CerealVeneta
CEREAL, SEED AND PULSE PROCESSING

Our Pubblications, Blog & Media

Article by
CerealVeneta´s R&D department.
The commitment to scientific and technological research is aimed at the continuous improvement of processes, products and solutions.

MICROBIOLOGICAL LOAD:

HOW TO MANAGE IT WITH THERMAL PROCESSING TREATMENTS VALIDATED BY LABORATORY ANALYSES

Preface

IN nature, no food is sterile, thus after a certain period of time it changes, and this happens even faster in foods rich in water, nutrients and micro-organisms. By acting on proteins, fats, sugars and other substances in food, micro-organisms change the characteristics, appearance and taste of food, decreasing its qualities.

Even during the various stages of processing, food is subject to contamination with bacteria, some of which may be pathogenic. The presence of even a few units of pathogenic bacteria in a foodstuff causes so-called food-borne diseases (FBDs), e.g. typhoid in the presence of *Salmonella Typhi* in an apparently healthy egg, while the growth of large quantities of non-pathogenic bacteria (total, mesophilic bacterial load) irreversibly alters the product through nutrient degradation and the release of endotoxins.

Food safety and consumer health

WHEN it comes to food safety and to protect consumer health, legislation distinguishes between two main categories of micro-organisms: pathogenic and alterative.

The former, due to its consequences on health, are strictly regulated in order to clearly establish whether or not a given food is safe (shigellosis, campylobacteriosis, salmonellosis, botulism, listeriosis...). The infection is generally transmitted from animals, such as sick calves, chickens and pigs, to humans through the consumption of “apparently healthy” eggs, milk and meat, the bacterial presence of which can only be detected by microbiological laboratory tests. Heat treatments and storage conditions at chilling or freezing temperatures reduce the biohazard in the intake of these foods.

Any product intended for human consumption must therefore be subject to strict limits. Compliance with the regulations imposed does not allow for exemptions for economic actors in the food sector.

A different approach is taken for non-pathogenic micro-organisms, which do not have a legally defined limit and sometimes no maximum range to comply with, presenting no risk to human health. Nevertheless, non-pathogenic micro-organisms present a risk of food spoilage that may affect consumer acceptability. Depending on the intrinsic characteristics of the product and the extent of the microbiological load, the action of altering micro-organisms leads to a premature decline in quality, which makes it impossible to comply with the declared shelf-life. The best known example is spoiled milk. No pathogenic germs are present here, save “normal” mesophiles that make the product unacceptable.

All foods therefore have an initial microbiological load and cereals are no exception. Although they naturally have a low humidity (10-15%) and water activity (0.5-0.7 a_W), they are still susceptible to changes caused by these micro-organisms.

Reducing bacteriological load in cereals, pulses and oilseeds

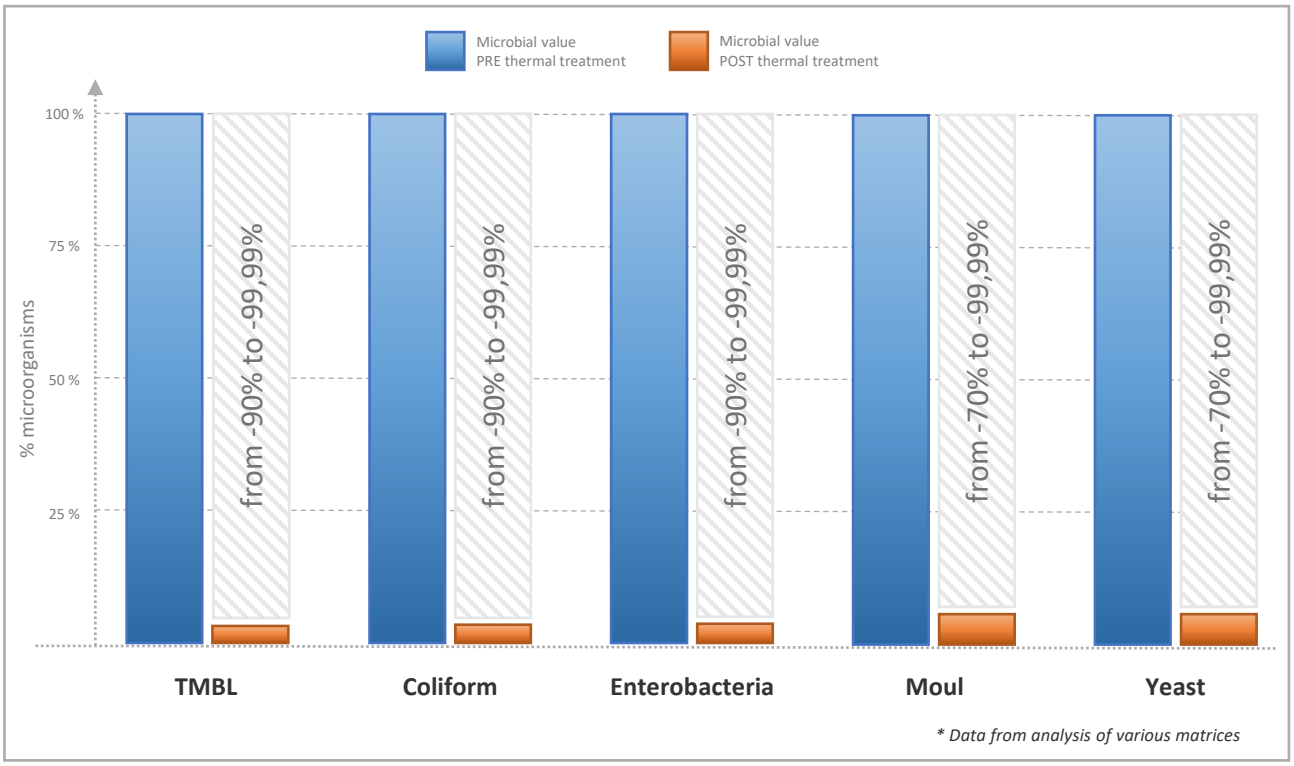
THE issue of bacterial load is one of the most problematic components in the processing of cereals, pulses and oilseeds, especially whole grains.

The high bacterial load in raw material and semi-finished products has repercussions on both the quality and palatability of the final product, as well as decreasing shelf life, due to generating off flavours, oxidative phenomena, bacterial and fungal proliferation.

In the cereal sector, thermal treatment processes are therefore used to inactivate the bacteria naturally present in raw materials. More advanced processes not only have a bactericidal effect, but also inactivate the enzyme load. By reducing water activity (down to 0.3 a_W) and humidity (down to 6%), an environment not conducive to microbial multiplication is created, improving product stability over time. If due care is taken at each stage of processing, thermal treatments that optimise the application of heat, and then optimise the subsequent cooling rate, also allow to eliminate the use of chemical additives for preservation.

The timing and temperature of heat treatments must be studied, tested and monitored on a product-by-product basis. Considering the initial microbiological load in seeds, it is possible to adjust the intensity of the treatment, which leads to excellent results in microbial elimination. The final bacterial load in the semi-finished product therefore varies depending on the intensity of the treatment, but also on the initial load present in the raw material.

By balancing time and temperature, a microbiological reduction of 2 to 6 logarithms can be achieved, while leaving the visual appearance of the seed unchanged *(see graph).*



Raw materials processed in this way are both microbiologically and enzymatically stable. The timing and temperature of thermal processing treatments are specific to each product as some seeds are very heat resistant, others brown immediately. However, very high temperatures are always discouraged to avoid the formation of acrylamide and other toxic degradative compounds.

It is therefore highly important for processors to be able to use raw materials and semi-finished products with low bacterial loads. Using quality ingredients has a positive impact on the final product for the consumer. This increases the perception of brand quality in the marketplace and, crucially, reduces the need for recalls.

The microbiology laboratory

THE microbiology laboratory is the body responsible for supervising and validating industrial bacterial reduction count processes. Its importance is absolute. It determines the presence or absence of any pathogenic species and the extent of enterobacteriaceae and mesophiles in raw materials and then in semi-finished products. From a health point of view, no pathogenic germs are acceptable in food.

At the same time, the utmost attention is paid to the Enterobacteriaceae family, which, having their natural habitat in the intestines of humans and various animals, is a sign of faecal contamination.

Unfortunately, the official selective medium for identifying Enterobacteriaceae used by accredited laboratories is not 100% selective, so many other bacterial species can grow, which cannot even be distinguished by the subsequent glucose fermentation and oxidase negative test. This is therefore not an "error" attributable to the various laboratories through carelessness or inexperience, but an inherent limitation of the analysis, even though the method is internationally accepted.

The emerging issue of reclassification of enterobacteriaceae

THE imperious development of molecular research, i.e. Genetic, is leading to major changes in the classification of bacteria, which was previously carried out only by shape, colouring, the presence of certain antigens and certain metabolic activities such as growth in a medium with bile.

Take ***Listeria*** for example, almost half of ***Listeria spp***. are being reclassified into other families and, ***Pantoea agglomerans***, a common enterobacteriacea formerly a part of the enterobacteriaceae family, is now classified under the ***erwiniaceae*** family *(source: NCBI, UniProt).* This makes it clear that there are important repercussions in day-to-day application. With regard to other taxonomic changes, however, such as that of ***Escherichia hermannii*** to ***Atlantibacter hermannii***, changes in name and gender did not lead to family changes, therefore the practical consequences are almost irrelevant.

Now, in order to determine the bacterial species growing in the Enterobacteriaceae medium, i.e. whether it is really an Enterobacteriaceae, it is necessary to perform a confirmatory test such as **PCR** or **MALDI-TOF**.

PCR | MALDI-ToF

THE precise identification of the bacterial species involved (where there are any doubts) is carried out by PCR or MALDI-TOF, starting with one or more colonies grown in the plate with a selective medium.

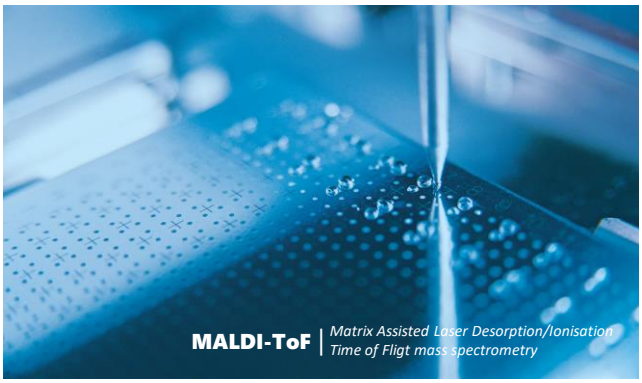
PCR is based on amplifying targeted parts of a DNA fragment for a given bacterial species *(Polymerase Chain Reaction).* It is the best technique, but expensive and relatively long.

The **MALDI-TOF** *(Matrix Assisted Laser Desorption/Ionisation - Time of Flight mass spectrometry)* is a cheaper and faster mass spectrometry technique with an accuracy not dissimilar to PCR, although slightly lower.

In this area, the vast scientific research on microbiological taxonomy can play tricks, as most microbiological databases for PCR and MALDI-TOF are out of date, thus identified bacterial species may be associated with the wrong family.

In fact, this happens quite frequently. It is therefore necessary for the R&D department of the company requesting these reference analyses to personally assess the actual taxonomic position of the bacteria identified by the laboratory in the most accredited databases worldwide. The official ***NBCI*** and ***UniProt*** websites can make this search easy.

PCR | *Polymerase Chain Reaction*



MALDI-ToF | *Matrix Assisted Laser Desorption/Ionisation Time of Fligt mass spectrometry*

Conclusions

WHILE the microbiological contamination of cereals and pulses is usually low compared to that of milk or fish, it has certain peculiarities. Raw material can come from various continents, each with diverse natural microbiological characteristics or human contamination, other than farmland. A simple change of origin of raw materials, even within the same country, is enough to find extremely different bacterial species, some of which can interfere with laboratory identifications. Not to mention some immensely large countries with little concern for the environment, where control of the land can have worrying aspects.

To realise the extent of the taxonomic issue and the difficulty of analysis, one need only consider that a healthy human has several thousand bacterial species in his or her body, and that it is estimated that there are something like 1000 billion bacterial species in the world! In contrast, there are some forty human pathogen species.

Requests for semi-finished products containing bacterial loads within certain limits must be aimed towards industrial processes through the use of technologies specifically tailored to the food matrix, and which are capable of reducing the TMBC and enterobacteriaceae, thus implementing control procedures which pay careful attention to the assessment and validation of the results of laboratory analyses: a non-compliant analysis for Enterobacteriaceae, following **PCR** or **MALDI-TOF** investigations may prove to be a false positive.

For industries concerned about bacterial load reduction, the best results are therefore obtained through the correct application of thermal processing treatments validated by laboratory analyses.