Review Article

Bio-preservation of foods: a review

Abstract:

Biopreservatives are commonly used in food products to satisfy the increasing demand of consumers with increasing advancement in food and technology. The foods with chemical preservatives are now a days being neglected by the people and they prefer products which are Generally regarded as safe (GRAS). Thus, as a result food industry is using naturally produced preservatives to increase the shelf life of product without any new technology. The most commonly used bio-preservatives are bacteriocins, essential oils, herbs and spices, vinegar, fermentation and sugar and salt. They exhibit growth inhibition of various micro-organisms when added at different concentrations so as to preserve food products. These preservatives have been tested under laboratory conditions to know their apt use. This review provides an overview of the importance of bio-preservatives as per the increasing demand of consumers.

Key words: Bio-preservation, Bacteriocins, Essential oils, Herbs and spices, Vinegar, fermentation, salt and sugar.

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Introduction:

The demand for production of food on a large scale is increasing tremendously due to increase in population and change in their food habits. Food products like meat and milk products are of perishable nature. Their shelf life can be increased by adding food preservatives which restrict the process of spoilage to some extent. The food industry is investigating more methods to preserve food products beyond the traditional methods which have been commonly used for thousands of years including curing, cooling, freezing, boiling, heating, sugaring, canning, pickling, fermentation and others. The new less invasive techniques are being developed such as use of high-pressure, pulse electric field, ultrasound, oscillating magnetic field, hurdle technology, hyperbaric pressure and UV treatment (Kim *et al*., 1995). Biopreservatives are the new alternatives derived from natural sources to preserve and enhance the keeping quality of food and well suited for food application with increasing demand of consumers for chemical free foods.

To extend the shelf life of products by use of natural preservatives or controlled micro biota and/or antimicrobial compounds obtained from microbes is referred to as bio-preservation. The natural food preservatives preserve the food by adjusting temperature, lowering the pH value, altering water activity (a_w) and settling the redox potential of the product (Sharif *et al*., 2017). Most commonly selected organism for bio-preservation are lactic acid bacteria (LAB) and their metabolites. The functional foods with natural ingredients promoting health instead of synthetic additives have been intensively commercialized by the food industry (Caleja *et al*., 2015; Carocho *et al*., 2014). During last

Comment [u2]: Check the format of gthe Journal on how to site intext References decade, the food industries are looking for new natural bio-preservatives which can promote human health besides acting as food preservative (Burt, 2004). The present communication provides an overview of the current scenario on bio-preservative compounds that are either actually being used industrially or under research and development stage.

Bacteriocins

Bacteriocins are secondary antimicrobial peptides that are ribosomally synthesized from the bacteria that exhibit antagonistic activity to some group of bacteria which can either be closely related or unrelated (Johnson *et al*., 2017). Colicin was the first discovered bacteriocin in 1952 from *Escherichia coli* and showed bactericidal effects by permeabilization of cell membrane, inhibiting cell wall synthesis and inhibiting DNase or RNase activity on bacteria that are closely related. The application of bacteriocins in various food products have been widely evaluated under different laboratory conditions. Majority of the bacteriocins used commercially are produced by metabolic activity of Lactic Acid Bacteria like *Lactobacillus acidophilus* which have status of "Generally regarded as safe (GRAS)" because protease can degrade them (Sharif 2017). Nisin and pediocin are the only commonly available bacteriocin in the food industry, produced by *L. lactis* and *Pediococcus acidilactici*, respectively. Bacteriocins produced from the bacterial strains exhibit the defensive advantage by competitive inhibition of the bacteria from other group for nutrition. In case of non-fermented food, bacteriocin producing bacterial strain used for their bio-preservation only when they do not have any undesirable effects on organoleptic properties of food. *L. brevis* is the most common spoilage organism in beer and is responsible for up to 52.5% contamination of beer. Nisin is a commercially used bacteriocin and is highly stable in the acidic solutions. It is incorporated in beer and is capable of killing 90% of the Gram positive bacteria without affecting the fermentation activity of Saccharomyces yeast (Müller-Auffermann *et al*., 2015). Lacticin 3147 is produced from the lactococcal strains, present in the Irish Kefir grain and is used in cheddar cheese manufacturing (Ryan *et al.*, 1996). Nisin is being widely used in research as a inhibitor of heat shocked spore of *Clostridium* and *Bacillus* strains in canned foods. Minimum inhibitory concentration to nisin to prevent the outgrowth of spores ranges from 3 to >5,000 IU/ml (Hurst, 1981). They inhibit various food borne pathogens. Properties of such bacteriocins and their usefulness against pathogenic organisms are detailed in Table 1.

Table 1. Classification and properties of bacteriocins

Class	**Bacteriocins**	**Source**	**Properties**	**Target food contaminants**	**Reference**
Class I (Lantibiotics)	Nisin	*Lactobacillus lactis subsp. Lactis*	Heat stable at low pH (2), resistant to trypsin, elastase, pepsin, carboxypeptidase A and	*Streptococcus thermophillus, Staphylococcus aureus, Bacillus cereus, Clostridium botulinum.*	Meghrous *et al.* (1999)

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			sensitive to α-chymotrypsin.		
	Lacticin	*L. lactis*	Stable at neutral and acidic pH	*L.monocytogenes*, *S.aureus* and *B.subtilis*	Ryan *et al*. (1999)
Class II	Pediocin PA-1	*Pediococcus acidilactici*	Stable at pH 4-6, resistant to DNAses, RNAses, lipase, catalase, lysozyme and phospholipase C.	*L.monocytogenes*, *Pediococcus pentosaceus*, *Lactobacillus helveticus*	Rodriguez *et al.* (2002)
	Enterocin 1071	*Enterococcus faecalis BFE 1071*	Sensitive to treatment with proteolytic enzymes	*Enterococcus feacalis*, *L.monocytogenes*, *L.innocua*	Balla *et al*. (2000)
	Enterocin EJ97	*E.faecalis* EJ97	Sensitive to proteolytic enzymes	*E. faecalis*, *Bacillus spp.*, *L. monocytogenes*, *Geobacillus stearothermophilus*, *S. aureus*	Galvez *et al*.(1998)
Class III (Bacteriolysin)	Lytic Bacteriocin (Enterolysin A)	*E. faecalis*	Causes cell damage by attacking peptidogylcan layer of cell wall in susceptible Gram-positive bacteria	*Enterococcus spp.*	Nilsen *et al.* (2003)
	Non lytic heat labile Bacteriocin (Helveticin J, Tisdysgalacticin)		Hamper glucose uptake and strave the bacteria, hence depleting energy reserve causing cell death	*Streptococcus pyogenes*	Heng *et al*.(2006)

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Class IV	Enterocin AS-48	*E. faecalis subsp. liquefaciens S-48*	Compatible with several chemical compounds like EDTA, lactic acid, per acetic acid, phosphoric acid, sodium hypochlorite, hyrdrocinnamic acid	*Bacillus spp., Geobacillus stearothermophilus,S.aureus, L. monocytogenes, Brocothrix thermosphacta*	Cobo Molinos *et al.* (2008)

Essential oils:

Also commonly known as volatile odoriferous oils, are extracted from different plant materials like roots, barks, flowers, fruits etc and are aromatic liquids (Tongnuanchan and Benjakul, 2014). The approach of using essential oils as natural bio preservatives was intended for the purpose for preventing rancidity of fats and possible prevention of chronic degenerative disease. The increasing demand of natural food additives as an alternate to the chemical preservatives, essential oils (Eos) are more used under the concept of hurdle technology with other food preservatives. Thus it aid as "green technology" in food market. Earlier, these oils were primarily used as medicines and now a day the food industry has adopted them as flavoring and coloring agents (Hyldgaard *et al.*, 2012). Colors of essential oils are due to the presence of indigenous pigments in plant material from where they are extracted. Because of their antimicrobial and antioxidant activities, they are widely and efficiently used in food products.

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Steam distillation is commonly used process for the production of essential oils at commercial scale. They constitute low molecular weight organic compounds with vast anti-microbial activity. They have been broadly classified as Terpene Hydrocarbon and oxygenated compounds with main active compounds like terpenes, terpenoids, phenylpropenes etc. Essential oils are more prone to enzymatic and chemical reactions like oxidation, cyclization, isomerization or dehydrogenation when not provided with protective compartmentation, which further leads to quality loss. Oxidized terpenoids as well as some aged essential oils known for organoleptic alterations, viscosity changes and even some skin sensitizing capacities (Woeber and Krombach 1969; Hagvall and others 2008; Skold and others 2008; Brared-Christensson and others 2010). Use of some of the essential oils as preservatives has been demonstrated by some of the researchers. The essential oils obtained from thyme and rosemary used in the preparation of Nile tilapia fillets showed preservation effect by reducing oxidative processes up to 96.5% from the 9th day of storage (Albarracín *et al.*, 2012). Two herbal essential oils i.e., curry leaves and cloves were added at the rate of 0.10 ppm and 0.20 ppm respectively to enhance the storage stability of burfi without interfering with the sensory acceptability of the product (Badola *et al.*, 2018).

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The classification, mechanism of action and other applications of essential oils are summarized in Table 2.

Table 2. Classification and properties of essential oils

Chemical Classification	**Example (Plant Origin)**	**Model Organism and MIC**	**Mechanism of Action**	**Reference**
Terpenes	p-Cymene (Oregano and Thyme)	*Staphylococcus aureus (1250* µg/ml), *Escherichia coli (2500* µg/ml)	Reduce cell mortality Decrease membrane potential Decrease membrane melting temperature	Cristani *et al.* (2007), Burt *et al.* (2007), Ultee *et al.* (2002).
	α-Terpinene (Oregano and Thyme)	*Staphylococcus aureus (2500-34000* µg/ml), *Escherichia coli (5000* µg/ml)	Decrease membrane melting temperature Decrease transition enthalpy Might disrupt the membrane of micro organism	Cristani *et al.* (2007), Carson and Riley (1995)
	Limonene (Lyme)	*Aspergillus flavus* (560 µg/ml), *A. parasiticus* (1130 µg/ml), *E. coli, S. aureus*	Reduce aflatoxin production Extra and intracellular damage to cells	Rammanee and Hong-pattrakere (2011)
Terpenoid	Carvacrol (Oregano and	*Candida* strains (75-100 µg/ml),	Transient Calcium ion	Kim *et al.* (1995), Helander *et al.*

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	Thyme)	*Bacillus cereus* (900 µg/ml), *Salmonella typhimurium* (150-250 µg/ml), *Listeria monocytogenes* (450-1500 µg/ml)	surge Act on specific signaling pathway Dissipated pH gradient & membrane potential Permeabilize cell membrane and vesicles	(1998), Cristani *et al* (2007), Gill and Holley (2006 a,b).
	Menthol (peppermint)	*C. albicans* (2500 µg/ml), *B. cereus* (1250 µg/ml), *Klebsiella pnuemoniae* (2500 µg/ml), *Pseudomonas aeruginosa* (2500 µg/ml), *Proteus vulgaris* (1250 µg/ml)	Disrupt membrane permeability, Alter intracellular components, Release cellular content	Iscan *et al.* (2002), Bassole *et al* (2010), Trom-Betta *et al.* (2005).
	Linalool (Basil and Citrus oil)	*C. albicans* (2145 µg/ml), *B. cereus* (1073 µg/ml), *C. jejuni* (515 µg/ml), *L. monocytogenes* (1000-2145 µg/ml), *S.* Typhimurium (1000 µg/ml)	Permeabilize cell membrane	Kim *et al* (1995), Carson and Riley (1995), Fisher and Philips (2006, 2008), Bagamboula *et al.* (2004).

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Phenylpropene Phenolic Aldehyde	Vanillin (Vanilla)	*L. innocua* (5325 µg/ml), Yeasts and molds (456-1460 µg/ml), *Lactobacillus plantarum* (11411 µg/ml), *E. coli* (2282 µg/ml)	Dissipates potassium and pH gradients, Activates ATP production in some cells	Fitzgerald *et al.* (2003, 2005)
	Cinnamaldehyde (cinnamon)	*B. cereus* (0.3 µg/ml) *B. thermosphacta* (0.84 µg/ml) *E. coli* (397-1322 µg/ml) *E. faecalis* (250 µg/ml)	Concentration dependent ATPase inhibition Inhibited histidine decarboxylase and cytokinesis Inhibiting cell wall synthesizing enzymes	Zemek *et al.* (1987), Helander *et al.* (1998), Kwon *et al.* (2003), Wendakoon and Morihiko (1995), Bang *et al.* (2000), Yamazaki *et al.* (2004)

Herbs and spices:

Plants have ability to synthesize various chemical compounds and these antimicrobial components enhance the defense mechanism of plant against natural infections. Many herbs and spices such as cloves (*Carophyllus aromaticus*), cinnamon, guar gum, mustard seed and garlic known to have antimicrobial properties. When added in food products, the antimicrobial properties associated with such herbs and spices offer them competency to prevent food from spoilage and exercise food safety by controlling growth of spoilage and pathogenic micro-organisms (Jack *et al.*, 1995). There are number of plants which are recognized as herbs or spices and are part of culinary practices followed in different countries to improve the sensory quality and shelf life of food products as well as owing to their nutritional, antimicrobial and health promoting properties . These properties are more intense in the extracts of spices rather than spices as such, due to the reason that spices release volatiles at a slower rate (Shelef *et al.*, 1980).

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The main advantage of using herbs and spices in food as preservatives is due to their 'generally recognized as safe' (GRAS) status because their use in human diet is time tested. Further they are usually free from chemical residues and therefore are potential alternatives to chemical additives (Reunanen and Saris 2003). Herbs and spices contribute to the total dietary phenolic intake as they are the sources of polyphenols. The herbs and spices have great antioxidant property and consumption of herbs like cloves and garlic has positive effect on human health and they also have anti carcinogenic effect. According to National Medical Research Council (NMRC), consumption of half or one clove of garlic decrease the cholesterol level up to 9% and consumption of 7.2 gm of aged garlic extract has been related with anticlotting and decrease in systolic blood pressure (Tapseel *et al.*, 2006). The mechanism of action for herbs and spices has not been completely understood irrespective of the clear expression of antimicrobial activity. Butylated hydroxyanisole (BHA) prevents the auto oxidation of lipids and is seen to inhibit growth of various Gram negative as well as Gram positive bacteria when added in different food products. In a study conducted by Shelef and Liang (1982), BHA added at a concentration of 5000 ppm in strained chicken and 1000 ppm in cooked rice exhibit growth inhibition of vegetative cells of *Bacillus* spp.

Vinegar

Vinegar is a liquid solution and one of the most typical pickling agents with 5%-10% acetic acid and it preserves food by anaerobic fermentation. It provides flavor to the product and also vitamins. The pH 4.6 is a distinguishing and characteristic feature of the process which doesn't allow the bacteria to grow and also kills them. Vinegar can be synthesized by alcoholic fermentation or the acetic acid fermentation. Vinegar provides an acidic medium to the food for preservation and improves the shelf life of the food product. Park *et al*. (2014) stored the blanched tea leaves for 4 days at 30°C in pickling solutions as mixture of soy sauce, water and vinegar in different concentrations. The different parameters like color, pH, hardness, antioxidant compound content, sensory evaluation, acidity, ABTS radical scavenging were determined during storage. Authors observed that the total acidity and pH increased consistently and thereby imparted preservative effect. Jang and coworkers (2006) conducted a study on Korean seasoned beef to examine vinegar and sake as preservation hurdles and detect their effect of sensory quality and microbial stability. They found that the combination of vinegar and sake did not improve the sensory quality however; microbial stability was improved both at 8°C and 20°C. Acetic acid has been used for decontamination of carcass to increase shelf life. Laboratory studies showed significant reduction of *E. coli* on rib-eye steaks treated with an acetic acid dip (Kotula and Thelappurate, 1994). Tzortzakis (2010) studied prevention of Anthracnose rot (*Colletotrichum coccodes*) in Tomato (*Lycopersicon esculentum*) by treatment with Vinegar (VIN), Chlorine (CHL), absolute ethyl alcohol (AEA) and origanum oil (ORI) when stored at 12°C with 95% relative humidity. After treatment with AEA and VIN, the growth of mycelium was accelerated and exposure to pure VIN-, ORI vapors and CHL- decreased germination of spore's in vitro up to 92%.

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Fermentation

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Fermentation process produces numbers of beneficial products with the bacteria which helps in reduction of food spoilage and renders the food free from pathogenic microorganisms and metabolites (Ganguly, 2013). The organisms used to serve this purpose mostly belong to the group of lactic acid bacteria (LAB). The metabolic compounds of these bacteria, such as organic acids are capable to exert antimicrobial properties as well as imparts unique flavor and texture to the food products (Lucera *et al.*, 2012). There are certain advantages of fermentation carried out by LAB over other methods of food preservation. These include comparatively more availability of nutrients and ease in carrying out the process which require almost nil to little energy. Tarhana, a Turkish fermented cereal food using baker's yeast and yoghurt bacteria as culture was studied to detect compositional changes in the organic acids developed during the fermentation phase of 3 days and subsequently stored for a period of 6 months. In a fermentation period of 3 days, lactic acid, titrable acidity, priopionic acid and pyruvic acid increased from 13.58 to 20.26 g/kg, 26.50 to 41.4 g/kg, 2.44 to 7.58 g/kg and 0.16 to 0.58 g/kg respectively and citric acid content reduced from 6.39 to 3.58 g/kg (Erbas *et al*., 2006). Gotcheva *et al*., (2006) monitored the fermentation of traditional Bulgarian beverage boza, a cereal based fermentation product prepared from both flour and whole wheat grains and the impact on product quality by raw material used for preparation. Yeast and Lactobacilli were the major organisms responsible for fermentation of boza and biochemical and physical changes were observed during initial 48 hour of fermentation. Glucose content was increased while viscosity, dry matter, pH and free amino nitrogen content were decreased. Two Nigerian fermented food products *Fufu* and *Ogi* from cassava and maize respectively were investigated for changes in pH, titrable acidity and temperature during fermentation by hetero fermentative *Lactobacillus* and *Leuconostoc*. pH decreased rapidly from 5.6 to 3.7 and 5.9 to 3.8 in *fufu* and *ogi* respectively and increase in temperature from 25 °C to 31 °C and 26 °C to 30 °C in *ogi* and *fufu* respectively (Oyedeji *et al*., 2013). A study conducted to stabilize meat cubes and minced meat with salt and glucose by process of fermentation by using culture of *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactococci lactis*. In an incubation of 24-36 hour at 37 °C and 30-42 hour at 30 °C, a significant reduction in *Escherichia coli*, *Salmonella* spp, coliforms and *Staphylococcus aureus* with a pH value of 4-4.2 was achieved (Sakhare and Narasimha Rao 2003). *Rhizopus oligosporus* was used to ferment oat tempe and whole grain barley to investigate the effects of different pretreatment like rolling, moistening, pearling, autoclaving and soaking on mineral and phytate content. Phytate content in both oat and barley was decreased by 74% and 89% respectively by the most effective pearling process (Eklund-Jonsson *et al*., 2006). Fermented fish paste was prepared by fermenting at a room temperature for 8 and 32 days with addition of 2% NaCl from dried anchovy fish. Water content and pH values were increased during fermentation. Fatty acids except DHA, EPA and stearic acid decreased (Anggo *et al*., 2015).

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Sugar and salt

Both salt and sugar exert food preservation effect by same mode. When added to the food product, it absorbs water and thereby inhibits the growth of micro organisms by restricting the water availability (Dwivedi *et al*., 2017). Sugar is added with salt to preserve fish and meat. It can be mixed with salt to form a dry mixture covering food or can be dissolved in liquid to make a brine to surround the food. Salt in water produces an isotonic condition for non marine organism at concentrations of 0.85-0.90%. When salt is used in high concentrations to fresh meat, it undergoes plasmolysis which results in drying of meat and leads to death of microbial cells. The non marine organism can be easily inhibited by <20% NaCl. Salt and sugar inhibit organisms at different concentrations. Sucrose needs about six times more concentration than salt to exhibit same degree of inhibition (Jay, 2012).

The research work conducted to study impact of sucrose solution on strawberry for three months at 15 days interval each. Data showed the physiochemical and organoleptic changes in various treatment groups (Khan *et al.*, 2015). A study was conducted to find out the survival of indicator and pathogenic microorganism in seven liquid sweeteners. The sweeteners were inoculated with various species of bacteria at the rate of 10^5 cells per gram. The micro organism showed a significant reduction in less than 3 days when the inoculated products with sweeteners held at normal holding temperature. The slowest rate of reduction was observed for *S. aureus* (Niroomand *et al.*, 1998). Different hypertonic sucrose solutions with or without NaCl were used to treat apple samples osmotically. The acceptability of products was reduced when 0.5% NaCl was added than that by addition of 0.5% sucrose (Sacchetti *et al*., 2001). Effects of addition of salt and sugar on the enzymatic activity and quality of grass carp fish (*Ctenopharyngodon idellus*) fillets were evaluated. The samples were grouped as uncured (CK), dry cured with 1.0% salt (T1) and dry cured with 1.0% salt and 1.0% sugar (T2). Authors observed that all curing treatments decreased TVB-N accumulation and improved the level of IMP in grass carp as compared to uncured samples. (Quin *et al.*, 2016). Salt is used in cheddar cheese to control the development of bitter flavor by inhibiting the proteolysis of β-casein in presence of 10 % NaCl and was significantly reduced by use of 5% NaCl. In presence of 5-10% NaCl, rate of proteolysis was maximum of αs-casein (Fox and Walley 1971). The survival of *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli O157:H7* and *Clostridium perfringens* in natural casings at different water activity (aw) levels was studied by preservation with NaCl to determine antimicrobial properties. The casings were stored at different temperatures in different brines with dry salt. The effect of salt was well sufficient to minimize the contamination of bacteria except the spores of *Clostridium* below acceptable level at aw 0.85 or decreased after a storage period of 30 days (Wijnker *et al*., 2006).

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