

PROCESSING INDUSTRIES INCLUDING PRODUCERS OF VEGETABLES AND SPICES

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THE METHODS USED FOR MONITORING THE MICROBIAL CONTAMINATION

Testing foods for pathogenic and spoilage bacteria is the cornerstone to ensuring a safe and wholesome food supply. The methods for monitoring of microbial contamination are based on the following principles:

- Cultivation of microbes in the presence of different energy sources,
- Analysis of their macromolecular composition and their metabolic by-products,
- Use of specific immunological reagents for bacterial classification and identification.

Mostly, the conventional detection methods like plate counting and rapid methods are preferred for the routine controls in order monitoring of microbial contamination. In the laboratory internationally accredited methods by U.S. Food and Drug Administration (FDA), AOAC International (AOAC), and

International Organisation for Standardization (ISO) are used in performing the microbiological analyses covering all stages in the food chain. The validation of the microbial detection method is another approach in order to improve accuracy of analysis in the governmental institutions. For the companies which do not have laboratory, the tests can be achieved by an accredited laboratory. The rapid methods as well as traditional methods are preferred in private sector laboratories. It is very important to know some aspects of microbiological safety in food technologies in every stage of processing and packaging. The traditional tests are used to detect the microbial contamination by taking the samples from critical control points in HACCP system, the process stages, finished products, working area, packing machines, trucks, air. The effectiveness of cleaning can be monitored using ATP tests. As example, the culture media (agar) used in private sector is:

- Nutrient agar for total bacterial count
- Endo Agar for coli form bacteria and *Escherichia coli* detection
- Lysine agar for wild yeast determination
- DTA (dextrose tryptone agar) for thermophilic and mesophilic bacteria such as *Bacillus subtilis*
- YGC Agar (yeast extract – glucose – chloramphenicol agar) for moulds and yeast determination
- Baird Parker agar for determination of staphylococci
- *Bacillus cereus* agar
- DCLS (Desoxycholate Citrate Lactose Sucrose) agar for *Salmonella*, *Shigella* and other *Enterobacteriaceae*.

In addition to these classical methods, rapid methods, e.g. electrical impedance measurement, are used for detection of micro-organisms. Membrane filtration, flow cytometry, polymerase chain reaction (PCR) based methods, pulsed-field gel electrophoresis (PFGE) and Fourier Transform Infrared (FTIR) are the other methods used for detection and enumeration of micro-organisms. On the other hand, as raw agricultural commodities, spices and herbs commonly harbour large numbers of bacteria and fungi including potential spoilage organisms. The manner and environment in which they are grown, harvested and handled, as well as the chemical nature of the spice, directly impacts its microbiological

quality. In general, roots, berries and herbs carry a greater microbiological load than the bark and seed items. Bacterial multiplication is not a concern if the products are sufficiently dried, stored and shipped under normal, dry conditions, however fungal spoilage may occur if the spices are subjected to improper storage. After harvest, various types of cleaning processes are used to progressively reduce the number and types of micro-organisms. Additional means are treatment with the ethylene oxide, high temperature steam or irradiation. Spices are subjected to different type of processes such as washing, peeling, curing, drying, fumigation, cleaning, grading and milling. Here follows a list of method to improve the product hygiene:

- Drying is the most important step to prevent the mould growth. Dried spices undergo extensive cleaning to remove extraneous matter such as dirt, stones, stalks, leaves and metallic contamination.
- Milling; during which a considerable reduction in the total bacterial load as a result of the increase in product temperature.
- Ethylene oxide fumigation; vegetative cells, including coliforms, *Eschericia coli* and *Salmonella* are eliminated, with low to moderate concentrations of bacterial spores typically remaining. Many factors affect the overall reduction in microbial counts, including the initial microbial type and load, the concentration of ethylene oxide, the temperature and relative humidity in the chamber, the physical and chemical nature of the spice and its moisture content.
- Irradiation of spices with gamma rays is a simple, safe and efficient method, allows the treatment of the products in their final packaging, which eliminates recontamination issues.
- Treatment with high temperature steam: is another efficient and economical method for reducing microbial populations of some spices without sacrificing appearance and flavour levels.

POTENTIAL ISSUES IN THE MICROBIAL DETECTION

The most important issues in the microbial detection are sampling and time requirement for the microbial tests. Sampling and analyzing time are important issues for choosing the detection methods. Rapid methods are used when the results are needed in a short period of time. Although cost effective and

sensitive, the conventional methods are generally time consuming and require several days to obtain test results. During this period, the raw materials cannot be used for the production. It requires laboratory clearance before using in production. Therefore, microbiological evaluation procedure should be organized with enough time. Some food products such as minimally processed foods have shorter shelf-life. It may lead to restricting the use of conventional testing methods for this sort of products. From this point, rapid tests are the alternative methods for the microbial detection. There is a growing interest in having rapid systems that are faster and less time consuming in laboratory routine, but on the other hand, problems still arise with interference from the food components. During the conventional culturing methods, there is a difficulty to detect the injured cells. Most rapid methods rely on culturing methods to recover injured cells and amplify the number of target cells. However, rapid methods are needed to be verified by conventional detection methods. That's the reason why conventional testing methods are still the most common methods in most laboratories in routine. The fouling of sensors for in-line detection of microorganisms is a distinct problem associated with sensor technology. Furthermore, it is not easy to sample from big surfaces or volumes. Sampling method may create problems if the raw material is powdered e.g. powder milk and powder eggs and put in bags of 25 or 50 kg. The sample to be taken should be representing the rest of the bulk or the test area. Microbiological samples must be taken from critical spots on food processing surfaces. Taking the samples to the laboratory in proper conditions, training of staff, contamination during sampling and detection limit for the microbiological tests are other significant issues. For example, we assume that 1 ml of inoculate is used. If the contamination is lower than 1 colony forming units (CFU)/ml, it is not possible to detect it. There is an exception in detection of coliform bacteria in process water that is done by filtration and the amount of sample is 100 ml so the detection limit is 1 CFU/100 ml. In sample preparation, homogenization is an important factor and either stomachers or blenders are used for this purpose. In plants, the technological procedures and HACCP are also important in terms of microbiological evaluation. At first, pH, aw and humidity tests are done before microbiological evaluation, as indicative analysis. Swab samples are also taken from the hands of workers and the surfaces. In government labs, the samples are taken by the ministry's inspectors and tested in regional labs in Turkey. In addition, generally, 5 samples are taken from each lot for microbiological analysis.

SAMPLING AND PREPARATION OF HERBS AND SPICES FOR ANALYSIS

The choice of the sampling plan for food depends on the spoilage and health hazards associated with the micro-organisms of concern and how the food will be handled and consumed after it is sampled. A three-class attribute sampling plan with five samples taken at random from each lot of material, as described by the International Commission on Microbiological Specifications for Foods (ICMSF), is appropriate for routine microbiological examinations for aerobic plate count bacteria, yeasts, moulds, coliforms and *E. coli*. As with any type of food sampling, aseptic techniques should be employed. Most of the spices can easily be sampled with sterile three zone powder samplers, needle point samplers, scoops or spoons. The samples (200 g each) should be placed in sterile, polyethylene sample bags that are clearly labelled, submitted to the laboratory and tested. Spice samples should be stored in a cool (<20.0 °C) and dry area (<60% humidity) before testing.

Procedure: Sample preparation and the initial dilution vary according to the nature of the material being examined. Whole berries, roots, bark and large seeds should be reduced to a moderate particle size before testing. Aseptically weigh 100 g of the sample into a sterile, dry blender jar. Blend the sample at the lowest speed for 30 s or more. Take special care not to generate excessive heat during the blending step for this may injure or destroy the micro-organisms. Initial dilution: a) Ground spices, herbs, seasonings and small whole seeds: b) whole and coarsely ground leafy herbs Prepare 1:10 dilution (a) 1:20 dilution (b). Aseptically weigh 11 ± 0.1 g of the sample into a sterile filter stomacher bag, polypropylene bottle or blender jar and adding 99 ± 2 ml (a) or 209 ± 2 ml of 0.1% peptone water (b) and then either stomach for 30 to 60 s, shake at least 25 times or blend for 2 min depending on the type of container. Methods: It is important to note that some spices and herbs may have inhibitory action to bacteria and fungi and may produce low counts on lower dilution plates and high counts on higher dilution plates because of the transfer of the antimicrobial compounds with the inoculum. It is necessary to prepare a sufficient number of serial dilutions to overcome this natural inhibitory effect and prevent the reporting of low counts.

FUTURE NEEDS FOR THE MONITORING OF MICROBIAL CONTAMINATION

Especially, more rapid and reliable methods are needed for food manufacturing plants and for monitoring microbial contamination. For example, biosensors or fluorescence techniques could be utilized since they offer high sensitivity, short collection times and capability of monitoring large areas/volume. In Turkey, SMEs should establish a laboratory consisting of at least minimum instruments for microbial detection such as coliform, total aerobic bacteria, etc. in their plant. The most important and target micro-organisms responsible from the spoilage of food products are needed to be analyzed in the plant. The product traceability is a key element to be developed for food safety systems. The ability of trace back may be one of the most important weapons in determining of origin of contaminated or adulterated food. The ICMSF recommends that spices should be treated as raw agricultural commodities and as such the ultimate use of products will dictate the specifications. For example black pepper that contains a high concentration of spore forming bacteria may be suitable as a table condiment for seasoning cooked foods that will be eaten immediately, but maybe unsuitable for canned food processor. More swab samples should be taken from the production plants and this control should be done more frequently. Swabs should be taken for *Listeria monocytogenes* from the food contact surfaces where the *L. monocytogenes* problem has been experienced. Swabs samples should also be taken for *Enterobacteriaceae* and *Salmonella* from abattoirs e.g. chicken slaughterhouse.

Government agencies, academia and industrial microbiologists should establish controls in-process in a Hazard Analysis and Critical Control Point (HACCP) system to assure the product integrity, rather than reliance on end-product testing for compliance to specifications. HACCP verification and validation activities must be improved. The strengthening of HACCP systems that encompass all stages of production, processing and distribution will serve to further enhance the microbial safety of these products. If any company extends its product portfolio, some revisions should be done in present monitoring system. As new technologies and tests are introduced into the complicated arena of laboratory testing, it becomes increasingly difficult for regulatory and advisory agencies to provide specific safety regulations and guidelines for each new situation. It is, therefore, the responsibility of the laboratory itself to develop its own guidelines

and work practices to ensure a safe work environment for all employees. In addition, educating of consumers as to the importance of correct food handling practices will also help to prevent the spoilage and illness incidents. Furthermore, the food poisoning network should be organized among the related authorities. Collaboration in this topic should also be improved between industry and universities.

METHODS USED FOR RISK ASSESSMENT

HACCP, ISO2200/2005, IEC 17025 and BRC standards methods are especially used in all countries. Some companies have own standards and/or cleaning and sanitation programs. As new technologies and tests are introduced into the complicated arena of laboratory testing, it becomes increasingly difficult for regulatory and advisory agencies to provide specific safety regulations and guidelines for each new situation. It is, therefore, the responsibility of the laboratory itself to develop its own guidelines and work practices to ensure a safe work environment for all employees. Risk assessment is carried out by the HACCP team that meets when necessary – such as a modification in the system and determines the risks that may appear and the procedures to keep those risks from becoming actual problems. For environmental safety risks we have a system based on the requirements of the ISO 14001 standard.

Risk Assessment in laboratory: In the laboratory, the risk assessment for each separate procedure and experiment are done. In the laboratory, we use routine tests such as counting of total aerobic, coliform, yeast and moulds. Sterilized equipment are used such as sterile pipettes, sterile petridishes. The plating of micro-organisms is conducted under laminar flow hood. To develop effective strategies that continually guarantee employees a safe work environment, the performance of risk assessments must be an integral and on-going part of laboratory operation. The risk assessments should be carried out at regular intervals, at least annually, but more frequently if problems are discovered. It should be performed whenever a change occurs in the laboratory such as a move or renovation, new worker, new infectious agent or new reagent, new piece of equipment. Tools useful in performing laboratory risk assessments are:

- Reviewing laboratory records
- Injury, illness, and surveillance reports

- Equipment maintenance records
- Employee training records
- Environmental monitoring records
- Formal inspections by certifying agencies
- Reviewing published materials, equipment manuals, manufacturers' bulletins and newsletters, product inserts, scientific journals, published safety manuals and guidelines
- Observing laboratory operation (requires knowledge of relevant literature and experience with similar activities).

PREVENTIVE MEASURES

The companies which have been producing dried vegetables and spices should have HACCP, GMP, SSOP and ISO 22000. Preventive measures in plant are: GMP, process design, cleaning and disinfection, hygiene monitoring, protective clothing, physical separation of raw and cooked products and pest control. Equipment contacting to product should be cleaned easily. There is a specialized hygiene team which has daily/weekly/monthly/yearly hygiene program in plants. Authorized personnel should employ cleaning in place (CIP) systems in cleaning for the critical equipment e.g. bioreactors and fermentation tanks as well as storage tanks for yeast production. The cleaning agents and/or disinfectants should be approved to use in food industry. In the laboratory, we follow the cleaning and disinfection procedures and we wear laboratory coats (protective clothing) all the time. Training of personnel is another significant issue. Workers should always wear the protective clothing when they are in the processing line. Education of employees about good hygiene practice and HACCP program should be done, periodically. It should include use of protective clothing and consideration of preventive measures to insure product safety. As for, work safety every department employs specific equipment. In the laboratory microbiology safety equipment is used e.g. sterile rooms, sterile air cabinets, sterile gloves and breathing masks.

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