Popular Biotechnology Lecture Series

In order to create awareness on basic concepts, recent advances and applications of biotechnology among students and teachers of schools, colleges and general public, a series comprising three popular biotechnology lectures is organised every year by PSCST. This year, the 16th Popular Biotechnology Lectures Series was organized on 18th March, 2010 at Chandigarh College of Technology, Landran, Mohali. The programme was supported by DBT-GOI. Dr. Jatinder Kaur Arora, Additional Director (Biotech), PSCST gave an overview of developments in biotechnology sector. Subsequently, three popular biotechnology lectures were delivered by experts viz. lecture on '*Biotechnology & Food Production*' by Dr. S. K. Soni, Associate Professor, Department of Microbiology, Panjab University, '*Food Safety & Standards – a perspective*' by Dr. Ajit Dua, Senior Scientist (Quality Management), Punjab Biotechnology Incubator and *'Biotechnological interventions in post harvest processing'* by Dr. Alkesh, Senior Scientific Officer, PSCST. The Lectures were followed by elaborate Question Answer Session. More than 300 students and teachers from the host as well as surrounding institutes participated in the programme. A booklet entitled 'Biotechnological Interventions in Food Production, Processing & Safety' edited by Dr. J. K. Arora and Dr. Dapinder K. Bakshi was also released during the programme.

Popular Biotechnology Lecture Series

Focus: Biotechnological Interventions in Food Production, Processing & Safety

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FOREWORD

Biotechnology is a frontline area of modern science having immense potential for the benefit of mankind. It is a multidisciplinary subject having diverse applications in the fields of agriculture, healthcare, animal science, environment and industry. In order to create awareness on basic concepts, recent advances and applications of biotechnology among students & teachers of schools, colleges, universities as well as general public, the Department of Biotechnology (DBT), Govt. of India supports the organization of Popular Biotechnology Lectures Series all over the country. Punjab State Council for Science & Technology has been organizing this lecture series in the state since 1995. This year, the theme of 16th Popular Biotechnology Lecture Series being organized by the Council is “Biotechnological Interventions in Food Production, Processing & Safety.” I hope the lectures compiled in this booklet will serve as useful resource material for students, teachers, research scholars and all others interested in the field.

Neelima Jerath
Executive Director
Punjab State Council for
Science & Technology

PREFACE

Biotechnology is playing an important role in agriculture and food production. This has been possible via advent of advanced techniques / technologies like in-vitro vegetative propagation of economically important plants, antisense technology, genetic engineering, fermentation technology etc. In order to enable the students & teachers to understand these advancements, three lectures viz. Biotechnology in Food Production contributed by Dr. V.K. Joshi; Biotechnological Interventions in Post Harvest Processing by Dr. Alkesh and Food Safety & Standards- a Perspective by Dr. Ajit Dua have been compiled in this booklet. We are grateful to the contributors for their painstaking efforts. The lectures have been edited to ensure easy comprehension. For those desirous of exploring the subject further, a 'Further Reading' section has also been included. We are grateful to Department of Biotechnology, Govt. of India for financial support and DBT-CTEP Management Cell, The Energy & Resource Institute for facilitating the organization of Popular Biotechnology Lecture Series by the Council and hence, the bringing out of this publication.

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BIOTECHNOLOGY IN FOOD PRODUCTION

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1. Introduction

Biotechnology is the exploitation of biochemical potential of plants, animals and microorganisms for medical, agricultural and industrial purposes. In reference to food production and processing, a biotechnologist would probably define biotechnology as "the application of biological organisms, systems or processes in manufacturing and service industries." It is a multidisciplinary field involving different subject areas viz. microbiology, biochemistry, molecular biology, genetics etc.

Biotechnology can significantly influence the food supply, including the production and preservation of raw materials and the alteration of their nutritional and functional properties. Improvement in agricultural production and food and nutrition situation depends on land, water and energy resources, which are generally considered as limited. These improvements in addition to the above also depend on renewable biological resources e.g. cultivated plants, domestic animals and micro-organisms. Biotechnological techniques are making significant contribution in production processes, propagation of new cultivated varieties, using microorganisms for producing useful substances, transforming food products, preserving food stuffs and improving their nutritional properties. Generally the food fermentation technology is recognized as traditional or conventional biotechnology, while that involving genetic engineering or recombinant DNA technology is known as modern biotechnology. Food biotechnology has been defined as "the application of biological techniques to food crops, animals and microorganisms to improve the quality, quantity, safety, ease of processing and production economics of food.

The application of modern biotechnology in genetic manipulation of industrially important microorganisms has been focused primarily on the improvement of processes and desirable metabolic by-products by mutation, directed selection and genetic recombination. Developments in various fields of biotechnology including bioengineering hold a wide scope in enormous areas of food production and processing.

2. Food production and biotechnology

World population has increased from about 4.5 billion in 1980, to about 5.8 billion in 1995 and about 6.5 billion today. It may reach 8.5 billion by 2035 and 10 billion by 2050. Food production and availability also had to increase commensurately.

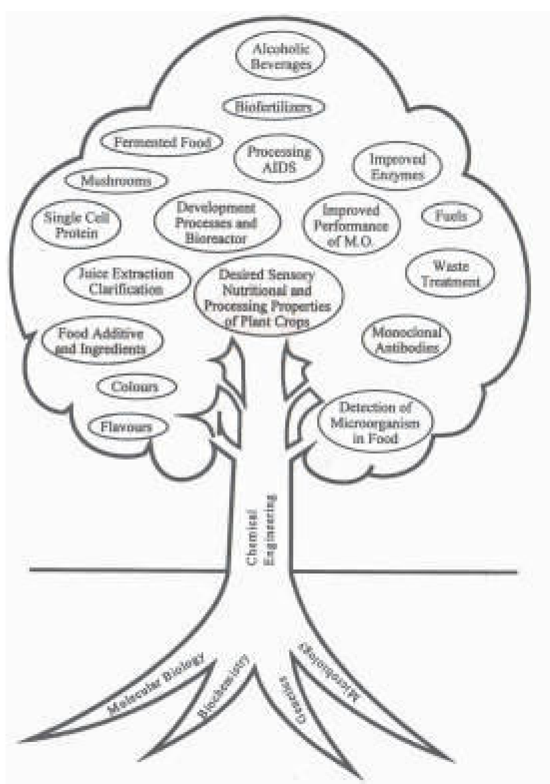

Fig.1. Tree of Biotechnology

Food crop production increased dramatically during 20 years of the Green Revolution and in the years beyond. FAO data shows that wheat yields rose 208% from 1960 to 2000, rice 109%, corn 157%, potato 78% and cassava 36% from increases in yield per hectare rather than increases in area under cultivation, and from increased adoption of modern varieties (up from 9% in 1970 to 63% by 1998). Future expansions, however, will need to be provided by land already in use. With world cereal demand predicted to increase by 50% over the next 20 years, the increased productivity must come from continued genetic improvement of food crops (both conventional and biotechnology) and a reduction in wastage from post harvest losses. Thus, we have to rely more heavily on biotechnology-related improvements in crop and animal yields. Biotechnology can also be employed to alter the nutritional and functional properties of raw materials.

2.1 Plant tissue culture: It is a major contribution of biotechnology capable of providing large plant populations in a relatively short time and in a limited space. In such populations, mutants can be produced that can be used for selection purposes. This technique has been increasingly used since 1965 by horticulturists and nursery men for producing large number of disease free saplings, particularly of annual plants e.g. a rasp-berry bush meristem can produce a progeny of 50,000 by *in vitro* culture, where as a progeny of 50 is obtained with conventional cutting techniques.

2.2 Genetic modification: Genetic modification has been used by humans for at least 10,000 years through selective breeding methods of crops and animals to achieve higher yields, disease resistance and quality. During the past 25-30 years, developments in biotechnology have enabled selected genes to be transferred within or from a species, or from one species to another in much shorter time, using molecular biology techniques including recombinant DNA technologies.

Modern genetic manipulation enables scientists to cut, copy and reassemble strands of DNA at specific locations in order to insert one or more genes for desirable characteristics, or remove gene(s) for undesirable characteristics. The techniques to produce recombinant DNA are now relatively routine laboratory procedures with highly specific outcomes. Modern recombinant genetic engineering techniques can be used to transfer genes from microorganisms, plants or animals into cells from each of these living forms. To enable modified cells to be easily recognised in the laboratory, marker genes coding for characteristics such as antibiotic resistance have been included, and this has led to the concern that these traits may be transferred into microorganisms in the human body (e.g. gut), thus increasing their resistance to therapeutic antibiotics. While scientific opinion about the risk of antibiotic resistance marker genes is that it is very low in most cases, their further use is to be discouraged until newer techniques become established.

Genetic modification of food raw materials offers several potential advantages and benefits as compared to traditional selective breeding techniques. It can provide more food in shorter span and hence is more economical, offers more precision in selecting desirable characteristics, allows more traits to be improved e.g. herbicide and insecticide tolerance, insect resistance, stress (e.g. drought), temperature and virus resistance, salt tolerance and better quality and shelf life of fresh fruits and vegetables.

The application of recombinant DNA technology has primarily helped in producing three major types of transgenic plants having improved performances. These are (1) Development of stress tolerant plants, (2) Development of plants having improved yield, (3) Transgenic plants as a source of biopharmaceuticals.

2.2.1 Development of stress tolerant plants

2.2.1.1 Plants resistant to environmental stress: Plants need to cope up with abiotic stresses such as drought, cold, heat and soils that are too acidic or salty to support plant growth. While plant breeders have successfully incorporated genetic resistance to biotic stresses into many crop plants through cross breeding, their success at creating crops resistant to abiotic stresses has been more limited, largely because few crops have close relatives with genes for resistance to these stresses. Therefore, rDNA technology is being increasingly used to develop crops that can tolerate difficult growing conditions. Genetically modified tomato and canola plants that tolerate salt levels 300 percent greater than non-genetically modified varieties have been developed. Similarly, many genes involved in cold, heat and drought tolerance found naturally in some plants and bacteria have been identified. In Mexico, maize and papaya have been produced that are tolerant to high levels of aluminum that significantly impedes crop plant productivity in many developing countries.

2.2.1.2 Herbicide resistant plants: Many effective broad spectrum herbicides do not distinguish between weeds and crops, but crop plants can be modified to make them resistant to herbicides, so as to eliminate weeds more selectively. For example, the herbicide RoundupTM contains active ingredient glyphosate, which kills plants by binding to the active site of the enzyme (EPSP synthase) which is critical for synthesis of aromatic amino acids. Roundup is an extremely effective herbicide but it kills almost all species of plants, including most crop plants. On the other hand, it is very safe for humans and animals because they do not have EPSP synthase. By using rDNA technology, modified EPSP synthase gene (that produced enzymes that were still functional but were not inhibited by glyphosate) has been introduced into crop plants such as cotton and soyabean. These genetically modified plants were found to be highly resistant to treatment with Roundup. Genes that provide resistance to other herbicides such as sulfonyl urease, gluphosinates etc. have also been developed and transferred to produce various transgenic plants.

2.2.1.3 Insect resistant plants: To minimize crop damage by insects, mites and nematodes, farmers use synthetic pesticides extensively which cause severe effects on human health and environment. The transgenic technology provides an alternative and innovative method to improve pest control management which is eco friendly, effective, sustainable and beneficial in term of yield. This involves genetic incorporation of toxic gene (product of which is lethal to insect) in to the plant. This kill the insects without use of dangerous insecticides. The first genes available for genetic engineering of crop plats for pest resistance were Cry genes (popularly known as Bt genes) from a bacterium *Bacillus thuringiensis*. These are specific to particular group of insect pests, and are not harmful to other useful insects like butter flies and silk worms. Transgenic crops (e.g. cotton, rice, maize, potato, brinjal, cauliflower, cabbage etc.) with Bt genes have been developed and such transgenic varieties proved effective in controlling the insect pests and it has been claimed worldwide that it has led to significant increase in yield along with dramatic reduction in pesticides use. The most notable example is Bt cotton (which contains CryIAc gene) that is resistant to a notorious insect pest Bollworm (*Hellicoverpa armigera*).

Biotechnology has also opened up new avenues for natural protection of plants by providing new biopesticides, such as microorganisms that are toxic to targeted crop pests but do not harm humans, animals, fish, birds or beneficial insects. One such microorganism is commonly found soil bacterium *Bacillus thuringiensis*. The spores of Bacillus thuringiensis (Bt) contain a crystalline protein (Cry) which breaks down to release a toxin, known as delta-endotoxin which is highly toxic to lepidopteran larvae. This toxin binds the intestinal lining and creates pores resulting in an ion imbalance, paralysis of the digestive system, and consequent death of the insect. *Bt* toxin sprays and powders have been in use for many years.

2.2.1.4 Disease resistance plants: Plants are susceptible to viral, bacterial and fungal diseases. Much progress has been made in evolving transgenic plants resistant to viruses. For example, expression of a gene that encodes the coat protein of tobacco mosaic virus (TMV) in transgenic tobacco plants has been shown to cause the plants to resist TMV infection. A number of other virus resistant plant species have been developed including squash and potatoes. Genetic engineering of crop plants for resistance to fungal and bacterial infections has been more difficult. However, by studying the protective genes that are expressed in naturally disease-resistant plants, an encouraging progress has been made. The proteins encoded by these so called pathogenesis related proteins (PR proteins) can, in some cases, provide limited disease protection in transgenic plants. There are several strategies for engineering plants for viral resistance and these utilize the genes from virus itself (e.g. the viral coat protein gene). The virus-derived resistance has given promising results in number of crop plants such as tobacco, tomato, potato, alfalfa, and papaya. Some virus resistant transgenic plants like papaya resistance to papaya ring spot virus have been commercialized in some countries.

2.2.2 Qualitative & quantitative improvement of crops

2.2.2.1 Increased productivity

In addition to increased crop productivity by using built-in protection against diseases, pests, environmental stresses and weeds to minimize losses, attempts are being made to use biotechnology to improve crop yields directly. Researchers at Japan's National Institute of Agrobiological Resources added maize photosynthesis genes to rice to increase its efficiency of converting sunlight to plant starch and increased yields by 30 percent. Crops that have better accessibility to the micronutrients they need are also being developed. Nitrogen is the critical limiting element for plant growth and researchers from many scientific disciplines are tearing apart the details of the symbiotic relationship that allows nitrogen-fixing bacteria to capture atmospheric nitrogen and provide it to the plants that harbor them in root nodules as given below:

- Plant geneticists in Hungary and England have identified the plant gene and protein that enable the plant to establish a relationship with nitrogen-fixing bacteria in the surrounding soil.
- Microbial geneticists at the University of Queensland have identified the bacterial gene that stimulates root nodule formation.
- Collaboration among molecular biologists in the European Union, United States and Canada yielded the complete genome sequence of one of the nitrogen-fixing bacteria species.
- Protein chemists have documented the precise structure of the bacterial enzyme that converts atmospheric nitrogen into a form that the plant can use.

2.2.2.2 Increase in quality of plant products

One of the most successful research efforts to change the characteristics of a plant produce was carried out with tomatoes. Tomatoes need to be picked while still green so that they are firm enough to withstand mechanical handling and transport. Unfortunately, they do not develop the same flavor and texture of vine ripened tomatoes. Softening of tomatoes and many other fruits is caused by the enzyme pectinase or polygalacturonase (PGA). This enzyme digests the pectin polysaccharide that cements the plant cells together. Softening of the fruit is caused, in part by this breakdown of pectin. In order to reduce the levels of PGA in ripening tomatoes, researchers placed the PGA gene in reverse orientation relative to the CaMV 35S promoter. This results in transcription of an antisense RNA that is complementary to the normal sense PGA mRNA. Although the exact mechanism is unknown, antisense RNA is able to arrest the translation of the endogenous PGA mRNA in the tomato fruit. Transgenic tomato plants that express an antisense PGA gene only have about 5 to 10% of normal PGA levels. Fruits of these plants have normal color and flavor but they soften more slowly and can be picked and processed after they are ripe. They also have a higher content of soluble solids and are therefore better than normal tomatoes for processed tomato products. Transgenic lines of potato having increased levels of starch also have been developed by introducing a gene construct that expresses a gene from bacteria that produce an enzyme that enhances starch biosynthesis. A promoter from a potato gene that encodes the major protein in potato tubers has been used, so that the expression of the introduced gene is limited to the tuber. Tubers accumulate approximately 3 to 5% more starch than normal potatoes and when they are deep fried, absorb less oil and yield chips having fewer calories.

In 1998, genetic engineers' team of Jawaharlal Nehru University, New Delhi, India, developed the transgenic potato converting a starchy potato into a whole-some nutritive food. These potatoes have much higher amino acid content than ordinary potatoes. To make these transgenic nutritive potatoes, they transferred the gene coding for seed storage protein named AMA 1 responsible for essential amino acids. They have also introduced the gene AMA 1 in a yeast *Schizo. pombe*, which can be fermented on commercial scale and used as a protein supplement in poultry feed. Raw material modification and improvement can be employed to increase stress resistance and improve their functionality and nutritional quality. The ability to convert polysaccharides and to impart specific structural changes can improve the functionality of carbohydrates in foods and increase product yield.

Recent experiments have demonstrated that it is possible to turn off the expression of certain genes in transgenic plants by introducing a gene constructed to generate antisense RNA.This allows expression of specific genes to be diminished, permitting their identification and assessment of their function in ripening. In post-harvest physiology, one of the main concerns for prolonging the shelf life of fresh fruits is to stop ethylene action and this is usually achieved by the use of controlled atmospheres (CA) or ethylene adsorbents like potassium permanganate or activated charcoal/vanadium oxide.

Genetic transformation has been utilized successfully to interfere with ethylene action. The transformation of tomato with either the ACC synthase or EFE gene in antisense dramatically reduced ethylene production. The resultant transgenic fruits change colour, but over a period of weeks do not over ripe or shrivel like the controls. When similar fruits were ripened in the presence of ethylene, this effect was ovecome and the fruit ripened in a similar way like that of normal control fruits. This would obviously be a desirable characteristic for many fruits in postharvest physiology. Some of the other value added transgenic crops include:

- Golden rice: containing beta carotene to overcome vitamin A deficiency in regions where rice is the staple food
- Canola containing high levels of oleic acids and laurate
- Barley containing feed enzymes
- Tomatoes which do not rot at room temperature
- Other vegetables and fruits with delayed ripening as well as modified flavour characteristics

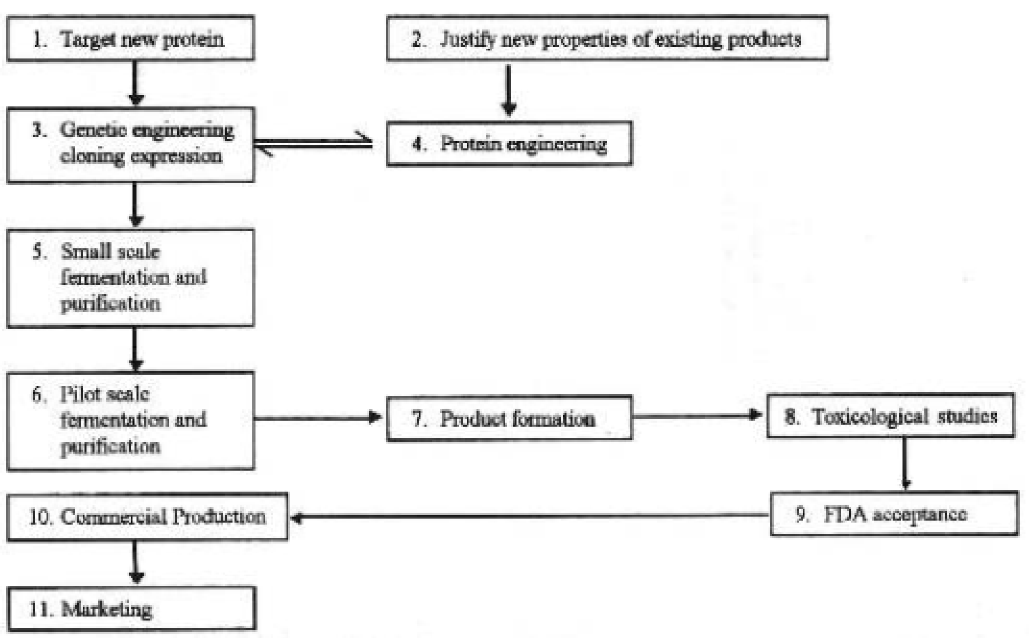

Fig.2. Flow chart for commercial development of recombinant food products

However, there are some concerns pertaining to GM crops & foods such as safety w.r.t. unintended consequences if any, environmental impact, potential allergenicity of novel proteins in GM foods and social impact in terms of their cost and public access.

2.2.3 Therapeutic proteins, enzymes and diagnostics

Transgenic plants can also be used for cost effective bulk production of a variety of proteins used in diagnostics for detecting human diseases and therapeutics for curing human and animal diseases. Given the right genes, plants can serve as bioreactors for production of modified or new compounds such as amino acids, proteins, vitamins, plastics, pharmaceuticals (peptides and proteins), drugs, and enzymes for food industry and so on. In this context, a potential and remarkable example is described below:

Edible vaccines

Crop plants serve as cost-effective bioreactors to express antigens which can be used as edible vaccines. The genes encoding antigenic proteins can be isolated from the pathogens and expressed in plants and such transgenic plants or their tissues producing antigens can be eaten for vaccination/immunization (edible vaccines). The expression of such antigenic proteins in crops like banana and tomato are useful for immunization of humans since banana and tomato fruits can be eaten raw. The edible vaccines that are produced in transgenic plants have great advantages like the alleviation of storage problems, easy delivery system by feeding and would be low cost as compared to recombinant vaccines produced by bacterial fermentation. Vaccinating people against dreadful diseases like cholera and hepatitis B by feeding them banana/ tomato, and vaccinating animals against important diseases such as foot and mouth disease by feeding them sugar beets could be a reality in the near future.

2.3 Metabolic engineering and secondary products

Plant biotechnology will lead to improved plant sources for production of valuable secondary metabolites. Over-expression of the gene which encodes for the first enzyme in a pathway generally results in higher levels of desired end product, and this has been successfully done for enhancement of taxol production from the transformed tissue cultures of *Taxus* sp. Another strategy involves use of *Agrobaccterium rhizogenes* to induce the excessive formation of secondary roots in plants that normally produce useful secondary metabolites in this region. Transgenic plants can be used as factories to produce polyhydroxy butyrate (PHB, biodegradable plastic). Genetically engineered *Arabidopsis* plants produced PHB globules exclusively in their chloroplasts without effecting plant growth and development.The large scale production of PHB may be easily achieved in tree plants like populus, where PHB can be extracted from leaves. Industry has already begun to explore the production of biodegradable plastics from transgenic plants.

2.4 Biotransformation

Biotransformation can be of importance in production of compounds by plant cells. The enzymatic conversion of a readily available or inexpensive precursor to a valuable product is called biotransformation. It is an appealing technology particularly when coupled with cell immobilization methods. Although plant cell possesses a diverse array of enzymatic capabilities, biotransformation should be considered only for the reactions that are highly plant specific. Multistep biotransformation can be achieved by utilizing a cell line that expresses a series of enzymatic activities. For the production of useful compounds, the mass culture of plant cells was mastered in 1976 by Japanese researchers. The destruction of plant cells for complete biosynthesis of useful compound as well as bioconversion of a given substance can be avoided by immobilizing them within the polymers and immobilized biocatalyst technology can be employed for the production of secondary plant metabolites.

2.5 Biofertilizers

Intensive work on biological atmospheric nitrogen fixation is underway with a view to improve the efficiency of this process which is imperative to the functioning of nitrogen cycle in the biosphere as well as in increasing the plant food productivity. Biofertilizers (preparations containing microbes benefiting the crops) are one of the important components in "organic food production" which has great potential in increasing the food production quantitatively as well as qualitatively. Benefical impacts of rhizobial inoculation on various legume crops' yield as well as protein yield have been achieved. Tomato fruit yield as well as the citric acid content improved significantly due to vesicular-arbuscular mycorrhizal fungi. Inoculation of soil with VAM-fungi improved the seed as well as protein yield of soybean. The creation of nitrogen fixing symbiotic associations with non-leguminous plants would have important agricultural applications and, in the longer run, there is immense scope for research on transfer of nif genes from bacteria to plants. The construction of self-transmissible plasmids bearing nif genes made it possible to convert non- N_2-fixing bacterial species into N_2 fixing. In order to improve the N_2-fixing efficiency, genetically screened and improved strains of *Rhizobium* will constitute more promising inoculants for cultivated legumes for better production as well as protein synthesis.

2.6 Production of plant proteins in microorganisms

The production of plant proteins commercially in microorganisms is economical. The research efforts are primarily directed towards the most effective production process by choosing the efficient microbial host, optimization of the stability of the genetically modified constructs to sustain the large scale fermentation, optimization of the expression of gene, optimization of secretion etc.

Thaumatin which is a sweet tasting protein synthesized in the fruits of African shrub *Thaumatococus danielli* Bonth, can be produced in genetically engineered *E.coli* and *S.cerevisiae* cells. It is not only 100,000 times sweeter than sugar on a molar basis, but also has flavour enhancing effects at subsweetness levels.

2.7 Food fermentaion technology

Fermentation is an age-old biotechnology which carries out the transformations in simple raw materials using either the phenomenon of growth of microorganisms and their activities or both and synthesizes amazing value added range of products. The science and technology of food fermentation is important as it involves all those processes where either the ultimate product is used directly as food, as an additive to food or a basic ingredient to the food or the byproduct formed during fermentation, their utilization, disposal or proper management. The additional advantages of fermentation technology are

- Produces value added food and adds variety to human diet.
- Potent tool for food preservation.
- Improves quality of the food products through flavour development, enrichment with various nutrients viz; vitamins, proteins, amino acids etc.
- The foods also become nutritious as the locked nutrients in the plant structures are released.
- Energy efficient than their counterparts, require less complex or costly equipments.
- Can recycle the natural materials and convert waste materials into useful products e.g. Single cell proteins, alcohols etc.
- Produces metabolite or by-produts having immense utility in the food industry.

2.7.1 Preservation of food

Fruits and vegetables are highly perishable commodities. Due to lack of adequate postharvest handling facilities & proper infrastructure, the post harvest losses due to spoilage are as high as 25-30 percent of value of the produce. Thus the preservation of such perishable commodities can be achieved through fermentation and dehydration which are the age old techniques. In contrast to other methods of preservation, where microorganisms are either killed or suppressed, in fermentation they are encouraged to grow, multiply and allowed to carry out metabolic activities. In case of milk, vegetables and some fruits (e.g. olives), fermentations are primarily aimed at preserving the nutrients of these foods (which otherwise will be quickly degraded and food would lose its palatability) and improving their digestibility (e.g. Soybeans are not readily consumable by humans even when cooked). While other food fermentations are primarily targetted at improving the taste and flavour of foods and increasing their protein contents simultaneously. Fermentation is widely used to preserve the perishables like milk, meat, fruits and vegetable foods. Fermented foods are important in the countries of South-East Asia since ancient times, where they contribute markedly to the protein component of the diet.

The preservative effect of fermentation contributes in the formation of ethanol, CO_2, bacteriocins, reutrin etc; and lowering of pH by lactic or acetic acid bacteria (e.g. bread making and alcoholic beverages). The yeast fermentation produces CO_2 resulting in lowering of pH which is complimented by the synthesis of metaboliltes (e.g. citric acid, keto-glutaric acid etc.). In the sour rye or sour white bread, the preservative effect is pronounced as besides yeast, lactic and acetic acid fermentation also takes place.The whole gamut of food fermentation is dependent on the microorganisms and their activity in the food. The yeasts are undoubtedly most important group of microorganisms exploited commercially by man in brewing industry. Besides alcoholic beverages, yeast has also been utilized in number of products. The heterologous molecular cloning in yeast *Saccharomyces s*pp. produced various proteins and peptides viz; somatostain, glucoamylase, a-glucanase, a-glucosidase, chymotrypsin, thaumatin etc. In most of the instances, the development of desired sensory qualitiles of indigenous food depends on the activities of two or more types of microorganisms. Naturally yeasts and molds are often present on fruits and vegetables which contribute to the development of proper flavours and aroma of fermented foods.

2.7.2 Alcoholic beverages

The manufacture of alcoholic beverages by various strains of microorganisms is based on the alcoholic fermentation converting the fermentable sugars of starch or the juice of a fruit to ethanol and other less abundant products. Wines are made principally from fruits like grapes or extract of other fruits, while the beers are prepared from cereal grains. The principle carbohydrate in cereal grains is starch, which is hydrolyzed through malting process. Malting induces the production of enzymes in grains. Variety of liquors viz; whisky, rum, gin, vodka etc. are produced by distillating the beer while brandy is produced from distillation of wine. In addition to the ethanol and other metabolites' production, the other biotechnological practices, involving chemical engineering and enzyme technology, have very important roles in the alcoholic beverage industry. The developments in this industry made it possible to select the yeast for producing particular type of beverage; to reduce the incidence of undesirable 'wild' yeasts; to optimize the brewing conditions viz; temperature, pH, aeration and use of yeast nutrients. The yeast strains of *Saccharomyces cerevisiae* and *S. carlsbergensis* are used in brewing. Modification and improvement of yeasts, through genetic engineering techniques such as protoplast fusion, recombinant DNA technology, could overcome low tolerance to ethanol and high substrate concentration (Table 1).

Table 1: Modification in industrial yeast strains using protoplast fusion technique

Fusion between	Characteristics/abilities modified in product
S.cerevisiae x S.diastaticus	More ethanol production Utilizing both starch & glucose
S.cerevisiae x S. mellis	Better fermentation (osmotolerant)
Saccharomyces x Kluyveromyces Spp. lactis	Better wort fermentation

For improvement or diversification of the traditional brewing process, new strains of *S. cerevisiae* can be produced through recombinant DNA technology. *S.cerevisiae* cannot ferment dextrins (polysaccharides) present in barley, however other yeast S.*diastaticus* can ferment the barley starch completely to alcohol through secretion of enzyme responsible for breaking down the dextrin. The fermentation of dextrin due to *S. diastaticus* imparts the unpleasant taste to beer. The transformant *S. cerevisiae* strain having DEX gene encoding amylo--1, glucosidase obtained from *S. diastaticus*, produces a low carbohydrate good taste beer by fermenting most of the dextrins for the production of ethanol.

It is worth mentioning that the fermentative production of alcohol has been acknowledged in the light of the successive increases of oil prices. The number of alcohol-producing plants are already being exploited or could be cultivated on large available surfaces for the production of ethanol (Table 2). The production as well as quality of produce of such plants can also be improved through various modern biotechnological processes. The biotechnological advances viz; use of immobilized enzymes or genetic recombination for developing higher yielding yeast and having certain other properties as discussed can improve the production of ethanol by fermentation.

Ethanol can also be manufactured by fermenting the waste material generated from food as well as other industries. Apple pomace (a waste) is produced in large quantities in most of the apple processing countries of the world. Theoretical yield of ethanol to the tune of 78% was obtained from apple pomace with fermentation by adding yeast obtained from apple pomace inducing the fermentation by adding yeast. A solid state fermentation process was developed involving the inoculation of apple pomace with commercial wine yeast, and separation of alcohol from spent pomace after fermentation, with butchi rotary vacuum evaporator Simultaneous production of ethanol and animal feed by solid state fermentation of apple pomace has been successful.

Table 2: Alcohol producing plants for ethanol production.

Name of plant	Storage carbohydrate
Cassava, cereals (especially maize potatoes).	Starch
Jerusalem artichokes	Inulin
Sugarcane, pine apple, sugar-beet and sweet sorghum.	Sucrose

3. Biotechnology and process waste management

During the production and processing of food, large volume of waste is produced which creates disposal & pollution problems. This also results in substantial loss of essential nutrients. Such waste can be used for various purposes. The use of microorganisms in the waste management has received a considerable attention which besides curtailing the environmental pollution produces the metabolites of economic importance viz; ethanol, proteins (single cell proteins, mycoproteins). The cultivation of algae on waste water from various food industries purifies the waste as well as produces a biomass rich in proteins and trace elements. The nature and scale of such microbial conversion depends on their economic viability factors and also on their degree of acceptance & suitability to the society.

The starch contained in waste from vegetable & food industries can be hydrolysed by enzymatic actions into dextrins and glucose which can further be used to produce ethanol by fermentation as well as fructose syrups. The complete hydrolysis of cellulose to glucose can be carried out by *Trichoderma reesii* through the enzyme cellulase. Microorganisms capable of degrading cellulose are numerous as well as ubiquitous in nature. The enzymes viz; amylases and cellulases together can be exploited in management of process waste through production of alcohols. Genetic recombination techniques can contribute to the isolation and development of high yielding amylase as well as cellulase producing strains in order to reduce disposal and pollution problems of the process wastes by converting them in alcohols, especially ethanol. Certain kinds of process waste from food industries can be converted into many useful value added products. Apple pomace from juice processing can either be used directly in bakery products and soups, preparation of jams, sauces, toffees as bulking agent, as animal feed or can be converted in to chemical energy e.g. butanol, ethanol, single cell proteins (SCP) etc. The use of process waste as substrate for production of SCP can solve the future food problem as well as address the problem of under nourishment especially in developing world. SCP as human food or animal feed was considered an alternative to cope up with the food shortage in Germany during First World War. Brewer's yeast and certain other food yeasts were incorporated in the soups and sausages. The desirable attributes of microorganisms for use as SCP on commercial scale are rapid growth, good quality high protein content, non-toxicity, should be easy to digest, good palatability, rich in various nutrients and low nucleic acid content.

BIOTECHNOLOGICAL INTERVENTIONS IN POST HARVEST PROCESSING

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1. Introduction

Biotechnology has historically been used in food processing e.g. in bread making, brewing etc. However, with the advent of modern biotechnology its potential is being explored in food processing sector especially in the areas of enzymes, food ingredients, food testing and post harvest management of horticultural crops.

The post harvest technology includes the activities like harvesting, handling, storage, processing, packaging, transportation and marketing. Insufficiency in any of these activities accounts for substantial post harvest losses, the magnitude of which depends on crops, country & year. It is important to mention that post harvest losses are one of the most significant factors limiting the agricultural production in the third world & developing countries. Whereas the technically advanced countries such as USA, Japan, Australia & European countries can apply relatively sophisticated technologies to minimize losses; developing countries cannot afford these. In addition many of the developing countries are situated in the Tropics, where high temperature & humidity exacerbate the problem.

1.1 Production statistics & post harvest losses

India is the second largest producer of fruits & vegetables in the world. It produces 63.5 MT of fruits and 125.8 MT of vegetables, which constitutes about 12 and 13 % of world fruit & vegetable production respectively. The bulk of this production is consumed fresh and substantial quantity ranging from 25-40% goes waste due to improper post harvest management. The cumulative annual loss of such wastage amounts to as high as Rs. 50,000 crores. India wastes fruits and vegetables every year equivalent to the annual consumption of the United Kingdom. Further, the processing of fruits & vegetables in India is estimated to be < 2.5% of the total production. Hence the reduction in post harvest losses needs to be addressed with utmost attention in order to put a vital link in place between production & consumption.

Why horticultural produce deteriorates after harvesting?

The fruits and vegetables are living entities & carry all the metabolic activities even after they are detached from their growing medium i.e harvested from parent plant. The major factors responsible for their spoilage are;

- *Biological Factors*: Respiration, Ethylene production, Compositional changes, Transpiration, Physiological and Pathological breakdown, Physical damage.
- *Environmental Factors*: Temperature, Relative Humidity, Atmospheric composition, Light & Other factors like chemicals (fungicides, growth regulators etc.)

2. Biotechnological applications in post harvest management

The Post harvest management of agricultural commodities includes maintenance of quality in terms of appearance, texture, flavour & nutrient value, to maintain food safety and reduce the losses along the supply chain between harvest & consumption. From Biotechnology point of view, it is therefore important to establish the nature of crops & particular problems before establishing the approach to be attempted. Modern genetic engineering techniques allow to cross the species barriers (and even kingdom barriers) and therefore genes that would normally be not accessible by conventional breeding can now be incorporated into plant species being targeted. The latest technologies which are being used to improve the properties of these commodities to bring about the improvement at post harvest levels are as:

a) Genetic Engineering (GE): Genetic manipulation by which a gene (usually defective) is replaced by a gene from an outside source, thus having a new combination of inherited properties to benefit to man. There are a number of ways through which genetic engineering is accomplished. Essentially, the process has five main steps.

- Isolation of the genes of interest
- Insertion of the genes into a transfer vector
- Transfer of the vector to the organism to be modified
- Transformation of the cells of the organism
- Selection of the genetically modified organism (GMO) from those that have not been successfully modified

b) Antisense Techniques: Antisense techniques are used to deactivate disease-causing or undesirable genes so that they cannot produce harmful or unwanted proteins.

2.1 Control of ripening

Ripening of fruits & vegetables involves large number of biochemical pathways that result in marked changes in texture, taste, colour & flavour. At molecular level, genes involved in ripening are tightly regulated in order to induce the right changes at the right time in highly coordinated manner. Most of the genetic engineering (GE) approaches attempted in order to improve the shelf life & general appearance of fruits have focused on the set of genes controlling fruit firmness (membrane & cell wall properties) & the ripening rate (ethylene production). These approaches target endogenous genes with vital functions in the ripening process aiming to down regulate their activity by gene silencing.

2.1.1 Control of fruit firmness

Pectin is an important component of plant cell wall that significantly contributes to fruit firmness. Softening is an important contributor to losses experienced during handling & transport of fruit which is due to degradation of pectin. Among the genes involved in firmness, the most extensively studied is the one coding for Polyglacturonase (PG), a cell wall enzyme that catalyze the hydrolysis of Polyglaturonic acid chains.

The lower level of this enzyme in fruit is helpful in increasing the shelf life. In tomato, the partial softening of the PG gene has been achieved by using sense & anti-sense technique. Low PG tomatoes obtained are resistant to cracking and splitting than regular fruit. Further, they had superior handling and transport characteristics as apparent from reduced degree of damage during these processes. The molecular analysis of transgenic tomato lines showed that fruit in which PG activity had decreased to less than 1% of normal levels contains larger polygalacturonic acid chain thereby increasing the cell wall adhesion & making fruit sturdier.

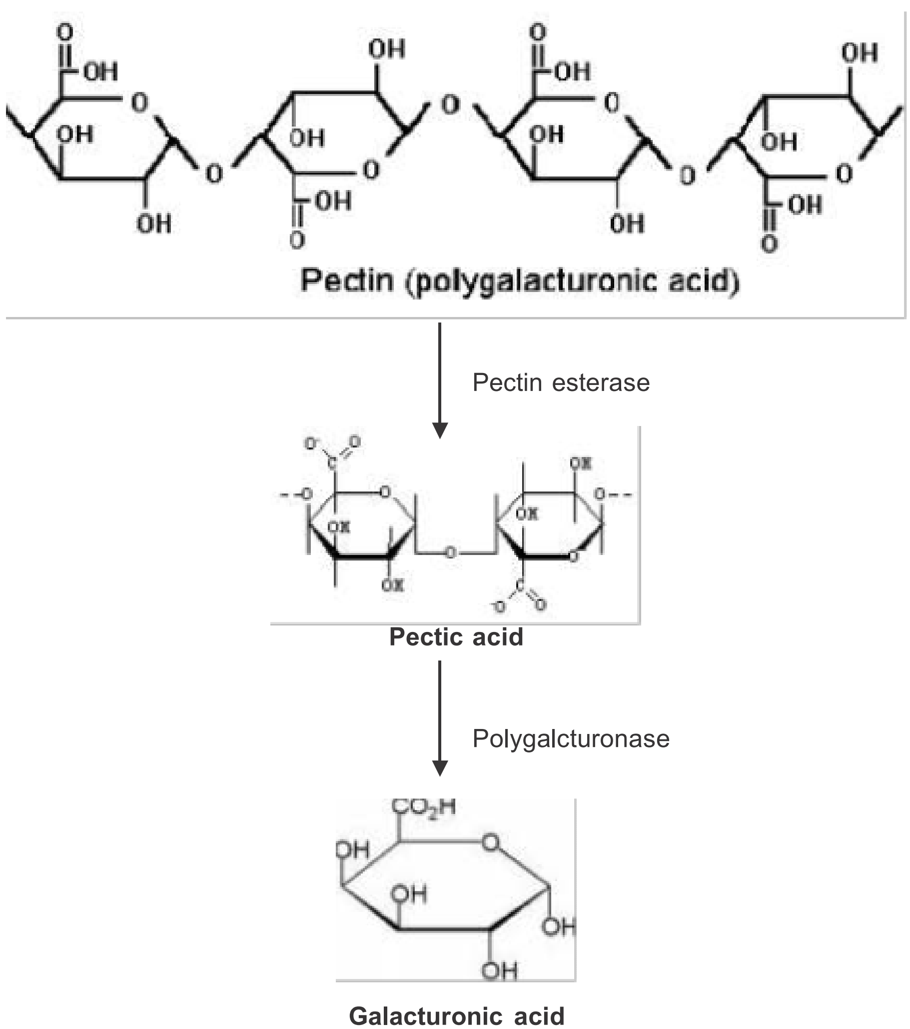

Figure1. Pectin degradation pathway

Agronomically, the effect of low PG can be translated in fruits that can be left on the vine for a longer time, therefore enhancing flavour as the softening process has been slightly delayed. The main commercial use of low PG tomato fruit has been in the processing industry. Transgenic low PG tomatoes show enhanced viscosity of the processed products & produce less waste.

"Flavr Savr" the commercial name of low PG tomatoes, marked an important milestone in Plant Biotechnology being first Genetically Modified (GM) plant to reach market, commercialized by Calgene in the USA in 1994. Zeneca & Associates are currently commercializing a tomato puree based on GM low PG tomatoes. It is important to remark that softening varies among all fruits and different species have been found to have different cell wall modifying enzymes like cellulase, pectinesterase, galacturanases etc. Therefore, it is not possible to advise a single universal strategy to control softening.

2.1.2 Control of ethylene synthesis & perception

Based on the response of ethylene, fruits are classified as Climacteric & Non Climacteric.

- *Climacteric fruits* refer to fruits that have high respiration rate during their ripening & are able to ripen even after harvest like apple, apricot, banana, mango, pears, tomato etc.
- *Non Climacteric* fruits are those which have no increase in respiration rate during ripening & do not ripen after harvest. They include fruits like citrus, grapes, cherries, strawberries etc.

Ethylene (C_2H_4) is a natural product of plant metabolism and is produced by all tissues. It is one of the simplest organic molecules with biological activity and only gaseous hormone known till date. In climacteric fruits, ethylene controls the onset & rate of ripening & hence, several strategies have been devised to interfere with either the rate of ethylene synthesis or its perception by the fruits. The elucidation of ethylene biosynthesis pathway has opened the doors for isolation & cloning of the corresponding genes.

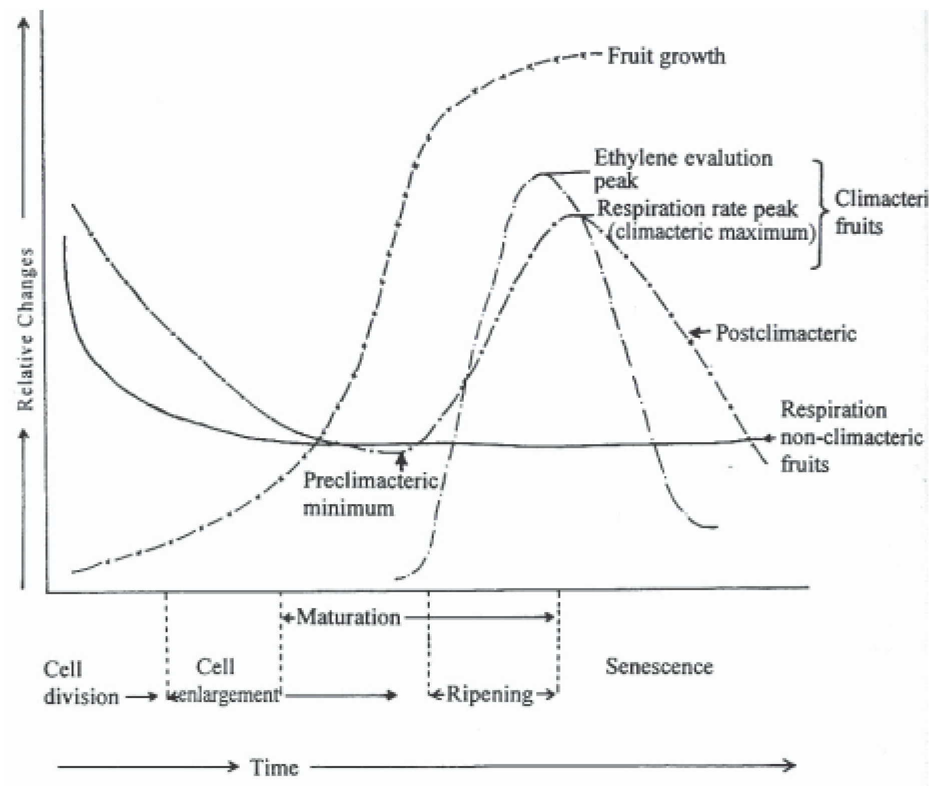

Figure 2. Changes in the fruit growth and respiration and ethylene evolution rates in climacteric & non- climacteric fruits.

Source: Wills *et al*. 1981

Aminocyclopropane carboxyalate (ACC) synthase & ACC oxidase are the main enzymes in this pathway controlling the last two steps in the production of ethylene. Both of them are encoded by the multigenes families & normally one or two members of the family are active in the fruit during ripening.

ACC synthase gene active during ripening of tomatoes (LEACC2) was silenced using anti sense technique effectively reducing the production of ethylene by the ripening fruit by 99.5%. While control fruits begin to produce ethylene 48-50 days after pollination & immediately undergo a respiratory burst, genetically modified tomatoes produced minimum level of ethylene & failed to produce the respiratory burst (atleast during 95 days).

Transgenic fruit started showing symptoms of chlorophyll degradation 10-20 days after the control fruit turned to yellow & eventually months later developed an orange colour, while control tomato fruit needed only 10 days for translation from mature green to red tomatoes. Further, instead of altering the level of enzyme controlling the biosynthesis of ethylene, two companies (Monsantto & Agritope) have opted for alternative strategies aimed at depleting the intermediate substrate of the pathway.

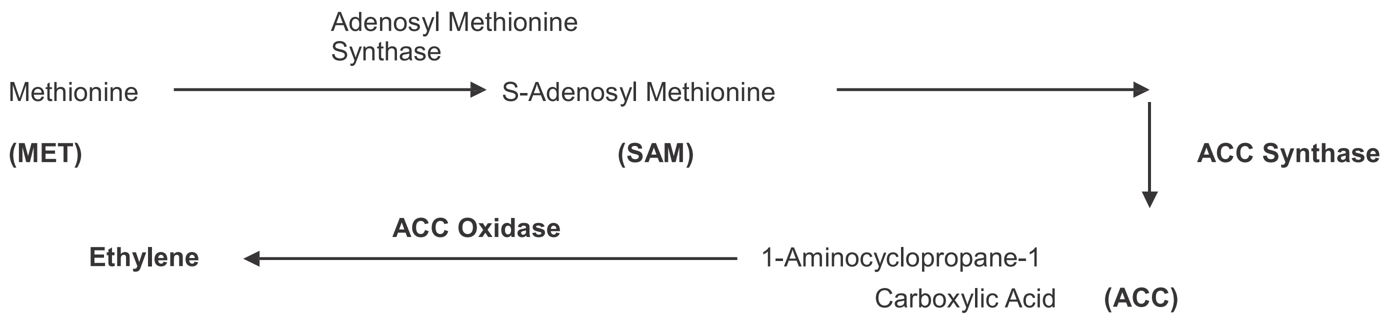

Figure 3. Ethylene Biosynthesis pathway

Monsanto has used a bacterial enzyme (ACC deaminase) to drain immediate precursor of ethylene (ACC) from the cell. Over expression of an ACC Deaminase gene in tomato plants led to marked depletion of the level of ACC & therefore reduced the availability of this precursor to be converted to C_2H_4. Out of all the independent transgenic lines obtained, the best one produced fruits with ethylene levels of only 10% of the control. Fruits picked from plant at breakage stage & stored at room temperature achieved fully red colour in 24 days for transgenic while it took 7 days in control. Softening behavior was also remarkably different in these, the control showing incidence of softening two weeks after picking while transgenic fruit remained firm for 5 months.

Agritope has used a bactriophage gene encoding S- Adenosyl Methionine (SAM) hydrolase, in conjuction with a ripening specific promoter, to hydrolyse the first intermediate of the ethylene Biosynthesis pathway in ripening cherry Tomato fruit. The transgenic fruit exhibited a delayed ripening phenotype & a reduction of spoilage due to over ripening.

Antisense techniques inhibited ACC oxidase level & concomitant ethylene production during ripening of cantaloupe “Chanterias” melons. This variety has excellent eating quality but poor storage capacity. GM plant produced < 1% of ethylene than that of control. Storage quality was extended, with transgenic fruit remaining fresh after 10 days at 25°C while the control fruit had spoiled. Further, low ethylene melon has considerably less sensitivity to chilling injury. This is an additional improvement since most tropical & subtropical fruits are sensitive to low temperature & this fact severely impairs their transport & storage potential causing significant losses. The antisense ACC Oxidase melon did not develop chilling injury when stored for up to 3 week at 2°C while control exhibited extensive damage.

An alternative to control ethylene production during ripening is to decrease the sensitivity of the fruit to the hormone. It has been established that during ripening, fruits not only increase the production of ethylene but they become more sensitive to it. The cloning of ethylene receptor (etrl1) has opened the door to the manipulation of ethylene perception instead of ethylene production. A mutated version of *etrl1* in Arabidopsis (etrl1) confers ethylene insensitivity as a genetically inheritable dominant trait. The same mutated gene has also been introduced into tomato & petunia conferring ethylene insensitivity & producing fruit that fails to ripen or flowers with extremely delayed senescence respectively. It is clear that complete ethylene insensitivity is not a desirable trait for a fruit since it would render the fruit unable to ripen even when exposed to ethylene & hence partial or induced insensitivity to ethylene could be commercially useful.

2.2 Modification of food processing properties

Wheat: It is commonly used to make bread and number of end products such as cakes, cookies, pastries etc. The improvement in processing properties of wheat grain w.r.t. two main properties of the grain which have been targeted to be modified by genetic engineering are:

- Gluten protein composition that may eventually determine the viscoelasticity of the dough.

- Grain hardness that has direct bearing on the milling and baking properties of the grain.

Visco-elasticity: For successful bread-making, dough made from wheat flour needs to have considerable elasticity. The elasticity is provided in part by seed storage proteins called high molecular weight (HMW) glutenin subunits**.** As wheat is a hexaploid, upto 6 possible genes for the HMW sub unit may be expected. However, the modification of 1 or 2 genes may be sufficient to bring the desirable change in the properties of the dough.

Softness: Grain hardness (soft or hard) of wheat is the single most characteristic which determines its utilization and marketability and two wheat proteins named puroindolines A and B, acting together in a 1:1 ratio, control wheat grain softness to some extent. In cereals with hard texture like corn and rice, these two proteins are not present in grain. Genetic modification of rice grain texture with wheat puroindoline genes has significantly increased the softness of rice grain.

Potato: Potato constitutes a basic food in diet of many western countries where it is commonly consumed as potato chips. Upon harvesting, potato tubers are frequently stored in cold stores to prevent the sprouting. During cold storage, part of starch of the tuber is converted into hexose. The increased hexose (glucose and fructose) induce the sweetening of the tuber and have adverse effect on the quality of the processed chips. Excess of hexose react with amino acid during frying causing a non-enzymatic browning of the chips. Increase in hexose sugars in cold stored tubers is the result of imbalance between the rate of their production from starch and rate of their degradation in glycolytic pathway. The accumulated hexose may be metabolically converted into sucrose that is eventually split into glucose and fructose by the action of enzyme invertase. Several strategies have been attempted with variable success to keep hexose level low in cold stored tubers. Genetic modification by gene encoding a protein that inhibits the invertase enzyme is the best alternative to overcome this problem. The presence of this protein in invertase enzyme would inhibit the hydrolysis of sucrose into hexose. Transgenic potato plants over expressing invertase inhibitor gene have been reported to reduced the hexose accumulation by 70% after cold treatment. Further, as expected, browning of the chips also prevented.

2.3 Biotechnology in the production of food additives

All the foods are perishable in nature and sooner or later their quality deteriorates and hence needs to be preserved or conserved by appropriate techniques. In post harvest processing, certain chemicals substances called food additives are used to retain the quality. They are defined as substances which are added to foods during processing to improve color, texture, flavor, or keeping qualities such as antioxidants, emulsifiers, thickeners, preservatives, and colorants.

Food additives have to keep pace with the fast growing food industry in India and elsewhere in World. With the increased awareness among the consumers regarding ill effects of chemicals, many of them being banned, the need for natural alternatives is being felt. Some of these additives are produced at present by biotechnological interventions like fermentation

2.3.1. Antimicrobial agents or preservatives

The health consciousness among consumers has increased pressure on food industry to reduce the use of synthetic/chemical food additives without compromising the food safety. Moreover, doubts about safety of some of traditional preservatives like nitrite, sulphur dioxide has revived the interest to use natural preservatives in food. Among these, bacteriocins and antimicrobial enzymes are gaining importance in food industry.

2.3.1.1 Bacteriocins

These are any proteinaceons compounds (usually a peptide) that have bactericidal action against a limited range of organisms which are usually closely related to the producer organisms. In 1928, NISIN was reported as first bacteriocin from Lactic Acid Bacteria (LAB). Since then, a number of bacteriocins from LAB having potential for use in food fermentation have been reported. The mechanism of inhibitory action of bacteriocins occurs in 2 distinct steps.

Step1: Attachment of bacteriocin with specific receptor of cell membrane of the target cell.

Step 2: Bacteriocin acts on cell membrane & causes lethal changes in sensitive strain.

Classification of Bacteriocins**:** They are classified into four groups as Class I, II, III & IV

Class I: They are called as Lantibiotics and consist of small membrane peptide (< 5 KDa) with 19 to more than 50 amino acids chains. They are further categorized in two subgroups as Linear & Circular due to the interchain positioning of these polycyclic structures. e.g Nisin, Lactocin etc.

Class II: These are the small (<10KDa) heat stable proteins. This is the largest group of bacteriocin & can be further subdivided to class IIA, class IIB & class IIC. e.g. Pediocin, Sakacin, Lactococcin, Divergicin etc.

Class III: This class of bacteriocin are large in size (>30KDa) and are heat labile.e.g. Helvicitin.

Class IV: This type of bacteriocins are composed of proteins plus one or more chemical moieties off lipid and carbohydrates.

Class I & II bacteriocins are membrane active peptides & form membrane channels thus destroying the integrity of cytoplasmic membrane. As a result, the membrane permeability is altered. This causes leakage of metabolites or dissipation of proton motive force.

Table 1: Bacteriocins producing microorganism & their inhibitory spectrum

Bacteriocin	Microorganism	Inhibitory spectrum
Subtilin	*Bacillus subtilis*	Gram +ve bacteria
Nisin	*Lactococcus Lactis subsp. Lactis*	Gram +ve bacteria
Lacticin	*Lactococcus lactis subsp. Lactis*	LAB, Clostridia
Pediocin	*Pediococcus pentosaceus*	LAB, Clostridia, Listeria, Staphylococci
Sakacin	*Lactobacillus bake*	LAB, Listeria
Microgard	*Propionibactrium hermani*	Gram –ve bacteria, some yeast & mold

Source:Singal & Kulkarni,1999

2.3.2 Acidulants

Substances used as additives that give a sharp taste to food. Acids have been used for centuries as important contributors to flavour and the acid environment they produce prevents the growth of many microorganisms. The different natural acidulants used in the food industry are discussed below:

2.3.2.1 Acetic acid (Vinegar)

The dilute aqueous solution of about 4% acetic acid in the form of vinegar has widespread application as an acidifier, flavour enhancer, flavouring agent, pH controlling agent, pickling agent. Vinegar, due to its antimicrobial properties finds its way in foods such as salad dressings, sauces, ketchup etc. It is also used in curing meat and canning of certain vegetables. Natural vinegar is produced by successive anaerobic alcoholic & aerobic acetous fermentations from sugar rich materials such as apple, grapes or malt.

Vinegar production involves two basic fermentation steps:

Alcoholic fermentation: Anaerobic conversion of sugar to alcohol and carbon dioxide through yeast enzymes.

$$\text{Glucose} \longrightarrow 2CO_2 + 2C_2H_5OH + 55\text{kcal}$$

Acetous fermentation: It is the acetic acid fermentation which is oxidative fermentation carried out by acetic acid bacteria known as *Acetobactor aceti*

$$C_2H_5OH + O_2 \longrightarrow CH_3COOH + H_2O + 116\text{kcal}$$

2.3.2.2 Citric acid: The traditional process of manufacturing citric acid was extraction from lemon juice and later from pine apple waste. *Aspergillus niger* was first potential mold introduced for citric acid production and is still the organism of choice on industrial scale. Commercially citric acid is produced from molasses by surface, solid state or submerged fermentation process.

$$C_6H_{12}O_6 + 1\tfrac{1}{2}\ O_2 \xrightarrow{A.niger} C_6H_8O_7 + 2H_2O$$

2.3.2.3 Fumaric acid : is produced by *Rhizopus nigriificans*. Many other microorganisms can also produce fumaric acid as an intermediate of TCA cycle. Screening of strain of *Rhizopus sp.* is utmost important as some strains of *Rhizopus* do not produce it while others produce fumaric acid or mixture of fumaric acid & lactic acid.

2.3.2.4 Lactic acid : is first ever synthesized acid using microorganisms. There are 2 types of Lactic Acid fermentations.

- *Homofermentative fermentation:* The fermentation is carried out by the microorganisms such as *L. bulgaricus, L. plantarum, L. delbruckii, Streptococus sp* and these bacteria are collectively called as Lactic Acid Bacteria. These microorganisms convert almost all the sugars (hexose) into Lactic acid with a yield of 93-95%.

- *Heterofermantative fermentation:* Carried out by the microorganism like *Leuconostoc mesentroides.* It is named so because alcohol & carbon dioxide are also produces in addition to Lactic acid.

$$\text{Glucose + ADP + Pi} \longrightarrow \text{Lactic acid + Ethanol + } CO_2 \text{ + ATP}$$

Fruit Washing &

↓

Grading Juice

↓

Extraction

↓

Alcoholic Fermentation (*S.cerevisiae*)

↓

Clarification

↓

Acetous Fermentation

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Raw Vinegar

↓

Aging

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Clarification, Packing, Pasteurization, Labeling

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Storage & Marketing

Figure 4: Unit operation for preparation of fruit vinegar

2.3.3 Enzymes for novel food application

Latest research developments in food industry have brought out many interesting & novel applications of enzymes as food additives. This is a promising area and commercial applications of these enzymes will provide long-needed solutions to the processors. Enzymes are gaining importance as processing aids for the production of high quality fruit juices, concentrates and processed foods. Microbial enzymes are preferred over plant and animal enzymes due to:

- Increased enzyme concentration by altering the environmental conditions and by recombinant technology.
- Easy to handle.
- Rapid growth rate of microorganism.
- Capacity to grow on inexpensive media.
- Conditions of the fermentation process can be chosen according to the physico- chemical properties of the enzymes.

The significant impact of the modern biotechnology in the food processing industry is the development of High Fructose Corn Syrup (HFCS). The production of High Fructose Corn Syrup (HFCS) involves three steps in which á-amylase, glucoamylase, gluco-isomerase act successively effecting the liquification and saccharification of corn starch and yielding approximately equimolar mixture of glucose & fructose. Further, application of biotechnology in food processing involves the modification of enzymes with respect to their primary structure which alters its target specificity, acidic conditions or thermostability.

Table 2. Enzymes & their application in food industry

Enzyme	Source	Applications
ß- Glucanase	*Trichoderma*	-Aids mash filteration in brewing by reducing viscosity
Acetolactate decarboxylase	*Aerobacter aerogenes*	-Reduces the production time of beer from 5 weeks to 2 weeks by avoiding the formation of diacetyl
á- Glucosyl transferase	*Protaminobacter robrum*	-Rearrangement of sucrose to isomaltulose, a non-carcinogenic nutritive sweetner
Sulphydryl oxidase	*Aspergillus niger*	-Strengthening weak dough from low gluten flour
Urease	*Lactobacillus fermentum*	-Reduces urethane, a carcinogenic in sake (Japanese alcoholic beverage prepared from rice)
á- Glactosidase	*Aspergillus niger*	-Modifies guar galactomannan to mimic locust bean gum
Amylases	Bacteria (*Bacillus Sp*.) Fungi (*Aspergillus sp.)*	-Starch hydrolysis into dextrin, maltose & glucose -Removal of starch -Saccharification of starch for alcohol production -Proper volume in baked goods
Cellulase	Fungi (*Trichoderma reesii*)	-Hydrolysis of cellulose into glucose
		-Ethanol production
Glucose isomerase	Bacteria	-Production of High Fructose Corn Syrup
Glucose oxidase & catalase	*Cornybacterium spp.*	-Removal of O2 & antisepsis of foods
Invertase	Yeast (*Sacharomyces cerevisae)*	-Production of invert sugar from sucrose
Naringinase	Fungi (*Aspergillus niger, Coniella diplodiella)*	-Sugar confectionary, chocolate -Debittering of fruit juices especially citrus
Pectinases	Fungi (*Aspergillus spp.*) (Polygalacturanases)	-Clarification of wines & fruit juices - Viscosity reduction in fruit processign
Proteases	Bacteria (*Bacillus spp.*)	-Dough production
	Fungi (*Aspergillus spp*.)	-Soy sauce
	Yeast(*Sacharomyces spp*)	-Lysis of plant proteins -Removal of protein clouds in fermentative beverages.
Pullulanases	Bacteria	-Peptone manufacturing
		- Beer production
		-Improve glucose & maltose processes.

Source: Raverkar & Joshi,2000

Furthermore, immobilization of enzymes increases its stability, allows easy separation of the product from enzymes and hence facilitates enzyme recycling.

2.3.4 Biocolours

Colour plays a special role in the food we eat. Possible reasons for use of colorants in food substances are to maintain: 1) Original food appearance even after processing and storage; 2) Uniformity of colour in processed food products thereby avoiding the seasonal variations. 3) Quality by intensifying the colour of food. 4) Flavor and light susceptible vitamins by making a light – screen support. The use of synthetic dyes in food has become a matter of concern in many parts of the World due to their controversial health impact. Moreover, in recent past, the increased awareness of consumers towards health foods has necessitated the development of natural food colours.

2.3.4.1 Source of biocolourants

Biocolourants come from variety of sources such as:

- Plants (Flower, fruits, seed, roots etc.)

- Animals (Cochineal, lac etc.)
- Microorganisms (*Monascus, Rhodotorulla, Bacillus, Archomobacter, Phaffia* etc.)

But few of them are available in sufficient quantities for commercial use as food colorants and mostly are of plant origin. For biotechnological production of such colorants, plants and microorganisms are more suitable due to understanding of proper cultural techniques and processing.

2.3.4.1.1 Major plant based natural colours

- **Annatto:** The yellow to orange colour of annatto is due to the presence of carotenoids (Bixin & norbixin) in the outer layer of seeds of *Bixa orellana.* Major uses of annatto include colouring of butter cheese & other dairy products.
- **Red Beet:** It is a dark red beet powder which is made from ripe red beets that are cooked, peeled, sliced, dried and then ground. The colouring compound in beet is betalains. Its applications include ice cream, yoghurt, cake mixes, gravy mixes, sausages, soft drink, fruit chews etc. Many of these products contain ascorbic acid as colour stabilizer.
- **Paprika:** The principal carotenoids which are responsible for red colour of the paprika are Capsanthin, capsorubin, zeaxanthin violaxanthin. Paprika oleoresin is mainly extracted from the pods. It is mainly used in colouring the sauces, ketchup etc.
- **Saffron:** Crocin & crocetine are the main carotenoids present in saffron (*Crocus sativa*) and are considered the most expensive colourants as well as spice. It has been used in many traditional fairness oils and extensively used in medication.
- **Turmeric:** It is bright yellow powder from the roots of traditional herbs *Curcuma longa*. The pigment responsible for colouration is a polyphenol called as curcuminiods. It is used for colouring curries, pickles, cooking oils, soups, baked goods etc.

2.3.4.1.2 Natural colour of animal origin

Carminic Acid: Cochineal insects (*Dactylopius coccus*) are soft-bodied, flat, oval-shaped scale insects. Carminic acid also called as cochineal constitutes about 17-24% of the weight of the dry insects which can be extracted from the insect's body and eggs and mixed with aluminum or calcium salts to make carmine dye. It is one of the most light- and heat-stable and oxidation-resistant of all the natural colorants. Its application in food industry together with ammonia includes sausages, processed poultry products, alcoholic beverages, bakery and dairy products.

2.3.4.1.3 Microbial biocolours

A) Molds

i) Monascus: "Ang-Khak" a traditional fermentation product in China is produced by fermenting rice with *Monascus purpureus (*also known as ang khak rice mold), which is subsequently grinded and its powder form is used as food colourant or as spice in cooking. The pigments responsible for colouration in *Monascus* are ankaflavine & monascine (Yellow), rubropunctatine & monascorubrine (Orange), rubropunctamine & monascorubramine (Purple). These pigments are secondary metabolites of *Monascus* fermentation & produced mainly in cell bound state. The variation in colour is influenced by the culture conditions, in particular by the pH value and the phosphorus and nitrogen source in the substrate. Annual consumption of this pigment has increased many folds in Asian countries and finds its application in processed meats products, marine products, tomato ketchup etc. But the Western World has not yet started its commercial exploitation due to ignorance & reluctance to change the opinion of food public agencies.

ii) *Blakeslea trispora:* This fungus thrives in symbiosis with tropical plants and many of its strains can produce high level of -carotene. The production of -carotene from this mold includes two steps, in first step, the glucose, corn steep liquor or whey are used as substrates for aerobic submerged fermentation to produce the biomass while in second stage, biomass is isolated and transformed into a form suitable for the isolation of -carotene. It is then extracted using ethyl acetate and subsequently purified & concentrated.

In 1995, Gist-Brocades, now DSM became the first company to produce -carotene from this fermentative source.

iii) Riboflavin: Also known as Vitamin B_2. It possesses yellow or yellow-orange colour and is being used as a food colourant & as nutrient supplement in food products. In food industry, it is used in baby foods, breakfast cereals, pasta, sauces and processed cheese etc. Various biotechnological processes have been developed for industrial scale production of riboflavin by using microorganisms like *Ashbya gossypi, Candida sp, Bacillus sp* etc. The Riboflavin fermentation could be classified into three categories;

Weak overproducers: 100mg/lt or less e.g. Bacterium *Clostridium acetobutylicum*

Moderate overproducers: upto 600mg/lt e.g. Yeast *Candida gulliiermundii*

Strong overproducers: over 1g/lt e.g. Fungi *Ashbya gossypi*

B) Yeast

i) *Xanthophyllomyces dendrorhous (Phaffia rhodozyma*)

This yeast is known for production of an astaxanthin pigment. It is widely distributed in nature and is principal pigment in crustacean and salmonids. These carotenoid pigments impart orange- red colour to farm animal species when supplemented in their feeds. For proper intestinal absorption of astaxanthin pigment by the animals, disruption of the cell wall of yeast (chemical, physical or enzymatic) is required prior to its addition in feed.

ii) Rhodotorula: The carotenoid pigments produced by this yeast are torulene, torularhodin & -carotene. *Rhodotorula* is a common environmental inhabitant and can be cultured from soil, water, and air samples.

2.3.5 Bioflavours

Natural flavours play an important role in determining the quality of food and beverages. Premature harvesting, extended storage and other food processing unit operations cause the loss of naturally present aroma compounds & hence, addition of flavour supplements to processed food is often required. The fast changing life styles, food habbits & increase in number of processed foods in the market have created a strong demand for flavours. As isolation of natural flavours from plants is limited, the alternative sources for natural flavours are being searched for. Therefore, the biotechnological production of aroma compounds using microorganisms, isolated enzymes or plant cell cultures, has received much attention in the recent past. Currently, about 100 flavour molecules of biological origin are available to the food industry. Among them, natural lactones represent one of the major large-volume flavour products.

The three options to meet the supply of natural flavours or flavour precursors in the industry are:

- collection from the wild plant population
- agricultural cultivation,
- plant tissue culture/ Microbes

Collection from the wild plant population is easiest way & has been in practice since ages but over collection of the same has endangered the plant species in many cases. The supply can be supplemented by agricultural cultivation of these wild plants but in some cases the wild populations require specific growth conditions which cannot be reproduced elsewhere. Although, cultivation of the plant or related species is clearly the most economic solution to the flavour supply but, the crop may suffer from pests and diseases, and adverse climatic conditions which can affect yield and quality. The plant may also be difficult to propagate and may require exacting condition for growth as in the case of vanilla pods. Under such conditions, plant cell culture technique may help to alleviate the pressure on the supply of natural flavours in a sustainable manner by helping with the propagation of the particular plant or the *de novo* microbial production of the flavour.

The commercial production of microbial bioflavours can be increased by the use of genetically modified microorganisms. The complete separation of these organisms from the volatiles during the product recovery step has raised the hopes of food industry that this technique will also be applicable in industrial flavour production in near future. Further, improvements will certainly be triggered by the enormous progress currently being made in the field of total genome sequencing. Moreover, the growing demand from the consumers side for 'natural' additives for food, feed and cosmetics, the commercial importance of biotechnologically produced flavours will certainly grow further in the near future.

2.3.5.1 Bottlenecks for industrial applications

- The microbial flavours are often present only in low concentrations in the fermentation broths, resulting in high costs of down-stream processing.
- Microbial flavours are much costlier than their synthetic counterpart.
- Volatility and low solubility in water of many flavours makes their recovery often difficult.
- Legal regulations are major obstacle for commercialization as it takes years to get clearance from authorities.

Table 3: Application of biotechnology in biocolourant production

Name of food grade biocolorants	Original source	Biotechnological source	Approaches for Large scale production
Monascorubramine	*Monascus purpurious*		Fermentation and bioprocess engineering
Astaxanthin	Plants	Fungus: *Xanthophyllomyces dendrorhous*	
		Algae: *Haematococcus lacustris*. *H. pluvialis* compactin resistant mutant	Fermentation and bioprocess engineering
Arpink red		Fungus: *Penicillium oxalicum* var. armeniaca CCM 8242	Fermentation and bioprocess engineering
ß-Carotene	*Daccus carota*	Fungus: *Blakeslea trispora, Phycomyces blakesleeanus car S* mutant	Fermentation and bioprocess engineering
		Algae: *Dunaliella salina, D. bardwil*	Organic farming and Integrated Crop Management (ICM)
		GM plant: Golden Rice	
Riboflavin	Milk	Moulds: *Ashbye gossypii, Eremothecium ashbyii, Ashbya gossypi*	Fermentation and bioprocess engineering
		Yeast: *Candida gulliermndii, Debaryomyces subglbosus*	
		Bacteria: *Clostridium acetobutylicum*	
Bixin and Norbixin	*Bixa orella*		Organic farming and ICM
Betanin	*Beta vulgaris*	Higher yielding plant generated through somaclonal variation	Organic farming and ICM
		Hairy root culture	Fermentation and bioprocess engineering
Canthaxanthin		Algae: *Haematococcus lacustris*	Fermentation and bioprocess engineering
		Bacteria: *Bradyrhizobium* sp.	
Carminic acid	*Dactylopius coccus*		Organic farming and ICM
Cyanidin and Peonidin	Ascherry, Canberry	Higher yielding plant generated through somaclonal variation	Organic farming and ICM Fermentation and bioprocess engineering
		Cell culture	
Acylated anthocyanins	Black Carrot		Organic farming and ICM
Lycopene	Tomato	GM fungus: *Fusarium sporotrichioides*	Fermentation and bioprocess engineering
		GM bacteria: *Erwnia uredovors*	
Lutain	*Tagetes erecta*		Organic farming and ICM
Cepsorubin	*Capsicum annuum*		Organic farming and ICM
Zeaxanthin	Corn	Bacteria: *Flavobacterium* sp.	Fermentation and bioprocess engineering
Curcumin	*Curcuma longa*		Organic farming and ICM
Chlorophyll	Spinach		Organic farming and ICM

Source: Chattopadhyay *et al. 2008*

Notwithstanding these difficulties, a number of flavour compounds are already being produced by micro-organisms on an industrial scale.

- BASF (Germany): started the microbial production of 4-decalactone, a peach aroma which is distributed by its subsidiary company Fritzsche, Dodge & Olcott. The process involves bioconversion by *Yarrnwia lipolytica* of castor oil, an oil that is pressed from the seeds of *Ricinus communis* and is composed of 80 % of a triglyceride of ricinoleic acid.
- Unilever: a UK based company is producing (R)-S-dodecanolide on commercial scale using baker's yeast and 5-ketododecanoic acid.3'. The lactone produced can be used as a butter flavour in margarines.
- Hercules Inc. an American company has produced Bulyric acid and ethyl butyrate microbiologically by using *Clostridium butyricum* that converts glucose under anaerobic conditions into butyric acid, the concentration of which can reach 1.2 % in the fermentation broth. Butyric acid, a component naturally present in butter and some cheeses, can used for natural cheese aroma.

Table 4: Microbial Production of Flavours

Type of Component	Micoorganisms	Odour
Pyrazines	*Psuedomonas sp. Streptomyces sp. Streptococus sp. Bacillus sp.*	Bell Papper, earthy, Peas & Potatoes
Terpenes	*Ceratocystic sp. Kluveromyces sp. Phellinus sp. Asocoidea sp.Lentinus sp.*	Floral, rose, fresh citrus, fruity
Lactones	*Ceratocystic sp.Saccharomyces sp. Candida sp. Sarcina sp.Cladosporium sp.*	Banana, fruity, peach, pear, rose
Esters	*Candida sp. Pseudomonas sp. Saccharomyces sp. Bacillus, Rhizopus*	Rose, fruity, pineapple, apple, mint
Diacetyl	*Leuconostoc sp. Streptococus sp.*	Butter like flavour

Source: Singal & Kulkarni,1999

2.3.6. Miscellaneous food additives

2.3.6.1 Antioxidants of microbial origin

Concern about the possible carcinogenic effect of the most of commonly used antioxidants, Butylated hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) has necessitated the development of natural antioxidants, a fact evidenced by intensive investigation on natural anti-oxidants during the past 15 years or so.

Glucose Oxidase : is the only commercial enzyme currently used as antioxidant for removal of O_2 in conjunction with catalase. The enzyme combination is extracted from *Aspergillus niger* & enjoys the GRAS status worldwide & has been shown to be effective in preventing off flavour development in various food products such as fruit juices, mayonnaise, salad dressings etc.

Alcohol oxidase : Retard off flavour development in emulsion containing fish oil.

Thiol oxidase or sulphydryl oxidase : catalyses the oxidation of thiols to disulphides, is naturally present in milk & along with lactopoeroxidase is suggested to exert an antioxidant effect.

2.3.6.2 Microbial polysaccharides as stablizers & thickners

Bacterial: Alginates (*Azotobacter vinelandii*), Gellan (*Pseudomonas elodea*), Xanthan (*Xanthomonas compestris*)

Fungal: Pollulan (*Aureobasidium pullulans*)

FOOD SAFETY AND STANDARDS: A PERSPECTIVE

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Food Safety is a concept that ensures that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use. It has emerged as an important global issue in wake of awareness of consumers and globalization of markets. In the developed countries, concerns about safe food have replaced those about adequate food. A food qualifies to be called as Safe Food is the one:

- which does not cause illness or injury when consumed as intended.
- which is free from chemical, physical or microbiological hazards.

1. Food safety concerns

Some foods are consumed as such while others are subjected to processing including cooking and storage before they are eaten. Some of the constituents of foods enhance nutritive value while others created during processing or added intentionally lower the nutritional value or are toxic. The food safety crisis exists in several parts of the world including India. Our food is devitalized, colored, filled with chemicals, drugs and synthetic ingredients, polluted by agricultural and environmental chemicals and grown on impoverished land puffed up by the use of chemical fertilizers and other aids. The major Food Safety Concerns are briefly described below:

1.1 Pesticide residues: The chemical pesticides played an important role in bringing the green revolution in the country. But, since last two decades, pesticides have become serious health concerns as some of them are not destroyed even after cooking and make their way in the food chain. Some of the pesticides are carcinogenic (cancer causing). Pesticide residues are found in ground water also due to seepage. When ingested, pesticides are absorbed by the small intestine. The fatty tissues distributed throughout the body store these pesticides which can damage vital organs like heart, brain, kidney and liver.

1.2 Heavy metals: Lead, Cadmium, Arsenic, Mercury, Selenium, Chromium etc are some of the heavy metals which contaminate food and water. The source can be environmental pollution, packaging material used for packaging the food, processing equipment, adulteration etc. These heavy metals beyond certain limits are highly toxic.

1.3 Adulterants: Adulteration of food may endanger health due to either addition of a deleterious substance or removal of a vital component. Adulteration may be intentional or unintentional. The intentional adulteration is a willful act intended to increase the profit margin while the incidental / unintentional contamination is usually due to ignorance, negligence or lack of proper facilities. Adulteration of food stuffs is commonly practised in developing countries as the consumers like to get maximum quantity for as low a price as possible. Melamine in milk and milk products, Sudan dye in red chilli powder, argemone oil in edible oils etc. are some of the examples of adulteration.

1.4 Environmental pollutants: The polluted irrigation water and soil used for cultivation of crops results in uptake of harmful substances by the crops. Some of the environmental pollutants like Poly Aromaic Hydrocabons (PAH) are carcinogenic (cancer causing) and others like methyl mercury, cyanide etc. are highly toxic.

1.5 Drug residues: Various types of antibiotics are used for disease control in animals. Further hormones like oxytocin are administered to milch animals to enhance the milk production. These drugs and hormones get into the milk and are left as residues. The milk & milk Products containing these are not safe for human consumption.

1.6 Microbial pathogens: Micro organisms are the root cause of food quality and safety problems. Food borne micro organisms which cause diseases in human beings are called Food Pathogens. They are responsible for food borne infections, some of which are so severe that they can lead to death. The examples of microbial food pathogens include *Salmonella, Shigella, Bacillus, Clostridium* etc.

1.7 Microbial toxins: The toxins produced by microorganisms are called microbial toxins. Those produced by bacteria are called bacterial toxins while fungal toxins are known as Mycotoxins. The ingestion of food products containing such toxins leads to foodborne Intoxication also referred to as food poisoning. *Staphylococcus aureus* and *Clostridium botulinum* are two examples which cause bacterial foodborne intoxication. Similarly Aflatoxin- a fungal toxin produced by *Aspergillus flavus* is a serious health concern and there are stringent regulations for levels of aflatoxins in different food and feed products.

1.8 Food additives: Food additives are defined as substances added intentionally to food, generally in small quantities, to improve its appearance, flavor, texture or storage properties. These include preservatives, antioxidants, bleaching and maturing agents, food colors, flavours, artificial sweeteners, nutritional supplements etc. These substances when added beyond permissible limits pose harm to health.

2. Food safety incidents

Highly publicized food safety incidents affect consumer perceptions, leading to changes in food purchasing patterns. Some of the popular Food Safety incidences reported are given in Table I.

Table I: Food Safety Incidences in Industrialized Countries

Year	Incidence	Country
1996	BSE (Mad Cow Disease)	Britain
1995 - 97	Avian Flu spreads to humans	Hong Kong , Taiwan
1999	Dioxin in animal feed	Belgium
2000	Food Poisoning- Dairy	Japan
2003 - 2004, 2008	Recurrences of Avian Flu	Mainly in South East Asia
2008	Melamine in Milk	China
2008	Dioxin in animal feed	Ireland

3. Food standards

To deal with food safety and consumer concerns, different countries of the world have their own food laws and regulations. These laws define food, food preservatives, food additives etc. and bar the sale of foods prepared under unhygienic conditions or containing any deleterious or unsafe substances. Food laws also specify standards of quality for various classes of foods and outline the conditions under which a food would be considered as adulterated or misbranded.

3.1 Indian food laws and standards

There are a number of food laws in India which are being implemented by various Ministries/Departments. A summary of various food laws being implemented under different ministries in India is given in Table – 2.

Some of the food laws are mandatory and some are voluntary. Various food laws which are operational in India are briefly discussed as under:-

3.1.1 Prevention of Food Adulteration Act (PFA)

The first central Act called the Prevention of Food Adulteration Act (PFA) was passed in 1954 and came into force from June 1, 1955. The objective of this act was to ensure that food articles sold to the consumers are pure and wholesome. The Act lays down specifications for various food products and is mandatory.

The act defines in specific words what is meant by a food adulterant and what shall be deemed to be an adulterated food. According to the Act, an article of food shall be deemed to be adulterated:

i. If the article sold by a vendor is not of the nature, substance or quality demanded by the purchaser and as it is represented to be.

ii. If it contains any other substance or processed as to injuriously affect the nature.

iii. If any inferior or cheaper substance has been substituted wholly or in part for the article.

iv. If the article had been prepared, packed or kept under unsanitary conditions whereby it has become contaminated or injurious to health.

v. If the article consists of any filthy, putrid, disgusting, rotten, decomposed or diseased animal or vegetable substance or is insect-infested or otherwise unfit for human consumption.

vi. If the article is obtained from a diseased animal.

vii. If the article contains any poisonous or other ingredient which renders its contents injurious to health.

viii. If the packaging material (container) of the article is composed of any poisonous or deleterious substance which renders its contents injurious to health.

ix. If any coloring matter other than as prescribed and in amounts not within the prescribed limits is present in the article.

x. If the article contains any prohibited preservative or permitted preservative in excess of the prescribed limits;

xi. If the quality or purity of the article falls below the prescribed standard or its constituents are present in quantities, which are in excess of the prescribed limits of variability.

Table 2: Food Laws under Different Ministries

Ministry	Food Law
Ministry of Health & Family Welfare	■ Prevention of Food Adulteration Act, 1954 (PFA) & Prevention of Food Adulteration Rules, 1955
Ministry of Agriculture	■ Milk & Milk Products Order - 1992 (MMPO)
	■ Insecticide Act- 1968
	■ Meat Food Products Order (MFPO) – 1973
Ministry of Food & Consumer Affairs	■ Essential Commodities Act, 1955
	■ Standards of Weights & Measures Act ,1976
	■ Standards of Weights & Measures (Packaged Commodities) Rules, 1977
	■ Consumer Protection Act, 1986
	■ BIS Act, 1986
Ministry of Commerce	■ Export (Quality Control & Inspection) Act 1937
	■ Agriculture Processed Food Export Development Authority Act (APEDA)
	■ Marine Products Export Development Authority Act (MPEDA)
Ministry of Food	■ Fruits & Vegetable Products (Control) Order - Processing Industries 1955 (FPO)
Ministry of Rural Development	■ Agricultural Produce (Grading & Marking) Act 1937 (AGMARK)
Ministry of Environment & Forests	■ Environment Protection Act (1986) Ecomark
Ministry of Science & Technology	■ Atomic Energy Act -1962
	■ Control of Irradiation of Food Rules, 1991
	■ Genetically Modified & Organic Foods
Ministry of HRD (Department of	■ Infant Milk Substitutes, Feeding Bottles & Infant Women & Child Welfare) Foods (Regulation of production, supply & distribution) Act -1992 & Rules, 1993 (IMS) Act.
Ministry of Food and Civil Supplies	■ The Vegetable Oil Products (Control) Order, 1947
	■ The Edible Oils Packaging (Regulation) Order, 1998
	■ The Solvent Extracted Oil, De oiled Meal, and Edible Flour (Control) Order, 1967

The act prohibits the manufacture, sale and distribution of not only adulterated foods but also foods contaminated with toxicants and misbranded foods. A food may be considered as misbranded if it has

i. False or misleading label

ii. Is offered for sale under the name of another food

iii. Imitates another food without the declaration on the label

iv. Label without the name of manufacturer or marketer

v. Label without information about the ingredients

vi. Label with wrong information about the ingredients

vii. Label without nutritional labeling as per norms

The persons found guilty under the act can be convicted. Severity of sentence depends on the gravity of the offence.

3.1.2 Fruit Products Order (FPO)

FPO lays down statutory minimum standards in respect of the quality of various fruit and vegetable products and processing facilities. Packing fruits and vegetables of a standard below the minimum prescribed standards is an offence punishable by law. Periodic inspection by government inspectors in registered establishments is carried out to ensure conformity of standards by processors.

3.1.3 Meat Food Products Order (MFPO)

MFPO makes it illegal to transport meat unless it has been prepared and processed according to the provisions of the order and carries the mark of description. It provides means to:

i. Detect and destroy meat of diseased animals.

ii. Ensures that the preparation and handling of meat and meat products be conducted in a clean and sanitary manner.

iii. Prevents the use of harmful substances in meat foods.

iv. See that every cut of meat is inspected before sale to ensure its wholesomeness.

MFPO also lays down rules and conditions for procedures to be adopted for the selection of disease-free animals, slaughter house practices and further treatment of the meat so as to maintain the meat in a wholesome manner, free of pathogens (disease producing organisms).

3.1.4 Milk and Milk Products Order (MMPO)

Govt. of India promulgated the Milk and Milk Product Order (MMPO) 1992 on 09.06.1992 under the provisions of Essential commodity Act, 1955. As per the provisions of this order, any person/dairy plant handling more than 10,000 liters of milk per day or 500 MT of milk solids per annum needs to be registered with the registering authority appointed by the Central Government. The main objective of the order is to maintain and increase the supply of liquid milk of desired quality in the interest of the general public and also for regulating the production, processing and distribution of milk and milk products.

3.1.5 Edible Oil Packaging (Regulation) Order, 1998 (EOP)

In order to ensure availability of safe and quality edible oils in packed form at pre-determined prices to the consumers, the Central Govt. promulgated on 17th September, 1998, an Edible Oils Packaging (Regulation) Order, 1998 under the Essential Commodities Act, to make packaging of edible oils sold in retail compulsory unless specifically exempted by the concerned State Govt.

3.1.6 Solvent Extracted Oil, De-oiled Meal and Edible Flour (Control) Order,1967

The Order is basically a quality control order to ensure that the solvent extracted oils in particular are not reached to the consumers for consumption before the same are refined and conform to the quality standards specified in the order for the purpose. Standards for the solvent (hexane), which is to be used for extraction of oil from the oil-bearing materials, have also been specified so as to eliminate possible contamination of oil from the solvent used.

3.1.7 Bureau of Indian Standards (BIS)

Bureau of Indian Standards (BIS), formerly known as Indian Standards Institute (ISI) came into being as ISI Act in 1952. BIS is a corporate body which includes representatives from the government, consumers and industry, as members. It formulates the Indian Standards for various products including vegetable & fruit products, spices & condiments, animal products, processed foods etc. Once these standards are adopted, manufacturers whose products conform to these standards are allowed to use an ISI mark on each unit of their product. The products are checked for quality by the Bureau of Indian Standards (BIS) in their own network of testing laboratories at Delhi, Mumbai, Kolkata, Chennai, Chandigarh and Patna or in a number of public and private laboratories recognized by them. ISI marked products guarantee optimum levels of quality, safety and performance.

3.1.8 The AGMARK Standard

The word 'AGMARK' is derivative of Agricultural Marketing. The AGMARK standard was set-up by the Directorate of Marketing and Inspection of the Government of India by introducing an Agricultural Produce Act in 1937. This Act defines quality of cereals, spices, oilseeds, oil, butter, ghee, legumes, eggs etc., and provides for the categorization of commodities into various grades depending on the degree of purity in each case. The grades incorporated are grade, 1, 2, 3 and 4 or special, good, fair and ordinary. These standards also specify the types of packaging to be used for different products.

The ISI Standards and the Agmark Standards have benefitted both the producer and the consumer. It is possible for the producer to realize better prices for the products with these certification marks, as it ensures quality and guarantees the wholesomeness of the product for the consumer.

3.1.9 Food Safety and Standards Act (FSSA) 2006

As, the food sector in India is governed by many laws under different ministries. This multiplicity in laws in food sector resulted in many problems in the area of maintenance of food standards. Need for a single regulatory body and an integrated food law was recognized as a remedy for this multiplicity of laws. The Food Safety and Standards Bill was introduced in 2005 with an objective to unify the food safety laws in India. Food safety and standards Act 2006 was passed by the parliament on 23rd August 2006.

Government of India is moving towards putting in place an integrated food law under Food Safety and Standards Act, in line with Codex specifications which are followed internationally. The main objectives of the Act are to:

i. Introduce a single statute relating to food.

ii. Provide for scientific development of the food processing industry.

iii. Meet the requirements of international trade

iv. Make the domestic industry competitive in the global market

v. Establish a single reference point for all matters relating to food safety and standards, by moving from multi-level, multi-departmental control to a single line of command

The Food Safety and Standards Authority is to regulate manufacture, storage, distribution, sale and import, to ensure availability of safe and wholesome food. The highlights of the act are :

i. Food Safety and Standards Authority (FSSA) to regulate the food sector.

ii. Food Safety and Standards Authority aided by several scientific panels and a central advisory committee will lay down standards for food safety.

iii. Standards for food safety will include specifications for ingredients, contaminants, pesticide residue, biological hazards, labels etc.

iv. State Commissioners of Food Safety and other local level officials will enforce the law.

v. Every entity in the food sector is required to get a license or a registration under the Act.

The Food Safety and Standards Act on implementation will replace the following existing Acts/Orders related to food:

i. The Prevention of Food Adulteration Act, 1954.

ii. The Fruit Products Order, 1955.

iii. The Meat Food Products Order, 1973.

iv. The Vegetable Oil Products (Control) Order, 1947.

v. The Edible Oils Packaging (Regulation) Order, 1998.

vi. The Solvent Extracted Oil, De oiled Meal, and Edible Flour (Control) Order, 1967.

vii. The Milk and Milk Products Order, 1992.

viii. Any other order issued under the Essential Commodities Act, 1955 relating to food.

The Food Safety and Standards Act aims to integrate the food safety laws in the country in order to systematically and scientifically develop the food processing industry and paradigm shift from a regulatory regime to self-compliance.

3.2 International Food Laws & Standards

3.2.1 Codex Alimentarius

The Codex Alimentarius Commission, a UN body, implements the joint Food Standards Programme of Food and Agriculture Organization (FAO) and World Health Organization (WHO). The purpose of which is to protect the health of consumers and ensure fair practices in the food trade. The Codex Alimentarius (Latin, meaning Food Law or Code) is a collection of internationally adopted Food Standards presented in a uniform manner. The Hazard Analysis Critical Control Point (HACCP) approach has been recognized by Codex as a tool for assessing hazards and establishing control systems that focus on preventive measures rather than relying primarily on end-product testing. The Codex HACCP and food-hygiene standards have been adopted by the Bureau of Indian Standards (BIS). Food processing units are being encouraged to adopt these systems on a voluntary basis.

3.2.2 BRC Global Standard –Food

BRC Global Standard –Food was set up in 1998 by British Retail Consortium (BRC), which is a leading trade Association for UK retailing. It is a food safety and quality management protocol based on Hazard Analysis and Critical Control Point (HACCP) System and designed for manufacturers of all types of food products. BRC Standards also establish general Good Manufacturing Practices (GMP) for food safety.

3.2.3 International Food Standards (IFS)

IFS was set up in 2002 by HDE (Hauptverband des Deutschen Einzelhandles), the German Retail Association. IFS is a food safety and quality management protocol based on HACCP that is designed for producers of all types food products. The IFS program allows two levels of certifications namely, foundation level and higher level. The foundation level is considered as the minimum requirements for the international food industry and the higher level is considered as a superior standard in the food industry. This division permits suppliers to implement more flexibility and encourages continuous improvement.

3.2.4 Safe Quality Food (SQF) Codes

Safe Quality Food (SQF) standards were originally established by the Western Australian Department of Agriculture in 1996, in response to the demands of the farming and small food manufacturing sectors for a quality assurance system that enables their businesses to meet regulatory food safety and commercial food criteria. In 2003, worldwide ownership of SQF standards was transferred to Food Marketing Institute (FMI), an American Retail Association. SQF program is intended to deal with complete food safety management systems. However, in comparison to BRC or IFS standards, it specifies requirements on quality management system and does not specify good practices nor HACCP plans (though it demands them). SQF 1000 code is for primary producers and deals with pre-farm gate production, harvesting, preparation of primary products, while SQF 2000 is for food industries and deals with raw materials & ingredients, processed or prepared foods, beverages and services.

3.2.5 Dutch HACCP Code

The Dutch National Board of Experts-HACCP set up Dutch HACCP code in 1996. The standard focuses on all operators along the food chain. Primary producers are neither explicitly included in, nor excluded from, the scope of the standard. It establishes requirements on quality management systems and HACCP systems, but not on good practices. The architecture of the requirements of the standard is close to the structure of HACCP code as described by Codex Alimentarius.

3.2.6 EurepGAP

Euro-Retailer Produce Association was created in 1997 by large European Retail Chains and was subsequently joined by large fresh produce suppliers and producers. EUREP has developed certification program named EUREP Good Agriculture Practices (EurepGAP). As compared to other international food safety standards, EurepGAP standards are specific to fresh products and concern farm companies directly. The following five different certification programs have been established:

- EurepGAP-Fruits and Vegetables
- EurepGAP-Integrated Farm Assurance
- EurepGAP-Integrated Aquaculture Assurance
- EurepGAP-Coffee
- EurepGAP-Flowers and Ornamentals

3.2.7 ISO 22000

ISO 22000 was released on August 30, 2005 with an objective to establish a single internationally recognized standard for food safety management systems. ISO 22000 has been set up by International Organization for Standardization (ISO). ISO 22000 applies to all food operators (feed producers, primary producers, manufacturers, transport and storage operators, retail and food service outlets and related organizations such as producers of equipments, packaging material, cleaning agents etc). It follows farm to fork approach.

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