

Microbial update

Yeasts & moulds

Produced as a service to the food industry by Oxoid Ltd.

Yeasts and moulds are prolific in the environment, making them a frequent contaminant of foods and drinks. As such, they are an extremely important group of organisms to the food industry.

Historically, this contamination has at times been beneficial to mankind, leading to the discovery of fermented drinks such as mead, wine and other alcoholic beverages.

We also have yeasts/moulds to thank for staples such as cheese and leavened bread. Indeed, a contaminating mould was responsible for one of the greatest medical advances in the 20th century, with Sir Alexander Fleming's discovery of penicillin.

More commonly, however, contamination with environmental yeasts/moulds is a heavy burden on the food industry, causing foods to spoil prematurely and reducing product quality. This spoilage may be clear to see, with visible growth on the surface of the product or other obvious changes, such as stringiness or cloudiness, or it may be invisible, resulting in gas production that can affect the taste, texture and/or smell of a product.

Growth characteristic

Yeasts and moulds belong to the fungal kingdom. Moulds are multi-cellular organisms, growing in thread-like filaments (known as hyphae) that spread to form distinctive mat-like colonies. They multiply by producing single cell spores that may settle on another food source and form a new colony.

Yeasts, on the other hand, are unicellular organisms that reproduce by budding (the formation of a small bud, or daughter cell, which grows and separates from the parent cell) or by binary fission.

The reason contaminating yeasts and moulds are such a problem to food manufacturers is that they are able to withstand a number of preserving techniques. Whilst these methods may be effective against the majority of contaminating bacteria, certain yeasts and moulds are still able to grow and proliferate:

1 Cold storage. Many yeast and moulds are able to withstand extremes in temperature. Certain yeasts, for example, can grow at temperatures as low as 0°C, allowing them to multiply despite refrigeration.

1 Acidification. Some species can also survive in acidic conditions, making them a particular challenge for manufacturers of fruit juices and fermented milk products.

1 Low water activity (a_W). Drying of foods has been used as a preserving technique for thousands of years. However, many yeasts and moulds are xerotolerant (able to grow in environments with an a_W as low as 0.65), making them a potential problem for products such as dried fruits, nuts, grains and spices. In addition, some species are osmophilic (able to withstand high sugar levels), with implications for bakery/confectionery products, or halophilic (able to withstand high salt levels), causing problems for manufacturers of dried/cured meats, for example.

1 Bactericidal treatments. Some treatments designed to minimise bacterial contamination, such as irradiation, high hydrostatic pressure or organic acid treatment, may not be effective against certain yeasts and moulds. Indeed, with the competition from the normal bacterial flora removed, certain species may flourish. *Zygosaccharomyces bailii*, an important spoilage yeast in wine, for example, is extremely resistant to commonly used organic acid food preservatives.

Faced with these challenges, it is important for food manufacturers to take precautions to minimise opportunities for yeast and mould contamination in their processes, ensuring that levels in products, raw materials and the environment fall within acceptable limits.

Sources of contamination

Effective preventative measures require a good understanding of potential routes of contamination. Since yeasts and moulds are naturally occurring and abundant in the environment, there are many possible sources that should be considered.

1 Air. This is an important source of contamination for yeasts and fungal spores, which may become airborne from, for example, soil, dust, drains, surfaces (such as floors, walls, ceilings, etc) or ventilation ducts.

Other factors that may influence the carriage of these organisms in the air include attachment to particles and the particle size, air flow and air disturbances. Certain sensitive operations (for example aseptic filling), therefore, may require control measures, such as air filtration, positive air pressure and regular microbiological monitoring.

1 Water. Environmental yeasts and moulds are frequently found in surface water and may also cause difficulties in factories with poorly maintained water systems. In particu-



Aspergillus.

lar, *Pichia* spp. have been isolated from water supplies and have long been recognised as a problem to the brewing industry.

1 Equipment and surfaces. Products may become contaminated by yeasts and moulds because of inadequately cleaned and sanitised processing equipment and surfaces. Certain equipment, such as the proportioning pumps, hoses and valves used in fruit juice plants, is notoriously difficult to clean and requires particular care and attention. Also, residues such as sugar left on processing surfaces have been known to lead to yeast contamination problems in bakeries.

1 Packaging. Another potential source of yeast/mould contamination is material used in packaging. Porous materials, such as cardboard, may be heavily contaminated and even foils and plastics can be contaminated at low levels. This may be exacerbated by static, caused by the unrolling and moulding of the material, attracting dust and other particles from the environment. Some methods used to decontaminate packaging materials include heat treatment, UV irradiation, hydrogen peroxide and gamma irradiation.

1 Raw materials. Having taken care to ensure that yeast/mould contamination is minimised throughout the manufacturing process, it is extremely important to have equal confidence in the quality of the raw materials, especially if they are being used in the manufacture of numerous large batches (such as the addition of rennet during cheese manufacture) or if they are added without further cooking or processing (such

as the addition of fruit pulp to yoghurts or fillings/coatings to cakes and pastries).

Due to the nature of some raw materials, it can be extremely difficult to keep contamination to a minimum, so regular testing and monitoring of yeast/mould levels in raw materials plays a crucial role in the management of contamination in the manufacturing process.

Raw materials should always be sourced from reliable suppliers and checked, along with the finished product, for yeast and mould contamination.

Enumeration

Food manufacturers use enumeration methods (counting the number of viable cells in a sample) to identify the levels of yeast and mould contamination in raw materials, finished products and the environment. These methods are invaluable in assuring that levels fall within acceptable limits, set by individual factories, and help in determining the shelf-life of products.

Traditional methods for the enumeration of yeasts and moulds are culture based. Many different culture media are available and the medium of choice is selected according to the type of food to be tested, as this may affect the growing conditions required and the type of organisms expected to grow. Culture plating methods are described in the FDA CFSAN Bacteriological Analytical Manual and in the draft ISO standard 21527.

Some commonly used media include:

1 Potato Dextrose Agar (PDA) is suitable for the isolation and enumeration of yeasts and moulds in dairy products or on the surface of fresh meats, cured meats and sausage products utilising a low pH for the preferential growth of yeasts and moulds. This medium is also suitable for the detection and enumeration of heat resistant moulds in thermally processed fruits and fruit products.

1 Oxytetracycline-Glucose-Yeast Extract Agar (OGYEA) is based on a formulation by Mossel et al who found that the use of oxytetracycline in a medium with a neutral pH suppresses bacterial growth, including lactobacilli, and gives increased counts of yeasts and moulds from a variety of food-stuffs. Very proteinaceous foods and higher incubation temperatures (~35°C), however, will inactivate oxytetracycline (for alternatives, see RBCA or DG18 below).

1 Rose-Bengal Chloramphenicol Agar (RBCA) is recommended for fresh proteinaceous foods whose associated flora consists mainly of Gram negative, rod shaped bacteria. The medium has a neutral pH and chloramphenicol is used as a selective agent to suppress the growth of bacteria. Because of the stability of chloramphenicol, RBCA is also suitable when higher and prolonged incubation temperatures are required. The Rose-Bengal dye facilitates visualisation and enumeration of colonies and helps control

the size of faster growing mould colonies, thus preventing the overgrowth of slower growing strains.

1 Dichloran Rose-Bengal Chloramphenicol Medium (DRBC) is a modification of RBCA based on the formulation described by King et al. By lowering pH, reducing Rose-Bengal content and adding Dichloran, DRBC is designed to further inhibit the growth of bacteria and spreading moulds such as *Rhizopus* and *Mucor* spp.

1 Sabouraud Agar, which may be supplemented with various carbohydrate sources such as maltose or dextrose, is often used for environmental samples and where identification by colonial morphology may be required. The medium uses a low pH to preferentially select for yeasts and moulds. Antibiotics such as chloramphenicol and cycloheximide may also be added to increase selectivity.

1 Dichloran-Glycerol Agar (DG18) is recommended for the enumeration and isolation of xerotolerant moulds from dried and semi-dried foods, such as dried fruits, meats and fish products, spices, confectionery, cereals and nuts. This formulation, described by Hocking and Pitt, contains glycerol to lower the water activity (aW) of the medium, and dichloran to inhibit spreading of mucoraceous fungi and to restrict the colony size of other genera.

1 Aspergillus Flavus Parasiticus Agar (AFPA) is based on the formulation described by Pitt, Hocking and Glenn and is recommended for the rapid detection and enumeration of *Aspergillus flavus* and *Aspergillus parasiticus*. These species develop an intense yellow/orange colouration on the reverse of the colonies, allowing them to be easily differentiated. Bacteria and rapidly growing fungi are inhibited by dichloran and chloramphenicol in the medium.

Culture methods for the enumeration of yeasts and moulds may take up to five days to perform. Whilst this remains the method of choice for many manufacturers, others are seeking faster results. This may be particularly important for manufacturers of fresh products with short shelf-lives, or where holding material until a negative result is achieved proves very costly. In these situations, a rapid yeast and mould result can prove extremely cost effective.

Rapid enumeration methods include:

1 Fluorescent microscopy is used by some drink manufacturers for the quantification of viable and non-viable yeasts in beverages. This technique provides faster results than culture based methods, but it is labour intensive and highly subjective.

1 Polymerase Chain Reaction (PCR) – a new PCR method, the BAX System Q7 from DuPont Qualicon is available for the threshold quantification of yeasts and moulds in foods, providing same day results for highly contaminated samples (>500cfu/g) or in 48 hours for lower levels of contamination (10-500cfu/g). This automated method is easy to perform, requiring no special expertise and has little hands-on time. Results are dis-

played as positive (above threshold) or negative (below threshold), with the target threshold set by individual laboratories and validated against historical plate counts for specific food products.

The system is accurate and reliable in detecting yeasts, moulds and spores, and has been evaluated using a variety of different food types.

Conclusion

Premature spoilage of foods can prove extremely costly to companies and retailers alike. Microbiological investigation to enumerate yeast and mould contamination in food and drink products is implemented to prevent such events, thereby protecting the reputations of the brand, the retail outlet and ultimately, the manufacturer.

Cultural methods – typically taking up to five days – may be replaced in the future by rapid methods in situations where time to result is important. 11

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