknown mean (μ). However, it can be calculated by means of the quantiles of the standard normal distribution z_{σ} = 0.9975:

$\mu = x_{99.75} - z_{\alpha} * \sigma$, which is in our case 1.75 log cfu/g = 4 log cfu/g – 2.81 * 0.8

This means that 2.5% of all sampling units of a lot of broiler carcasses with a mean concentration μ = 1.75 log cfu/g and a standard deviation σ = 0.8 of *Listeria monocytogenes* are expected to exceed the predefined PO ≤ 4 log cfu/g.

The next step is to decide the most suitable MC so that the PO is accomplished. This MC should be based on the establishment of a microbiological limit (m) such that the sampling plan is feasible in reality. This decision corresponds to food safety managers and food operators, in such a way the sampling procedure can be effectively done and PO is accomplished.

By setting 2 log cfu/g as value of m; if 1 sample is taken from the lot, the probability of acceptance (P_{accp}) is 0.62, while there is a probability of 0.38 to reject the lot (P_{rej}). P_{accp} is understood as the probability that 1 sample taken from the lot is below m (2 log cfu/g).

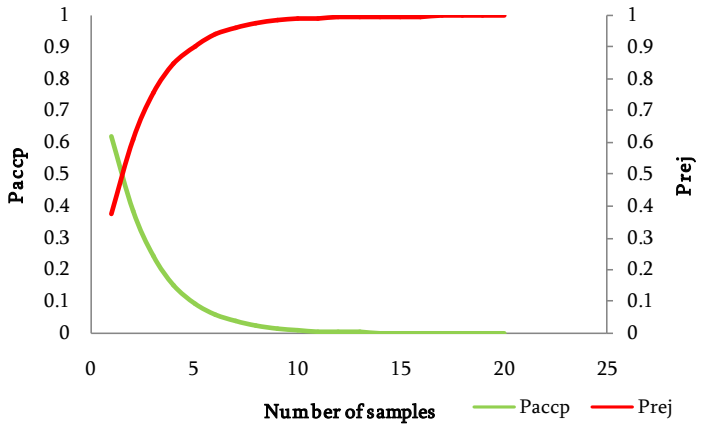

Fig. 1. Illustration of the probability of acceptance (P_{accp}) and rejection (P_{rej}) of the food lot if 1-20 samples are taken.

Figure 1 shows the P_{accp} and P_{rej} for 1-20 samples taken from the lot. If the confidence limit with which a non compliant lot should be rejected is set at 95%, 7 samples must be tested (0.62^{7} = 0.035).

Please note that several other aspects of an MC and the underlying sampling plan need to be additionally defined, such as the microbiological characteristics of the food/lot concerned, the analytical method used etc.

If alternative MC are set, the number of samples can vary, as shown in Table 1. This would give alternative designs of the sampling plan that can detect/reject non compliant lots with the same confidence.

Table 1. Number of samples required to reject the food lot (95% CL) by setting different microbiological limits (m, log cfu/g) for a two-class sampling plan (c is assumed to be 0)

m (cfu/g)	m (log cfu/g)	n
10	1.0	2
31.62	1.5	4
100	**2.0**	**7**
316.23	2.5	16
1000	3.0	50
3162.28	3.5	208

For reference purposes, the sampling plan can be formulated as indicated on Table 2.

Table 2. Sampling plan formulated

Analysis	Standard/Guideline			Assessment	
	n	c	m	Satisfactory	Unsatisfactory
Pathogen	7	0	2	<m/g	> m/g in any of the subsamples tested

III – Establishment of an MC from a PO that is set in prevalence or proportion terms

In this case, the PO will be established at any point of the food chain using bacterial prevalence (i.e. analytical tests to verify presence/absence of the microorganism in a certain quantity of lots).

As an example, we consider absence of the pathogen in the tested sample after an enrichment technique is carried out. A PO can be set as the absence of the pathogen in ≤20% of the samples. In other words, the minimum proportion of non contaminated units in the food lot should be higher than 80%.

The first step is to calculate the P_{accp} of the food lot by taking n samples. P_{accp} would be the probability that, if taking n samples, the proportion of contaminated units is lower than the established PO (≤ 20%). In this case, the contamination rate is 20%. Therefore, the probability of having a negative sample would be 1-0.20 = 0.80.

In the following table, several values of n are presented, corresponding to different probabilities of having negative samples:

Subsequently, a decision must be made regarding the level of confidence of the sampling plan, to accept or reject the lot. In this case, a 95% probability is deemed to be appropriate.

Given the PO, there is a less than 5% probability that lots with a 20% contamination rate or higher would be accepted by a sampling plan with n = 14 samples (0.044).

Alternatively, the negative binomial distribution can be used: = NEGBINOMDIST (0; 14; 0.8) = 0.044, where 0 reflects the number of defective units tolerated in the lot; 14 is the number if samples required to reject defective lots, and 0.8 is the probability of non contaminated units tolerated.

In such a case the number of sample is unrealistic; we should note that additional requirements may be defined before establishing a practical sampling plan. If the concentration of the pathogen is relatively high, it can be detected by using traditional enumeration methods (i.e. ISO). For that specific case, a two-class sampling plan can be applied. If this sampling plan is too stringent (i.e. it has a very high discriminatory power to accept/reject lots), the value of c should be different from 0; or alternatively, a three-class sampling plan can be formulated.

Table 3. Sampling plan formulated

Analysis	**Standard/Guideline**			**Assessment**	
	n	c	m	Satisfactory	Unsatisfactory
Pathogen	14	0	absence	Not detected	Present

IV – Establishment of an MC from an FSO that is set in qualitative terms to non detectable concentration values

In this example, an FSO is set at time of consumption as the maximum concentration that can be present in a food in order to not produce adverse effects for human health.

Subsequently, a PO can be articulated in one or more food chain steps so that the established FSO does not exceed. Once POs are established, suitable MC should be defined for the verification of lots meeting the PO.

For the purpose of this example, we assumed that the FSO has been set as no more than 1% of the lot units will have a pathogen concentration higher than 10 cfu/g.

Firstly, we must decide about the candidate distribution for the pathogen. As in the previous examples, we could start with a log normal distribution where the estimated standard deviation is equal to 0.95. We can proceed in this case in the same way as explained in the first example; i.e. determining the mean concentration of the lot units that would exactly comply with the suggested FSO.

Lots with a mean concentration of -1.21 log cfu/g would match the established FSO (10 cfu/g = 1 log cfu/g).

$P_{\text{normal, cumulative}}$ (1; -1.21; 0.95) = 0.99

A sampling plan based on quantitative analysis seems not practical in this case, because a very high number of samples (298) would be necessary to reject the lot at 95% CL.

Our aim is to determine whether the mean log concentration in the lot is such that less than 1% of the units exceed the FSO (Van Schothorst *et al.,* 2009).

If we consider the as overall probability of detecting a cell from any sample drawn in the lot as the product of that concentration occurs in the lot and the probability of detecting a cell (based on sample size), we are following a Poisson Log normal approach.

Therefore, in such a case, a quantitative test should be moved to a qualitative test (with enrichment). If we consider a 25 g sample, the probability to detect/reject the lot if we take 1 sample is 0.6497.

The following P_{rej} values can be calculated for n samples:

Table 4. Resulting probabilities of rejection at different values of n

n	P_{reject}
1	0.650
2	0.877
3	0.957
4	0.985
5	0.995

In this case, to reject a lot with 95% CL, 3 samples of 25 g each should be taken.

It is noted that this approach is applicable to verify the compliance with an FSO; which is defined as the maximum allowable concentration at time of consumption.

The mean log concentration can be derived at earlier points in the food chain to evaluate the compliance with a PO.

To determine Performance Criteria it can be applied the inequation proposed by the ICMSF (2002) and Zwietering *et al.* (2010). The inequation, in a few words, considers the effect of different processes and sub processes along the food chain (growth, inactivation, cross contamination, etc.) to reach a FSO:

$$H_0 + \sum I + \sum R \leq FSO \quad (1)$$

H_0 is the initial population of microorganisms, I is a factor of increase and R is a factor of reduction. The terms are expressed in log.

If we consider a reduction of 0.59 log (sd = 0.27) and an increase during storage of 1.1 log (sd = 0.8), then the initial concentration (H0) will be:

$FSO = H_0 - R + I$

$H_0 = FSO + R - I = -1.21 + 0.59 - 1.1 = -1.72$

$s^2(FSO) = s^2(H0) + s^2(R) + s^2(I)$

$s^2(H_0) = s^2(FSO) - s^2(R) - s^2(I) = 0.952 - 0.272 - 0.82 = 0.19$

$s(H_0) = 0.435$

A lot containing an initial mean log concentration equal to -1.72 log cfu/g and a standard deviation of 0.435 has a 99% probability of having a concentration below 1 cfu/g.

Given the values of the lognormal distributions for reduction (R) and increase (I) this PO can be well articulated with the established FSO.

Finally, a suitable MC must be set in order to reject the lot by means of sampling. The microbiological limit (m) chosen is absence of the pathogen in 25 g.

The probability of one sample being negative (mean = -1.72 log cfu/g; sd = 0.435 log cfu/g) is 0.426. Thus, if one sample is taken, the probability of rejecting a non-compliant lot is 42.6%.

The following P_{rej} values can be calculated for n samples:

Table 5. Resulting probabilities of rejection at different values of n

n	P_{reject}
1	0.426
2	0.671
3	0.811
4	0.892
5	0.938
6	0.964

Therefore, in order to reject the lot at 95% CL, 6 samples must be taken.

Given the increases and decreases (with their variability) of the pathogen level after the PO, these lots would comply with the FSO (≤1% of units below 10 cfu/g) at time of consumption.

Table 6. Sampling plan formulated

	n	c	m	Satisfactory	Unsatisfactory
Pathogen	6	0	Absence*	Not detected	Present

*after sample enrichment

V – Conclusions

In this study, microbiological criteria (MC) were derived from risk management metrics for three different situations. In order to illustrate the process, data needs and risk management decisions are required when operationalizing a PO/FSO. In all three cases, MCs could successfully be established, but to do so required specific data. When such data were not available, estimations or informed risk management decisions/assumptions were made regarding key parameters. In addition, risk management decisions relating to the discriminatory power of an MC should be made. For some specific cases, underlying distribution of the microbial contamination is needed and information regarding variability within and between lots. While a risk management metric relates the stringency for hazard control at a specific point in the food chain with public health protection, the MC derived from it allows one to verify in practice whether the food safety management system in place at the relevant point in the food chain actually meets the required stringency. In many cases, ICMSF schemes still offer a too high number of samples to be analyzed to ensure that FSO is accomplished. However, they constitute valid risk-based approaches for examining food lots.

Acknowledgments

This work was financed by the EU project KBBE 222738 BASELINE and by the Research Group AGR-170 HIBRO of the "Andalusia Plan of Research, Innovation and Development" (PAIDI).

References

Codex Alimentarius Commission (CAC), 1997. *Principles for the establishment and application of microbiological criteria for foods*. CAC/GL-21. FAO, Rome.

Codex Alimentarius Commission (CAC), 2004. *General guidelines on sampling*. CAC/GL 50-2004.

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union No. 1, 2005. http://eu.vlex.com/vid/microbiological-criteria-foodstuffs-24560853.

European Food Safety Authority (EFSA), 2007. Opinion of the Scientific Panel on Biological Hazards on microbiological criteria and targets based on risk analysis. In: *The EFSA Journal,* 462, pp. 1-29.

International Commission on Microbiological Specifications for Foods (ICMSF), 2002. *Microorganisms in Food 7. Microbiological testing in food safety management*. NY: Kluwer Academic/Plenum.

Regulation (EC) no 178/2002 of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Union No. 1, 2002. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:031:0001:0024:EN:PDF.

Stringer M., 2005. Summary report. Food safety objectives—role in microbiological food safety management. In: *Food Control,* 16, pp. 775-794.

Van Schothorst M., Zwietering M.H., Ross T., Buchanan R.L., Cole M.B. and International Commission on Microbiological Specifications for Foods (ICMSF), 2009. Relating microbiological criteria to food safety objectives and performance objectives. In: *Food Control,* 20, pp. 967-979.

Whiting R.C., Rainosek A., Buchanan R.L., Miliotis M., LaBarre D., Long W., Ruple A., and Schaub S., 2006. Determining the microbiological criteria for lot rejection from the performance objective or food safety objective. In: *International Journal of Food Microbiology,* 110, pp. 263-267.

Zwietering M.H., Stewart C.M., Whiting R.C. and International Commission on Microbiological Specifications for Foods (ICMSF), 2010. Validation of control measures in a food chain using the FSO concept. In: *Food Control,* 21, pp. 1716-1722.