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Microbiological Assessment of Food Quality

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Abstract. Microbiological quality assessment is an integral part of any product development as it gives a clue to the safety and keeping quality of the food. The studies include raw materials, on-line studies to nomitor processing, finished product and storage studies. Methodologies are also examined to develop and suggest newer and better methods. Future priority areas of work are also discussed.

1. Indtroduction

The large number of processed foods already developed and also those that are being developed by DFRL, Mysore to meet the specific and special requirements of the Services are of differing shelf-life and are therefore of different types. Some of them like compressed bars[1], fudge bars[2] and preserved *chapaties*[3] are in a ready to eat form and are not subjected to any terminal heating before consumption. Others like dehydrated soups or hot beverages require slight reconstitution—the addition of hot water. Still some others like dehydrated convenience mixes require very limited cooking. Shelf-life, safety and acceptability or deterioration on storage of these products depend to a great extent upon the microflora which have survived the processing treatment and remain in the food. Therefore prevention of contamination of prepared foods by micro-organisms which are potentially capable of causing illness as well as considerations of microbiological quality standards form an integral part of a food product development service. Formulation and maintenance of specifications of the product within prescribed tolerance during all stages of material handling, preparation, processing and packaging therefore, is a device to assure the quality. There is a growing awareness that the finished product is as good as the ingredients it is made from and a long life during storage depends upon the factors mentioned above. Thus microbiological examinations are aimed at :

(1) Improving the quality of the product in all relevant aspects,

(2) Ensuring a safe food for the user, and

(3) Taking precautionary measures for enhancing the keeping quality of the product.

2.0 Significance in Different Foods

Foods differ much in their nature, pH value, moisture content, osmotic pressure, etc. Therefore the types of micro-organisms of significance in different foods will also be different and what may be a suitable technique for one food may be quite unsuitable for another. For example, in the case of canned foods or other thermal processed foods the food must be particularly examined for thermophilic sporulating bacteria and anerobes which can withstand the autoclaving temperatures as also for leakers while in the case of compressed bars which should be examined for the total bacterial flora, coliforms, pathogens and fungi. Micro-organisms multiply very fast and hence sampling has to be done with the minimum delay. As many factors as possible must be standardised. However, there is as yet no microbiological specification for any food which may be called international. The difficulties lie in reaching an agreement on the type of foods to be standardised, relevent organisms, methods of enumeration or detection, and appropriate numerical values.

It is not possible to make a fixed examination plan for each foodstuff. It would be much too extensive and still not exact. Instead, some general guidelines for different groups of foods are used. For such grouping several factors should be considered, eg. origin of the foodstuff (meat, fish, vegetable etc.), the technology used for its production (heat treated, dehydrated, preservative used, irradiated, comminuted etc.), packaging temperature and, period and atmosphere of storage.

2.1 *Sampling plan*

Before commencing the laboratory examination, it should be ensured that the samples are treated and handled as uniformly as possible from the stage of collection to examination. The sample should be representative of the foodstuff concerned both regarding size and composition. It should be collected aseptically. Laboratory examination should start immediately after collection of the sample. Wherever this is not possible, sample should be stored and transported under refrigeration.

Many methods of analysis require some preparation of the sample. The main consideration is to get the micro-organisms into a homogenous suspension so that they can be pippetted; but if the food is solid, it is necessary to blend the food with a diluent to obtain a suspension.

With this in view, considerable amount of work has been carried out in the laboratory over the past 22 years. The raw materials were studied for their level and type of contamination as processing requirements are interrelated. On-line studies

are carried out to monitor processing and to pin point critical stages of contamination. In order to understand the behaviour of the residual flora during storage and to predict shelf-life, storage studies were also carried out. The work carried out falls into three different categories :

1. Assessment of the quality of raw material,
2. Quality of finished products and their behaviour on storage,
3. Improvement in methods and techniques.

2.2 *Assessment of Raw Material Quality*

The raw materials were assessed for their quality so that substandard materials could be rejected as the product quality is always related to the quality of the starting material. The extent and types of micro-organisms in a number of food materials were studied[4].

(i) *Meat* : Extensive studies were carried out on meat[5-11]. The various types of contamination occurring naturally and fluctuations in their profile with time, temperature and packaging were investigated. In raw mutton exposed under the tropical conditions, gram positives were in larger number. The normal flora consisted mainly of *Micrococcus* followed by *Bacillus* sp, *Staphylococcus*, *Acinetobacter* and *Pseudomonas* in that order.

(ii) *Dry rations* : The dry rations like wheat, wheat flour, rice and different types of pulses stored in Army warehouses located in hot, humid climatic regions are generally infested with fungi and extensive studies have been carried out in this laboratory on this aspect[12,13]. The level of contamination was highest during rainy season and lowest in winter and was attributed to relative humidity. *Aspergillus niger*, *A. fumigatus*, *Rhizopus nigricans* and species of *Mucor* and *Penicillium* were found occurring commonly in most of the samples. Amongst these, members of *Aspergillus* dominated in the rainy season followed by *Penicillium*, *Rhizopus nigricans* dominated in the winter months. Toxigenic moulds isolated include species of *Fusarium*, *Aspergillus ochraceous*, *Aspergillus* sp, *A. fumigatus*, *A. flavus*, *A. candidus* and *A. nidulans*. The food materials were screened for mycotoxins by the TLC method[14]. Presence of sterigmatocystin at a concentration of 5-10 μg/kg were detected in wheat, wheat flour, green gram dal, Bengal gram dal, arhar dal and black gram dal. Alfatoxin B, varying from 10-20 μg/kg was present in wheat flour, sooji, rice and arhar dal. From all commodities where sterigmatocystin and alfatoxin were detected, *A. flavus* and a few unidentified species of *Aspergillus* were isolated. Even when the food samples were autoclaved at 10 psi for 15 min. there was no noticeable decrease in the aflatoxin or sterigmatocystin concentration thereby showing that the effect of cooking on these mycotoxins was negligible. Therefore, although the total concentration present was at biologically insignificant levels, the very fact that they are not destroyed by cooking and body enzymes, points out to their risk by cumulative effect.

(iii) *Spices* : Spices which form a component of many of the items developed in the laboratory were found to be sources of extensive microbial contamination with staphylococci and coliforms being present in many in high numbers indicating poor hygienic conditions during preparation[15]. The work indicated that there is an urgent need for more standardised methods of harvesting, drying, milling etc.[16,17]

2.3 *Quality of Finished Products and their Behaviour on Storage*

(*i*) *Preserved chapaties and parottas* : Work carried out with preserved chapaties and parottas[17,18,19] showed that the product could be made free of pathogens, indicator organisms and moulds and also within reasonably low microbial profile if proper hygienic precautions are taken. The organisms that survive were found to be all gram positive and catalase positive rods mostly belonging to the genus *Bacillus*[19]. The distinctive features of the organisms were that they were starch and protein hydrolysing, could grow fermentatively, could tolerate appreciable levels of salt, could grow in the presence of 0.1 per cent sorbic acid, were capable of forming spores that can withstand the time-temperature treatment the packages are subjected to and could grow over a wide temperature range, but poorly at low pH. Thus the factors contributing to the effectiveness of this technique are (1) low initial microbial load (2) low pH (3) immediate packaging to prevent secondary contamination (4) subsequent in-pack heat treatment to kill the post baking contamination during handling and (5) the presence of sorbic acid arresting any possible growth of survivors. Unless these points are well taken care of, the product will not have the expected shelf-life and may spoil much earlier. Microbiological specifications have been suggested[17].

Table 1. Suggested standards for preserved chapaties

Total mesophiles	...	Below 8000/g
Total thermophiles	...	Below 5000/g
Moulds	...	Nil
Yeasts	...	Not more than 100/g

Coliforms, coagulase positive staphylococci and salmonella absent in 10 g.

(*ii*) *Dehydrated mixes* : Studies with dehydrated mixes showed that thermophiles, coliforms, staphylococci, thermophilic flat sour spores, aerobic spore formers and moulds were present in almost all the batches analysed[17]. *Salmonella* and *Clostridium* were absent in all the batches. The deydrated mixes were reconstituted and analysed for their microbial status immediately on reconstituting and after keeping them exposed for one hr as this is the maximum time lag that can take place in the unit kitchen after reconstitution. The data show that the load is considerably reduced on reconstitution and coliforms and moulds were totally destroyed.

(*iii*) *Omelette mix and AFD Meat* : An instantised convenience mix for omelette for use of the Defence personnel was developed by this laboratory[20]. Detailed microbiological studies were carried out on this product, based on which microbiological standards were recommended to the Technical Standardisation Committee for adoption. Similar studies were carried out with accelerated freeze dried mutton also[7,21].

Table 2. Suggested standard for omelette mix

Total aerobic count	...	Not to exceed 75000/g
Coliform count	...	Not to exceed 100/g
Yeasts and moulds	...	Not to exceed 50/g

Test for pathogens should be negative in 50 g samples.

(*iv*) *Compressed bars* : Compressed bars incorporating processed cereals, pulses, vegetables or mutton mince—the ingredients of traditional Indian meals—sweet and saltish compositions of both vegetarian and non-vegetarian types were extensively studied for monitoring their microbiological quality[17]. The high coliform count in the case of banana rice bar was traced to the spray dried powder and the post processing handling of the bars. Attempts to control this showed that heating the packaged bars in an oven at 80°C for 1½ hours could bring the coliform count to negligible levels. This did not impart any adverse effect on the organoleptic quality of the item and therefore was introduced as a regular step[7,21] in the manufacture of the item.

(*v*) *Inpack processed foods* : Considerable work was done on the inpack processing of foods of Indian palate[22,23]. Initially repeated analysis pointed out the non-uniformity in the quality of the packaging materials used. With the use of new indigenously manufactured polypropylene, this problem has been controlled.

(vi) *Canned foods* : A wide variety of canned foods to suit to the taste of Indian palate has been developed in this laboratory. Microbiological studies were essential to decide the processing requirements. The effect of different concentrations, salt, sugar and changes in pH on the survival of spores were studied [24,25]. Improvement in the quality of the product and at the same time saving in energy could be obtained by incorporating nisin[26].

(*vii*) *Intermediate moisture foods* : Intermediate moisture fruits, vegetables and fish were studied in detail[17,27,28,29] and it was found that the ingredients and formulations were within safe limits for direct consumption. The organisms that survived, were found to be only certain strains of osmophilic yeasts and staphlococci[29]. Factors responsible for controlling their growth in this product were also studied. Hygienic precautions to be taken and the conditions of preparation of modified canned butter were enumerated[30].

(*viii*) *Frozen foods* : A number of frozen foods were developed and their microbiology evaluated. Conditions of storage and effects of time, temperature abuse were

also worked out[31]. Studies on the enterotoxigenic staphylococci indicated that a large number of foods considered, comparatively as safe are not all that safe[32,33].

2.4 *Improvement in Methods and Techniques*

Intensive studies were carried out on methods and techniques used in microbiological analysis used in microbiological analysis, as these form the basis on which the analytical results are dependent upon. The existing specifications were re-examined to see whether the results obtained could be depended upon or they require modification. Based on the work on the microbiological analysis of canned meat the existing ISI and ASC specifications were modified[34].

(*i*) *Sublethal injury* : Work on sublethal injury showed that such cells are very vulnerable and require great care in demonstration. If this is neglected, one will get a falsely favourable impression of the hygienic quality or safety of the product. The detailed studies in this line showed that 1-2 hours stay in a non-nutrient medium will refreshen and recoup the stressed cells without appreciable multiplication, as the lag phase is rarely so brief[35]. This differed from organism to organism and process to process. In the case of *Bacillus stearothermophilus*—the flat sour organism, it was seen that the surviving spores were increasingly sensitive to bromo cresol purple used in the medium and starch or charcoal had to be added to the medium[25]. In the case of some organisms of public health significance like *Staphylococcus aureus*, *E. coli*, *Salmonella* etc, certain resuscitation treatments were required for the recovery[36,37] Although there were no obvious changes in colony appearance and gram stain, wide alterations in the biochemical/immunological properties were noticeable. Salt tolerance showed distinctive changes. Enterotoxin producing ability was reduced; so also coagulase, phosphatase and TNase activity. The antibiotic sensitivity showed a much changed pattern[38]. Studies with fungi showed that sublethally injured cells showed increasing sensitivity to preservatives[39].

Work carried out on the enumeration of fungi from food materials stressed the need for change in the standard methods[40,41,42].

(*ii*) *Selection of diluent* : Studies also indicated that selection of diluent should be made after knowing the type of food, eg., physiological saline which is the most commonly used diluent may have a direct fatal effect on obligately halophilic bacteria. For examination of highly salted products, the contents of *NaCl* in the preparatory solution should be from 4-10 per cent along with 0.1 per cent peptone. Cultivation of these halophilic bacteria shall also take place in salt containing media and it may be necessary to cultivate in an atmospere of controlled low humidity viz, 75 per cent R. H. Therefore, before starting the laboratory examination, one should always try to ensure that the samples are treated and handled as uniformly as possible at all stages from collection to examination.

3.0 Future Needs

Work carried out over the years have clearly identified some future thrust areas of research.

3.1 *Rapid Methods of Identification*

The conventional methods of enumeration and detection techniques are very time taking (2-7 days) and cumbersome. It is, therefore, not surprising that attempts are being made all over the world to devise some rapid methods so that interpretations can be made quickly and decisions hastened. Work being carried out in the laboratory in this direction involving enzymes gram negative endotoxins and other serological and immunological methods should be intensified. Investigations based on instrumentation also require to be taken up intensively.

3.2 *Sublethal Injury*

Focus on this aspect is being increasingly directed on account of modifications required in conventional methods to resuscitate the sublethally injured cells. The work carried out in this line in the laboratory points out the need for more concerted efforts in this area.

3.3 *Tests for Newer and Lesser Known Hazardous Microorganisms*

As newer food resources like marine and animals, wild berries and vegetables, and newer processing techniques continue to be explored, there is also the risk of newer or lesser known hazardous organisms becoming increasingly important in food surveillance. As such, there is an increasing awareness, that organisms earlier never considered as etiological agents of food borne infections and intoxications, are being implicated of late. Therefore, much work need be directed in this line also.

3.4 *Food Transmitted Viral Diseases*

Similarly relatively very little is known about the transmission of viruses by food in comparison with our knowledge of the behaviour of bacteria. Also there is very little information regarding their survival in foods during various types of processing. Epidemiological studies have indicated that Polio virus, Influenza virus, Echo virus, foot and mouth disease virus, Hepatitis A virus etc. may be transmitted by foods. Therefore the environmental factors affecting their survival in foods under various processing conditions and their transmission in food systems require to be investigated. The raw food will be a greater vehicle of transmission. Some simple and sensitive methods for their detection and isolation from foods are required to be evolved.

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Food is the walking Stick in the life long Journey