PRODUCTION OF EXTERNAL REFERENCE MATERIALS IN FOOD MICROBIOLOGY

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INTRODUCTION

Regular proficiency-testing schemes are useful for allowing laboratories to demonstrated the reliability of their results at a given moment, but do not permit laboratories to check the trueness of their results at any time. This is why BIPEA develops new external reference materials (ERMs), particularly in the field of food microbiology.

The ERMs could have several purposes:

i) qualification of operators for laboratories accredited according to the requirements of ISO standard IEC 17025 [1]

ii) validation of alternative methods according to ISO 16140 standard [2] or internal methods

iii) determination of reproducibility and repeatability of reference methods [3, 4, and 5].

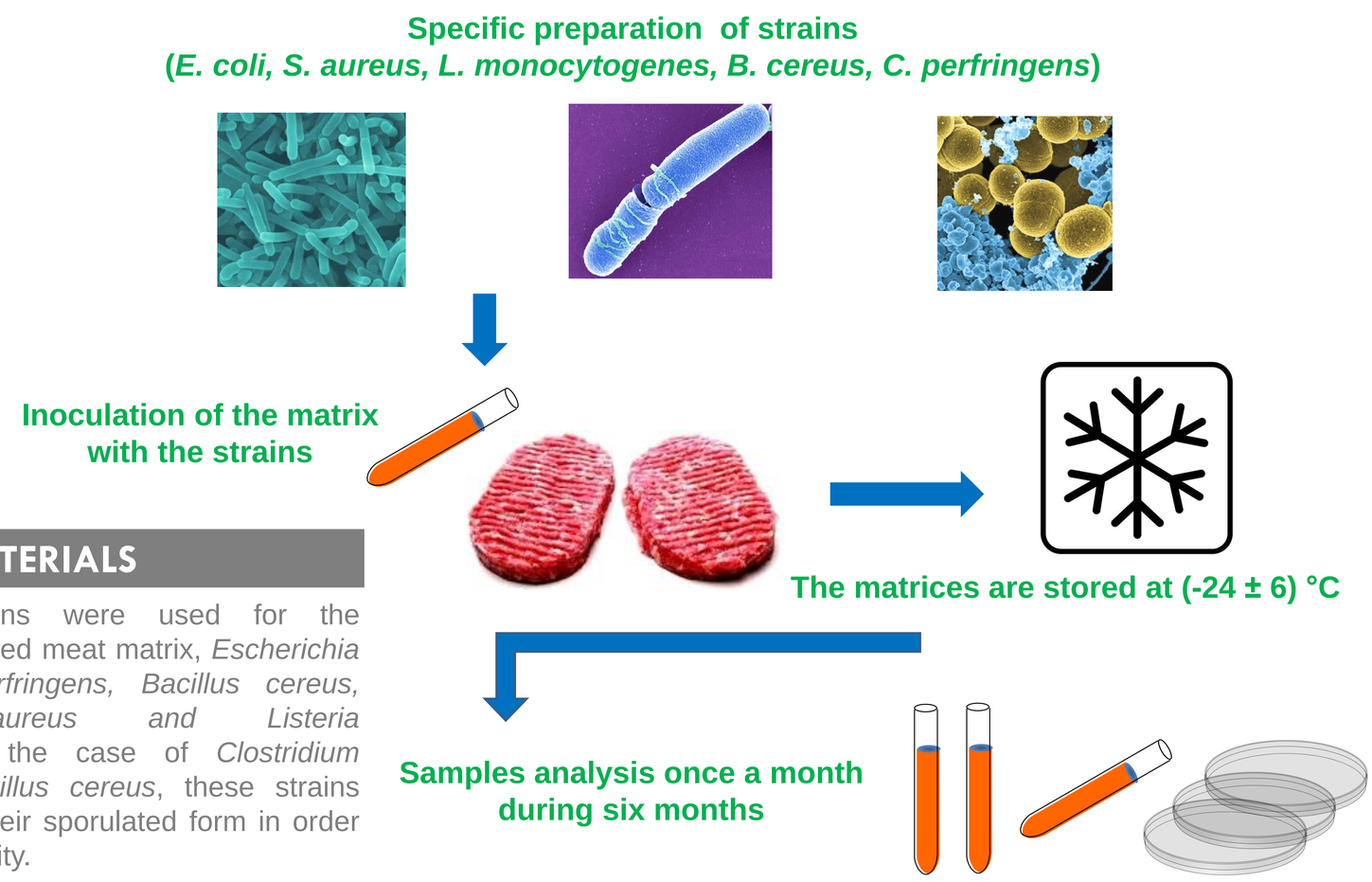
This article describes the different stages of the implementation of ERMs in minced meat matrix contaminated with several bacterial strains stored at (-24 ± 6) °C.

METHODS

The minced meat was divided into 25g test-portions in sterile stomacher bags. These bags were then sealed and sterilized by ionization.

Each bag was then inoculated individually from a pool of tested bacterial strains. The target concentrations of the spiking suspensions were obtained by carrying out dilutions from the primary suspensions.

The test-portions were analyzed at different time intervals according to the ISO methods for each analytical parameter (total viable count, *Escherichia coli*, Coliforms, Enterobacteria, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*).



EXPERIMENTAL DESIGN

Sterile minced meat, divided into 25g test-portions, was contaminated with several bacterial strains at given concentrations. Three of these test-portions were analyzed each month over a period of 6 months. To be used as ERMs, it has to be demonstrated the homogeneity between these test-portions and their stability over time, with a storage at (-24 ± 6) °C.

MATERIALS

Five bacterial strains were used for the contamination of minced meat matrix, *Escherichia coli, Clostridium perfringens, Bacillus cereus, Staphylococcus aureus and Listeria monocytogenes*. In the case of *Clostridium perfringens* and *Bacillus cereus*, these strains have been used in their sporulated form in order to increase their stability.

RESULTS and DISCUSSION

Rate of recovery

The test-portions were contaminated at levels close to 10^3 CFU/g for all tested microorganisms except for Clostridium perfringens (2.10^2 CFU/g) and Bacillus cereus (5.10^2 CFU/g). On day D0, which corresponds to the day of preparation of the test-portions, it is observed that recovery rates reache 100% except for *Listeria monocytogenes*, which was recovered at a 90% rate.

Coefficient of variation - Homogeneity

The coefficients of variation in the study show the reliability that can be attributed to the assigned values which are compared to assess the stability of the test-portions. From an analytical point of view, the coefficients of variation allowed sorting out those whose analysis is under control and those which raised difficulties.

From what can be observed, the CVs obtained are all less than 30%, whatever the parameter. The inter-samples variation is therefore sufficiently low to conclude that these test-portions are homogeneous.

Stability

In addition to the development of homogeneous test-portions, one of the aims of this study was to determine over which period of time these test-portions remain stable. Since microorganisms are living organisms, their concentration can be expected to evolve over time. It may increase, if the elements necessary for their multiplication are present in the matrix, or, quite the opposite, decrease due to the death of these microorganisms. However, some techniques such as freezing can stabilize their concentration. Indeed, freezing has the effect of slowing down, or even almost halting, the processes allowing them to live and develop without causing their death. Therefore, it was decided to store our test-portions at - (24 ± 6) °C, in order to stabilize as much as possible the concentration of the bacterial strains within the food matrix.

The obtained results confirm the effect of the negative temperature on the stability of our test-portions whose concentration in microorganisms does not vary by more than 0.5 log between the analysis of these test-portions at D0 and their analysis after several months of freezing.

		D0	M1	M2	M3	M4	M5	M6
Total viable Count	mean concentration [CFU/g]	3,6E+03	3,2E+03	2,7E+03	2,5E+03	1,8E+03	1,7E+03	1,6E+03
	mean concentration (log[CFU/g])	3,56	3,51	3,43	3,40	3,26	3,23	3,20
	Coefficient of variation (%)	3,9	4,4	15,3	11,6	14,8	0,5	2,2
Escherichia coli	mean concentration [CFU/g]	1,1E+03	9,1E+02	7,5E+02	6,7E+02	5,7E+02	4,4E+02	4,4E+02
	mean concentration (log[CFU/g])	3,04	2,96	2,88	2,83	2,76	2,64	2,64
	Coefficient of variation (%)	7,5	5,7	5,6	7,5	9,7	5,2	12,5
Coliforms	mean concentration [CFU/g]	1,0E+03	8,8E+02	8,2E+02	6,9E+02	5,1E+02	4,3E+02	3,5E+02
	mean concentration (log[CFU/g])	3,00	2,94	2,91	2,84	2,71	2,63	2,54
	Coefficient of variation (%)	8,4	6,4	7,1	3,8	14,3	5,6	11,8
Enterobacteria	mean concentration [CFU/g]	1,0E+03	8,5E+02	8,1E+02	7,5E+02	4,8E+02	4,5E+02	3,6E+02
	mean concentration (log[CFU/g])	3,00	2,93	2,91	2,88	2,68	2,65	2,56
	Coefficient of variation (%)	5,6	3,2	8,5	9,7	14,7	5,4	7,8
Clostridium perfringens	mean concentration [CFU/g]	2,0E+02	2,0E+02	2,2E+02	2,0E+02	2,2E+02	2,0E+02	1,8E+02
	mean concentration (log[CFU/g])	2,30	2,30	2,34	2,30	2,34	2,30	2,26
	Coefficient of variation (%)	5,3	9,1	16,6	10,3	8,3	11,3	7,9
Bacillus cereus	mean concentration [CFU/g]	5,1E+02	4,9E+02	4,0E+02	3,6E+02	3,1E+02	2,7E+02	2,4E+02
	mean concentration (log[CFU/g])	2,71	2,69	2,60	2,56	2,49	2,43	2,38
	Coefficient of variation (%)	4,1	9,4	2,3	3,9	1,6	3,0	7,7
Staphylococcus aureus	mean concentration [CFU/g]	1,1E+03	9,4E+02	7,4E+02	6,6E+02	5,2E+02	4,9E+02	5,0E+02
	mean concentration (log[CFU/g])	3,04	2,97	2,87	2,82	2,72	2,69	2,70
	Coefficient of variation (%)	4,8	7,7	7,8	3,5	21,5	6,7	9,3
Listeria monocytogenes	mean concentration [CFU/g]	9,3E+02	8,7E+02	6,9E+02	6,9E+02	5,0E+02	5,0E+02	5,0E+02
	mean concentration (log[CFU/g])	2,97	2,94	2,84	2,84	2,70	2,70	2,70
	Coefficient of variation (%)	2,9	5,8	6,0	8,9	22,2	15,0	8,5

Table 1 – Obtained results for the analysis of test-portions of minced meat contaminated with several bacterial strains over a period of 6 months after storage at (-24 ± 6) ° C.

CONCLUSION

The results of the counting of the various microorganisms present in the minced meat after 6 months of storage at (-24 ± 6) °C show satisfactory stability of the test-portions with a difference of extreme values less than 0.5 log for all the analytical parameters. The homogeneity of the test-portions is also satisfactory with coefficients of variation below 30% for all the analytical parameters.

The results of homogeneity and stability of the test-portions are therefore in accordance with the expected ones: satisfactory homogeneity and stability for 6 months at (-24 ± 6) °C.

This study enabled the implementation of a test production for trustworthy ERM's storage and shipping conditions.

Eight analytical parameters can be studied at any time by the laboratories to check their performance: total viable count, *Escherichia coli*, Coliforms, Enterobacteria, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes*.

REFERENCES

[1] ISO/IEC 17025:2005 - General requirements for the competence of testing and calibration laboratories

[2] 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.

[3] ISO 5725-6:1994 - Accuracy (trueness and precision) of measurement methods and results -- Part 6: Use in practice of accuracy values.

[4] ISO 5725-1:1994 - Accuracy (trueness and precision) of measurement methods and results -- Part 1: General principles and definitions.

[5] ISO 5725-2:1994 - Accuracy (trueness and precision) of measurement methods and results -- Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.