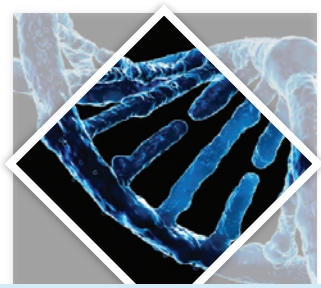


Number 1

FOCUSING on advanced microbial profiling

by Dr Greg Jones, Microbiologist, Campden BRI. www.campdenbri.co.uk

Advanced microbial profiling (AMP) is a powerful DNA technique that we use at Campden BRI which enables us to determine the unique mix of micro-organisms in a sample (its microbiome) without needing to culture them in the laboratory. We have recently shown that the chicken from different cutting plants can be traced to its source by studying its microflora using AMP.

A poultry company provided us with 20 packs of pre-cut poultry portions from two of its processing plants, as part of a project investigating the applications of AMP to the food industry. We extracted the DNA from poultry samples, amplified the 16S rDNA gene using PCR and sequenced the DNA.

The 16S rDNA gene can be used to identify the genus of a bacteria. The 16S rDNA gene is only present in bacteria so it means the sequenced DNA is not conflated with chicken DNA. The sequenced DNA was compared against a database to identify all the bacteria present in the sample.

Fig. 1 shows the abundance of the different genus of bacteria identified in the chicken samples taken from the two plants.

Samples taken from Site 1 have a higher proportion of Bifidobacterium and Faecalibacterium species, whereas samples taken from Site 2 have a higher proportion of Acinetobacter and Arthrobacter species.

The differences observed can be better displayed as a dendrogram (Fig. 2). A dendrogram provides a way to visually describe the relationships between bacteria.

Fig. 2 shows that there is a clear difference in the microbiome at the two sites.

AMP is a valuable tool that can be used to interrogate the microbiome of a sample to provide information about its origin. Populations can also be compared to show how individual populations are related to other populations.

In the example given in Figs. 1 and 2, there is a clear difference between the sample sets. The results suggest that the microflora from Site 1 originates from the chicken, whereas the microflora at Site 2 is more likely to originate from soil.

By comparing the populations one can also conclude that each site has produced chicken batches with very different microflora, and this can be used to identify the origin of a sample.

The power of AMP is only starting to be realised, but this case study demonstrates the ability to use populations of microbes to pinpoint the origin of food samples.

The technique also has applications in many areas of food manufacture where tracking the source of microbial populations is important, such as shelf life analysis, spoilage investigations or authenticity studies.

It can also be used to investigate the effects of changes in manufacturing practice. For example, if the chicken-derived flora is found in final product, controls and hygiene practices can be changed to prevent this happening. The advanced microbial profiling used here could then be used to monitor the effects of those changes. ■

Advantages of AMP over traditional culturing techniques:

- Identifies organisms that are difficult or impossible to culture in the laboratory
- Identifies both healthy and injured organisms
- Can analyse dozens of samples simultaneously
- Can analyse thousands of microbial marker genes simultaneously in a single sample

Potential uses of AMP

- Spoilage investigations
- Shelf life analysis
- Hygiene monitoring
- Authenticity testing

Fig. 1. Sample composition at taxonomic level 'Genus' (top 99% of genera across all samples).

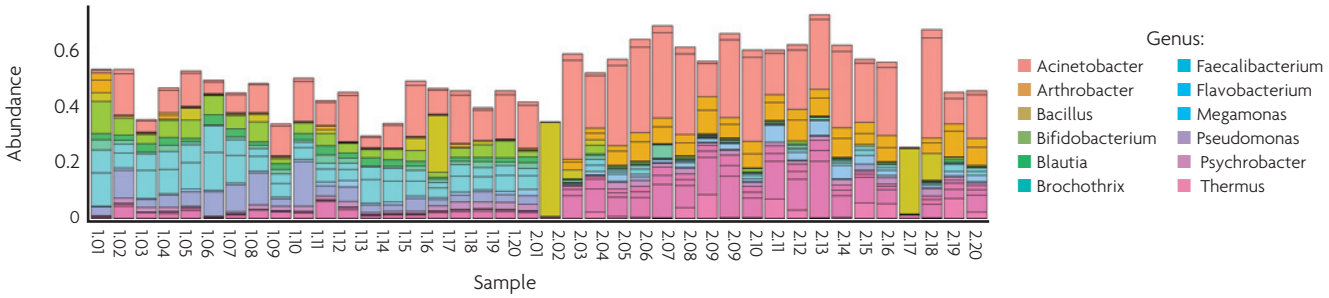
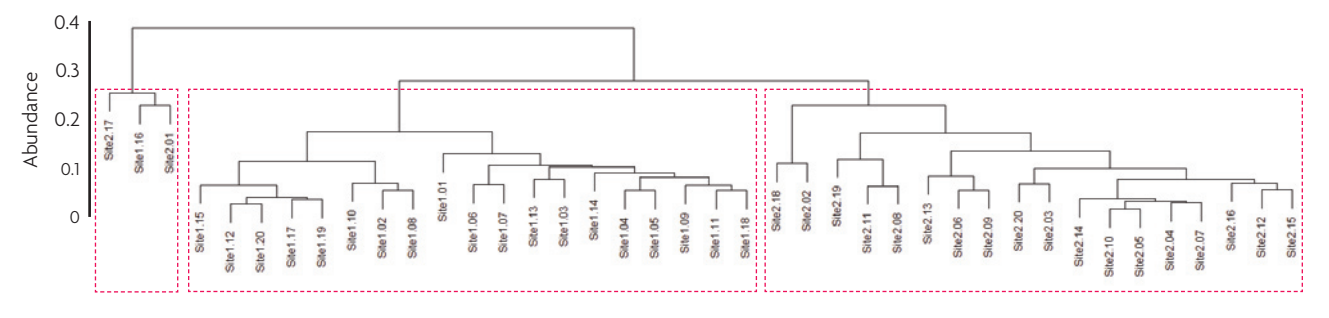


Fig. 2. This cluster dendrogram shows the genetic similarity of samples taken at the two chicken processing plants. Samples that are clustered contain more genetically similar microbial populations.



Number 2

FOCUSING on E coli 0157: H7 and other STEC

by Julie Archer, Microbiological Analytical Services Manager, Campden BRI. www.campdenbri.co.uk

The presence of Escherichia coli species over the years has sometimes been considered as an indicator of faecal contamination, as E. coli originates within the gut of animals. This is a great over simplification as E. coli can originate from many environmental sources and it would be better characterised as a general hygiene indicator.

The significance of E. coli strains can be greater than hygiene indicators, as there are a diverse range of strains in the E. coli species. Most E. coli are harmless, but a small number are pathogenic and are of importance to the food industry as food poisoning pathogens; these pathogenic E. colis are highly virulent with a low infective dose.

Their transmission is by the faecal-oral route and most cases are caused by the cross contamination of the micro-organisms onto raw vegetables, red meat and raw milk.

The various strains of E. coli are differentiated based on their serological type, of which there are over 200 'O' serotypes, and around 30 'H' antigenic types. Some specific serotypes can produce toxins and have other pathogenicity factors that make them serious human pathogens.

The first-known strain of this type found to be associated with a number of large outbreaks was E. coli O157: H7, when it was associated with the consumption of undercooked raw meats, for example undercooked burgers.

In 1993 E. coli O157:H7 was found to be responsible for an outbreak at the Jack in the Box restaurants in the USA, when over 700 people were infected, many were hospitalised and four children died.

E coli O157: H7 can cause acute severe bloody diarrhoea and abdominal cramps as it attacks the human gut wall. Toxins are produced in the gut, which if they reach the bloodstream can cause damage to the kidneys, called Haemolytic Uraemic Syndrome (HUS). The frequency and severity of the disease has led to this becoming the specific strain of E. coli most commonly tested for by the food industry for many years.

It is clear there are concerns in the industry relating to these pathogenic E. colis, so Campden BRI has a UKAS accredited next day test for E. coli O157: H7 to provide the industry with quick and reliable results for the qualitative detection of E. coli O157 (including H7) in red meats.

The toxins of these E. coli are referred to as Shiga-like toxins, encoded for by two genes Stx1 and Stx2. This group of E. coli are called either Shigatoxin producing E. coli (STEC) or Verocytotoxin producing E. coli (VTEC).

Whilst E. coli O157:H7 is perhaps the most well-known, many non O157 STEC have been implicated in numerous food outbreaks, and so their significance should not be underestimated.

Indeed, the largest outbreak in Europe of STEC was by E. coli O104:H4 in 2011, when 3,950 people were affected and 53 died. The source of the outbreak was traced back to fenugreek seeds imported from Egypt. Prior to this outbreak, only a single case had been associated with E. coli O104: H4.

The rapid emergence of this seemingly new E. coli O104: H4 demonstrates the requirement for the continued vigilance when working in the area of the non-O157 STEC, as others of significance may emerge in the future.

In a European Union Summary Report on trends and sources of foodborne outbreaks, data was compiled for the distribution of confirmed cases of human STEC infections in Europe during the period of 2011-2013. The report found the number of infections caused by non O157 serogroups to exceed those of O157, demonstrating the epidemiological significance of the non O157 serogroups of E. coli. Testing of specified foods (raw red meats in the USA, and sprouted seeds in Europe) for several serogroups of STEC is now compulsory in the USA and Europe. In Europe, meat must be tested for the 'Big 6': E. coli O26, O103, O111, O145, O157 and 0104. In the USA, the same applies except that serogroups O45 and O121 are tested for instead of O104.

The methods need to be very specific since only 6-7 E. coli serogroups out of all the existing possible serogroups need to be tested for and so typically DNA based PCR methods are used. These PCR methods detect the Stx 1 and Stx 2 genes, which whilst being complicated to run and interpret, allow for the specificity required. It is very important to remember that these presumptive positive PCR results should be treated with caution since they are only presumptive positives. As the PCR is done using a sample taken from an overnight enrichment broth and not from a colony on a plate, a positive PCR result does not necessarily mean the micro-organism is present.

Sometimes fragments of DNA from other strains or species can combine and provide false positive results. To confirm any presumptive positive PCR results, you must also isolate the STEC E. coli by growing it on a plate and do the PCR using a colony of the isolated strain.

A draft UK Working Party Policy for STEC has been produced by the UK Food Standards Agency (FSA). It provides recommendations to help Food Business Operators understand and manage the risks associated with the detection of STEC and advises at what stage of testing the action needs to be taken.

Here at Campden BRI we have a UKAS accredited PCR based method for the detection of both the European Big 6 and the USA Top 7 STEC detection in red meats. We also have a containment Class Level 3 testing facility on site (required to handle STEC E. colis due to their high virulence), so we can also confirm any presumptive results. This enables companies to take action on a confirmed result.

The STEC E. coli will pose greater challenges to the food industry in future. Companies will want answers to questions such as: Which foods are at risk from contamination? Which serotypes should I look for? What do I do if I detect it? And how can I control the presence of the organism?

Testing helps us to understand prevalence and leads to better, more directed control measures. ■

Number 3

FOCUSING on Bacillus (pathogen, spoiler or just annoying resident)

by Suzanne Jordan, Molecular Methods Manager, Campden BRI.
www.campdenbri.co.uk

The Bacillus genus is a diverse group of organisms which can cause food safety and quality issues. Bacillus cereus is one of the few bacteria that causes illness with the toxins they produce. Natural insecticidal toxins formed by Bacillus thuringiensis are used to control insects on fresh produce. Other Bacillus species spoil food with enzymes that create an undesirable product appearance, odour or texture.

Bacillus spp are spore-producing Gram-positive rods. Spores help bacteria to survive harsh conditions such as heat treatments, dehydration and acidic environments. The genus Bacillus comprises 268 species, found in soil, sediments, dust and vegetation. Many Bacillus species are important to the food industry for different reasons:

- Pathogens capable of causing food poisoning.
- Natural pesticides used to control insect pests on fresh produce.
- Growth of spoilage organisms that lead to the formation of undesirable product appearance, odour or texture of food products.

Pathogenic Bacillus

The predominant foodborne pathogen in the Bacillus family is Bacillus cereus. Although rice is thought to be the main transmission route for B. cereus, other foods have been associated with outbreaks, including pasta, meat or vegetable dishes, dairy products, soups, sauces and sweet pastry products. B. cereus food poisoning is caused by toxins rather than the organisms themselves. Two types of toxin are formed by some isolates of B. cereus – emetic and/or diarrhoeal toxins. Emetic toxin (cereulide) causes nausea and vomiting, with symptoms typically occurring one to five hours after ingestion of foods containing the toxin.

The emetic toxins are pre-formed in foods and are known to be pH stable and relatively heat resistant. Assays are available to detect cereulide in foods including commercial detection kits as well as an instrument based test using liquid chromatography-tandem mass spectrometry which is suitable for all food types.

The symptoms of B. cereus diarrhoeal toxin are abdominal pain and watery diarrhoea, which generally occur between 8-16 hours after eating contaminated food. Unlike cereulide, the diarrhoeal toxins are formed by B. cereus in the gut after ingestion of contaminated food, however they are known to be inactivated at low temperatures and pHs. It is thought that the infective dose of B. cereus needed to cause diarrhoeal symptoms is 10^5-10^7 cells. It is thought that at least two toxins are potentially involved in the diarrhoeal syndrome, however the exact mode of action of the toxins has not been confirmed.

Other members of the Bacillus genus have also been associated with food poisoning. Some strains of B. subtilis and B. lichenifomis are known to produce toxins that can cause illness. Limited information is available on the toxins produced by B. subtilis and B. lichenifomis, including the infective dose.

B. subtilis or B. licheniformis can cause ropy bread by breaking down the crumb structure and forming strands known as 'rope' that give the bread either a sweet or fruity odour. B. subtilis or B. licheniformis can either be present in the flour and survive the baking process or can be introduced post bake through contact with contaminated surfaces.

Bacillus biopesticides

Some Bacillus species are beneficial for the industry, such as the natural biopesticides used with fresh produce to control insects. Several commercial biopesticides are available on the market, many of which contain subspecies of Bacillus thuringiensis.

Each subspecies produces proteins that are toxic to a specific target insect population. The toxins are formed when Bacillus thuringiensis starts to produce spores, and many biopesticides are a mixture of spores and insecticidal proteins. When the toxins are consumed by insects, they become activated in the gut and kill the insect.

Most biopesticides contain subspecies of B. thuringiensis which is very closely related to B. cereus. The high level of similarity between these two species can cause issues with microbiological analysis of fresh produce. Studies have shown that B. thuringiensis can grow to produce typical colonies on the media used to detect B. cereus in foods.

This means that the numbers of B. cereus could be overestimated if biopesticides containing B. thuringiensis were applied to the fresh produce being tested. At present, there are limited ways to identify B. thuringiensis colonies and the most commonly used method is to detect the insecticidal proteins (by microscopy) or the genetic code that encodes for the toxin specific molecules by polymerase chain reaction (PCR). Microscopy based analysis can be a lengthy process as it is reliant on the culture producing spores which can take many days. Molecular approaches such as PCR offer a much more rapid result, however most of the genes responsible for toxin formation are specific to the B. thuringiensis and biopesticide applied to the fresh produce.

Food spoilage

Bacillus spp. cause many spoilage defects, including 'rope' in bread, bitty cream and thinning of soups and sauces.

Ropy bread is caused by B. subtilis or B. licheniformis breaking down crumb structure and forms strands known as 'rope' that give the bread either a sweet or fruity odour.

B. subtilis or B. licheniformis can either be present in the flour and survive the baking process or can be introduced post bake through contact with contaminated surfaces.

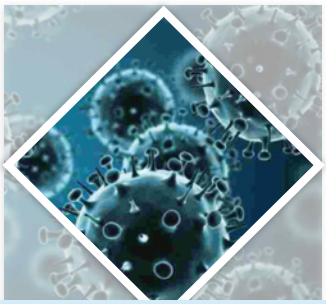
Bitty cream is a common defect in milk and cream and is thought to be caused by the enzyme (lecithnase) formed by B. cereus. The lecithinase degrades fat globules and creates fat and protein particles that float on the surface of hot drinks and coat the surfaces of crockery and glasses.

Thinning of sauces and soups occurs when B. pumilus, B. licheniformis, B. subtilis, and B. megaterium produce amylase that degrade starch molecules used for product thickening. Our research has shown that Bacillus pumilus, B. licheniformis, B. subtilis, and B. megaterium have a greater tolerance to heat treatments in soups compared to broth systems.

Conclusion

The Bacillus species create many safety and quality challenges for the food industry. To stop Bacillus cells and spores causing food safety and spoilage issues you need to sufficiently process your product and control its cooling and storage.

Advances in molecular techniques provide additional tools to monitor Bacillus and its toxins in foods and factory environments. Campden BRI has developed protocols to validate heat processes, troubleshoot spoilage and contamination issues and set product shelf life. ■



Number 4

FOCUSING on viruses: size doesn't matter

by Martin D'Agostino, Campden BRI. www.campdenbri.co.uk

Viruses are the most abundant organisms on earth. It is estimated that there are more viruses on earth than there are stars in the universe, and that if you were to stack them one on top of the other they would reach a distance that would take over 200 light years to travel. That is impressive for something you can not see.

These tiny capsules containing DNA or RNA are responsible for some of the worst illnesses mankind has ever experienced and even today they still have a global impact regardless of the advances in medicine.

When the subject of viruses comes up we may often think of viruses such as the common flu, or the more dangerous ones such as Ebola or Polio virus. We rarely associate viruses as being a hazard in everyday items – like our food, for example a lettuce or strawberry.

The reality is that food can become contaminated with and transmit viruses such as Norovirus, Hepatitis A and Hepatitis E.

These viruses cause infection when consumed, even when products are contaminated with low levels. They are very contagious and it is estimated that Norovirus can cause illness with as few as 18 virus particles.

These viruses are in the range of 30-40nm in size – approximately 2,000 times smaller than the diameter of a human hair and they can not be seen using a light microscope – to see them you need to use an electron microscope.

Viruses are non-living and are very simple in structure – they have a nucleic acid core which is contained within a protein coat called a capsid. They are only able to replicate when inside specific host cells. During infection the virus is introduced into its host cell where it proceeds to utilise the cell's machinery to replicate itself many times over. This is when the problems start and, for Norovirus, symptoms can start to appear within 24-48 hours. Hepatitis A and E viruses take a lot longer before symptoms appear – the incubation period can be up to six weeks.

Viruses can be a hazard in everyday food items, such as strawberries.

Transmission of the viruses

It is not a nice thought, but the main transmission route for enteric viruses such as Norovirus or Hepatitis A virus is by the faecal-oral route. It is important to remember that you can not see these viruses – you could fit enough of them on the head of a pin to make up to 1,000 people ill, so it is unsurprising that poor hand hygiene accounts for a lot of the problems.

Just to make things worse, the person who is infectious may not even know – you can start to shed the viruses before symptoms appear, for long periods of time after the symptoms have gone or even if you are asymptomatic.

Perhaps a food handler who is shedding Norovirus has touched your food before serving it to you – what if they have not washed their hands properly?

The viruses can also remain infective for a long time in the open environment. They may survive the sewage treatment process and enter waterways which are then used to irrigate crops, or, to prepare pesticide preparations which are then sprayed onto leaf or berry crops.

Contaminated handler's hands may transfer viruses onto the fresh foods. Shellfish may become contaminated by viruses by being farmed in waters that have been contaminated with human sewage, by run off from agricultural land or pig farms.

Why do we mention pig farms? Hepatitis E virus is not commonly transmitted via the faecal-oral route, rather it is considered a zoonotic pathogen.

Zoonotic pathogens are those which originate from animals, in this case mainly from pigs and/or boars. Hepatitis E does not cause harmful symptoms in pigs and can therefore go undetected. If ingested they can lead to a long-term illness in humans. Hepatitis E virus can be found in minimally processed pork products, or in products containing raw pig liver.

Over the last 10 years in the UK, there has been a significant increase in the number of non-travel related cases of Hepatitis E infection. This has been linked to consumption of imported pork products which have not undergone any type of heat treatment.

Controlling viruses: is it possible?

How do we stop viruses from getting into the foods we eat if we cannot see them, yet they cause us to get ill – sometimes very quickly? Probably the easiest control measure is good hygiene, both personal and environmental.

Simply washing our hands properly with soap and hot water is the most effective means of removing viruses from contaminated hands. If workers are in a field picking fruit, are there adequate toilet and handwashing facilities nearby? If so, are they cleaned regularly? Is the handwashing process monitored?

Bear in mind that a lot of the produce we consume is imported from different countries where diseases like Hepatitis A are endemic and where clean water may not be readily available, especially in the middle of a field.

Other questions we could ask are regarding safety checks, for example, if non-potable water is used for irrigation (a large percentage of UK produce is irrigated using surface water), how often is it checked for microbiological quality? What is being tested? Are specific tests for viruses included? The presence of E. coli does not necessarily relate to the presence or absence of viruses.

What can be done?

As we may begin to realise, the virus issue is large. Many in the food industry are aware of the virus issues, many are not, and few know what to do about it. We cannot easily culture the target viruses, so it is difficult to assess the effectiveness of food control strategies, such as heat treatments, so the issue is sometimes ignored.

That is where Campden BRI can help. Methods to detect viruses in foods do exist – there is an International Standard available for the detection and quantification of Norovirus and Hepatitis A virus in foods, including fresh and frozen produce and shellfish.

Campden BRI is the only UK laboratory to have been awarded UKAS accreditation for the detection of Norovirus and hepatitis A virus in fresh and frozen produce.

This method only detects the genomic material and therefore does not indicate if the virus is infectious.

However, what a positive result does tell you is that at some point before sampling, the food has come into contact with contents from the human gut – faeces or vomit, and that should not be happening.

So, what can be done if your product tests positive for one of these viruses? This depends on what is being tested and at what stage the sampling is done. If a positive result is found at the pre-harvesting stage, the food has been contaminated early during primary production.

This could be due to factors mentioned above, such as contaminated irrigation water or contaminated pesticide preparation water. If a positive is found on a food which is sold at retail, the line of investigation will be much longer because the food could have become contaminated at any handling point. Companies may need to improve the cleaning of food-contact surfaces, monitor the health of food handlers and treat non-potable irrigation waters. Vaccinating against Hepatitis A virus and subjecting the foods to post harvest treatments such as UV are other options to consider.

Understanding how viruses differ from bacterial hazards and the potential measures to control them is key. Campden BRI can provide bespoke training in all aspects of foodborne viruses and advise on how to improve food safety management plans to include viruses as hazards. Campden BRI can also validate control strategies such as heat treatments or produce decontamination technologies, using culturable surrogate organisms for the target viruses. ■

Number 5

FOCUSING on challenge testing

by Rob Limburn, Heat Resistance Research and Disinfectant Testing Manager, Campden BRI. www.campdenbri.co.uk

A huge variety of thermal processes are used by food manufacturing companies worldwide to create safe and stable products for the consumer by achieving a defined reduction in pathogens or spoilage organisms. Generalised and industry-specific guidance on thermal processes appropriate for cooked foods is widely available.

Significant emphasis is, rightly, placed on the proper validation of such measures in the HACCP system of food safety management to ensure that thermal processes are routinely effective when properly implemented. The validation of these control measures is even more important with the enormous and ever-increasing range of different product types being produced by food businesses. This includes the increasing demand for reformulation to reduce levels of salt, sugar and fat and the use of alternative sources of protein, fibre and other nutrients.

To accurately validate the efficacy of a cooking process as a kill step, the following must be considered:

- Which micro-organism(s) should be targeted? This choice will depend upon the intrinsic characteristics of the product, such as water activity, pH, salt level and its intended shelf life and storage conditions. These factors will determine which micro-organisms will survive or grow in a given product, and therefore the chosen target organism should be the most heat resistant.

- Definition of product and process parameters: The chosen product and process parameters should reflect the realistic worst case in the manufacturing scenario to be validated – for example lowest moisture/a_w, temperature, shortest cook time/residence time and lowest humidity.

- Once the parameters for validation have been defined, the process can be assessed to determine the lethality achieved for the chosen target organism

Process assessment

For batch processes applied to moist products, the validation of a thermal kill step can be achieved by carefully measuring the temperature throughout the process and using this data to determine the pasteurisation values achieved against a defined process and z-value. However, there are many scenarios where temperature measurement alone may not be sufficient to demonstrate the lethal effect of a thermal process.

These scenarios include:

- When processing low moisture foods.

- When processing products whose constituent ingredients may offer protection to target organisms (such as fat and sugar).

- Some continuous processes where it may be difficult to determine the product temperature over time.

- Where organisms are observed to survive a previously validated process.

In these instances, microbiological challenge tests are required to directly demonstrate the range of lethality achieved by a given process towards a target organism.

Challenge tests are extremely versatile techniques which demonstrate the log reduction achieved within real manufacturing situations. A typical cooking process challenge test would usually involve the following stages:

- Stage 1: Consideration of suitable target and surrogate organisms.

- Stage 2: Surrogate qualification.

- Stage 3: On-site or pilot-scale microbiological challenge test.

A laboratory comparison of the heat resistance of the target and surrogate organisms is made in the product to be assessed. Multiple strains of the target organism are often screened to select the most resistant strain for comparison.

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These typically include isolates or outbreak strains from the product type under assessment and known heat-resistant reference strains. Comparative heat resistance data is used to confirm the suitability and correlation of the surrogate organism with the target across a range of temperature appropriate for the process under assessment.

Challenge testing

The product is carefully chosen to represent the worst-case scenario in terms of its physical properties, such as water activity, pH, viscosity, particle size and flow characteristics.

The process parameters selected for challenge testing should reflect realistic, worst-case conditions expected during normal processing, such as maximum amount of product in the processing equipment, the minimum residence time and minimum temperature settings.

To establish these conditions, it is often necessary to determine the temperature distribution within processing equipment, allowing samples to be targeted to areas or channels within the equipment which would be expected to deliver the least severe process.

Samples of the product to be validated are inoculated with the qualified surrogate organism. Inoculation is done without altering the physical properties of the product, such as pH and water activity, and the inoculation reflects the way in which natural contamination would be expected to occur (surface associated, uniformly distributed, etc).

Inoculum can also be concentrated at chosen areas of a product, such as the slowest heating point so that log reduction calculated for the process reflects the areas exposed to the least severe process. This is often achieved by using alginate particles – small (usually 1-3mm) particles of inoculated product.

Food dye is added to the alginate particles to aid recovery. The advantage of this approach is that inoculum can be easily targeted and recovered without any loss or dilution of bacteria in the carrier product.

The inoculated food samples are then introduced to the processing equipment so that they are exposed to the full thermal process.

This part of the challenge test is carefully designed to ensure that the bacteria can easily be identified and recovered, whilst simultaneously ensuring that they are exposed to a truly representative process.

Enough samples should be included in the challenge test to allow a statistically robust demonstration of the distribution of lethality (i.e. the range of log reductions) of the process to the test organism, and the challenge test should be performed over at least duplicate processes to allow for batch to batch process variations.

Analysis and interpretation

Challenge test samples are analysed as soon as possible after processing to prevent possible regrowth of surviving micro-organisms.

'Transport controls' are included as part of the challenge test. 'Transport controls' are samples which are not processed but are prepared, stored, transported and analysed under the same conditions as the processed samples.

This allows us to calculate the thermal process lethality in isolation, discounting any die off which may be attributed to storage and transport of samples.

The log reduction achieved is calculated by subtracting the log CFU count from each processed sample from the mean (or in some cases, the lowest) transport control value. Interpretation of challenge test results often requires experience and should not be attempted without a sound knowledge of thermal process microbiology.

Planned, executed and interpreted correctly, microbiological challenge testing provides robust, 'real life' data to support the efficacy of control measures. ■

Number 6

FOCUSING on determining shelf-life

by Linda Everis, Microbiologist & Principal Research Officer, Campden BRI. www.campdenbri.co.uk

From a technical perspective, product shelf-life is the time after production that a product remains safe and retains the desirable attributes of product quality with respect to chemical, sensory and microbiological characteristics. It needs to be free from foodborne pathogens and maintain an acceptable level of spoilage organisms.

It is illegal to sell food which has deteriorated during storage so as to be injurious to health, or if its quality has deteriorated beyond that which would normally be acceptable.

Assigning the correct shelf-life requires a great deal of thought. Obviously, if it is too long there is the potential for food spoilage or growth of food pathogens – and the product will not meet the requirements of food safety legislation – but if it is too short (i.e. over cautious) then manufacturing costs and wastage may increase, and profit margins drop.

Consequently, it is important to assign the shelf-life in a systematic and scientific manner, taking all relevant factors into consideration. It is also important to re-evaluate shelf-life when products are reformulated – even minor changes in product formulation can have a substantial impact on the growth of micro-organisms.

Changing or reducing levels of a preservative, or reducing the salt, sugar or fat levels or types can all impact on shelf-life.

Factors affecting shelf-life

Manufacturing, product formulation and storage conditions all have an influence on shelf-life, so the effect of these on the growth of target micro-organisms must be considered.

Table 1. Factors affecting microbial shelf-life.

- Raw material quality
- Heat process
- Product formulation – pH (acidity), salt level or water activity and preservatives
- Distribution and storage times and conditions
- Packaging, including gas atmosphere
- Consumer handling

The food business operator should have enough product knowledge to be able to determine which factors will limit its shelf-life, and the approximate time for which the product will remain fit for consumption (i.e. days, months or years).

This could be by comparison with similar products with a clear insight into the differences between these 'similar' products and the product in question.

When microbiological issues are important, there are three basic approaches that are used to assess product shelf-life:

- Shelf-life trials.
- Challenge tests.
- Predictive microbiology.

Each has a key role to play in assuring the safety of the product shelf-life chosen.

Shelf-life trials

How long does this product remain within the designated quality parameters during normal production and storage conditions?

That is the question that shelf-life trials are designed to answer. These tests assess only the growth of naturally present micro-organisms in the product batch being tested. These trials do not determine the potential for growth of foodborne pathogens because it is unlikely that pathogens would be present in the product.

The shelf-life of food products is determined in a logical sequence of events:

- Kitchen/pilot scale assessment: the product and process characteristics are defined and a target shelf-life decided.
- Factory scale trials: the majority of laboratory testing is performed on batches of product produced under routine manufacturing conditions and where the shelf-life of the product is assigned. During this stage, the product is stored under conditions to which it is likely to be exposed during retail distribution and examined for any changes in levels of target micro-organisms.
- Full scale production: any changes to the shelf-life are monitored.

It is important to note that the shelf-life determined in these studies is only relevant to the product formulation and storage conditions used and cannot be extrapolated to other conditions.

Challenge testing

Will the product formulation and storage conditions control growth of pathogens (or spoilage organisms) during the designated shelf-life if they were present in the ingredients or contaminated the food during manufacture? Challenge testing answers this question.

With challenge testing, a food is deliberately inoculated with the relevant organisms and the growth of the organism is studied under controlled laboratory conditions.

The advantage of this technique is that it provides data to answer the 'What if?' questions that may not be answered during shelf-life studies, for example:

- What would happen if Listeria monocytogenes contaminated my product after cooking?
- What would happen if a preservative resistant yeast survived the processing?

Predictive microbiology

Predictive microbiology uses computer simulations to predict the likely growth of spoilage organisms or food pathogens in different product formulations or storage conditions. It provides a rapid answer for use in new product development and troubleshooting situations. Data can be obtained on the length of lag time, rate of growth and the time taken to reach a target number of organisms. In addition, many models can be used to predict the effect of fluctuating temperature profiles that may be seen during shelf-life.

Conclusion

The shelf-life of food products is influenced by microbiological, chemical and sensory considerations and, in some cases, legislative requirements. It needs to be determined by applying sound scientific principles that can take into account all the relevant formulation, manufacturing, distribution and storage factors.

Shelf-life is unique to the product and storage conditions tested and cannot be extrapolated to other products or storage conditions. Assigning the correct shelf-life can be the key to the commercial success of a new product and should be carried out in the early stages of new product development. ■

If you want to learn more about shelf-life testing and how to approach it, you might be interested in Campden BRI's Setting shelf-life: How to do it better seminar on 4th June 2019.

More information on challenge testing is available in:

Betts, G.D. (2010) Challenge testing protocols for assessing the safety and quality of food and drink. Guideline No. 63, Campden BRI, Chipping Campden, Gloucestershire, GL55 6LD.

More information on shelf-life testing is available in:

Betts, G.D., Brown, H.M. and Everis, L.K. (2004) Evaluation of product shelf-life for chilled foods. Guideline No. 46, Campden BRI, Chipping Campden, Gloucestershire, GL55 6LD.

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