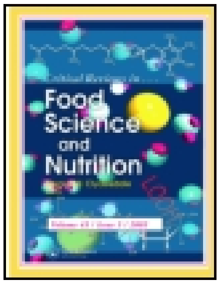

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Novel trends to revolutionize preservation and packaging of fruits/fruit products: Microbiological and nanotechnological perspectives

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Novel trends to revolutionize preservation and packaging of fruits/fruit products: Microbiological and nanotechnological perspectives

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Contents

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Abstract

Fruit preservation and packaging have been practiced since ages to maintain the constant supply of seasonal fruits over lengthened periods round the year. However, health and safety issues have attracted attention in recent decades. The safety and quality assurance of packaged fruits/fruit products are vital concerns in present day world-wide integrated food supply chains. The growing demand of minimally or unprocessed packaged fruits has further aggravated the safety concerns which fuelled in extensive research with objectives to develop novel techniques of food processing, preservation and packaging as well as for rapid, accurate and early detection of contaminant products/microbes. Nevertheless fruits and fruit based products have yet to observe a panoramic introduction. Tropics and subtropics are the stellar producers of a variety of fruits; majority if not all are perishable and prone to postharvest decay. This evoked the opportunity to critically review the global scenario of emerging and novel techniques for fruit preservation and packaging, hence providing insight for their future implementation. This review would survey key nanotechnology innovations applied in preservation, packaging, safety and storage of fruits and fruit based products. The challenges and pros and cons of wider application of these

innovative techniques, their commercial potential and consumer acceptability have also been discussed.

Keywords: edible films, fruits, HACCP, lab-on-a-chip, nanoceutical, nanotechnology

Introduction

Fruits have always been the essential dietary supplement being the natural sources of different vitamins, minerals, fibers and phytochemicals like flavanoids, the health benefits of which are well established (Table 1). Fruits are known to have presumed significance of reduction in the risk of certain types of cancer, cardiovascular diseases, and stroke probably due to increased antioxidant capacity of the plasma by fruit consumption (Hassimotto *et al.,* 2009). The fruit vitamins are usually the water-soluble vitamins *viz.* vitamin B complex and C as well as fat soluble vitamins A, E and K. Majority fruits contain high amounts of potassium, calcium, magnesium, sodium, phosphorus, iron and zinc apart from special minerals like selenium, copper and iodine.

The fruit fibers are of two types *viz.* soluble and insoluble, with the later type to be largely indigestible portions of the fruit present in the fruit skin that function as ‘roughage’ and increases the bulk. Chemically, the fruit fibers are cell wall components that include cellulose, lignin, hemicellulose, pectin, gums and mucilages (Ramulu and Rao 2003). These fibers act as a sponge due to their high water holding capacity and are known to regulate the bowel movements as well as reduce the blood cholesterol level. The role of fruit fibers and antioxidants along with oleic acid or monounsaturated fatty acid content in mediterranean-style diet is tremendous to reduce inflammation, and corresponding coronary events in middle aged adults (Basu *et al.,* 2006). Moreover, Hermsdroff and coworkers (2010) reported significant reduction in the levels of markers of inflammation like C-reactive protein (CRP) and homocysteine concentrations as well as decrease in the mRNA expression of certain WBC pro-inflammatory markers in healthy young adults due to consumption of high fruit and vegetable rich diet.

Fruit flavanoids are a group of phytochemicals that have enormous pharmacological and medicinal importance. The particular mention is for the citrus fruits that are rich in flavanoids like tangeretin, rutin, nobiletin etc, which help in alleviating several types of skin, mouth, lungs, stomach and colon cancers. Consumption of fruits rich in beta-carotene, vitamins C and E result in significant reduction in oral and pharyngeal, esophageal and breast cancer risks. The polyphenols and flavanoids in fruits work as signal molecules and alter the gut microecology directly by effectively inhibiting the adherence of pathogenic microbes to gut surface as well as indirectly by increasing the number of beneficial gut micorflora through enhanced adherence of probiotic microbes on gut surface (Parker *et al.*, 2008).

The consumption and importance of fruits is getting emphasized in today's balanced diet schedules though the actual working principles and mechanisms behind the positive impacts of consumption of fruits or fruits products have to be deciphered at the molecular (genomic, proteomic and metabolomics) scale. Since the inception of techniques for determining the mechanisms or principles behind the processes related to food preservation and safety, the present century has witnessed extensive applications of several novel tools like advanced fluorescence-, electron-, scanning probe microscopes and ultrasensitive sensors/probes to determine microbiological and nutritive status of preserved or processed foods. Microbiologically, fruit surfaces and few tissues harbor certain resident mciroflora which exhibit alteration in diversity profiles according to the age of a particular fruit as well as different types of microbes may be acquired from the tools and techniques employed during processing, preservation and storage of the fruit/fruit product till its consumption by the consumer. The revised and novel fruit packaging methods have lengthened the shelflife and availability of seasonal fruits/products. The fruit safety issues are being catered by development of highly specific sensors for rapid identification of known as well as emerging pathogens contaminating or spoiling the preserved or processed fruits even in very low concentrations not in range that could be detected by conventional techniques. This review aims at providing an overview of the modified or novel trends in deciphering the biotic and intrinsic factors for intricate fruit-microbe cross-talks and interactions, identification of postharvest storage, packaging, preservation and safety techniques and commercial status of novel products (Figure 1). The usefulness of any new approach in fruit preservation and packaging has been considered in its ability to retain the original physical, biochemical and organoleptic properties of different fruits and to provide the physiological and health benefits of fruit consumption.

Fruit Microbiology:

Microbiologically, fruits are not sterile and there are plenty of preservation and safety issues regarding the microbiology of fruits (Kalia and Gupta 2006). Studying the number, diversity as well as spatial distribution of normal/contaminating/pathogenic microbes present on the surface, in fissures/internal tissues of fruits is useful for maintenance of fruit quality, shelf life as well as safety. There are several microbiological benefits of consumption of fruits and fruit products. Nyanga *et al.* (2007) studies show that ripe, unripe and even dried unprocessed fruits contain enormous microbial diversity which varies according to the condition of the fruit.

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The natural microflora of fruits, lactic acid bacteria, streptococci, certain yeasts and yeast-like fungi are known to maintain the fruit quality (Rezende *et al.*, 2009). The presence of beneficial microbes further enhance the positive benefits imparted by consumption of fruits which is evidently observed in case of consumption of fermented fruit beverages. The benefits could also be enhanced by combining probiotics with fruit and vegetables like immobilization of *Lactobacillus* on apple/quince pieces (Kourkoutas *et al.*, 2005) and vacuum impregnation of probiotic cultures of *Lactobacillus*/*Saccharomyces* in commercial fruit juices (Betoret *et al.*, 2003). Fruit juices possess antimicrobial compounds that can curb the growth of several human gut pathogenic microbes *viz. Listeria monocytogenes*, *Salmonella enteritidis* and *Escherichia coli* O157:H7 at low temperature by causing significant damage in cell cytoplasm as revealed by transmission electron microscope studies (Raybaudi-Massilia *et al.*, 2009).

Microbial processing of fruits using microbes particularly fermentation, study of novel antimicrobial/microbistatic packaging systems, tracking/tracing the level of microbial contaminants in the fresh/processed produce and the positive impacts on the human gut microflora on consumption of fruits are most important study areas for fruit microbiology.

Nanomicrobiology: It is a conjugate burgeoning discipline encompassing the application of tools and techniques of nanotechnology to study microbes, their interactions and applications (Dufrene 2004, Alsteens *et al.*, 2009). Fruit microbiology could be converged to incorporate novel nanoscience/nanotechnology tools and techniques for fashioning Fruit Nanomicrobiology involving nanoscale studies of the individual components of fruit(s), fruit microbes (be it normal or contaminating microflora) and their interactions with the fruit surfaces; novel methods of decreasing the contaminating microbial load in processed products like fruit juices using nanoparticles or nanofilters, development of innovative, interactive functional fruit-based products using nanotextured nutraceuticals i.e. nanoceuticals, smart/intelligent packaging of the minimally processed fruits/processed fruit juices or fruit based beverages, sensing and tracing the early signs of fruit spoilage using nanobio-sensors and on product nanobarcoding using quantum dots or other functionalized nanoparticles.

Nanotechnology tools to decipher fruit-microbe interactions: Nanotechnology has revolutionized and definitely would alter our basic understanding of the mechanisms or materials and processes or products and hence the differences at macro-, micro- and nanoscopic levels in the present and coming decades. The two sister disciplines of nanoscience and nanotechnology have gathered several advanced tools, techniques and products that are instrumental in embracing, correlating as well as interpreting diverse information. Among these, the electron and scanning probe microscopes are the foremost tools for high resolution imaging to unravel petite information at the nanoscale i.e. nanobioimaging, which could be used to comprehend the behavior of certain microbes towards abiotic and biotic factors premiere in upscaling and enhancing the performance of microbial cell factories for production of particular product (like fermentation for production of alcohol, organic acids, antibiotics, vitamins, essential protein products) as well as to decipher the interaction and preference of a pathogenic microbe towards a specific surface (cell-cell communication and interactomics).

With the invention of electron microscopes (EM) by Knoll and Ruska in early 1930s, the basic tools of high resolution imaging were put forth to the cytologists aiming at explorations at the ultrastructural levels within a cell. The true real time nanoscale resolutions for imaging microbial cell surfaces were possible only after the invention of Atomic Force Microscope

(AFM) by Binnig, Gerber and Quate in 1986 (Binnig *et al.*, 1986). This is a virtual imaging tool which lacks lens system and basically produces image by physically raster scanning of the surfaces at nanoscale in real-time by a micorcantilever probe fabricated from monocrystal of silicon or silicon nitride without involving strenuous sample processing (Dufrene 2002, 2004, Muller and Dufrene 2008). The AFM belongs to a large family of Scanning Probe Microscopes (SPM) and several advancements in the original AFM have enhanced the number of variants (Magnetic Force Microscope, Dynamic Force Microscope, Lateral Force Microscope and many more) as well as the type of information that could be obtained at nanoscale be it nM range of features in x-, y- or z-scales, nN range of forces (van der Waal, magnetic) and nA/nV of current/voltage on the surface of a biological sample. The physical scanning feature of AFM has led to development of this as a tool for manipulation of biological matter/surfaces at the subnanometer scales and in deciphering the physico-chemical properties (like friction, stickiness, viscoelasticity, weak surface forces, chemical groups) of the surfaces (Yang *et al.*, 2007), in exploring mechanisms of specific interaction preferences among the microbe-host modules (molecular recognition, receptor-ligand interactions (Puntheeranurak *et al.*, 2006, Li *et al.*, 2007), protein folding and self-assembly dynamics (Eibl and Moy 2005)) and in highly sensitive detection of bioanalytes (at pico to femtomolar concentrations) real-time in physiologically active microbial cells (Dufrene 2008). The mechanism of antagonism could be deciphered at the molecular and even nanoscale level by using the antibody labelled Transmission Electron Microscopy, marker sandwiched fluorescence or confocal laser scanning microscopy and Dynamic Force Microscopy using tapping mode. These techniques would also be able to fish out prime interaction molecules (glycoproteins, receptor-ligands) as well as their location on the surfaces of the antagonistic microbes. The information yielded by various SPMs is particularly very useful in defining the mechanisms of interaction of the pathogenic microbes (fruit spoilage microbes, fruit contaminating opportunistic microbes, human pathogenic microbes) which could be implemented in developing novel control and eradication protocols regarding the product quality and safety issues pertaining to fruit postharvest handling, preservation and processing industries.

The structural and material properties of fruit greatly affect the fruit quality including the microscopic histological and cellular features as well as nanoscopic middle lamella and plasmodesmata spatial distributions (though fruit structure is difficult to investigate owing to uneven spatial distribution of distinct structural features). This calls for the application of predictive multiscale models which can predict and relate the material properties and structural micro or nanoscale geometry of a fruit to its macroscopic properties and quality. Though this technique of microscale modeling is at infancy and not very useful for the whole fruits as there exists arbitrariness in the geometry of biological microstructures but it would definitely be useful for the engineered products derived from fruits (Mebatsion *et al.*, 2008). The same predictive mathematical models can be used to predict the number of pathogenic microbes like bacteria (Valdramidis *et al.*, 2006), yeast (Wang *et al.*, 2004, Tchango *et al.*, 1997), fungi (Gibson and Hocking 1997) and viruses (Deboosere *et al.*, 2010) on the fruit surface for estimating the consequences of food handling and processing operations on growth, survival and inactivation of microbes such as the foodborne pathogens (McMeeken *et al.*, 2008). This would be a proactive technique among HACCP protocols that would help in describing the microbial behavior in order to prevent food spoilage and food-borne illnesses.

The internal structure and material properties of the fruits could be easily deciphered by high resolution imaging tools like Environmental Scanning EM, High Resolution-Transmission EM, Confocal Laser Scanning Microscope (CLSM) and AFM. These versatile techniques can be used to study the structure and molecular bonding of the individual molecules/macromolecules, like plant storage polysaccharide (starch), plant cell wall polysaccharide (cellulose), fungal cell wall polysaccharide (chitin), middle lamella material (pectin), DNA, proteins at the nanoscale which definitely would have strong positive impact and applications in predicting models that can be applied to improve shelf life and quality of fruits (Yang *et al.*, 2007).

Microbiological control of postharvest decay of fruits:

The microbiology of the fruit is an essential element for minimizing the postharvest losses of the fresh fruits. Deciphering the predominant microbial diversity transits or shifts would be instrumental in identification of the proficient pathogen of a particular fruit under a specific set of abiotic conditions prevailing during the processing and storage of the fresh fruit. Better economic returns by fruit growers and retailers can only be harnessed if the enormous postharvest losses could be decreased or curbed during handling and supply chain of fruits. Molds or fungi are the major causative agents of postharvest fruit decay of stored fruits and thus have to be eradicated using synthetic fungicides which are either sprayed or topically applied (dipping treatment) to stored fruits (Sharma *et al.*, 2009). The cost of pesticide treatment, evolution of resistant pathogenic strain/species and concerns for pesticide residues on or in fruit tissues emphasizes the need for alternative techniques that are environmentally benign as well as show effectiveness at par to the commercially available fungicidal formulations. Use of biological control agents is one such alternative technique to reduce post-harvest losses (Mari *et al.,* 2007).

Though fruits are equipped with special cuticular outer surface composed of lipid-wax cutin to physically ward off pathogens from entering the inner tissues but still the fruit pathogens tend to encroach deeper in the physical protective barriers by producing certain extracellular adhesives to cling on the cutin layer followed by its dissolution to infect the internal tissues. The interaction properties of pathogen like adhesiveness and removal from the fruit surfaces are largely affected by variation in the type of cutin fatty acids/waxes or even epicuticular wax quantity as this leads to alteration in the physical and chemical properties of the surface (Pierzynowska-Korniak *et al.,* 2002). Even the uniformity in thickness of the cutin coating significantly affects the surface toughness (Spotts *et al.,* 2009).

The other protective barrier on the fruit surface is in form of native microflora (microbiological) comprised of wild yeasts and certain bacteria which ward off in general all types of the bacterial and fungal pathogens (Figure 2). Several native microbes have been reported to act as potential postharvest microbial control agents by either decreasing or even eliminating the postharvest decay of fruits. A study conducted by Ukuku *et al.* (2004) indicated the positive role of native microflora to avoid contamination and growth of *Listeria monocytogenes* in fresh cut melons. Similar study by Teixido *et al.* (1998) showed that presence of yeast culture *Candida sake* significantly reduced the populations of *Cladosporium* and *Penicillium* during long time cold storage and ambient shelf-life storage conditions. Likewise Xu *et al.* (2008) have demonstrated the biocontrol potential of a variety of yeasts genera namely *Pichia membranaefaciens, Candida guilliermondii, Cryptococcus laurentii, Rhodotorula glutinis* against fungal pathogen *Monilinia fructicola* that causes peach fruit decay. These yeasts control the levels of protein carbonylation and mitigate *Monilinia* induced oxidative damage to curb decay in peach fruits.

Native yeasts are the most striking agents possessing biological control potential by virtue of an array of antagonistic mechanisms. The mechanism of antagonism may differ depending upon the extent of participation or interaction by the antagnostic chemical or feature. It could be either ‘active’ in terms of secretion of antimicrobial compounds like antibiotics, cell wall degrading enzymes and induction of host resistance to curb the growth of other microbial pathogens or ‘passive’ in terms of competition for space and nutrition with pathogens (Janisiewicz and Korsten 2002, Chan and Tian 2005, Sharma *et al.*, 2009). Further the active and passive mechanisms may be categorized as physical (competition for space and nutrients Janisiewicz *et al.*, 2000), chemical (production of cell-wall degrading hydrolytic enzymes, Olofse *et al.*, 2009), biochemical (resistance to oxidative stress, Castoria *et al.*, 2003, induction of an antioxidant defense response, Xu and Tian 2008), antimicrobial (direct interactions with the pathogen, Chan and Tian 2005) and physiological (induction of host resistance, Zhao *et al.*, 2008). Several novel mechanisms have been reported by researchers that either inhibit the pathogen spore germination and vegetative growth or directly kill the vegetative cells by producing active antimicrobial diffusible compounds. A report by Sipiczki (2006) demonstrates the antagnostic activity of red-maroon pigment-producing *Metschnikowia* strains against filamentous fungi, yeasts, and bacteria which has been hypothesized to be based on inhibition of growth of sensitive microorganisms by depletion of free iron in the medium due to iron binding pigment formation by the yeast strain other than the siderphore.

Though the postharvest fruit biocontrol tools and technologies have advanced yet the true commercial successes of a potential biocontrol agent is yet to happen. Majority of microbial antagonists have protective activity in small (3-5 mm deep) puncture wounds, however the commercial success of a biocontrol agent resides in curative activity in a wide range of wounds like bruises, scrapes, broken stems or broken epidermal hairs in several different commodities for pathogens with different etiologies (Drobya *et al.,* 2009). Largely the paucity of information on the diversity of the native fruit protective surface microflora of most of the fruits (grapes and apples being most elaborately studied till date) is responsible for screening out a multiple pathogen infection controlling agent for different types of fruit surfaces. Now that some information is available on the microbiological barrier from different fruit surfaces (Janisiewicz *et al.,* 2010) it is now possible to identify microbial culture(s) possessing multiple antagnostic properties. Nantawanit *et al.* (2010) have reported the biocontrol of chili anthracnose (*Colletotrichum capsici*) by inoculation of *Pichia guilliermondii* that initiates a spectrum of antimicrobial activities by itself or in the chili fruit such as enhanced phenylalanine ammonia-lyase, chitinase, and β-1,3-glucanase activities and the accumulation of capsidiol phytoalexin to combat the infection. A larger spectrum of pathogen control can be envisaged by combining the traditional physical and chemical methods with the novel biological control agents. Zhang *et al.,* (2009) have observed reduction in the disease incidence and lesion diameter of blue mold (*Penicillium expansum*) decay of pears by combined application of chemical methyl jasmonate (200 μM) and yeast culture *Rhodotorula glutinis* ($1x10^8$ CFU ml^{-1}) after incubation for 7 d at 20^0C. Similar use of calcium chloride alongwith yeast *Candida membrifaciens* has been reported to efficiently decrease the lesion formation by mold *Penicillium expansum* in apples

(Gholamnejad *et al.*, 2010). Janisiewicz and Conway (2010) have recently reviewed the combined use of physical, chemical and biological control agents for controlling postharvest decay of fruits.

Novel fruit preservation and processing techniques:

As fruits are seasonal perishable commodities, processing and preservation of fruits is age old practice. Microbial quality of fruits or fruit products has to be maintained at various levels of processing and packaging. Though production practices have a tremendous effect on the quality of fruits at harvest, on postharvest quality, and on shelf life but their preservation and processing is carried out to decrease or eradicate the contaminating microbial load. The common techniques of physical-, chemical- and radiation- processing and preservation can been integrated and improvised for increasing the quality and benefits imparted by the product. The application of a combination of preservative factors has given rise to a new concept in food processing and preservation termed as ‘Hurdle Technology’ (Lee 2004) and could exhibit effective control of a spectrum of pathogenic microbes (Mahapatra *et al.*, 2005). The commencing sections discuss about the recent studies and innovations leading to development of novel techniques and also improvements in the existing techniques based on scientific and technological advances in microbiology and nanotechnology.

Radiation preservation: Radiation preservation of whole fruits and fruit juices is nonthermal processing technique to effectively inactivate foodborne pathogens on whole fruit surface and fruit juices. In particular, UV ionizing radiation is most widely utilized for disinfection of fruit/liquid fruit products (Farkas and Mohacsi-Farkas 2011). Shama and Alderson (2005) have provided a novel method of UV hormesis i.e. application of low doses of UV to induce stress responses like production of anti-fungal compounds and delayed ripening in fruits or fresh produce. The short UV exposure of fruits causes reduction in the disease incidence and extends the shelf life. A radiation disinfestation of fruits using radiation dose upto 2 kGy has been most extensively utilized in Ukraine (Fan 2005). However, the non-ionizing radiations like radiowaves have also been used to enhance pathogen eradication. Ukuku and Geveke (2010) have utilized combination of Radio Frequency Electric Fields (RFEF) and UV-light treatments to inactivate bacteria in liquid foods and reported better performance of RFEF treatment in terms of causing more injury to the bacterial cells leading to more leakage of intracellular nucleic acid and proteins into a suspension that absorb UV light (so termed as UV-substances) than cells treated with UV-light alone.

Modified atmosphere packaging and its variants: There are a number of new strategies available for the preservation and processing of fruits, among which conjugate techniques involving application of more than two conventional strategies are the foremost. Conventionally, modified atmosphere packaging (MAP) is most commonly used for fresh cut fruits like pomegranate arils, apple, kiwifruit, honeydew, pineapple (Jayas and Jeyamkondan 2002, Soliva-Fortuny and Martin-Belloso 2003, Ayhan and Esturk 2009). Timmon (2005) has reported better product quality and enhanced shelf life of fresh cut fruits by using approximately 3 to 5% O_2 and 5 to 10% CO_2 within the package which slows down the deterioration of product. Now-a-days MAP technique has been used in combination with physical, chemical or radiation techniques. The texture and quality of fresh fruits packaged by using MAP technique could be enhanced by treating the fresh fruits with essential oils having antimicrobial properties. A study on the fresh sweet cherry fruits revealed that treatment with antifungal essential oils like eugenol, thymol or menthol imparts certain positive benefits on several quality parameters. The treated fruits exhibited reduced weight loss, enhanced delaying in colour changes and maintenance of fruit firmness compared to control fruits which may be attributed to the reduction in the action of cell wall degrading enzymes in the treated fruits (Serranoa *et al.*, 2005). Apart from essential oils, aromatic compounds (e.g. hexanal, 2-(E)-hexenal and hexyl acetate) hold a good promise for their use as shelf-life enhancers to impart better safety due to antimicrobial action towards the gram negative bacteria (Lanciotti *et al.*, 2004).

Bacteriocin based biopreservation: 'Bacteriocins', peptides having antimicrobial activity of bacterial origin, are best suited candidates for food biopreservation as their use would help in retaining the organoleptic and nutritional properties of particularly the fresh produce or the minimally processed fruits and also would help to reduce the practice of use of chemical preservatives and intense heat treatments for preservation (Leverentz *et al.*, 2003, Galvez *et al.*, 2007). These may be categorized into two groups i.e. broad or narrow spectrum bacteriocins depending on the kinds of microbe being targeted or controlled. Broad-spectrum bacteriocins include enterocins, colicins and lantibiocins that affect bacterial genera belonging to a larger group and thus have a wider use. Narrow-spectrum bacteriocins, however, specifically or selectively inhibit high-risk bacteria in fruits like *Listeria monocytogenes* without affecting harmless microbiota in fresh cut minimally processed (MP) fruits with high sugar and moisture content like honeydew melons, berries, apple etc for which narrow spectrum bcateriocins are of practical importance for extending shelf life (Leverentz *et al.*, 2003). Thus these peptides have a future as preservatives, shelf-life extenders, additives or ingredients that could be produced *in situ* by bacteriocinogenic starters, adjunct or protective cultures (Galvez *et al.*, 2007). Certain broad spectrum bacteriocins that are available for commercial applications are nisin, pediocin PA-1/AcH, lacticin 3147, enterocin AS-48 or variacin. Penney *et al.* (2004) however reported that bacteriocin nisin application did not prevent the growth of spoilage causing microbes in fruit yogurt made with minimally-processed wild blueberries rather they advocated the application of phytopreservatives such as vanillin as 'natural' antimicrobial agents in minimally processed fruit yogurt.

Edible films: Due to the high water content in certain whole and majority of fresh-cut fruits, biggest problem of discoloration and loss of quality occurs due to action of gases and contaminating bacteria and molds in conventional packaging systems. Fresh-cut fruit tissue deteriorates more rapidly than intact fruits which may be due to increased activity of wound-induced enzymes that act on cell walls and membranes of the cut fruits (Karakurt and Huber 2003). Some other biochemical changes also occur that cause deterioration in the tissues of fresh cut fruits (Toivonen and Brummell 2008). These problems could be sorted by developing edible films that act as barrier to minimize water loss (Bourlieu *et al.*, 2009) and are more efficient in controlling gaseous exchange that also delays the ethylene mediated senescence of respiring fruits. Moreover, value addition of edible films may equip these to perform multiple functions like ability to eradicate spoilage causing microbes on inclusion of an antimicrobial agent, ability to increase the types of flavor etc. Usually edible films are eco-friendly coatings composed of biodegradable polymers like cellulose, starch, wax that reduce the requirement of stringent conventional packaging, protects fruits from spoilage, extends the shelf life as well as help in ecofriendly removal of the wastes or by-products of the food industry due to bioconversion into value-added film forming components. Edible films not only improve the product stability, but are useful in maintaining the product quality and safety apart from creating a light weight packaging system having spectrum of transparent or nearly transparent packages for increased consumer convenience.

The edible coatings could be single, bilayered or multilayered i.e. composite coatings formed by depositing one type of material (like polysaccharide/protein) followed by deposition of another type (like lipid) and this imparts better protection from microbial spoilage, controls water loss/gain and gaseous exchange from the surface layers. Several studies have reported the enhancement in the hydration efficiency of the minimally processed fresh cut fruits like apple slices coated with low methoxyl pectin coatings (Lenart and Dabrowska 1999) and strawberries coated with sodium alginate, carrageenan, or guar gum solutions (Matuska *et al.*, 2006).

New advancements have to be welcomed for improving functionality and performance of the edible films to develop new genre of edible films that can better maintain the quality, shelf-life and naturalness of the fresh and MP fruits (Vargas *et al.*, 2008). The principle benefit of the barrier films could be improved by enhancing the coating properties by incorporating nanosized organic or inorganic materials, biological or synthetic matrices to fabricate nanocomposite films that exhibit better barrier, mechanical and functional properties and thus lengthen/maintain the quality of the fresh produce for longer periods desirable for storage and transportation time lags. The novel edible films can be value-added by addition of functional ingredients as encapsulated nutraceuticals like vitamins, water-insoluble flavanoids and other flavor/color enhancing phytochemicals, antioxidants like anthocyanins, carotenoids for avoiding discoloration of the cut surface and antimicrobial agents like bacteriocins (natural), biogenic nanoparticles of silver, titanium or zinc (inorganic synthesized) to curb the growth of spoilage causing microbes (Rojas-

Grau *et al.*, 2009, Janjarasskul and Krochta 2010, Oms-Oilu *et al.*, 2010). Edible coatings of the minimally processed fruits can contain antibrowning agents (Lee *et al.*, 2003, Perez-Gago *et al.*, 2006) and texture enhancers like $CaCl_2$ (Le Tien *et al.*, 2001, Toivonen and Brummell 2008). The bioactive packaging of whole/MP fruits particularly involves the incorporation of antimicrobials like the extracellularly secreted bacteriocins of microbial origin in the packaging material which is much useful to curb biofilm formation by spoilage causing/pathogenic bacteria in case of the cut fruits.

Nanocomposites for packaging: Nanocomposite materials include one-dimensional, two-dimensional, three-dimensional and amorphous materials made of distinctly dissimilar components that are mixed at the nanometer scale (Rhim *et al.*, 2006, de Azeredo 2009, Ma *et al.*, 2009). Compared to the conventional packaging materials, nanocomposites have several additional benefits like enhanced strength or elasticity, improved biodegradability and better control over gaseous molecules which are required for developing better performing packaging materials. Traditionally, nanocomposite material is composed of three different types of components *viz.*, the matrix material, filler and the filler interface material with at least one of them in nanoscale order.

There are basically two types of polymers that could be used as composite matrix material *viz.* the petrochemical based polymers (like polyamides, nylons, polyolefins, polystyrene, ethylene-vinylacetate copolymer, epoxy resins, polyurethane, polyimides and polyethylene terephthalate) and the biopolymers (polysaccharides, proteins, lipids and their composites). The bionanocomposites consisting of natural polymers/biopolymers, organic/inorganic filler (less than 5% by weight) and interfacial materials, exhibit elaborated benefits of biocompatibility and biodegradability which landmark their extensive applications in whole and fresh cut fruit packaging. The biopolymers that could be used as matrices for designing nanocomposites generally include polysaccharides like, cellulose and cellulose derivates, starch and starch derivatives, pectin, chitosan, alginate, carrageenan and different types of natural gums *viz.* xanthan, guar and gum Arabic; organic acids like poly lactic acid, polyhydroxy butyric acid, proteins like zein, gluten, soy protein, peanut protein, and cotton-seed protein (plant origin), casein and whey protein (animal origin) and lipids particularly a variety of waxes and fatty acids (Rhim *et al.,* 2006, de Azeredo 2009). The particulate or the filler component that is to be added to the matrix to enhance its barrier properties, usually are nano-sized particles with high aspect ratio and are as diverse in nature and characteristics as the matrix material itself. These may be of different types ranging from inorganic nanoparticles of clay such as montmorillonite or kalonite clay particles (Mangiacapra *et al.,* 2006), carbon nanotubes, nanoparticles of noble metals like silver-, silver-zeolite or gold-nanoparticles, nano zincoxide (Ma *et al.*, 2009), nanoSiO_2 to organic (nanocellulose fibers (Sozer and Kokini 2009), cellulose nanocrystals (Habibi and Dufresne 2008), starch (Ma *et al.*, 2009), chitin/chitosan (Chang *et al.*, 2010)). The nanoclay incorporated bionanocomposites are now being popular due to better control over gaseous exchange which is prerequisite for maintaining the quality of the fresh produce (Observatory NANO 2010). These nanocomposites could be synthesized by methods like exfoliation/adsorption and *in situ* intercalative polymerization which enhance the tortourosity of the path gas molecules have to follow to reach and react with the produce surface components (Gacitua *et al.*, 2005). A comprehensive overview of the various nanopackaging materials i.e. edible films, nanocomposite, bionanocomposite and biodegradable nanocomposite films has been provided by Miller and Senjen (2008). Among the above discussed biopolymer matrix materials, polylactic acid (PLA) (Tingaut *et al.,* 2010) has the highest potential for commercialization followed by cellulose and polyhydroxy butyric acid because of the ease of production/availability and scaling up for commercial production (Janjarasskul and Krochta 2010). The fruit and vegetable purees are the other major components that could be used to fabricate durable and cost effective bionanocomposites (de Azeredo 2009). There are many reports which suggest the higher organoleptic and fruit safety qualities of the minimally processed fruits by using the nanopacking materials over the conventional packaging (Baldwin 1994, McHugh and Senesi 2000, Stevens 2002, Avella *et al.,* 2007, Sothornvit and Pitak 2007, Sothornvit and Rodsamran 2008, Li *et al.,* 2009). Li *et al.* (2009) reported better physico-chemical and sensory qualities of Chinese jujubi fruit packed using nanocomposites over the conventional fruit packaging.

Fruit juice/beverage pasteurization and alternatives: The fruit juices and fruit based beverages can be efficiently pasteurized on a mass scale by the conventional thermal processes though these methods plunder away the nutritional and organoleptic characteristics of the final product. The thermal methods are also required for the concentration and clarification of the fruit juices resulting in loss of certain nutrients and sensory characteristics of the juices for instance the aroma compounds (more than 6000 compounds impart aroma in different fruits; approximately 200 compounds responsible for the distinct refreshing aroma in juices of passion fruit and oranges) are either destroyed or chemically modified during the temperature dependent processing techniques (Pereira *et al.,* 2005). However, the thermal treatments become mandatory for the fruits rich in soluble solid content and low in acidity, the two factors which make the fruit pulp vulnerable to microbial contamination and growth (Cassano *et al.*, 2007).

Pasteurization is not enough to eradicate all types of spoilage causing microbes. Occurrence and growth of *Propionibacterium cyclohexanicum* in a variety of pasteurized fruit juices of orange, apple, grapefruit, pineapple, cranberry and tomato at temperature ranging from 4 to 40^0C has been reported by Walker and Phillips (2009). The commercially pasteurized fruit juices of orange, grapefruit and apple fruits most likely contain the extreme heat resistant, acid tolerant and endospore forming spoilage microbe *Alicyclobacillus acidoterrestris* due to which it debars elimination by standard heat treatments (Silva and Gibbs 2001). Thus the pasteurization methods have to be conjugated with the application of antimicrobials like essential oils to prevent the germination of *A. acidoterrestris* spores after pasteurization. However, the use of microwave ovens for pasteurization of the fruit juices may better maintain the nutritional qualities of juice in comparison to the traditional pasteurization techniques (Cinquanta *et al.*, 2010).

There are several alternative pasteurization technologies available which not only extend the shelf life but also enhance the microbial safety of fresh juices while preserving organoleptic and nutritional qualities in terms of presence of antioxidants. The major non-thermal preservation techniques include the application of ultra-violet radiation, pulsed electric field (PEF) and ultra sound treatments which can be used for decreasing the natural microbial load of a variety of fruit juices, purees and fruit based beverages (Devlieghere *et al.*, 2004, Tiwari *et al.*, 2009). The principle of UV light action is well known that involves the eradication of the microbes by causing formation of intra-strand thymine dimers in DNA (Gould 1996, de Cruiji

1997, Yaar and Gilchrest 2007). The PEF technique is based on the principle of inactivation of microorganisms due to alteration in the cell membrane structure that results in pore formation in the membrane leading to cell death by oozing out of the cytosol through the pores (Zhang *et al.*, 1995a). It involves applying short pulses of electric field to fruit juices placed between two electrodes at low temperature during processing which results in cell death of common pathogenic microbes such as *Salmonella enteritidis, Listeria monocytogenes, E. coli* K 12 and *E. coli* O157:H7 in a variety of fruit juices (Zhang *et al.*, 1995b, Sentandru *et al.*, 2006, Aguilo-Aguayo *et al.*, 2009, Valappil *et al.*, 2009, Oms-Oliu *et al.*, 2009). The freshness of the fruit juices also depends on the physical properties like color or viscosity as well as the retention of aroma flavor compounds which could be better maintained by high intensity PEF (HIPEF) over the thermal pasteurization protocols (Aguilo-Aguayo *et al.*, 2010a, 2010b). Valappil *et al.* (2009) observed that PEF better retained the nutrients and a range of volatile aroma imparting compounds in apple cider as well as maintained the acceptable microbiological status pertaining to *E. coli* K 12 counts after four weeks of incubation with respect to the thermal and UV treatments. The microbial inactivation is enhanced due to the additional impact by applying PEF technique along with the antimicrobials like bacteriocins (nisin), essential oils and other compounds (Pol *et al.*, 2000, Mosqueda-Melgar *et al.*, 2008a, Mosqueda-Melgar *et al.*, 2008b). Moreover, the benefits of HIPEF treatment can be transcended over to fruit juice blended beverages. Morales-de la Pena *et al.* (2010) have reported HIPEF to be a better alternative method over conventional thermal pasteurization for maintenance of antioxidant/nutritional/quality parameters of fruit juice blended soymilk beverage. They also reported that HIPEF technique ensures better microbiological stability. The ultrasound treatment also results in cellular disruption so would be a nice alternative technique for extending the shelf life and organoleptic properties of fruit juices, sauces, purees, and dairy products (Corrales *et al.*, 2008, Vilkhu *et al.*, 2008, Gomez *et al.*, 2009) but it is more useful when applied in conjunction with thermal or pressure treatments (Raso *et al.*, 1998).

The advanced techniques including the nanotechnological tools and methods would be more valuable for the beverage industry particularly for fruit juices or the fruit based beverages as these products require reduction in the amount (concentration) or level of dissolved contaminants as well as fine filtration of the particles (clarification). Common fruit juice concentration techniques include the thermal treatments, reverse osmosis, membrane distillation and osmotic distillation (Vaillant *et al.*, 2001, Matta *et al.*, 2004, Vaillant *et al.*, 2005, Cassano *et al.*, 2007, Jesus *et al.*, 2007, Gurak *et al.*, 2010). These techniques suffer from one or other limitations which could be circumvented by applying advanced techniques of ultra- and nano-filteration in conjugation with the prior techniques for preconcentrating the juice contents. Cassano *et al.* (2003) have reported that ultrafiltration of citrus and carrot juices better retained the color and aroma in comparison to the thermal concentration technique as well maintained the total antioxidant activity with respect to the fresh juice. Thus the application of integrated membrane process would yield fruit juice concentrates of high nutritional value and quality in reduced time and that too at room temperature. A similar application of crossflow microfilteration with osmotic evaporation has been reported to obtain better quality juice from discarded melon fruits which would be a useful technique to overcome the product losses due to fresh quality issues (Vaillant *et al.*, 2005).

Nanofilteration is an innovative technique for concentration of different types of juices like grapes (Ferrarini *et al.*, 2001), apple and pear juices (Warcsok *et al.*, 2004, Carrin *et al.*, 2007) as well as for recovery of aromas from fruit juices (Daufin *et al.*, 1998) and decolorization of the dark compounds from long term stored fruit juices (Carrin *et al.*, 2007). It is defined as novel membrane cross-flow filtration technique involving use of nanofilters (nominal pore size 1 nm) using active principle ranging between ultrafiltration and reverse osmosis. The advantages of nanofilteration over the above techniques are based on integrated membrane application for fruit concentration such as reduction and simplification of the clarification process, better efficiency, speed and economy of separation of fine particles having molecular weight ranging from 20 to 180 weight units. The separation efficiency of nanofilteration depends on the sieving properties of the membrane through which the juice has to be pressure-driven, though the pressure is lower than what is required for reverse osmosis that can damage the juice as well as on the charge or the Donnan effect (Warcsok *et al.*, 2004).

Nanotechnology products:

Nanotechnology has led to the development of certain products which could be termed as 'Nanofoods' owing to their production lineage or processing or packaging involving components used/delivered at the nanoscale. The advancements in nanofood sector have landed onto the development of interactive-, designer-, customized-, intelligent- foods that could be lauded for their ability to proficiently ameliorate sensorial, health and economical benefits on consumption (Kim and Kwak 2004, Bowman *et al.*, 2010). The advent of nanotechnology in food sector has most likely surged to multibillion dollar global industry having USA, Japan and China as the key producers as well as consumers of the nanofood products (Joseph and Morrison 2006). Apart from development of the nanofoods; nanoscientific tools and techniques have been instrumental in fabricating novel sensing/tracking devices that offer rapid, easy, cost effective and sequential/whole time tracking of the spoilage conditions in packaged lots without sampling of the cartons. These innovations can also be applied to fruit value addition, preservation and packaging.

***Addition of Nanoceuticals*:** A phytochemical or nutraceutical, is a product isolated or purified from foods having demonstrable physiological benefit or may provide protection against chronic disease, are generally consumed as medicine and are not usually associated with foods (Brower 1998, Kalra 2003). Nanoceuticals are the nutraceuticals or dietary supplements like vitamins, minerals, polyphenols like flavanoids, which, if adsorbed onto nanocarriers or reduced in size to nano level exhibit larger surface area that not only enhances their disponibility in the medium but also enhances the absorption of the product theoretically due to greater traverse indexes for travel across the membranes and other barrier systems in the body. The nanoceutical product brands are at an alarming arouse due to little government control over analysis of efficacy and consumer health safety of old routinely used supplements from the new genre of nanosized products (Huang *et al.*, 2009). Though a relatively new arena for the food nanotechnology, there are an estimated 44 nanoceuticals in market as dietary supplements (Erickson 2009).

Several hydrophobic beneficial plant chemicals are poorly absorbed by body on consumption. On changing the pharmacokinetics and biodistribution of such plant chemicals through nanotexturization or nanoencapsulation can enhance their bioavailability and absorption. A study by Yen *et al.* (2009) has revealed the enhanced performance of a water insoluble phytochemical by adsorption onto nanoparticles. Naringenin (4′,5,7-trihydroxyflavanone), a natural flavonoid aglycone of naringin, is widely distributed in several fruits like cherries, citrus fruits and grapefruit. It possesses excellent free radical scavenging ability and pharmacological activities which are responsible for anti-tumor (Gates *et al.*, 2007), anti-inflammatory and hepatoprotective properties (Yen *et al.*, 2009). Gao *et al.* (2006) have reported the stimulation of the DNA repair system in prostate cancerous cells by the citrus flavonoid naringenin. Isolation of naringenin followed by its adsorption on polymer nanoparticles or nanoencapsulation (involves enclosing an active ingredient in a nanoscale capsule, Shelke 2005) enhanced the disponibility of this water insoluble compound on oral administration and effectively improved the release of naringenin. This resulted in better hepatoprotection mediated by antiapoptotic and antioxidant properties of naringenin (Yen *et al.*, 2009). The immunomodulatory effects of consumption of fruits and the fruit extracted flavanoids have also been documented as for example lime juice extract on activated human mononuclear cells (Gharagozloo and Ghaderi 2001). Similarly, Catoni *et al.* (2008) have also documented the positive effect of flavanoids on humoral immune response in frugivorous birds. Moreover, they have also showed the active selection of the fruit containing high amounts of flavanoids by blackcarp frugivorous birds over other fruits which enhance their immune response w.r.t control birds after an immune-challenge.

Nanoceuticals are expected to revolutionize the availability of product brands and types in the food pharmaceuticals or nutraceuticals and the cosmetics (anti-aging or sun screen manufacturing) industries because of their health benefits, product improvement or value addition and easy detectability (Erickson 2009). The major benefits of nanoceuticals include enhanced nutrient absorption, elevated brain related functions and general improvement in the health-promotive physiology, sensorial benefit like better product textural values (enhanced colour, flavor, taste, consistency due to reduced use of preservatives) (Srinivas *et al.*, 2010). The product benefits include the development of designer food i.e. customized design of the food supplement for targeted nutrition keeping in mind the age or health needs of the individual, development of interactive food that can release the active compounds on solvent activation or signaling due to changes in pH, temperature, irradiation, or osmotic shock, development of functional foods etc (Huang *et al.*, 2010).

Nanoceuticals have been supplemented in the fruit juices and fruit based beverages or fortified nutritional drinks. Addition of micro/nanoencapsulated probiotic bacteria in fruit juices (Sekhon 2010) or nanoencapsulated nutraceuticals like phytochemicals, flavanoids, Co-enzyme Q10 (Huang *et al.*, 2009) or supplementation of fruit juices with nano-iron or nano-zinc (Miller and Senjen 2008), would help in enhancing the health and sensorial benefits of the product which could extend towards creation of customized fruit drinks due to controlled release and better dispersion and absorption of water-insoluble food ingredients and additives (Huang *et al.*, 2010). A major breakthrough would be increasing the bioavailability of essential micronutrients *viz.* iron and zinc by using the fruit juice or juice blends with nano-textured iron/zinc (Miller and Senjen 2008).

Smart/active/intelligent packaging**:** The new era packaging modules are intriguingly complex networks involving better usage of computer assisted control systems for identification, sorting, response and tracking/tracing of various abiotic and biotic factors responsible for spoilage. There are two basic types of packaging systems *viz.* the smart or intelligent and the active packaging both of which are required for wholesome information on the status of the food regarding the nutritional as well as safety parameters (Appendini and Hotchkiss 2002). Srinivas *et al.* (2010)

have provided well annotated and gathered recent information on various aspects related to food nanotechnology including the active and intelligent packaging. The 'active packaging systems' involve the use of special packaging material that contains performance enhancing subsidiary constituents within or on surface/headspace of the package system particularly equipping it to provide protection (like protection against oxygen, ethylene and moisture) by controlling various biochemical/physiological reactions occurring inside the package or even reacting with the packed product (maintenance of food quality) and may even improve the quality of the product since its packaging (Robertson 2006). Thus the active packaging system alters in response to the triggering event like changed gas concentration due to respiration by the fruit/fruit surface. In general, during storage periods, the active packaging technique may involve active monitoring of the concentration of various volatile compounds and gases inside/in headspace of the package by altering the package permeation properties to maintain the freshness, firmness and color quality parameters of fruits in particular. Though it may also improve the quality of the packed produce during storage by active translocation of food additives like antioxidant polyphenols (flavanoids), flavor enhancing straight chain and aromatic aroma compounds (hexanal, hexyl acetate) and biocides (like bacteriocins of microbial origin, zinc/silver/magnesium oxide nanoparticles) in miniscule amounts from packaging material to packed product (Cooksey 2010). The active packaging material primarily include a variety of polymers, natural or synthetic in origin that contain a large diversity of particles, fibers, and other structures/compounds embedded or incorporated in the packaging material to yield a functional composite. Siro and Plackett (2010) have reported the use of microfibrillated cellulose for developing novel nanocomposite materials. The nanocomposites discussed in the earlier section have enormous applications for generation of active packaging materials. The active packaging system consists of gas scavengers or gas absorbers (moisture/humidity, ethylene carbondioxide, oxygen and off flavor/odor abosorbers) and emitters (ethanol, suphur dioxide, humidity, carbondioxide, organic acid, flavor, antioxidant, pesticide emitters) among which moisture/humidity and ethylene scavengers and carbondioxide, organic acid, flavor, antioxidant and biocide emitters could be used for fruit active packaging.

The antimicrobial active packaging is getting popular for the fresh-cut and minimally processed fruit packaging and involves the use of both volatile as well as non-volatile antimicrobials of microbial/plant origins. These antimicrobials could be either adsorbed or coated on the inner surface of the matrix polymer material, chemically immobilized to polymer, incorporated directly into polymer or added to package as sachet or pads (Appendini and Hotchkiss 2002). An *et al.* (2008) have demonstrated extension of the shelf-life of asparagus coated with silver nanoparticles-PVP coatings on common cold storage temperatures of 2^0C and 10^0C. Similarly, a study by Jin *et al.* (2009) explores the benefits of incorporation of the zinc oxide nanoparticles suspended in polyvinylprolidone gel for killing of *Listeria monocytogenes, Salmonella enteritidis* and *Escherichia coli* O157:H7. Apart from application of nanoparticles the bioactive food packaging relies on application of antimicrobial compounds of microbial or plant origin for curbing the growth of microbes in packaged food. Bacteriocins, the antimicrobials of microbial origin (discussed in section novel fruit preservation and processing protocols) could be incorporated in the packaging material particularly in case of the cut fruits as these would curb the growth of pathogenic bacteria (Janjarasskul and Krochta 2010). Other compounds like organic acids and antibrowning agents could be incorporated in the packaging materials to enhance the spectrum for maintenance of organoleptic properties of the packed product. Eswaranandam *et al.* (2006) incorporated malic and lactic acid into soy protein coatings to extend the shelf life of fresh-cut cantaloupe melon.

Intelligent packaging systems includes labels/portable equipments/quality markers incorporated into, or printed onto a food packaging material that identify, monitor and trace various aspects of food, report the conditions inside/outside of the package regarding the quality, tampering, time-temperature abuse throughout the supply chain and help consumer in decision making (Yam 2000, de Jong *et al.*, 2005, Han *et al.*, 2005). These systems primarily include the time-temperature (can trace back information about the time of temperature abuse), gas (indicate alteration in gaseous components particularly oxygen and carbondioxide gases colourimetrically by a chemical or enzymatic reaction), light (optically variable films containing photosensitive inks), physical shock, microwave doneness (consist of thermochromic inks which change color on heating and can detect readiness of foods on microwave heating), leakage and microbial spoilage/pathogen indicators (include the bio- and nanosensors for pathogen detection which are discussed in the following section) as well as the tracking/tracing instruments like radio frequency identity (RFID) tags (small antenna connected microchips for providing tracking and identification information, could be integrated with the time-temperature indicator or microbial biosensor to record and store data) (Yam *et al.*, 2005, Gander 2007). Thus intelligent packaging not only offers to identify, monitor and trace the history regarding various factors/parameters for ensured better quality of the packaged product but also ensure efficient information flow by offering innovative communicative functions (Dainelli *et al.*, 2008).

Nanosensors and nanoprobes for pathogen detection**:** Nanotechnology is at the forefront in the field of biosensors fabrication. Because of their size, nanosensors, nanoprobes and other nanosystems are revolutionizing the fields of chemical and biological analysis (Viswanathan and Radecki 2008). The sensors are the devices which receive or respond to a signal or a stimulus. A biosensor is a special sensor device that integrates a biological/biochemical element with a physicochemical transducer to produce an electronic signal proportional to a single analyte which is conveyed to a detector. Thus biosensor typically consists of three basic components *viz,* bioreceptor (could be a microorganism, tissue, cell, organelle, cellular macromolecules like nucleic acid i.e. DNA or RNA, enzyme, enzyme component, receptor, antibody etc), transducer (acts as an interface, measuring the physical change that occurs with the reaction at the bioreceptor which is transformed into measurable electrical output and includes electrode, thermistor, photon counter, piezoelectric device etc) and the detector (a microprocessor that amplifies and analyzes the signals sent by transducer and transfer data to data displayer or storage unit and include piezoelectric, electrochemical, optical and calorimetric detectors). A variety of biosensors could be developed by combining different types of basic three components. However, biosensors could be classified in to five basic types on the basis of the type of detector as calorimetric, potentiometric, amperometric, optical and acoustic wave biosensors. In food analysis, biosensors in preservation and processing industries have elaborate applications as reviewed by a number of researchers (Milardovi *et al.,* 2000, Prodromidis and Karayannis 2002, Amine *et al.,* 2006, Valadez *et al.,* 2009, Viswanathan *et al.,* 2009).

Nanosensors are nothing but modified biosensors at nanoscale i.e. contain one or two components be it receptor, transducer or detector. These are novel sensing devices that are equipped with rapid, portable, highly sensitive and specific, robust, largely automated, high throughput, user-friendly, less power hungry and less labour-intensive equipments that can respond to record environmental changes (temperature, humidity, oxygen exposure) and detect microbial contamination or their toxic active products/degradation products or other contaminants (heavy metals, pesticide residues, radionucleids) in samples be it food, water or soil (Bouwmeester *et al.,* 2009, Kalia and Gosal 2011).

The general components of a typical nanosensor are- at least one nanoscale receptor or blocker which may be free in aqueous environment or may be adsorbed onto a matrix material such as fluorescent quantum dots or other metal/non-metal nanoparticles, specific doping with whole/partly/conjugate molecule which actually targets out the specific component on/secreted out by the microbe such as specific antibody(ies) to receptor molecule(s) on cell surface, an amplifier and converter that amplifies the signals (be it force measured in nano- to micro-newtons, hydrogen ion concentration, milli-range current/volts) and converts them for display in concentration (for deducing toxin or other metabolite level) or number (for microbial count). There are a variety of nanosensors but in general there exists three categories depending on the type of measurement performed by the nanosensor (Figure 3).

Another novelty of nanosensors lies in their potential to be placed *in situ* in the sample particularly the food items like fresh cut minimally processed fruits or fruit juices, sea foods, grains which helps these sensors to sense even minute amount of the spoiling gases and toxins (Falasconi *et al.,* 2005, Cusano *et al.,* 2008, Gurlo 2011) or minimum number of pathogens (Strohsahl *et al.,* 2009) well ahead of the appearance of visual early signs of spoilage due to great portability, high sensitivity and rapid analysis features. Integrating the electronic tongue nanosensors in conventional packaging materials like polyethene would be user friendly as the nanosensor would not only detect substances present in very low concentrations (may be in parts per trillion) but also would trigger color changes in food packages to alert the consumer that food has been spoiled. However, the electronic noses have largely been utilized for wine discrimination in the fermentation wine/beverage industry. An excellent review on the use of nanomaterials for generation of novel chemo- and bio-sensors by Liu (2008) refers to the application of a variety of semiconducting metal-oxide-based nanowires or nanotubes for developing gas/humidity/immunosensors with special overview on development of electrochemical sensors using titanium nanotubes.

A wide variety of microbes particularly the bacteria and viruses could be detected in aqueous solutions by using fluorescent quantum dots labeled with pathogen specific fragmented antibodies coupled with a quencher-surrogate molecule (Kumar *et al.*, 2008). The design of this system allows sensing of even very low numbers of the targeted pathogen or the active chemical produced by the pathogen and employs fluorescent resonance energy transfer (FRET) reactions between fluorescent nanoparticles and organic quencher molecules that could detect common fruit contaminating pathogens like *E coli* 0571H7. Quencher-surrogate complex inhibits the fluorescence in the absence of target bacteria in the environment, however, in presence of the target pathogen equilibrium reactions will cause the displacement of the quencher labeled target which let the quantum dots to freely fluoresce and emit energy at their characteristic wave lengths. Though the original model system has been used for the detection of biological threat contaminants or pathogens in buildings (Kumar *et al.*, 2008) but the system would also be useful for the detection of pathogen loads (both qualitatively and quantitatively) in the aqueous fruit juices and other fruit based beverages.

To increase the sensitivity and lower the range of the target pathogen detection, nanomaterials like quantum dots/nanoparticles could be conjugated with nanowires/nanorods/nanoshells to fabricate novel nanosensors. Bosoon *et al.* (2007) have reported the fabrication of a novel bio-functional nanosensor using hetero-nanorods i.e. gold sputtered silica nanorods containing special fluorescent dye conjugated with anti-*Salmonella* antibody for detection of *Salmonella*. The high aspect ratio of the Si nanorods would allow production of an enhanced fluorescence signal by large number of fluorescent dye molecules attached to the Si nanorods on specific attachment with the *Salmonella* bacteria in the sample detectable with fluorescent microscopic imaging. Similarly, this nanosensor could be used to detect other foodborne pathogenic bacteria like *E. coli*, *Listeria monocytogenes, Vibrio chloerae*, *Shigella* sp., certain viral particles like Hepatitis A and C virus, in fruits or fruit based product(s) for safety and security applications.

Another novel easy-to-use nanotool has been fabricated from Nickel nanowires for fast, reliable and decentralized sensing of carbohydrates in form of a disposable electrochemical detectors on carbon-screen printed electrodes (CSPEs) having dimensions of about 330 nm diameter and 6µm length (Garcia and Escarpa 2011). A similar report of use of electrocatalytic properties of Nickel and Nickel-Copper nanowires for highly sensitive and class selective determination of monosaccharide index in honey has been reported by Garcia and Escarpa (2011). Nanosensors could also be designed from nanoshells imprinted with dipicolinc acid (DPA) that have been used to detect *Bacillus subtilis* spores in samples particularly to identify

Bacillus contamination in preserved fruit juices or fermented fruit beverages (Gultekin *et al.*, 2010). The use of nanomaterials in biosensors allows the application of many new signal transduction technologies in their manufacture.

Apart from the use of quantum dots, nanoparticles, nanowires, nanorods or nanoshells, another innovative type of nanobiosensors include nanocantilevers i.e. nanoscale probes fabricated from monocrystalline silicon or silicon nitride or platinum/iridium and many more types of metal crystals (Illic *et al.,* 2001, Gupta *et al.,* 2004 a, b, Gfeller *et al.,* 2005). These cantilevers possess specific spring or force constants by virtue of their weight and length parameters and exhibit specific free amplitude vibrations. On adsorbption or sticking of a molecule or cell, the weight of cantilever increases and this alters its vibrational frequency. This is the basic principle behind the use of nanocantilevers for pathogen detection as the cantilever will vibrate at various frequencies depending on the biomass of the pathogenic organisms (Gfeller *et al.,* 2005). BioFinger is a nanocantilever based nanosensor that can detect pathogens in food and water by sensing the ligand–receptor interactions (Sozer and Kokini 2008). The same vibrational phenomena is the basis for obtaining information about different types of interaction forces between a variety of molecules including the cellular macromolecules *viz.* DNA, RNA, proteins, carbohydrates etc and help in detecting the biological-binding interactions through physical and/or electromechanical signaling (Hall 2002). The common types of these interactions are antigen-antibody, receptor-ligand, substrate-enzyme complexes, enzyme-cofactor, DNA-protein/enzyme, RNA-protein/enzyme etc. These ultra-sensitive devices have a great ability to unravel the interaction forces among molecules which could be implemented to detect or identify

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microbially synthesized and released toxins as well as traces of antibiotic present in processed fruit products.

An advanced generation of sensors and probes include the Microelectromechanical systems (MEMS/BioMEMS), Nanoelectromechanical systems (NEMS) and Lab-on-a-chip devices. These devices are based on microfluidics and micro/nanofabrication techniques and contain moving parts ranging from nano- to milli-meter scale. These are considered to be sensitive, more specific, low cost, energy-efficient, robust and fast not only for real-time analysis and display but also for possessing ability to monitor various factors of the storage environment for maintaining better product quality and shelf-life as well as can communicate through various frequency levels allowing for highly integrated sensor applications which are important for locating and monitoring contamination or spoilage due to altered packaging and storage conditions. Though the research and reports in non-medical applications for the rapid and accurate identification and quantification of microbial pathogens and their toxins/deterrents are now increasing however, the medical applications of all types of bio-/nano-sensors and other nanotechnological devices are enormous (Kaittanis *et al.*, 2010).

Commercialization: The present scenario: Nanotechnology is a big revolution in science and technology holding enormous societal and economic implications. Nanotechnology has permeated to every discipline of food and agriculture with the development of nanofoods and nanopackaging systems that are speculated to exhibit large impact on the health and purchase behaviors of consumers. Several individual as well as company survey reports have provided the estimates on market application of nanotechnological products (approximately 150-600 nanofoods, 400-500 nanofood packaging applications) that are presently catering the needs of the consumers (Daniells 2007, Reynolds 2007) (Table 2). An estimated cost of US$6 billion will be obtained by sales of nanofoods worldwide with soaring interest of world's largest food processing, packaging, marketing and supply companies over globe in nanotechnological advancements for foods. In terms of investments in R&D, manufacture and figures of sales and purchase of nano-based products United States of America leads followed by Japan and China and then the whole Europe (Joseph and Morrison 2006).

***Safety issues and public response/concerns*:** The markets of industrialized developed nations are on surge with arrival of nanotechnology food products including nanofoods and nanopackaged/processed foods owing to growing concerns about the benefits, risks and socio-economic costs of diet-related diseases (Hailu *et al.*, 2009). Though the health conscious consumers incline towards functional or nutritionally improved foods yet there is a bit of reluctance to accept experimental food items nanopackaged or nanoprocessed fruits in particular. Similar to the transgenic food products, the nanoproducts have the concerns for grey goo that clouds the popularization and harnessing commercial benefits of the fruits of novel technological tools and products (Lopez-Gomez *et al.,* 2009). This cannot be denied that by including nanomaterials for development of novel packaging and preservation products for food particularly whole or minimally processed fruits, a higher environmental and health risks are involved due to release of the nanoparticles having new chemical and physical properties and substantial variation from normal macro particles of the same composition and thus a risk of interaction with the living systems to result in unexpected toxicity (Das *et al.*, 2009).

There exist a number of factors that alter or affect the consumers' buying behavior and aptitude towards novel products. A study done by Fischer and Frewer (2009) suggest that the consumer perception about particular food stuff not only affects the buying behavior but also the health and other physiological benefits imparted by the food item. The consumers have an alarming concern regarding the safety issues for the novel manufactured packaging products and preservation techniques and also give enormous weightage to the verification of the health claims and health risks of exposure to new food products through governmental agencies (Hailu *et al.*, 2009). However the government or non-government agencies should devote wholly/operationally to deal with the safety issues, rules for adulteration or over health benefit claims are required to be properly developed, popularized and implemented. Novel synthesized nanomaterials or other nanoproducts must be subject to rigorous nano-specific health and environmental impact assessment and should be demonstrated safe prior to approval for commercial use in fruits, fruit-packaging or fruit contact materials by the competent agencies. There is increased public awareness and demand for complete accessibility of methodologies and relevant data related to safety assessments i.e. in the public domain. All manufactured nano ingredients must be clearly indicated on product labels to allow members of the public to make an informed choice about product use. Thus a flexible legislative framework and appropriate testing methods are required for supporting this highly innovative field (Dainelli *et al.*, 2008). Joseph and Morrison (2006) have provided information in tabular format regarding the regulations applicable to use of nanotechnology in food sector in the European Union.

Moreover, there is an ardent need for a more fundamental understanding to enable design and large scale manufacturing of the nanoproducts with desired specifications i.e. scaling-up of the research concepts to commercial applications (Janjarasskul and Krochta 2010). The results of a research report by Siegrist *et al.* (2008) suggest that the consumers assume nanotechnology food packaging to be safer over consuming nanofoods which empathies the involvement of public concern for development of a nanofood/nanofruit product. The product naturalness particularly significant for whole and MP-fruits and trust regarding the safety are significant factors that highly influence the perceived risk and the perceived benefit of nanotechnology in food processing and packaging for any consumer. Since the consumer acceptance is prerequisite for development of successful food products, the consumers' attitude towards the novel foods should be taken in account at an early stage of product development (Siegrist 2008).

Food safety is a global health goal for which a variety of analytical and maintenance tools or devices like biosensors (Amine *et al.*, 2006, Frometa 2006, Velusamy *et al.*, 2010), nanosensors (Cui *et al.*, 2001, Baeummer 2004), BioMEMS (Gfeller *et al.*, 2005), NEMS (Chaudhary and Gupta 2009) and many more lab-on-a-chip devices are now been developed which can combine a biological/biochemical/physical element with a physical signal that can be translated into an indication of the safety or quality of the fruit/fruit based products (Table 3). These novel food safety indicators like nanosensors or nanoprobes are integral components of the smart packaging systems and are liable to legal and safety challenges due to deliberate interactions with the food. The downstream users i.e. consumers have to be informed and made aware of regarding interpretation of the information provided by the smart packaging devices, information about the intentional or accidental ingestion of these products and the efficacy of the packaging material (Dainelli *et al.*, 2008).

Fruit safety and HACCP:

There are several food safety related issues and problems as microbes; be it beneficial, contaminating or spoilage; are the integral part of the elaborate fruit ecology. The fruit safety problems have became explicit owing to dramatic changes in the inclination of the end consumers for fresh or minimally processed fruits/products as well as globalization of food markets and demand for proactive measures to reduce incidences of food borne illnesses. This has invoked an alarming demand for definite standards to ascertain the microbiological quality of the product fit for consumption. The microbiological fitness of the ready to eat fresh/minimally processed fruits, processed and preserved fruit/fruit products should not be considered as an end point revealer rather a proactive approach using preventive measures for alleviation or decrease in the viable cell counts of spoilage or contaminating pathogenic microbes should be aimed to minimize the nutritive, health and financial losses. The Hazard and Critical Control Points (HACCP) is one such proactive systematic protocol involving identification of certain Critical Control Points (CCP) which can be easily modulated to reduce or eliminate the risks of physical, chemical or biological hazards followed by stringent implementation of few practices to at least minimize or fully avoid contamination of pathogenic microbes. As identification of specific hazards throughout the entire processing chain is involved, HACCP aims on preventative measures for hazard control to assure the quality and safety of the food. This includes analysis of raw material sources and usage, processing equipment, operating practices, packaging and storage, together with marketing and conditions for intended use.

Usually the general Good Agricultural and Hygienic Practices (GAP/GHP) have to be followed for production of safe foods. However, if there is contamination by food pathogenic or spoilage causing microflora then certain signature features or products have to be identified for ascertaining or overruling the presence or absence of specific etiological agent. Fruits being vulnerable to spoilage or contamination by pathogens can act as foremost vehicles for food borne illnesses particularly the gastrointestinal illnesses of bacterial etiology followed by protozoal parasite caused outbreaks unless the proactive strategies are employed stringently. Apart from HACCP certain new protocols for the identification of CCP have been formulated, applied and comparatively assessed w.r.t HACCP (Ropkins and Beck 2000).

HACCP systems are extremely important as a part of the changing quality requirements in international trade. HACCP standards have been developed in many countries which vary widely with different levels of auditing and hence the HACCP certification. Here are few HACCP systems used in different countries; Food hygiene-HACCP system IS 15000:1998 (India), Singapore Standard 444 (Singapore), SABS 0330 (South Africa), Food safety version September 2002 (Netherlands), National Standard of Ukraine 4161-2003 (Ukraine), Turkish Standard TS 13001-March 2003 (Turkey), National Standard Agency SNI:01-4852-1998 (Indonesia). The Ministry of Food Processing Industries, Goverenment of India, provides grants covering up to 50% of the cost toward the implementation of Total Quality Management (TQM) including HACCP certification (Gupta 2005).

Inspite of a plethora of variations in standards, globally four standards nmely BRC, Dutch HACCP code, SQF 2000 code and International Standard for Auditing Food Suppliers (IFS) have been benchmarked. Tapia *et al* (2009) have reviewed the implementation of HACCP strategies for production of safe fresh-cut fruits and vegetables in particular. Apart from the benchmarked standards, the variations in auditing and standards of various HACCP systems have been harmonized to develop ISO 22000. Varzakas and Arvanitoyannis (2007) have compared the HACCP protocols with the new Food safety Management System termed

ISO22000. The ISO22000 is more flexible due to introduction of less number of CCP in comparison to HACCP and thus provides rapid prediction of microbial growth behaviors.

Future perspectives:

The fruits are natural commodities that are superlative in their nutritional properties but require processing and preservation for long term availability. With the advent of novel protocols in science and technology pertaining to cellular structure and microbiology of fruits it is now possible to enhance the shelf-life of the fresh produce during storage and transportation for long term availability of fruits or their products. New trends to enhance the understanding of the basic structures and mechanisms among integrated networks at the cellular and molecular levels are being applied to descramble the information required for fabricating innovative products of multifunctional properties by reverse engineering.

With the advent of fully automatized packaging, detection and status analyzers, stringent regulations have been implemented to minimize losses by spoilage and transmission of foodborne human pathogens that further relatively minimized the pre and post-harvest losses as well as fruit safety concerns. The bioactive packaging is one among the customized packaging techniques that would help in shelf-life extension of minimally processed fruits by using either the biocontrol microbes (bacteriophages, bacteria, yeast or molds) (Garcia *et al.,* 2008, Guenther *et al.,* 2009, Heringa *et al.,* 2010, Coffey *et al.,* 2010) or antimicrobial compounds of microbial origin (bacteriocins) or any other products (gold or silver nanoparticles) to curb the growth of unwanted spoilage or opportunistic contaminating microbes. The *Listeria* bacteriophage Listex^{TM} P100 has already been commercialized to preserve fruit juices (EBI press release 2010). Food contact surfaces can also be effectively cleaned by using phages against *Listeria*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Salmonella*, *E. coli* and *Campylobacter* (Hagens and Offerhaus 2008). Moreover, these bacteriophages can also serve as rapid and sensitive tools for detection of pathogenic bacteria throughout the food chain owing to their high specificity for the host (Garcia-Aljaro *et al*., 2009).

Recent half decade has observed the fabrication and application of more sensitive, highly accurate, less power hungry, cost effective, real-time/online and portable alternatives i.e. nano-sensors or -probes for rapid detection of microbial pathogens *in vivo* (discussed in detail in nanosensor section). Large scale production, environmental and safety concerns as well as active commercialization of these sensors is still in debate and has to be followed up for development of rapid identification and safety protocols to be followed at the global scale. Similar concerns are to be abolished for both production as well as commercialization of innovative and intelligent packaging materials that are non-migratory and safe for environment and public release. Identification of the microbially synthesized or secreted volatile compounds, signature peptides, glycoproteins, glycan etc using chromatographic and mass spectrometery techniques is another burgeoning arena in detection of pathogenic or spoilage microbes. Bianchi *et al.* (2010) have reported application of gas chromatography–Mass spectrometery (GC-MS) of volatile compound profile for early detection of *Alicyclobacillus acidoterrestris* in spoiled juice. Another type of spectroscopic technique, the Fourier transform infrared spectroscopy (FT-IRS) could be used to correctly identify the pure as well as mixed cultures of several spoilage causing *Alicyclobacillus* spp. and human pathogenic *E. coli* microbes in fruit juice samples on the basis of unique spectral features of various components of the microbial cells (Al-Qadiri *et al.,* 2006).

Conclusions:

Every field in science and technology is being transformed by the percolation of novel protocols, tools and techniques of innovative fields of nanotechnology, and fruit- processing, preservation and microbiology are not exceptions. Nanotechnological innovations have equipped us with the manipulation and manufacturing prowess at nanoscales which have provided freedom for development of tailor-made designer products with potentials like quickness, high sensitivity and spectrum of functional properties that could be integrated for generation, maintenance and storage of information regarding various aspects of food. Since the postharvest losses are still enormous, probably nanotechnological advancements will help us in lowering down the loss i.e. extension of the shelf life necessary for long term storage and transportation periods. The losses may be attributed to spoilage causing microbes and other environmental factors by either manipulation of the abiotic and biotic factors or by intelligent ardent sensing prior to appearance of visible sigs of spoilage in fresh/minimally processed/processed products. Above all miniaturization, portability, accuracy and sensitivity are the prime features of materials and tools developed by nanoscience and nanotechnology which are definitely required for *in vivo* placement and integration in complex networks to obtain multiple functions and hence these areas of research have been dealt in this overview.

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Table 1. Nutritional components and dietary benefits of some fruits

Name of the fruit	**Chemical nature**	**Name of the active chemical**	**Mode of action**	**Benefits imparted**	**Reference/Source**
Acai	Flavanoids, anthocyanin, protein, omega-6 and omega-9 fatty acids, Vitamin B1, B2, B3, Vitamin E, Vitamin C, dietary fiber	Cyanadin-3-glucoside, Oleic and linolenic acids	Antioxidants	Anti-aging properties, strong heart health benefits including protective effect on the heart and cardiovascular system, role in lowering cholesterol levels in blood, may regenerate skin and stabilize muscle contraction	www.xyngularian.com/uploads/Xyngularwhitepaper.pdf http://www.natural-weight-loss-programs.com/monavie.htm Lichtenthaler *et al* (2005)
sApple	Flavonoids, polyphenols, Vitamin C,	Quercetin, catechin, phloridzin	Antioxidants	Improves heart health, provides protection against cancer and	Boyer and Liu (2004) Liu *et al* (2005)

	galacturonic acid, malic acid, tartaric acid, insoluble/soluble fiber	and chlorogenic acid, Pectin		asthma removes toxic substances from the body, helps prevent spoilage of protein matter in the intestine, help prevent liver disturbances, improve digestion, lower cholesterol, reduce skin diseases, strengthening of the blood.	
Berries	Flavanoids	Quercetin	Antioxidants	Reduction in several types of cancer	www.mass.gov/agr/massgrown/annotated_bibliography.pdf
Blue berries	Flavanoids, anthocyanin, Vitamin C	Anthocyanidins,	Antioxidants	Enhances visual acuity, protection against macular degeneration, cardioprotective,	Andres-Lacueva *et al* (2005) Gates *et al* (2007)

				promotes gastrointestinal health, protects against colon and ovarian cancer,	
Citrus fruits	Flavanoids, flavonol glycosides, kaempferol-related compounds, Vitamin C, Folate, citric acid	Tangeretin, rutin, nobiletin, Naringenin	Antioxidants Antibiotic	Protection against Rheumatoid Arthritis, Limonins support optimal health, help fight cancers of the mouth, skin, lung, breast, stomach and colon, protective against the contraction of cholera	www.mass.gov/agr/massgrown/annotated_bibliography.pdf Rodrigues *et al* (2000) Gharagozlo and Ghaderi (2001) Gao *et al* (2006) Manners (2007)
Grapes (*Vitis venifera*)	Flavanoids, Polyphenol, Saponins	Resveratrol, Pterostilbene	Anti-initiating, anti-promoting	Reduction in prostrate and colon cancer, reduced severity of inflammatory conditions, such as asthma, osteoarthritis, and rheumatoid	Eng *et al* (2003) Donnelly *et al* (2004) Turner *et al* (2006)

				arthritis	
Grape fruit	dietary carotenoids or Vitamin A, Vitamin B1, B5 and B6, Vitamin C, dietary fiber, Folate	Lycopene	Antioxidant	Lowers cholesterol, support heart health and protect from heart diseases, keep the flexibility of heart muscles, lower risk of Alzheimer's disease, drug bioavailabilty	Dahan and Altman (2004) Hassimotto *et al* (2005) Dai *et al* (2006) Gorinstein *et al* (2006)
Pears	Vitamin C and K, malic and citric acid	Dietary fiber	Antioxidants	Protection from free radicals, promotes cardiovascular and colon health, protection against macular degeneration and post menopausal breast cancer	http://www.whfoods.com

Table 2. Commercialized nanotechnology products used for preservation and packaging of food particularly fruits/fruit based products

Company/Supplier	Product name	Product description	Action/reference
Packaging			
Bayer	Durethan® KU 2-2601 plastic wrapping,	Nanoparticles of silica in a polymer-based nanocomposite	Nanoparticles of silica in the plastic prevent the penetration of oxygen and gas of the wrapping, extending the product's shelf life
SongSing Nanotechnology	Nano ZnO Plastic Wrap;	Nanoparticles of zinc oxide Antibacterial, UV-protected food wrap.	http://www.ssnano.net/ehtml/detail1.php?productid=79
Nanobiosensors Absorbers/Indicators			
Kraft	Nano-sensor based 'electronic tongue'	able to 'taste' chemicals to the level of parts per trillion and then guide chemical release	Control the release of smell, taste and nutraceuticals into food products in response to the preferences of individual consumers (de Wolfe 2009).

CSP Technologies	Multiple absorbers and indicators	Polymer capable of releasing ingredients into the food or beverage in response to external stimuli	Control over humidity, oxygen, bacteria, odour and even the flavour of the food itself (LeGood and Clarke 2006)
Life Lines Technology	FreshCheck	Polymer able to identify and monitor temperature changes w.r.t time	Time-Temperature indicator for perishables (LeGood and Clarke 2006)
Nanofruit drinks			
High Vive.com	Beverage Fortified fruit juice	300nm iron (Sun Active Fe)	http://www.highvive.com/sunactiveiron.htm
Jamba Juice Hawaii	Beverage "Daily Vitamin Boost" Fortified fruit juice	300 nm iron (Sun Active Fe) 22 essential vitamins and minerals	http://jambajuicehawaii.com/vita-boost.asp

Adapted from Miller and Senjen (2008)

Table 3: Microfluidics based analytical systems for identification of adulteration or authenticity of sample fruit/fruit product

S.No	Target/analyte in sample	Sample pretreatment required and detection technique	Reference
A.	**Capillary-electrophoresis microchip analysis**		
1	Toxic alkaloids in apple juice	Off-chip filtration and dilution followed by detection using UV-absorbance spectra	Newman et al., 2008
2	Dyes in juice	Off-chip extraction, filtration and dilution followed by electrochemical detection	Lee et al., 2008
3	Antioxidants, vitamins and flavours in apples	Off-chip extraction, pulverization, macerated, dilution and filtration using ultrasensitive carbon nanotubes	Crevillen et al., 2007
4	Natural antioxidants in apples and pear skin and pulp	Off-chip extraction, dilution and filtration followed by class-selective electrochemical index and individual antioxidant determination approach	Kovachev et al., 2010

5	Total isoflavones & antioxidant in apples and pears	Off-chip extraction and filtration using MW-CNTs for electrochemical detection by flow injection and separation electrokinetic driven systems	Crevillen et al., 2009
6	Nitrite in vegetables and fruits	Off-chip extraction, filtration and dilution followed by chemiluminiscence detection	He et al., 2007
B.	**Nucleic acid based microchip analysis (Genomic Microchips)**		
1.	Identification of fruit (apple, blueberry, elderberry, grape, pear and pomegranate) used to make fruit pulps/purees	Off-chip extraction of extract DNA from fruit juices followed by PCR-RFLP amplicon analysis	Clarke et al., 2008
2.	Arbutin and ascorbic acid in pear pulps and commercial juices	Juice filtration or solid-liquid extraction and filtration followed by off-chip electrochemical detection to separate target antioxidant couples.	Blasco et al., 2005

3.	Detection of mandarin juices in orange juice	Off-chip extraction and filtration using Polymerase Chain Reaction (PCR)	Scott and Knight, 2009
4.	*Alicyclobacillus* species *viz.*, *Alicyclobacillus acidocaldarius, Alicyclobacillus acidoterrestris,* and *Alicyclobacillus cycloheptanicus* in fruit juice	DNA microarray chip to rapidly detect by hybridization of genomic DNA with random probes	Jang et al., 2011
C.	**Antibody based microchip analysis (Microfluidic immunosensor)**		
1.	*E. coli* cells in apple juice without any pre-enrichment	Capturing by polyclonal antibodies (anti-*E. coli*) biosorbed onto nanospheres or nanorice through a protein-A layer and detection by SERS (limit 10^3 cells/mL)	Naja et al., 2010

2.	*E. coli* cells in fruit juice	Cellulosic membrane sample platform for adsorption followed by detection using integrated circuit biochip by sandwich immunoassay with Cy5-labeled antibody probes	Stokes et al., 2001
3.	Atrazine weedicide in orange juice	Immobilization of affinity proteins (protein A and G) on silicon microchip surfaces and detection by chemiluminiscence	Yakovleva et al., 2003
4.	Phenoxyl-type *N*-methylcarbamate pesticides (carbaryl, carbofuran and propoxur) in fruit juices	Pre-column hydrolysis of pesticides and derivatization of their hydrolytic metabolites with dansyl chloride followed by detection using HPLC with peroxyoxalate-chemiluminescence	**Orejuela and Silva, 2003**
5.	*Botrytis cinerea* in Apple tissues (@ 0.02 µg ml^{-1})	Screen-printed microfluidic modified with carbon nanotube	Baldo et al., 2009
6.	Ochratoxin A (OTA) in *Aspergillus ochraceus* contaminated	competitive indirect immunoassay method based on use of anti-OTA monoclonal antibodies immobilized	Baldo et al., 2011

	apples	on 3-aminopropyl-modified magnetic nanoparticles as platform	
D.	**Enzyme based microchip analysis (Proteomic microchips)**		
1.	Develop insight on relation between enzymatic pectin conversions and firmness and viscosity in whole kiwi fruit/ fruit juice	Plant methylesterase and PME inhibitor in kiwi fruit SPR based chip	Jolie et al., 2010
2.	Atrazine weedicide in orange juice	On-chip microdialysis followed by detection of atrazine immobilized on silicon by horseradish peroxidase (HRP), catalyzing the chemiluminescent oxidation of luminol/p-iodophenol	**Yakovleva et al., 2002**

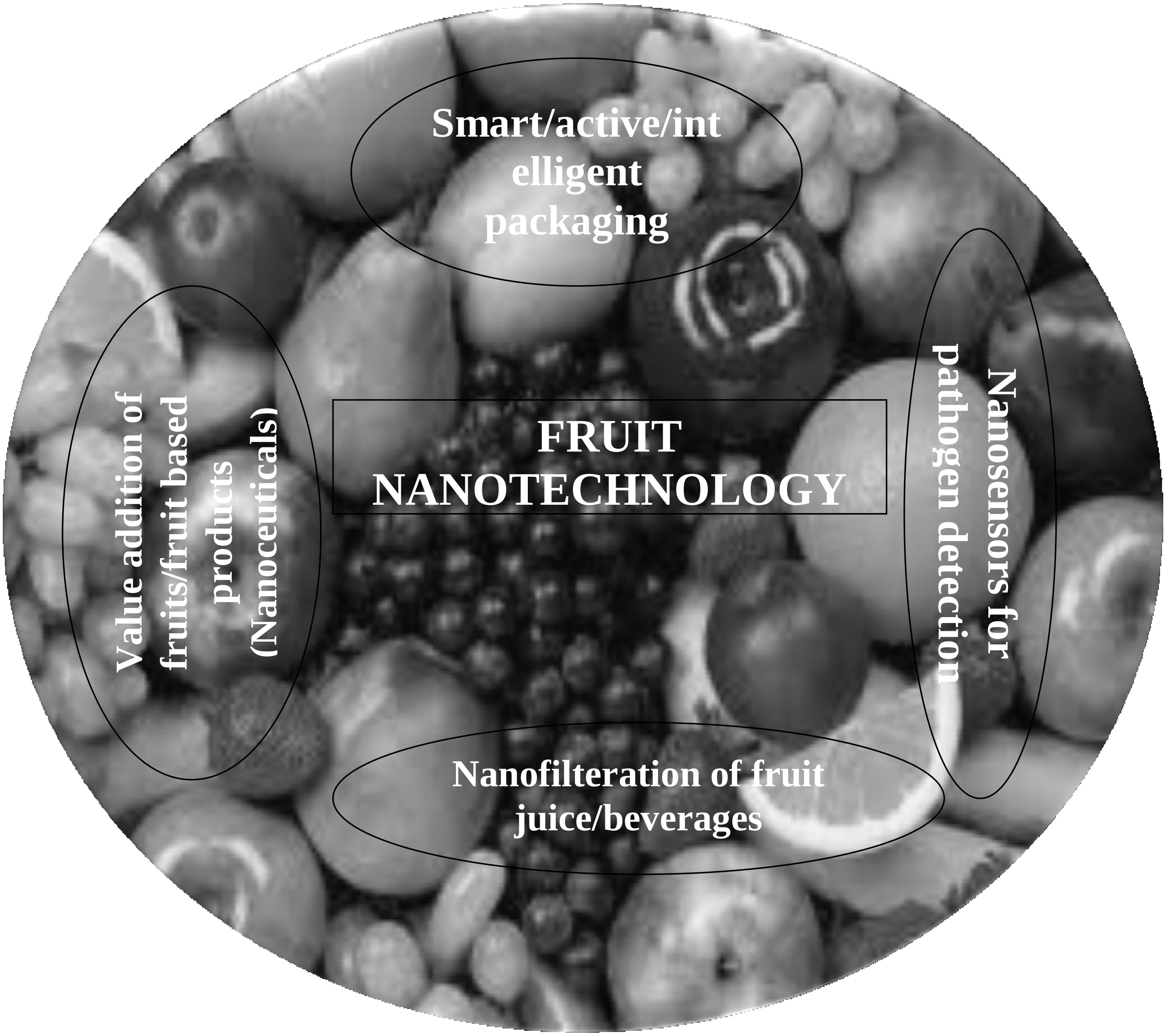

Figure 1. Overview of nanotechnology applications in fruit processing, packaging and pathogen detection

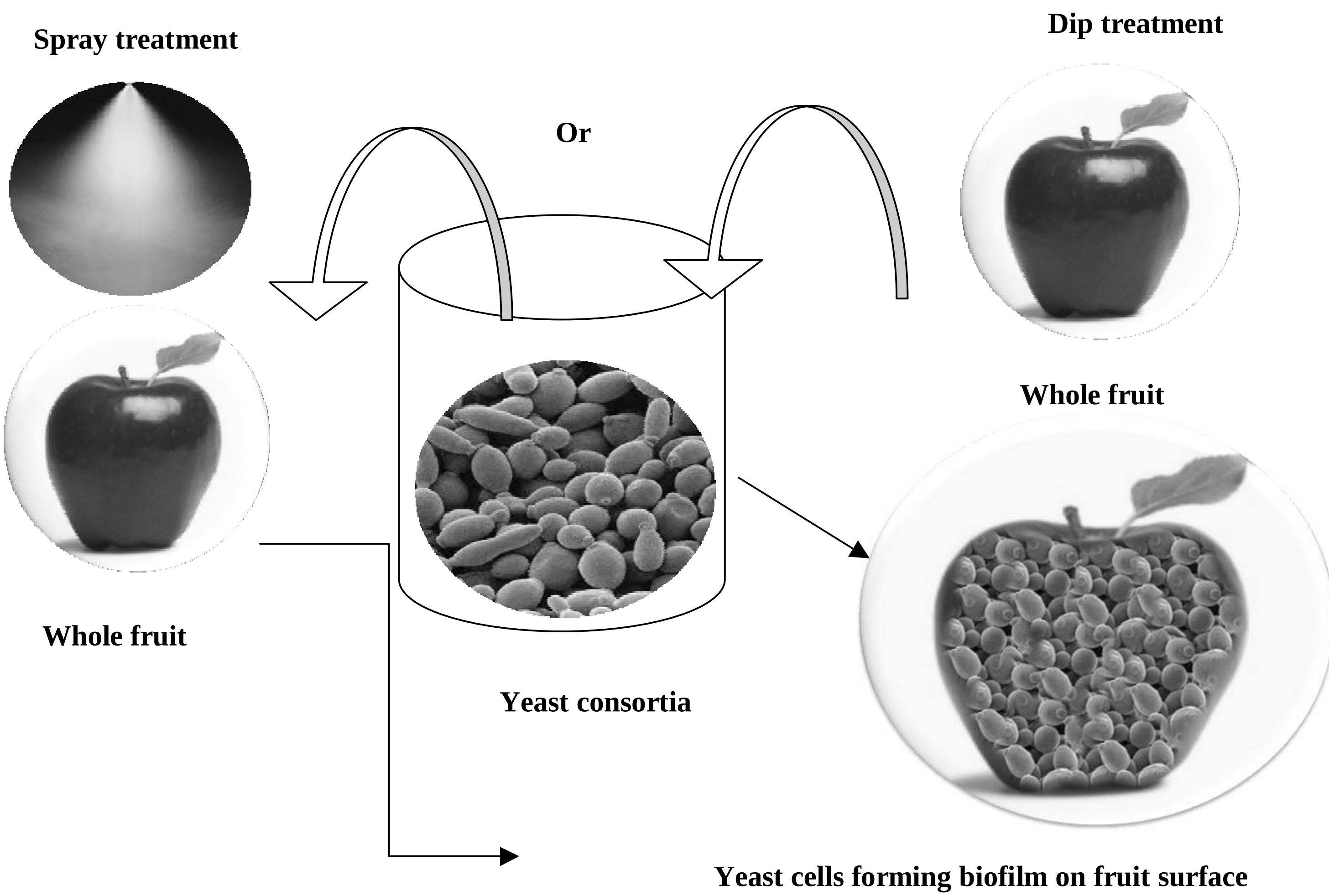

Figure 2. Schematic diagram of the yeast consortia treatment for postharvest decay control of fruits (Adapted from Sharma *et al* 2009)

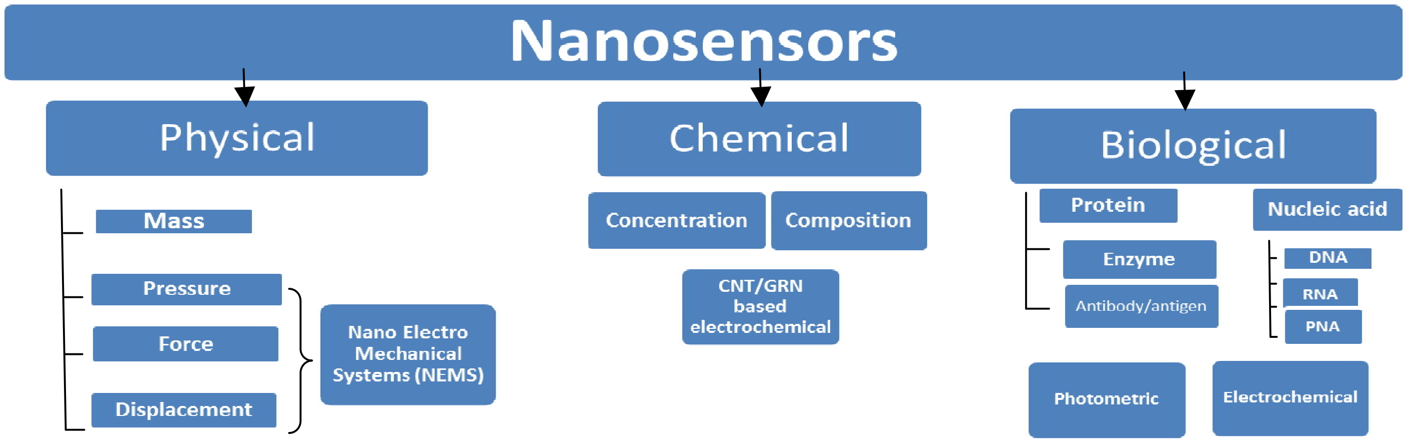

Figure 3. Types of nanosensors being used/ evaluated for assessing presence of different molecules or cells in fruit samples (Adapted from Akyildiz and Jornet 2010)