Shelf-life analysis and challenge testing in foodstuffs

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Shelf-life analysis and challenge testing are not synonymous; they target different parameters of foodstuffs. Determination of shelf-life is a 'holistic' approach to the overall microbiological characteristics of the food under normal or specified storage conditions, entailing sensory analysis (acceptability for a consumer), development of the 'total' microflora, and presence/growth of food poisoning micro-organisms.

These analyses should be on products produced under Good Manufacturing Practices (GMP) and subject to an effective HACCP.

Changes in the formulation, or processing or storage conditions, will affect the initial microflora and its development during storage, and therefore the time to undesirable changes, i.e. spoilage or safety issues.

Challenge testing

In contrast, challenge testing is used to determine whether the food could support the growth of specific microbes, usually food poisoning organisms, if inadvertently contaminated during or after manufacture, or during inappropriate storage.

By using variations in the intrinsic parameters of the food (for example pH, water activity), and in the conditions imposed on the food (extrinsic parameters, for example temperature, gas atmosphere) challenge testing may be the basis for the development of mathematical, predictive models.

These can then be used to predict the behaviour of the challenge organisms in the challenged food matrices under certain conditions. Construction and application of such models can be a powerful tool to minimise the necessity for conducting challenge trials, and many models are already available on the internet.

In shelf-life determinations, one must consider the probable microflora of the raw ingredients, the effects of formulations and processing on these organisms during manufacture, the intrinsic characteristics of the food and the storage conditions. All these parameters will determine the types of micro-organisms that may form the initial microflora, its development during storage, and the likely type of spoilage that will occur.

Define the method

These considerations should then be used to define the microbes to be sought and methods to be used during the microbiological shelf-life determinations.

It is not sufficient to determine just 'total viable counts' at a single temperature in a general purpose medium, as the media and methods used and incubation conditions (for example pH, temperature and gas atmosphere), should be representative of the characteristics and storage conditions of the food.

This is especially important in the case of chill-stored foods, as some psychrophilic spoilage organisms have a very low maximum temperature for growth (ca. 22°C) and are readily killed by pour plating methods, or even diluents that are too warm, for example Photobacterium phosphoreum in iced fish, and some strains of clostridia in vacuum packaged minced meat.

The media used for assessment of the microflora must be chosen carefully.

As the microbial flora develops, pH and gas atmosphere should be determined, since any changes in these can effect changes in the composition of the microbial population that may not be detected by the analytical methods initially chosen.

An example is the accumulation of carbon dioxide in wrapped (even in 'cling film') packages of meat (up to ca. 8-10% v/v), leading to a preponderance of enterobacteria rather than the expected pseudomonads.

Such modifications in the microflora will also be reflected in the type of spoilage (odours, appearance) that develops. An important part of the determination of shelf-life of foods, must be analyses for pathogens, even though the manufacturing process may have an effective HACCP system in place and the producing plant operated under GMP.

Analytical methods for pathogens should follow accepted and validated standard methods, for example ISO methods, but appropriate resuscitation methods should be considered for those foods and processes in which pathogens may have been sub-lethally damaged.

When pathogens are detected, their growth and potential for toxin production must be the prime determinant of the acceptability of the food, and requires that the microbiological status of the ingredients, production methods, validation steps of the HACCP system etc, must be carefully checked and changed where necessary, to eliminate these hazards.

The type of spoilage detected by a taste panel or the ultimate consumer, depends on the type of microflora that develops in the product and the accumulation of its metabolic end-products.

Although spoilage is generally detectable when microbial numbers reach ca. 10^{6-7}cfu/g or mL, it is not only microbial numbers that determine shelf-life, as in some cases quite low numbers of microbes can result in distinctive spoilage odours, for example ammoniacal (trimethylamine) odours in spoiling cod by Photobacterium phosphoreum.

Evaluation panel

The effective end of shelf-life should be determined by an evaluation panel (tasted only if proven pathogen-free) and then correlated with microbial types and numbers.

'Best before' dates are generally set when approximately 75-85% of the end of shelf-life has expired, thus allowing the consumer some time to store before consumption.

The intrinsic and extrinsic parameters of a food product, will largely determine the growth and/or toxin production by food-borne pathogens, according to their individual implicit characteristics. For a risk assessment, it is essential to know the values of the food characteristics and the limits for growth/toxin production of the pathogens.

However, if the interactions of the various parameters with regard to microbial growth is not well understood in relation to

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pathogens, nor specifically available in the predictive models, challenge testing is indicated.

The choice of appropriate challenge organisms, or indeed strains of the challenge organisms, is based on knowledge of the intrinsic and extrinsic characteristics of the food and the implicit factors of challenge organisms, generally pathogens.

A laboratory inoculated batch of the challenged food is stored under both the intended storage conditions, and under mild abuse conditions (for example slightly elevated chill temperature, usually ca. 8°C) and determinations of the numbers of the inoculated organisms, carried out during the intended and slightly extended storage time.

Additionally, the intrinsic characteristics of the food being challenged, may also be modified to represent small variations in for example pH, level of acids, water activity, levels of preservatives, that may occur during normal manufacturing conditions.

In addition to the choice of an appropriate challenge organism or specific strain(s), it is important to consider the growth and/or pre-treatment of the inoculum in terms of adaptation to the environment it will experience on inoculation into the food.

For example, in the case of psychrotrophic pathogens, for example Listeria monocytogenes, the temperature of growth of the inoculum must be considered, since it has been clearly demonstrated that previous low temperature growth of the organism, rather than at near-optimal temperature, promotes a much more rapid adaptation to and subsequent growth in, chill-stored foods.

Similarly, for challenging sauces, pickles and mayonnaises, the challenge organisms are required, in the CIMSCEE code, to be pre-adapted to the concentrations of acetic acid and pH values of these products.

Realistic results

Such pre-adaptations can be a lengthy process, but are essential if the results of the challenge tests are to be realistic. In some cases, for example determination of thermal death, it may be desirable to allow some growth of the chosen organism(s) in the food matrix, prior to carrying out thermal death trials.

Also the growth phase of the inoculum culture must be considered and recorded; lag- or log-phase cells are much more sensitive to heat than stationary phase cells. Even the length of time spent in the stationary phase, can be important.

The most appropriate strains of challenge organisms, are those that have been previously isolated from that food or beverage, and possessed or had developed specific characteristics permitting growth or survival in that type of food.

However, subsequent cultivation or storage of that strain must have been such as to maintain those characteristics, for example tolerance of low pH, a particular acid, resistance to preservative(s), or high sugar content (low Aw).

It is debatable whether to use a number of single defined strains as challenge inocula, or to use a mixture of strains, but grown individually, that may represent a 'worst case' scenario.

For Gram-negative organisms, competitive growth in a mixed strain inoculum, where one strain becomes dominant in the food through the phenomenon of 'Quorum Sensing' (QS), can occur, although it can be argued that this could occur in a 'natural' situation since the natural Gram-negative microflora could also exert a QS influence on a challenge inoculum.

Natural isolates

If natural isolates from the food type being challenged, are available, these would be the best choice for 'worst case scenarios'.

Whilst there are no specific guidelines for preparation of the challenge inoculum, certain methodologies need to be considered with respect to the food being inoculated and the trials undertaken.

For beverages, suspending a centrifuged pellet of cells in the beverage at higher concentrations than required to inoculate the product, and further dilutions in the beverage, would be adequate.

This would obviate any problems resulting from alterations in pH, dilution of salts or sugars (altering aW) or any natural or added anti-microbials. In the case of solid foods, this is not possible and suspending the inoculum in a diluent that closely mimics the aqueous phase of the food, i.e. pH, type of acid, salt and/or sugar content (aW), antimicrobials, etc, would be ideal. If time and conditions permit, perhaps allow the inoculum to equilibrate in the diluent, under non-growth conditions, before inoculating the food.

The inoculation procedure should be in small volumes (ca. 0.05mL) distributed evenly throughout the food sample, and if possible by manipulating the food contained within a plastic bag.

Samples of the inoculated food taken at suitable intervals during storage, should be of a sufficiently large amount (ca. 25g) and well replicated (at least triplicate samples at each sampling interval, and a trial repeated at least twice on independent occasions), to minimise any uneven distribution of the inoculum or growth conditions within the food.

Identifying organisms

A major problem with inoculated food trials, is that of being able to identify and selectively count the inoculated organism(s).

Highly selective indicator media may not be appropriate due to the lethal effects of the selective agents on already stressed cells, and distinguishing the inoculated organisms from the natural microflora may present difficulties.

Although specifically marked strains, for example antibiotic resistant strains, or possessing some additional biochemical characteristic such as Green Fluorescent Protein (GFP) or luminescence, may be a suitable approach, but the presence of these markers must be demonstrated not to result in growth or death characteristics differing from those of the parent (native) cells.

During the validation of predictive mathematical models in real food systems (at Leatherhead Food Research), double antibiotic-resistant strains of some pathogens were developed and used successfully.

For inoculated-food trials in food plants under realistic processing conditions, it would not be permitted to use pathogenic strains and the use of surrogate strains must be considered. Examples of these are Listeria innocua for L. monocytogenes or Clostridium sporogenes for Cl. botulinum.

However, in the latter case, Cl. sporogenes as a mesophile, cannot be used as a surrogate for the non-proteolytic, psychrotrophic types B and E strains).

As in the case of specifically marked strains, it is essential to demonstrate that the surrogate strains(s) possess growth/survival/death characteristics closely similar to those of the pathogen.

Trial limitations

There are considerable limitations in the use of challenge trials for proving the safety and stability of a food product. These limitations may be summarised as being expensive in planning, laboratory effort and analyses of results, but the major drawback is that results obtained are only applicable to the particular set of conditions tested.

This latter limitation has been largely overcome by the development of predictive, mathematical models, from many challenge experiments in broth culture systems, and validated by trials in 'real food' systems.

A major set of predictive models, mainly for foodborne pathogens, is available for free download from the Institute of Food Research, Norwich, UK and has been extended by incorporation of models from the USA by Whiting and Buchanan, and from Australia by Ratkowsky, Ross and McMeekin, and is being continuously expanded and refined, to include more conditions (for example modified atmospheres) and non-pathogens, for example some food spoilage microbes such as yeasts.

Similarly, the CIMSCEE equation should be used to predict whether changes in pH and ingredient concentrations will result in a stable and safe pickle, sauce or mayonnaise.

The use of such models is strongly recommended before any alterations to food product formulations or processing are put into practice. ■