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# Bio-Preservation and the Food Industry: An Overview

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**A B S T R A C T**

The unending needs and demands for chemical free preservatives in food industry are on the increase due to the facts that diseases like cancer and complications from oxidative stress have been linked to the xenobiotics we eat in foods as preservatives. There is need for safe means of preserving our foods without side effects and that is why the world needs bio-preservative in all forms to augment both the nutritional properties and shelf life of food products. Bio-preservatives like bacteriocins produced from organisms with GRAS status, essential oils, vinegar, herbs/spices and sugar/salt reviewed in this work have shown credible antimicrobial properties against spoilage/food borne pathogenic and toxigenic microorganisms thus served as good bio-preservative agents suitable for a dispensation yearning for green solution areas of food improvement and preservation. This review provides an overview of the importance of bio-preservatives in food safety and nutritional augmentation needed in food industry as a whole.

## Introduction

The heterotrophic nature of man has made food indispensable for his survival, as human population grow, so is the demand for food production. Humans and animals are not capable of producing macromolecules needed for their fortification and growth so they rely on autotrophs to get nourishment and energy (Vaishali *et al.*, 2019). Food might be synthetic or natural product consisting all necessary or moderate source of nutrition but unfortunately, food are also perishable products that requires protection from spoilage at every stage required until the finished stage in order to give or improve their shelf-life (Rasooli, 2007).

Food products like milk/milk products or generally food product containing animal proteins have very low shelf life which can be increased by adding preservatives which check deterioration and spoilage to some extent (Vaishali *et al.*, 2019). Some synthetic preservatives could have a long term danger towards man, and the need for more green solution within the confines of food bio-preservation is inevitable. Although over the years, food biotechnology has been exploiting methods beyond traditional routines of preservation which have their limitations. Even the modern methods which include curling, sugaring, canning, pickling, fermentation to mention a few are still not enough to proffer pivotal solution in areas where nutrition have to be augmented along with the shelf life (Sharif *et al.*, 2017; Vaishali *et al.*, 2019).

Furthermore, techniques like high-pressure, hurdle technology, pulse electric field, ultrasound, oscillating magnetic field, hyperbaric pressure and UV treatment (Kim *et al.*, 1995; Rasooli, 2007) still have their limiting effect on the probiotics and prebiotics present in the food product. So on the evidence of these intricacy incurred by modern preservative techniques, there is need for a probiotic-sustaining approach that will improve the shelf life and also augment the nutritional properties of the food product, and that is why the world need bio-preservative, a green solution to food products with low shelf life (Sharif *et al.*, 2017; Vaishali *et al.*, 2019).

Sweet and benign poisonous chemicals used in food in the name of preservation have led to lots of long term effects leading to cancer and oxidative stress, these facts have led to ever increasing demands for food product that are chemical free. Bio-preservative application is suited for food enrichment and production.

## Bio-preservatives

Bio-preservatives are organic-derived antimicrobial agents aimed at enhancing the nutritional properties, market value and shelf life of food products. The use of controlled microbiota or antimicrobial organic compounds like bacteriocins (secondary metabolites from bacteria especially lactic acid bacteria) is referred to as bio-preservation. Bio-preservative is the emerging alternatives gotten from natural sources to preserve and augment food products to ensure good quality and acceptance in biotechnology. The controlled microbiota or antimicrobial organic compounds prolong the shelf life of the food through the lowering of pH value, altering water activity (aw) and settling the redox potential of the product (Sharif *et al.*, 2017).

The advancements in food biotechnology have ignited curiosity in looking for novel bio-preservatives which can promote human health and market value asides acting as bio-preservative (Burt, 2004). The nutritional and functional food products with organic additives and bio-preservatives have been highly commercialized by food industry in this dispensation because of their health promoting properties (Carocho *et al.*, 2014). Lactic acid bacteria (LAB) and their metabolites are the most used organism for bio-preservation. This review is aimed at illuminating on bio-preservative compounds that are being used industrially, the ones that are under reported/utilized and the ones in developmental stage in biotechnology.

## Forms of Bio-Preservatives

### Secondary Metabolites

#### Bacteriocins

Bacteriocins are secondary metabolites known to be antimicrobial peptides ribosomally secreted by some bacteria. Bacteria that produce bacteriocin as their secondary metabolites are known to be antagonistic against some other bacteria that are either closely related or unrelated (Johnson *et al.*, 2017). The reactive oxygen species i.e hydroxyl (−OH) groups present in the organic compounds in bacteriocins react with the bacterial cell membrane, causing disruption its organic structures and eventually leading to leakage of its components, which eventually leads to their bacteriocidal or bacteriostatic action (Quinto *et al*., 2019).

The application of bacteriocins as biopreservative in some food products have been tremendously evaluated under various innovative laboratory conditions. Bacteriocinogenic protective cultures have be employed in prolonging shelf life of fermented and non-fermented food products through the inhibition of pathogenic bacteria that cause spoilage (Galvez *et al.,* 2007). In case of non-fermented food, bacteriocin producing bacterial strain selected for their bio-preservative potential only if they do not have any undesirable effects on the market value and organoleptic properties of food. Furthermore, the bacteriocins commercially used in food production must be produced by organisms with GRAS status (Generally regarded as safe) e.g Lactic Acid Bacteria like *Lactobacillus acidophilus* have GRAS status (Sharif *et al.*, 2017).

## Classification of Bacteriocins

### Bacteriocin from Gram-Positive Bacteria

### Class I

These are post-translationally modified bacteriocin, they are linear/globular peptides containing lanthionine, β-methyl lanthionine and dehydrated amino acids.

### Types of Class I bacteriocins

Nisin A: is a bacteriocin produced by *Lactobacillus lactis* (Field *et al*., 2012) and it has molecular weight of 3352.

Nisin U: is a bacteriocin produced by *Streptococcus uberis* (Wirawan *et al*., 2006) with molecular weight of 3029

Nisin Z: is a bacteriocin produced by *Lactococcus lactis* (Mulders *et al*., 1991) with molecular weight of 3493.

In this dispensation, Nisin and pediacin are some of the commonly available bacteriocin used in food technology, they are produced by *Lactobacillus lactis* and *Pediococcus acidilactici*, respectively (Vaishali *et al.,* 2019). These Bacteriocins possess defensive advantage through competitive inhibition of bacteria from other group for nutrition. It has been reported that Nisin is highly stable in acidic solutions and it is capable of killing 90% of the Gram positive bacteria without affecting the fermentative activity of the fermenting yeast (*Saccharomyces cerevisea*). Nisin have bactericidal potential against various food borne pathogens and are also used as inhibitor of heat shocked spore of *Clostridium* and *Bacillus strains* in canned foods (Muller-Auffermann *et al.,* 2015; Vaishali *et al.,* 2019). The reported Minimum inhibitory concentration to nisin as an antimicrobial agent used in preventing spores outgrowth ranges from 3 to >5,000 IU/ml (Vaishali *et al.*, 2019).

### Mersacidin

Mersacidin is a bacteriocin produced by *Bacillus sp.* Y85, 54728 with molecular weight 1824.

### Labyrinthopeptin

Labyrinthopeptin is a bacteriocin produced by *Actinomadura sp.* (Meindl *et al*., 2010) with molecular weight 1922.

### Subtilosin A

Subtilosin A is a bacteriocin produced by *Bacillus subtillis* 168, with molecular weight 3399.

### Class II

Class II bacteriocins are heat stable, heterogenous, non-lanthionine-containing, unmodified class of small peptides.

### Class IIa: (pediocin PA-1like bacteriocins)

### Pediacin PA-1

Pediacin PA-1 is a bacteriocin produced by *Pediococcus acidilactici* (Henderson *et al*., 1992) with molecular weight 4629

### Carnobacteriocin x

Carnobacteriocin x is a bacteriocin produced by *Carnobacterium maltaromaticum* C2 (Tulini *et al*., 2014) with molecular weight 3602.

### Class IIb

Class IIb are composed of two peptides.

### Lactacin F

Lactacin F is a bacteriocin produced by *Lactobacillus spp.*, with molecular weight 4755

### ABP-118

ABP-118 is a bacteriocin produced by *Lactobacillus salivarius subsp. Salivarius* U118with molecular weight 4096.

### Class IIc (circular peptides)

### Carnacyclin A

Carnacyclin A is a bacteriocin produced by *Carnobacterium maltaromaticum* UAL307 (Martin-visscher *et al*., 2008) with molecular weight 5862.

### Enterocin AS-48

Enterocin AS-48 is a bacteriocin produced by *Enterococcus faecalis* and *Enterococcus sp.*, with molecular weight 7149, they can be isolated as Enterocins A and B. Various pathogenic bacteria like *Clostridium tyrobutyricum, C. sporogenes, Listeria monocytogenes* and *Staphylococcus aureus* have been reported to be inhibited by Enterocins A and B (Kubasova *et al.*, 2019).

### Class IId (linear, non-pediocin like, single peptide)

### Epidermicin

Epidermicin is a bacteriocin produced by *Staphylococcus epidermidis* N101 (Sandford and Upton, 2012) with molecular weight of 6074

### Lactococcin A

Lactococcin A is a bacteriocin produced by *Lactococcus lactis subsp. Cremoris* with molecular weight of 5778.

### Class III

These are groups of bacteriocin that are heat stable proteins with high molecular weight.

### Casseicin 80

Casseicin 80 is a bacteriocin produced by *Lactobacillus cassei B80* with molecular weight of ~42000.

### Enterolisin A

Enterolisin A is a bacteriocin produced by *Enterococcus faecalis* LMG 2333 (Nilsen *et al*., 2003) with molecular weight 34501

### Helveticin J

Helveticin J is a bacteriocin produced by *Lactobacillus helveticus* 481 with molecular weight 37511.

### Bacteriocin from Gram negative bacteria

### Colicins

In 1952, Colicin was discovered from *Escherichia coli*. Colicin is a bacteriocin that binds to the membrane of cell or other cytosolic targets leading to permeabilization of such cell membrane, inhibition of cell wall synthesis and inhibition of DNase or RNase activity on closely related bacteria even to a producing species, in order to mitigate competitors for nutrients and space (Garcia-Bayona *et al.*, 2017). Colicins are categorized in three domains:

Amino-terminal translocation (T) domain: which is implicated in the transfer across the outer membrane via the translocator protein

Central receptor-binding (R) domain: which is bound with a bacterial outer membrane receptor. Carboxy-terminal cytotoxic (C) domain: which has antibacterial activity (Cascales *et al.*, 2007;

Kleanthous, 2010). In order to avoid bacteriocidal-poisoning by self-produced colicins, specificity immunity proteins are simultaneously produced by the producing bacterium to inactivate colicins (Kleanthous, 2010; Shih-Chun *et al*., 2014.). The colicin sensitive strains are known by the presence of an outer membrane colicins recognition receptors protein located at the surface and the translocators protein system, these allow the transportation of the colicin into the bacteria eventually leading to death, but for the colicin resistant strains, a particular colicin, non-receptor protein is present instead. Furthermore, bacteria that are deficient of translocator protein system are classified as tolerant strains and the ones that produce immunity proteins are classified as immune strains. Immune, resistant and tolerant strains are not affected nor killed by corresponding colicins (Kleanthous, 2010; Shih-Chun *et al*., 2014.).

## Microcins

Microcins are bacteriocins predominantly produced by Enterobacteriaceae, they are ribosomal synthesized hydrophobic antimicrobial peptides (<10 kDa) with low molecular weight and they show great tolerance to extreme pH, heat, and proteases (Rebuffat, 2012). Microcins are produced as precursor peptides, including N-terminal leader peptide and core peptides and are secreted outside the bacteria through the Type I ABC (ATP binding cassette) transporter secretion system, which is composed of a number of proteins (Duquesne *et al*., 2007). The antimicrobial mechanisms of microcins are diverse, ranging from pore-forming type, the nuclease type which includes such as DNase and RNase functions, inhibitors of protein synthesis types to DNA replication inhibitors types (Severinov *et al*. 2007). Microcins classification is listed in Table 3.

## Fermentation

Fermentation is a biochemical process which encourages the viability of probiotics that in turn helps in enhancing food nutritional value and shelf life. The secondary metabolites of this probiotics are antimicrobial in nature thus help to inhibit pathogenic organisms and their metabolites (Vaishali *et al.*, 2019). The probiotics mostly used to trigger this biochemical process are lactic acid bacteria (LAB). The secondary metabolites of these lactic acid bacteria have antimicrobial properties and also their organic metabolic compounds help in imparting unique organoleptic properties to the food products (Lucera *et al*., 2012).

Some food product are subjected to fermentation in order to increase their shelf lives, milk product, cereals product and even root and tubers product are often subjected to fermentation processes locally in order to improve the nutritional value and shelf life. Two Nigerian fermented food products from cassava and maize i.e Fufu and Ogi respectively are good examples of food product subjected to fermentation to achieve the afore mention properties (Oyedeji *et al*., 2013).

Sakhare and Narasimha (2003) reported the preservation of minced meat with glucose and salt perfected through fermentation. The authors subjected the sample to 24-36 hour at 37°C and 30-42 hours of incubation at 30°C with pH value of 4.0-4.2, using viable culture of *Lactococci lactis*, *Lactobacillus casei* and *Lactobacillus plantarm* as inoculum. After the incubation period, a significant reduction in *Staphylococcus aureus*, coliforms like *Escherichia coli* and also *Salmonella spp*. were observed and that was achieved when compared with the control (Sakhare and Narasimha, 2003).

## Essential Oil

Essential oil are organic extracts with low molecular weight from various plant materials like barks, flowers, fruits and roots to mention a few. Essential oils are also commonly known as volatile odoriferous oils, constituting different family of low molecular weight organic compounds with enormous antimicrobial activity (Trombetta *et al.,* 2005; Tongnuanchan and Benjakul, 2014). The active compounds can be divided into three major categories based on their chemical structure: terpenes, terpenoids, phenylpropenes, and others like Allyl-isothiocyanate and allicin (Hyldgaard *et al.,* 2012).

## Forms of Essential Oils

### Terpenes

Terpenes are essential oil with hydrocarbons backbone produced in the cytoplasm of plant cells; they are formed from combination of several isoprene units ($C_5H_8$) and the synthesis starting from acetyl-CoA, are through the mevalonic acid pathway (Caballero *et al*., 2003). The main forms of terpenes are monoterpenes ($C_10H_{16}$) and sesquiterpene ($C_{15}H_{24}$), although other forms with longer chains exist e.g diterpenes ($C_{20}H_{32}$) and triterpenes ($C_{30}H_{40}$) to mention a few. Under monoterpenes form we have: α-pinene, limonene, sabinene, and P-cymene while under sesquiterpenes we have γ-terpinene and β-caryophyllene.

Terpenes generally represent a group of essential oil with low quality of antimicrobial activity. Koutsoudaki *et al.,* (2005) reported the comparison effects of α-pinene, β-pinene, p-cymene, limonene, β-myrcene, γ-terpinene and β-caryophyllene on *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli,* and it was observed that their bactericidal potentials were absent or very low. Bagamboula *et al.,* (2004) reported that p-cymene extracted from thyme had no bactericidal effect on several Gram-negative microbes even at high concentration of 85700μg/mL concentration (Bagamboula *et al*., 2004). Dorman and Deans, (2000) also reported the poor to none bactericidal effects of α-pinene, limonene, (+)-sabinene, β-pinene, δ- α- terpinene and 3-carene and on different pathogenic bacteria.

### Terpenoids

The terpenoids are a large group of compounds known for their antimicrobial potentials against many microorganisms. Terpenoids are biochemically modified terpenes through enzymes that move or remove methyl groups and add oxygen molecules (Caballero *et al*., 2003). Terpenoids can be subdivided into alcohols, aldehydes, ketones, esters, ethers, phenols, and epoxides. Examples of terpenoids are: citronellal, thymol, carvacrol, carvone, borneol, linalool, linalyl acetate, piperitone, menthol, and geraniol; carvacrol and thymol are the most active mono-terpenoids identified so far. The antimicrobial potentials of most terpenoids are based on their functional groups, and on the evidence of this the presence of hydroxyl group of phenolic terpenoids and delocalized electrons are crucial feat for antimicrobial activity (Ultee *et al*., 2002; Ben Arfa *et al*., 2006; Veldhuizen *et al*., 2006). Furthermore, the antimicrobial activity of terpenoids can also be linked to its phenolic constituents (Aligiannis *et al*., 2001; Kalemba and Kunicka, 2003; Rhayour *et al*., 2003).

Dorman and Deans (2000) reported the investigation of the effect of many terpenoids against twenty five bacteria, and the results showed that all terpenoid compounds showed a great bactericidal activity but borneol and carvacrol methyl ester. Bassolé *et al*., (2010) also reported the bactericidal activity of carvacrol, thymol, linalool, and menthol were investigated against *Listeria monocytogenes, Enterobacter aerogenes, E. coli,* and *Pseudomonas aeruginosa* respectively. The most active compounds were carvacrol and thymol with highest MIC of 300 and 800μg/mL respectively (Bassolé *et al*., 2010).

### Phenylpropenes

Phenylpropenes are essential oil from organic compounds known as phenylpropanoids that are produced from the amino acid precursor phenylalanine in plants. Phenylpropanoids is a name carved out from the six-carbon aromatic phenol group and the three-carbon propene tail of cinnamic acid, formed in the first step of phenylpropanoid biosynthesis. The phenylpropenes constitute small part of essential oils and some have been extensively studied e.g cinnamaldehyde, eugenol, vanillin, isoeugenol and safrole (Hyldgaard *et al.*, 2012). The antimicrobial potentials of phenylpropenes is dependent on number and forms of substituents on the aromatic ring, selected test organisms, growth medium, pH and temperature to mention a few (Pauli and Kubeczka, 2010; Hyldgaard *et al.*, 2012).

Dorman and Deans (2000) investigated the bactericidal potentials of eugenol against twenty five pathogenic bacteria and it was observed that only one strain was resistant (Dorman and Deans, 2000). Phenylpropene, vanillin, inhibits bacteria and yeasts also a range of mycelial inhibition was observed in toxigenic molds (Fitzgerald *et al*., 2003, 2004, 2005; Rupasinghe *et al*., 2006; Hyldgaard *et al.*, 2012).

Essential oils are mostly used under the approach of hurdle technology with other food preservatives (Hyldgaard *et al.*, 2012; Tongnuanchan and Benjakul, 2014). Earlier, essential oils were primarily used as medicines but recently the food industries have adopted them as bio-preservative, flavoring and coloring agents (Hyldgaard *et al.*, 2012).

The usage of essential oils as bio preservatives was purposely targeted at preventing rancidity of fats and possible prevention of chronic degenerative disease and because of their antimicrobial and antioxidant activities, they are efficiently used in food products. Furthermore, with main active compounds like phenylpropenes, terpenes, terpenoids to mention a few, essential oil have been categorized as Terpene Hydrocarbon and oxygenated compounds.

When not properly stored, essential oils are prone to dehydrogenation, isomerization, cyclization or oxidation which eventually results in quality loss (Skold *et al*., 2008; Brared-Christensson *et al*., 2009). Some researchers have reported the use of some essential oils as bio-preservatives; essential oils from curry leaves and cloves were researched at the rate of 0.10 and 0.20 ppm respectively to augment the storage stability of Burfi without interfering with the organoleptic quality and acceptability of the product (Badola *et al*., 2018).

## Herbs and Spices

Plants contain a plethora of phyto-chemicals that constitute organic compounds/functional groups with antimicrobial potentials, feats that enhance plants defensive mechanism against infections and could also confer this properties on the food they are introduced too as additives or bio-preservatives (Jack *et al*., 1995; Vaishali *et al*., 2019).

Spice are regarded as aromatic vegetable-substance either in the broken, ground or whole form, which plays a major role of seasoning rather than nutrition in food and from which no portion of any volatile oil or other flavouring has been removed (Sung *et al*., 2012).

It has been reported that over 100 varieties of herbs and spices such as ginger (*Zingiber officinalle*), cloves (*Carophyllus aromaticus*), turmeric (*Curcuma longa*) (Radwan *et al.*, 2014), lemon grass (*Cymbopogan citrates*) (Prasad *et al.*, 2011), mint (*Metha piperita*) (Tyagi *et al.*, 2013), balm (*Melissa officinalis*) (Moradkhani *et al.*, 2010) and garlic (*Allium sativum*) (Yadav and Singh, 2004) to mention a few are known to have bactericidal or bacteriostatic properties (Prasad *et al.*, 2011; Vaishali *et al*., 2019). Plants (herbs or spices) in forms of dried seed, root, bark, fruit, husks or even vegetable parts have been used in culinary practices to improve the nutritional qualities, organoleptic qualities and shelf life of food products throughout all the countries of the world.

The antimicrobial compounds in these herbs or spices are more available in the extracts of the spices than the whole spices as such, due to the fact that the spices release volatiles at a slower rate (Vaishali *et al*., 2019). "Generally recognized as safe' (GRAS) status of herbs and spices are the main reasons for their usage in food as additives and bio-preservatives, furthermore, they are excellent alternatives to chemical additives because they are free from chemical residues (Rodriguez and Guevara, 2002). Herbs and spices are good source of polyphenols and thus contribute to the total dietary phenolic consumption. The herbs and spices have great free radical scavenging property; they help mitigate the risk of oxidative stress and as a result reduce the risk of cancers and all other complications associated with accumulation of free radicals in the body (Vaishali *et al*., 2019).

The mechanism of actions for some herbs or spices have not been completely researched within the confines of their bactericidal or bacteriostatic activities but Butylated hydroxyanisole (BHA) isolated from some spices and herbs have been reported to prevent the auto oxidation of lipids and thus observed to show inhibitory potentials against the growth of various Gram positive and Gram negative bacteria when added as additive or conscious bio-preservative (Badola *et al*., 2018; Vaishali *et al*., 2019). Badola *et al*., (2018) reported a study on Butylated hydroxyanisole antimicrobial properties against the vegetative cells of *Bacillus spp*. at concentrations of 5000 ppm and 1000 ppm in strained chicken and cooked rice respectively.

**Table.1** Classification of Colicins (Group A)

| Group A Colicins | Molecular weight | Producing strains | Antibacterial activities | Reference |
|---|---|---|---|---|
| **A** | 62989 | *Citrobacter freudii* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010 |
| **E1** | 57279 | *Escherichia coli* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **DF13** | 59293 | *Escherichia coli* | 16SrRNase | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **K** | 59611 | *Escherichia coli* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **N** | 41696 | *Escherihia coli* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **S4** | 54085 | *Escherichia coli* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **U** | 66289 | *Shigella boydii* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **28b** | 47505 | *Serratia marcescens* | Pore-forming | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **E2** | 61561 | *Escherichia coli, Shigella sonnei* | DNase | Ursino *et al*., 2002 |
| **E3** | 57960 | *Escherichia coli* | 16S rRNase | Cascales *et al*., 2007; Kleanthous, 2010; Shih-Chun *et al*., 2014. |
| **E5** | 58254 | *Escherichia coli* | tRNase | Shih-Chun *et al*., 2014. |
| **E6** | 58011 | *Escherichia coli* | 16S rRNase | Shih-Chun *et al*., 2014. |
| **E7** | 61349 | *Escherichia coli* | Dnase | Shih-Chun *et al*., 2014. |
| **E8** | ~70000 | *Escherichia coli* | Dnase | Shih-Chun *et al*., 2014. |
| **E9** | 61587 | *Escherichia coli* | Dnase | Shih-Chun *et al*., 2014. |

**Table.2** Classification of Colicins (Group B)

| Group B Colicins | Molecular weight | Producing strains | Antibacterial Activities | Reference |
|---|---|---|---|---|
| **B** | 54742 | *Escherichia coli* | Pore-forming | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |
| **La** | 69429 | *Escherichia coli* | Pore-forming | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |
| **Ib** | 69923 | *Escherichia coli* | Pore-forming | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |
| **5** | 53137 | *Escherichia coli* | Pore-forming | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |
| **10** | 53342 | *Escherichia coli* | Pore-forming | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |
| **D** | 74683 | *Escherichia coli* | TRNase | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |
| **M** | 29453 | *Escherichia coli* | peptidoglycan | Ursino *et al*., 2002; Shih-Chun *et al*., 2014. |

**Table.3** Classification for Gram-Negative Microcins

| Classification | Microcinss | Molecular weight | Characteristics | Producing strains | Reference |
|---|---|---|---|---|---|
| **Class I** | B17<br>C7<br>C51<br>D93<br>J25 | 3094<br>1177<br>1177<br>1000<br>2107 | Possess peptides with low molecular weight | *Escherichia coli* | Wilson *et al*., 2003; Severinov *et al*., 2007; Collin *et al*., 2013. |
| **Class IIa** | L<br>n/24<br>v | 8884<br>7274<br>8741 | They required more than one genes to synthesized and arrange functional peptides | *Escherichia coli* | Pans *et al*., 2004; Corsini *et al*., 2010 |
| **Class IIb** | E492<br>H47<br>M | 7886<br>7284<br>4865 | Linear peptides with post-tralational modifications or not at C-terminal | *Escherichia coli* | Pons *et al*., 2002; Vassiliadis *et al*., 2010 |

**Table.4** Lists of some Common Herbs and Spices Used as Bio-preservatives against some Pathogenic Organisms

| Scientific names | Common names | Functional antimicrobial compounds | Test microorganisms (Bacteria) | Test organisms (fungi) | References |
|---|---|---|---|---|---|
| ***Aframomum melegueta*** | grain of paradise | Gingerol | *Escherichia coli, Salmonella spp.* | *Aspergillus niger* | Nneka and Jude, 2012; Gottardi *et al.*, 2016. |
| ***Alium cepa***<br>***Alium sativum***<br>***Alium schoenoprasum*** | onion<br>garlic<br>chives | Allicin, Diallyl sulphide | *Staphylococcus aureus, Salmonella typhii, Bacillus cereus, Bacillus subtillis, Escherichia coli* | - | Yadav and Singh, 2004; Rattanachakunsopon and shirshova *et al.*, 2013 |
| ***Angelica archangelica*** | Angelica | A-Pinene, limnene, phellandrene and δ-3-Carene. | *Escherichia coli, Staphylococcus aureus* | - | Fratemale *et al.*, 2014 |
| ***Alpinia galangal*** | Greater galangal | Galango-isoflavonoid, β-sitosterol, Galangin, β-caryophyllene, β-selinene | *S. typhimurium, Staphylococcus aureus, Bacillus subtilis* | *Aspergillus niger* | Kaushik *et al.*, 2011 |
| ***Boesenbergia rotunda*** | Fingerroot | Pinostrobin, pinocembrin, cardamonin, boesenbergin A, Boesenbergin B, camphor, linalool, camphene | *Bacillus cereus, Staphylococcus aureus, Lactobacillus plantarum* | *Candida albican* | Eng-Chong *et al.*, 2012 |
| ***Ceratonia siliqua*** | Carob tree | Nonadecane, heneicosane, farmesol, camphor | *Bacillus cereus, Stapylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* | *Aspergillus niger* | Hsouna *et al.*, 2011 |
| ***Coriandum sativum*** | Coriander | Dodecenal, 1-Decanol ergosterol | *Staphylococcus epidermidis, staphylococcua aureus, Pseudomonas aeruginosa* | - | Bharti *et al.*, 2012 |
| ***Curcuma longa*** | Turmeric | Curcumin | *Salmonella typhi, lostridium sp. Staphylococcus* | *Candida albican* | Radwan *et al.*, 2014; Gottardi *et al.*, 2016. |

| | | | | | |
|---|---|---|---|---|---|
| | | | *aureus, Escherichia coli, Y. enterocolitica, P. notatum,* | | |
| ***Cuminum cyminum*** | Cumin | Cuminal | *Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas fluorescens, Staphylococcus aureus* | *Aspergillus niger, Candida albican* | Sethi *et al*., 2012; Gottardi *et al*., 2016. |
| ***Cymbopogon citrates*** | Lemon grass | Citral, myrcene, linolool, farnesol | *Escherichia coli* | *Candida albican* | Vazirian *et al*., 2012; Gottardi *et al*., 2016. |
| ***Foeniculum vulgare*** | Fennel | Anethole | *Bacillus cereus, Bacillus subtillis, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae* | *Aspergillus niger* | Ceylan and Fung, 2004; Gottardi *et al*., 2016. |
| ***Garcinia indica*** | Kokum | Garcinol | *Escherichia coli, Staphylococcus aureus, Bacillus cereus* | *Candida albican* | Bumalai and Eswaraiah, 2011 |
| ***Lippia adoensis*** | Koseret | Linalool, germacrene D | *Staphylococcus aureus* | *Candida albican* | Folashade and Egharevba, 2012; Gottardi *et al*., 2016. |
| ***Melissa officinalis*** | Balm | Neral, citronellal, isomenthone, menthone, β-caryophyllene, carvacrol | *Shigella sonnei* | - | Moradkhani *et al*., 2010 |
| ***Mentha piperita*** | Mint | Menthol; 1,8-cineole | *Escherichia coli, pseudomonas aeruginosa, Streptococcus faecalis, Staphylococcus aureus* | *Candida albican* | Sharafi *et al*., 2010; Tyagi *et al*., 2013; Gottardi *et al*., 2016. |
| ***Myristica fragrans*** | Nutmeg | Myisticin, sabinene, β-pinene | *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa,* | *Aspergillus niger* | Rancic *et al*., 2005; Gottardi *et al*., 2016. |

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|---|---|---|---|---|---|
| | | | *Clostridium spp* | | |
| ***Murraya keonigii*** | Curry leaf | Murrayanol, Murrayacine, Methanine | *Staphulococcus sp.,* | - | Handral *et al*., 2012 |
| ***Ocimum basilicum*** | Basil | 1,8-Cineole, Linalool, methyl chavicol | *Escherichia coli, Sataphylococcus aureus, S. typhimurium, Clostridium botulinum* | *Candida albicans* | Shirazi *et al*., 2014; Gottardi *et al*., 2016. |
| ***Pimpinella anisum*** | Anise | Anethole | - | *Aspergillus ochraceus, Fusarium moniliforme* | Gottardi *et al*., 2016. |
| ***Syzygium aromaticum*** | Clove | Eugenol | *Escheichia coli, Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Citrobacter freundii* | - | Yadav and Singh, 004; Naveena *et al*., 2006; Gottardi *et al*., 2016. |
| ***Thymus vulgaris*** | Thyme | Thymol, cinnamaldehyde | *Pseudomonas putida, Listeria monocytogenes* | - | Gottardi *et al*., 2016. |

## Vinegar

The usage of natural preservative is a necessary alternative to chemical alternative in food production. Natural preservative like vinegar has been popular for its ability to preserve food product and also augment the nutritional properties alongside with the aroma or flavour although its strong smell and taste limits its versatility as bio-preservative in food production (Park *et al*., 2014; Vaishali *et al*., 2019).

Vinegar is produced by the acetic acid fermentation or alcoholic fermentation; a solution containing a typical pickling agents with about 5%-10% acetic acid acetic, a known preservative with ability to inhibit the growth of microorganisms in food products by altering water activity or pH in the product (Jang *et al*., 2014; Vaishali *et al*., 2019).

Park *et al*., (2014) reported the bio-preservative potentials of vinegar on stored blanched tea leaves for 4 days at 30ºC preserved in pickling solutions containing mixtures of vinegar, soy sauce and water at varied concentrations. After the storage period, Park *et al*., (2014) observed that the pH increased consistently within the confines of acidity and thereby conferred preservative effect on the product.

Jang *et al*., (2006) also reported the organoleptic and bio-preservative potentials of vinegar and sake on Korean seasoned beef. It was found that the mixture of vinegar and sake as bio-preservative did not improve the organoleptic quality of the Korean seasoned beef, however; microbial stability was achieved both at 8ºC and 20ºC.

Vinegar mixture has been used for decontamination of meats from especially coliforms like *E. coli* and food spoilage microbes to prolong shelf life (Tzortzakis, 2010), preservation of vegetables like tomato (*Lycopersicon esculentum*) to prevent Anthracnose rot caused by *Colletotrichum coccodes* (Vaishali *et al*., 2019).

## Sugar and Salt

Locally and industrially, salt and sugar are used nakedly for food preservation, within the confines of their usage when added to food products, some salts and sugar absorbs water and thus restricting water availability to the microorganisms leading to death and also, salt dissolved in water at high concentration produces an hypertonic solution and when introduced to the food it creates an environment that induces osmotic pressure on the cell wall of some microorganisms, this also leads to death of such organism and as a result check spoilage of such food product (Dwivedi *et al*., 2017; Vaishali *et al.,* 2019). Locally, table salt (sodium chloride) is used to preserve fermented food products like locust beans, even smoked fish and meat to mention a few. Furthermore, sugar can be mixed with salt to form a covering for some food products or could be dissolved in water to form brine in which some kind of food products are submerged (Vaishali *et al.,* 2019).

According to Khan *et al.,* (2015) it was reported that Potassium sorbate, Sodium benzoate and sucrose solution were found highly effective in the treatment of strawberry fruits for 15 days interval (Khan *et al*., 2015). Wijnker *et al*., (2006) reported the preservative measure of casings with NaCl against five pathogenic organisms; *Staphylococcus aureus, Escherichia coli, E. coli* O157:H7, *Salmonella typhimurium, Listeria monocytogenes,* and *Clostridium perfringens* isolated from natural casings at different water activity levels. At different temperature with dry salt in different brines, the casings were stored for 30 days. The antimicrobial effect of salt inhibited all the pathogenic bacteria but the spores of *Clostridium* which was below the acceptable level at aw 0.85 or reduced after 30 days of the storage period (Wijnker *et al*., 2006).

Conclusively the old and traditional methods of bio-preservatives have been improved upon by the new methods triggered by technological advancement and research, the use of organisms' secondary metabolites, essential oils, herbs/spices, vinegar and fermentation have opened doors beyond shelf life improvement to nutritional bio-augmentation of food products. Especially fermentation triggered by probiotics which leads to some immensely complex interactions between these beneficial microorganisms and the food matrix they are fermenting, this illuminates areas with potential worth exploiting well beyond the confines of shelf life extension, on the evidence of this facts which also spread to other biopreservative methods mention in this review, it is safe to say all the bio-preservative methods reviewed have been proven to be green, eco-friendly and very effective within the confines of the criteria guiding their usefulness and there are lots of avenues for technological improvements in areas of bio-preservation and food biotechnology as a whole.

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