Bioprotection: Natural Food Preservatives

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Abstract- *The major goal of any food processing and storage technique is to guaranty safe and high-quality food products. In order to achieve improved safety against food pathogens, food industries make use of chemical preservatives or physical treatments such as high temperatures. These preservation techniques have many drawbacks such as the proven toxicity of some chemical preservatives and the alteration of the organoleptic and nutritional properties of foods. Research has begun to focus on natural preservatives from plants, animal and microbial sources as alternative means of meeting consumers demand for fresh and healthy food products with high safety and nutritional values. The ability of the natural product to enhance food quality and extend shelf life without significantly altering the sensory properties of the product is referred to as bioprotection. This paper seeks to highlight causes and factors of food deterioration, food preservation methods and sheds light on the bioprotective action of essential oils, antimicrobial peptides and organic acids and their efficacy as compared to chemical preservatives. It also highlights the present challenges of the food industry in their usage and gives recommendations for growing research. The numerous experimental applications of essential oils, enzymes, bacteriocins, chitosans and organic acids to various fresh perishable foods demonstrate that they are well suited to be utilized as preservatives in foods and in combination with other preservative methods could be often valid alternatives to exclusive synthetic food additives.*

Keywords- Biopreservation, Deterioration, Food; Mode of Action and Natural

I. INTRODUCTION

Food, in any form it comes, plays an essential role in the overall well being of man. Food was historically derived by either hunting and gathering or agriculture. The growing population of the world and so called 'modernization' of today have made these options limited. Today, the majority of the food energy required by the ever increasing population of the world is supplied by the food industry.

Various techniques and processes have been employed in agriculture to optimize the yield and make more food available to the growing population. Also, in a bid to preserve the left-over for reasons such as transportation and food security, various food processing methods have developed in the last century (Lucera *et al*., 2012). At their most fundamental levels, all food related processes from harvest to digestion are ways of bringing about, or preventing, biochemical changes. Controlling these changes is the major concern of food scientists, technologists and biochemists. These biochemical changes if not controlled would lead to deterioration, spoilage, waste and ultimately hunger. Understanding the components of food, and the mechanisms by which these biochemical changes occur has brought light to various preservative techniques and products that have been able to successfully combat the changes that occur internally or externally (Simpson *et al*., 2012). A major goal of any food process and storage is to ensure safe and high-quality food. Proper storage and process extends the shelf-life of food, which depends on the food type, packaging and storage conditions, particularly temperature and humidity (Abdulmumeen *et al*., 2012).

Natural and artificial preservative methods are employed by every manufacturer during processing, in order to achieve improved food safety against pathogens and extend shelf life of many products (Nath *et al.,* 2013). All food products except the ones growing in our kitchen garden have food preservatives and enhancers (Singh *et al*., 2010). Preservation techniques such as the very effective chemical preservatives and physical treatments (e.g. high temperatures) have many drawbacks, which include the proven toxicity of the chemical preservatives and the alteration of the organoleptic and nutritional properties of foods (Abdulmumeen *et al.,* 2012; Anand and Sati, 2013; Islam *et al*., 2015).

In recent years, a rise in demand for 'natural products', that is, food preserved with natural additives in replacement of chemical additives has been observed, due to a growing consumer awareness towards the health aspects and concern for synthetic chemical additives (Singh *et al.,* 2010). Improvements in food preservative methods and products is becoming increasingly important as modern consumer trends and food legislations are requiring more high quality, preservative-free, safe but mildly processed foods with extended shelf-life (Mohanka and Priyanka, 2014). As an alternative to certain disadvantages associated with chemical preservatives, food industries are paying more emphasis on the use of natural preservatives and other 'natural food products' thus targeting the development of more healthy products due to consumer‘s demands for decrease of chemical preservatives. Increasing demand for green food products with high safety and nutritional values by consumers has caused research for a few years now, to focus on the use of plant; animal and microbial sources to meet this need, a process referred to as bioprotection (Lucera *et al.,* 2012; Mohanka and Priyanka, 2014).

II. FOOD DETERIORIATION

Many food products are perishable by nature and require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life (Lucera *et al.,* 2012). A thorough understanding of the composition of food, and the mechanisms by which these biochemical changes occur has helped to develop various preservative techniques and products that have been able successfully combat the changes that occur internally or externally (Simpson *et al.,* 2012).

Food begins to deteriorate once harvested. Breakdown of food composition leads to final loss of its qualities until it finally becomes unfit for consumption. The process of becoming undesirable is referred to as deterioration (Featherstone, 2008). Food spoils as a result of undesirable chemical, biological or physical action, rendering it unfit for consumption. Two major factors have been implicated as the cause of food spoilage:

- Natural deterioration in foods (Autolysis) and;
- Contamination by micro-organisms (Bio-deterioration)

A. Autolytic Deterioration

Biochemical changes are associated with the change in food texture once removed from its natural source. This self-destruction is termed as autolysis. When animal or vegetable material is removed from its natural source of energy and nutrient supply, chemical changes begin to occur which lead to deterioration in its structure. Chemical components of the food, including its lipids, proteins and carbohydrates begin to break down into monomers. The rate at which the chemical reactions takes place depends on many factors, such as, water activity, temperature, pH, exposure to light or oxygen. Chemical reactions responsible for the major chemical changes which occur during the processing and storage of foods and lead to deterioration in sensory quality are catalyzed by various enzymes. Each food component experiences breakdown leading to rancidity, softening and browning. A few other autolytic reactions however are non-enzymic, such as Maillard browning (Simpson *et al.*, 2012) and autoxidation, majorly undergone by lipids, which lead to oxidative rancidity

B. Biodeterioration

Before spoilage can occur organisms which are capable of altering the components of a product in-situ must first be introduced via raw materials, the processing plant, packaging materials, operatives or elsewhere in the environment. Although spoilage does not necessarily depend upon the growth of these contaminants it is generally facilitated if the formulation and the ambient conditions of temperature and humidity encourage their multiplication.

Significant activities of organisms (microbes, insects, rodents or bird) as a result of their metabolic processes within a food item, leading to undesirable changes in the composition of the food material is referred to as bio-deterioration (Hueck, 1968; Featherstone, 2008). However, when harnessed by man, biodeterioration can be used as a tool to recover waste material and make it more acceptable (Allsop *et al.,* 2004).

Bio-deterioration can be artificially classified based on the mode of the deterioration, that is, chemical and physical (Allsop *et al.,* 2004; Featherstone, 2008). Living organisms involved in bio-deterioration are known as *bio-deteriogens.*

C. Promoting Factors of Food Deterioration

Various biochemical factors promote food deterioration and an understanding of these factors has helped in the formulation of the various preservative techniques being employed today.

The major ones include: Composition of food, water activity (a_w), acidity (pH), concentration of sugars, oxygen tension and temperature.

III. FOOD PRESERVATION

The process or techniques employed to prevent or slow down food spoilage is known as food preservation. This is the major objective of any food processing operation (Abdulmumeen *et al.,* 2012). Food scientists and engineers utilize a variety of processes that aim to exclude air, moisture, and microorganisms, or to provide environments in which organisms that might cause spoilage cannot survive using methods involving heat, cold, and automated mechanical devices (Barbosa-Canovas, 2005).

Food preservation is basically done for three reasons: to preserve the natural characteristics of food, to preserve the quality and appearance of the food and to increase the shelf value of food for storage.

Over the years, food preservative techniques have involved two methods that are focused on dealing with the problem of contamination by micro-organisms and enzymatic reactions. The methods according to Mudambi *et al*., 2006 and Sultanbawa (2011) are:

- Bacteriostatic methods, which incapacitate the micro-organism and make them unable to grow, as well as inactivate deteriorative enzymes using techniques such as: smoking, freezing, dehydration or chemical preservatives, that serve as growth or activity inhibitors.
- Bactericidal methods, which involve total destruction of micro-organism at extremely high temperatures and pressure, using techniques such as canning, cooking, irradiation etc.

A. Chemical Preservatives

According to Singh *et al.* (2010), Preservative products can be classified into two:

- Artificial preservatives, made from synthetic chemical substances added to or sprayed on food materials and medications. The exact definition is however problematic, as natural compounds are the basis of many artificial preservatives. Artificial preservatives maybe organic (derived from a living organism), such as benzoates, propionates or inorganic, such as sulfites and nitrites/nitrates.
- Natural Preservatives, chemical constituents extracted from natural sources (plants, animals or microbes), with intrinsic ability to protect against microbial growth. They include the essential oil constituents, flavonoids, phenolic compounds, etc.

Chemically synthesized preservatives have been classically used to decrease both microbial spoiling and oxidative deterioration of food. Their common acidic function helps to raise the acidity of the food, which kills the micro-organisms. However, in recent years, as consumers are demanding partial or complete substitution of chemically synthesized preservatives due to their possible adverse health effects. This fact has lead to an increasing interest in developing more ‘natural’ alternatives in order to enhance food shelf-life and safety (Santas, *et al.*, 2010). Most commonly, chemical preservatives are derived from acids and their main preservative function is that they raise the acidity of foods which kills microorganisms.

IV. BIOPROTECTION: A TOOL FOR BIOPRESERVATION

Bioprotection as described by Vignolo *et al.* (2012) is ‘the ability of the natural product to enhance food quality and extend shelf life without significantly altering the sensory properties of the product’ (Vignolo *et al.,* 2012). Bioprotective measures make use of natural products and methods to prevent deterioration of food quality and destruction of food products. In addition, bioprotection targets the use of advanced technologies, tools and systems that use negligible chemicals to eliminate the impact of targeted pest and disease. In order to maintain access to international markets, invasive pests must be prevented from crossing borders, and existing pests and diseases must be controlled without leaving problematic chemical residues on produce for export.

Bioprotection serves as a promising tool for biopreservation, which is a means of controlling and extending shelf life and quality of food using natural methods. Biological preservation (biopreservation) is fast gaining attention in recent years as the environmental and consumer-friendly solution in combating the problems of food preservation (Ananou *et al.,* 2007; Vignolo *et al.,* 2012).

V. NATURAL PRESERVATIVES

With consumers becoming increasingly aware of the health implications of foods and the growing demand for natural products, functional foods and nutraceuticals, new ingredients are being pervasively introduced into the food supply at a rapid pace (Simpson *et al.,* 2012; Singh *et al.,* 2010).

Natural products can also be prepared by chemical synthesis (both semi synthesis and total synthesis). Natural preservatives are chemical agents derived from plants, animals, microbes and their metabolites that prevent the decomposition of products by any means (Singh *et al.,* 2010). The mode of action of these natural preservatives is same as the chemical or common methods of preservation which includes - inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in the foodstuffs.

The natural preservatives have been categorized into four types:

- **Plant derived products as preservatives** – these products are chemicals present in various parts of the plants produced for their protection from pathogens in the environment. These chemical constituents differ in composition from plant to plant. Examples include: essential oil- Gammaterpinene and alpha hydroxyl acids obtained from the dried outer peel of the ripe fruit, floral water and seed extracts of Citrus species (*Citrus limonum* (Lemon) and *Citrus aurantium* var. *Dulcis* (sweet orange)), found to be active against *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

- **Animal derived products as preservatives** – Extra- or Intra- cellular secretions are made by certain animals that have protective functions and serve as preservatives in their crude or processed form. Example includes: Chitosan, an extracellular biopolymer derived from crustaceans and cell wall of fungi, found to have antimicrobial effect against fungi, bacteria, etc. Chitosan solutions are used as biopesticides and anti-ripeners (to reduce respiration rate of fruits) (Pawar *et al.,* 2011; Saputra *et al.,* 2009). Also, many natural proteins derived from animals after hydrolysis, yield a peptide fragments of diverse biological activities, including antimicrobial, using the 'tailoring and modelling' strategy.

- **Certain microbes and/or their metabolites** – preservatives derived from microbes have been the major focus of biopreservation. Preservative agents here include the use of the microbes or their metabolites itself as preservative. Example includes: bacteriocins, an antimicrobial peptide produced by lactic acid bacteria (LAB), an organism well known to have antagonistic behavior toward closely related bacteria and undesirable harmful microorganisms (Vignolo *et al.,* 2012). Nisin is another example of an presently used industrial preservative, effective against heat-resistant bacteria spores of *Clostridium botulinum* and against food-borne pathogens such as *L.mococytogenes, S.aureus* or *B. cereus.*

- **Certain antimicrobial constituents of other foods** – these are preservatives derived from raw or already processed food. They include examples already classified under antimicrobial derived from animal proteins, such as lysozyme derived from egg white. Another example known as 'fermentate', a product obtained from a red mold species, *Monascus purpureus,* cultivated on starch containing substrates such as rice and used as a preservative in meat products. It shows antimicrobial effect against *Bacillus, Streptococcus, Staphylococcus* and *Pseudomonas*

Note: A more comprehensive list of each type of bio-derived preservatives can be found in the article by Pawar *et al*., 2011.

Artificial and natural preservatives can be categorized into three general types based on their mode of action (Anand and Sati, 2013; Pawar *et al*., 2011):

1. ***Antimicrobials:*** These chemical agents create an environment non-conducive for the growth of bacteria, yeasts, molds or fungi, thereby inhibiting them.

2. ***Antioxidants:*** They reduce the potential rancidity. Three types include: <u>True antioxidants</u> such as Butylated hydroxytoluene (BHT) and Butylated hydroxyanisole (BHA) which block chain reactions by reacting with free radicals; <u>Reducing agents</u> such as ascorbic acid have lower redox potential than the material/product they are protecting and; <u>Antioxidant synergists</u> such as Sodium edetate enhance the effects of other antioxidants.

3. ***Antienzymatics and Chelators,*** where antienzymatics help in slowing down natural enzymatic processes, such as ripening that occur in foodstuffs after harvest, chelating agents such as EDTA and ferulic acid (which serves as a natural preservative enhancer).

VI. MODE OF BIOPROTECTION

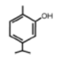
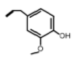
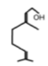
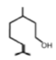
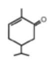
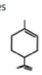
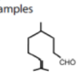
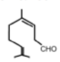
The mode of action of the natural preservatives can be discussed under the following broad headings: essential oils, antimicrobial peptides and organic acids

A. Essential Oils

These are made from a very complex mixture of volatile molecules that are produced by the secondary metabolism of aromatic and medicinal plants (Faleiro, 2011). Essential oils (EO) are complex mixtures of up to 45 different constituents. Essential oil constituents are a diverse family of low molecular weight organic compounds with large differences in antimicrobial activity. The active compounds can be divided into four groups according to their chemical structure: terpenes, terpenoids, phenylpropenes, and ―others (Hyldgaard *et al.,* 2012). The four groups and structural examples are fully elucidated by Hyldgaard *et al.,* 2012. Quite a number of essential oils have been studied for their mode of action. However, a study on each of the almost 45 different constituents in each essential oil might reveal details of their preservative nature that could have been concealed when studying a mixture of these constituents (Hyldgaard *et al.,* 2012).

For the basis of this work I would be focusing on the general hypothesized mechanisms by which two essential oil constituents carvacrol and thymol act as antimicrobials and anti-oxidative.

Table 1: Classification of aromatic molecules according to their chemical function (Source: Saad *et al.,* 2013)

Aromatic component	Molecular structure	Physico-chemical characteristics	Content in some plants' essential oils (%)
Phenols	Examples  Carvacrol (CAS No. 499-75-2)	Density: 1.07 g/ml MW: 164.2 g/mol	Carvacrol in thyme [*Thymus vulgaris* (Lamiaceae)] 33% and in origan [*Origanum vulgare* (Lamiaceae)] 76%
	 Eugenol (CAS No. 97-53-0)	Density: 0.98 g/ml MW: 150.2 g/mol	Eugenol in clove [*Syzygium aromaticum* (Myrtaceae)] 82%, in bay rum tree [*Pimenta racemosa* (Myrtaceae)] 60% and in pepper [*Pimenta dioica* (Pimenta dioica)] 54%
Terpenic alcohols	Examples  Geraniol (CAS No. 106-24-1)	Density: 0.88 g/ml MW: 154.3 g/mol	Geraniol in palmarosa [*Cymbopogon martinii* (Poaceae)] 75–95%, in *Helichrysum* spp. (Asteraceae) 80–90%, in citronella java [*Cymbopogon winterianus* (Poaceae)] 12–18% and in *Cymbopogon nardus* (Poaceae) 20–40%
	 Citronellol (CAS No. 106-22-9)	Density: 0.86 g/ml MW: 156.3 g/mol	Citronellol in citronella java [*Cymbopogon winterianus* (Poaceae)] 11–15% and in *Cymbopogon nardus* (Poaceae) 10–20%
Cetones	Examples  Carvone (CAS No. 99-49-0)	Density: 0.96 g/ml MW: 150.2 g/mol	Carvone in caraway [*Carum carvi* (Apiaceae)] 50%
Aliphatic hydrocarbons, sesquiterpenes	Examples  Limonene (CAS No. 5989-54-8)	Density: 0.96 g/ml MW: 150.2 g/mol	Limonene in caraway [*Carum carvi* (Apiaceae)] 45%
Terpenic aldehydes	Examples  Citronellal (CAS No. 106-23-0)	Density: 0.89 g/ml MW: 154.3 g/mol	Citronellal in citronella java [*Cymbopogon winterianus* (Poaceae)] 35–45% and in lemon eucalyptus [*Eucalyptus citriodora* (Myrtaceae)] 90%
	 Neral and geranial (CAS No. 5392-40-5)	Density: 0.89 g/ml MW: 154.3 g/mol	Citral in lemongrass [*Cymbopogon citratus* (Poaceae)] 70–80% and in lemon balm [*Melissa officinalis* (Lamiaceae)] 50%

Antimicrobial: The antimicrobial actions of EO are linked to one of the most important EO characteristics, its hydrophobicity resulting in increased cell permeability and consequent leaking of cell constituents. A disrupted cell structure would lead to a cascade of other reactions due to other affected cellular structures. The most elucidated action concerns the components of oregano and thyme EOs, carvacrol and thymol..

Carvacrol is a phenolic monoterpenoid. Together with its closely related isomer thymol, it is one of the most extensively studied essential oil constituents. The antimicrobial effect of carvacrol is expected to be similar to that of thymol, causing structural and functional damages to the cell membrane (Hyldgaard *et al.*, 2012). Although carvacrol affects the outer membrane, its site of action is thought to be the cytoplasmic membrane, resulting in passive transport of ions across the membrane. Carvacrol has a hydroxyl group that has been proposed to function as a transmembrane carrier of monovalent cations across the membrane, carrying H+ into the cell cytoplasm and transporting K+ back out (Hyldgaard *et al.*, 2012; Saad *et al.*, 2013). The hypothesis of carvacrol mechanism of action as a transmembrane proton carrier is further explained and illustrated below in Fig 1. However, it is also proposed by another, Hyldgaard *et al.* (2012) that the relatively high activity of a non-hydroxyl compound ruled it out as the main mode of action of carvacrol. It is recognized that the action of carvacrols results in the release of the lipopolysaccharides from gram negative bacteria with the consequent cell membrane permeability increase and ATP loss (Faleiro, 2011). Membrane permeabilization by carvacrol has been confirmed by monitoring the efflux of H+, K+, carboxyfluorescein and ATP, and the influx of nucleic acid stains (Hyldgaard *et al.*, 2012). However, the presence of the phenolic group appears to be more important for the antimicrobial activity than expansion of the membrane.

Thymol, as carvacrol, contains a phenolic group and a system of delocalized electrons; therefore it has strong antimicrobial activity. Studies have shown that thymol also interacts with cell membranes. The interaction affects membrane permeability, and this has been documented by loss of membrane potential, cellular uptake of ethidium bromide, and leakage of potassium ions, ATP, and carboxyfluorescein (Hyldgaard *et al.*, 2012).

Consequently, measurement of antibacterial activities of carvacrol, thymol, cymene, menthol and methyl carvacrol demonstrated that the hydroxyl group and the presence of a system of delocalized electrons are important elements for antibacterial activity (Saad *et al.*, 2013).

The general proposed mechanism of essential oil can be summarized in figure 2.

Antioxidant: A constituent of garlic essential oil is allicin (diallyl thiosulfinate). It is the biologically active compound mainly found in the garlic extracts with antibacterial and antifungal properties. However, 2-propenesulfenic acid is really responsible for the antioxidant property of garlic and not the allicin. Figure 3a demonstrates the mechanism of the radical scavenging activity of the allicin. The radical-scavenging activity of allicin involves H-atom transfer to a peroxyl radical from the methylene of the allyl group on the divalent sulfur. Figure 3b demonstrates an alternative mechanism, where the radical scavenging activity of allicin can be accounted for 2-propenesulfenic acid, which is produced from allicin by Cope elimination. 2-Propenesulfenic acid is reported to be over 1000 times more reactive toward ·OOH radicals than allicin (Nimse and Pal, 2015).

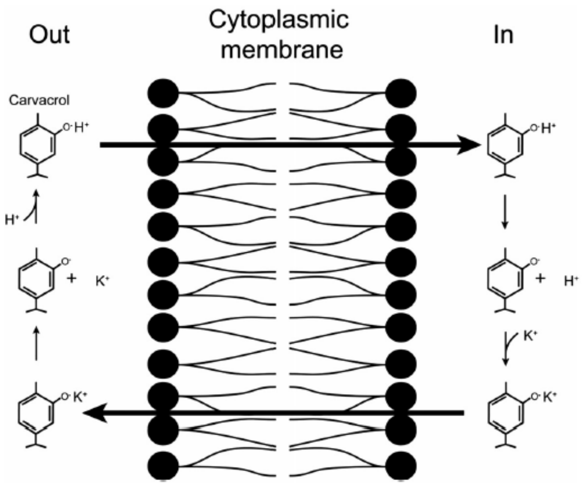


Fig 1: A representation of the proposed carvacrol mechanism of action. Undissociated carvacrol diffuses through cytoplasmic membrane to the cytoplasm where it dissociates, releasing its proton. In the cytoplasm, carvacrol attaches a potassium ion (or another ion), which will then be transported across the cytoplasmic membrane to the external environment. Once outside the cytoplamic membrane a proton is again fixed on carvacrol, which releases the potassium ion. In its protonated (undissociated) form, carvacrol is ready to diffuse again across the cytoplasmic and dissociates, releasing the proton into the cytoplasm. (Source: Saad *et al.,* 2013)

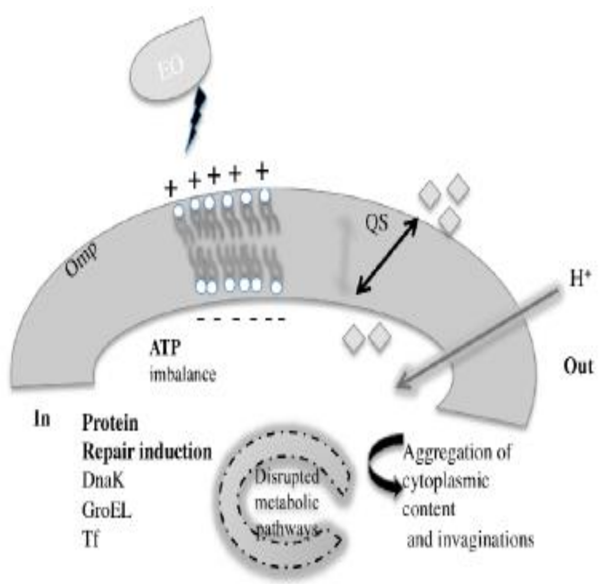


Fig 2: A description of bacterial cell structures and cellular processes disrupted by the action of essential oils or their components. Cells treated with EOs become more permeable to protons which brings about an ATP imbalance, inducing the synthesis of chaperones. Metabolic pathways can be injured (Source: Faleiro, 2011). (Omp- Outer membrane protein); (QS-quorum sensing).

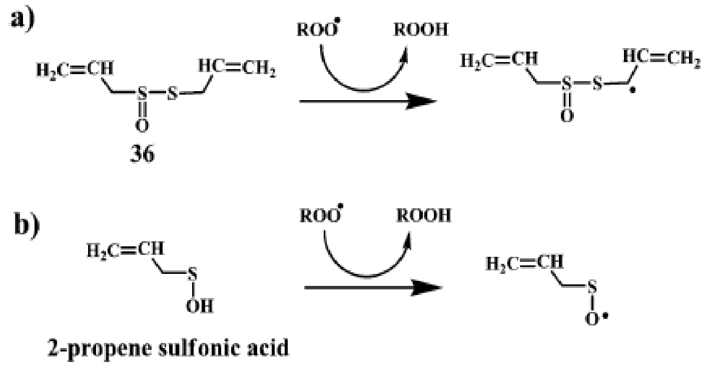


Fig 3: Mechanism for the radical trapping activity of (a) allicin and (b) 2-propenesulfenic acid

B. Antimicrobial Peptides (AMPs)/ Proteins

These are widely distributed in animals, plants and microbes as effectors of innate immunity. AMPs are usually small, amphipathic and typically carry an overall positive charge (+2 or greater) due to a relative excess of amino acids arginine and lysine (Galvez *et al.,* 2014; McDermott, 2009). The peptides show a broad spectrum of antimicrobial activity and have many additional effects on mammalian cell behaviors. Animal sources of AMPs are majorly categorized into defensins and cathelicidin (McDermott, 2009). Plants also produce a variety of AMPs, many of which can be grouped in different classes: thionins, defensins, lipid transfer proteins, cyclotides and snakins (Galvez *et al.,* 2014).

Various AMPs differ significantly in their ability to kill specific bacteria. Most of the bacteriocins are synthesized by the ribosomal and are bactericidal, with the exception of a few. The primary site of AMP action is the microbial membrane, disruption of which leads to permeabilization, loss of essential intracellular components and death. A number of different mechanisms have been proposed, but all have electrostatic interaction between the positively charged AMP and the negatively charged microbial membrane as the initial interaction. Negatively charged molecules on the outer membrane of Gram-negative bacteria include phosphate groups on lipopolysaccharide, whereas Gram-positive organisms have teichoic acids.

Nisin, an approved polypeptide (microbial derived AMP) has been proposed to act on the cytoplasmic membranes of Gram-positive bacteria to cause lesions and following nisin treatment, whole or intact sensitive cells and membrane vesicles exhibit efflux of amino acids and cations. Loss of these substances depletes proton motive force, which ultimately interferes with cellular biosynthesis. These events result in collapse of the membrane potential and ultimately cause cellular death (Jeevratnam *et al.,* 2005). Other mechanisms of bacteriocins are explained in the figure 4 below (Yang *et al.,* 2014).

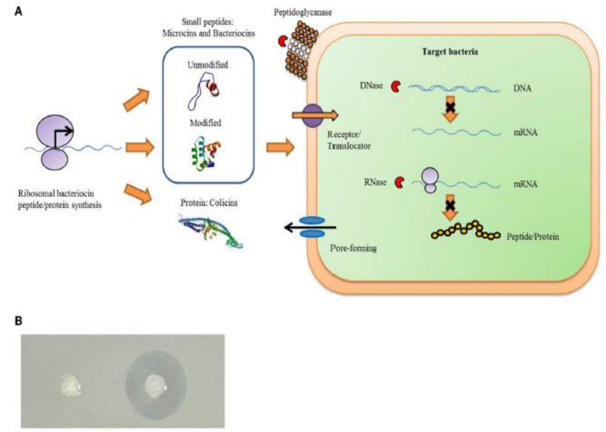


Fig 4: *Bacteriocins function as effectors of innate immunity:*

Many forms of bacteriocins are produced by Gram-positive and Gram-negative bacteria which gives them the ability to inhibit the growth of other sensitive bacteria. (A) Describes the general production process of bacteriocins and its antibacterial activity. **Bacteriocins** are peptides or proteins released by bacteriocin-producing bacteria, from the ribosomal. Sensitive bacteria, have receptors corresponding to the bacteriocins when they come in contact, hence carrying out its bactericidal activity by either using mechanisms such as the pore-forming type, nuclease type with DNase and RNase function, and peptidoglycanase type etc. Subtilisin, is an unmodified bacteriocin form of AS-48 and coilcin, structures of small molecular weight. According to Yang *et al.* (2014), these structural diagrams were taken from the protein data bank. (B) An inhibition zone (right colony) is observed when a bacteriocin-producing strain grows on a sensitive bacteria LB soft agar, which is however absent in the bacteriocin non-producing strain (left colony). (Source: Yang *et al.,* 2014)

Lysosyme also called N-acetylmuramidase or muramidase, lysozyme is a hydrolyse-type enzyme (antimicrobial protein) that catalyses the breakdown of peptidoglycan polymers of bacterial cell wall at the β 1-4 bond between N-acetylmuramic (NAM) acid and N-acetylglucosamine (NAG) residues, thereby lysing sensitive bacteria. It has been successfully purified from various plant, animal, microbial (bacteria, virus and fungi) materials (Benkerroum, 2008). Figure 4.5 describes the mechanism of lysozyme in details.

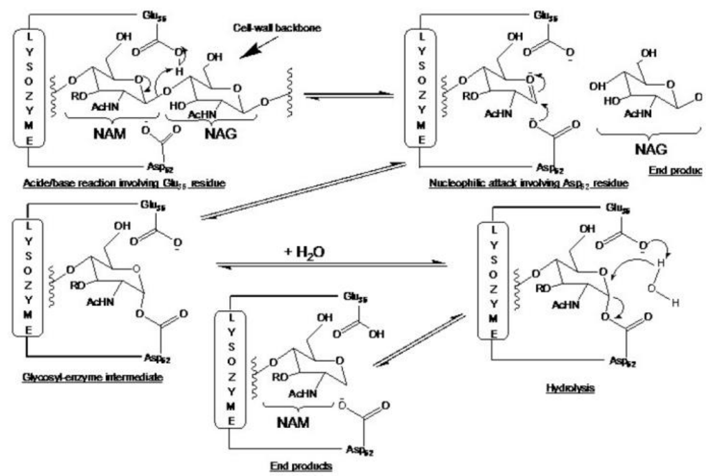


Fig 5: The mechanism of chicken egg white lysozyme in the breakdown reaction is described

C. Organic Acids

The most common classical preservative agents are the weak organic acids, for example acetic, lactic, benzoic and sorbic acid. These molecules inhibit the outgrowth of both bacterial and fungal such as cells and sorbic acid is also reported to inhibit the germination and outgrowth of bacterial spores (Brul and Coote, 1999).

The efficiency of an organic acid to inhibit the growth of a microorganism depends on its pKa value, which describes the pH value at which the acid is available 50% in its dissociated and undissociated form respectively. Only in its un-dissociated form the organic acid has its antimicrobial power as they can pass through the walls of bacteria and fungi and alter their metabolism. Preservatives have optimal inhibitory activity at low pH because this favors the uncharged, undissociated state of the molecule which is freely permeable across the plasma membrane and is thus able to enter the cell (Brul and Coote, 1999).

Therefore, inhibition of growth by weak acid has been proposed to be due to a number of actions including, membrane disruption, inhibition of essential metabolic reactions stress on intracellular pH homeostasis and the accumulation of toxic anions. In yeasts, it has also been proposed that the actual inhibitory action of weak acid preservatives could be due to the induction of an energetically expensive stress response that attempts to restore homeostasis, and results in the reduction of available energy pools for growth and other essential metabolic functions.

Citric acid is an excellent chelating agent, binding metals. The dominant use of citric acid is as a flavoring and preservative in food and beverages, especially soft drinks. As a chelator and an anti-enzymatic it blocks the natural ripening and enzymatic processes that occur.

D. Industrial Applications of Natural Preservatives

- Monoacylglycerols have increased the shelf life of various foods including soy sauce, sausages, cakes, and noodles
- Efficacy of oregano EO was comparable with chlorine as a decontamination treatment for ready-to-eat carrots
- Carvacrol, which has strong antimicrobial properties, has been used to preserve various foods, such as baked food products (up to 15.74 ppm), non-alcoholic beverages (up to 28.54 ppm/0.18mM), and chewing gum (Saad *et al.*, 2013)
- Nisin has been used to inhibit microbial growth in poultry foods such as milk, orange juice and tomato juice and for increasing the shelf life of chicken and meat without altering sensory properties of the product
- Bacteriophages specific for Salmonella serotypes have also been used *on* various food substrates such as sprouted seeds and animal skins and carcasses (e.g Salmofresh cocktail).
- Antimicrobial agents derived from essential oils are interesting candidates for development of activated films or packagings.
- Film-coating applications have been reported for meat, fish, poultry, bread, cheese, fruits, and vegetables. Chitosan are also satisfactorily used as fruit coating film.
- Ergothioneine, patented and commercialized by a German company is being used as a preservative in foods and beverages.
- Pro-food deals in commercialization of Epsilon-polylysin, a natural antimicrobial.

E. Challenges with the Use of Natural Preservatives

- In the case of essential oils, despite their great potential, their use in food preservation remains limited mainly due to their intense aroma and toxicity problems. Several authors have reported changes in the organoleptic properties of the food when these oils are used.
- The use of essential oils remains expensive, so from an economic point of view this preservation strategy needs further enhancement.
- A critical understanding of the type of spoilage and pathogenic microorganisms present in the food in relation to the antimicrobial effect of plant extracts is needed, as it is not the same for all microorganisms.
- Incorporation of plant antimicrobials in food can give rise to the growth and virulence of certain pathogens due to the changes in microbial ecology. It is critical to understand the effect of plant extracts on the behaviour of these microbial population in complex food systems.
- The growing environment, period of harvest, storage of the source plant and extraction procedures used influences the levels of active components responsible for antimicrobial activity and the sustainable supply of the antimicrobial compound. Hence, challenging its use as a functional food ingredient (Sultanbawa, 2011).
- Antimicrobial effect of plant extracts is not the same for all microorganisms
- Higher concentrations of the natural preservatives as compared to the synthetic counterparts are needed which would have significant effects on the organoleptic properties.

VII. RECOMMENDATIONS

In order to further promote the application of natural active compounds at the industrial level, it is necessary to have a good understanding of the mechanism by which antibacterial agents operate. For many natural compounds this information is still lacking. Better understanding of the modes by which antimicrobials can control microorganisms, should provide solid grounds for engineering new and upgraded derivatives with optimized potency, stability and cost of production. I recommend the following;

1. **Micro- and nano-encapsulation of active compounds-** To minimize the required doses and improve the effectiveness of active coatings enriched with essential oils, interesting options would be the use of these modes of encapsulation.
2. **Bioanalytics and Metabolomics** - The use of DNA microarray analysis on identified potential sources of natural preservatives for further elucidation of gene expression, pathways of metabolism and molecular mechanism of action of their natural active agents of preservation. This will then give us a better understanding of the modes by which antimicrobials can control

microorganisms and should provide solid grounds for engineering new and upgraded derivatives with optimized potency and stability.

3. **Combinatorial biosynthesis**- which is the combination of metabolic pathways from different micro-organisms at the genetic level, where genes from different microorganisms are combined for the production of new and interesting plant secondary metabolites. It involves engineering the biosynthetic pathway of certain natural active preservatives into a less complex host cell. However, the elucidation of the entire biosynthetic pathway is required. This technique has already been successfully reported for various plant secondary metabolites (Khan *et al.,* 2009) and could be channeled in the discovery and production of natural preservatives, in order to deal with the challenge of economic viability i.e. cost of production.

4. **Bio-engineering-** More focus on the possible use of bioreactors to enhance production of these natural active ingredients. Bioreactors, an upcoming prospect for production of secondary metabolites in medicinal plant research would also be a potential and viable tool for the large scale synthesis of natural active compounds in bio-preservation.

5. **Hurdle technology- T**he hurdle concept was introduced by Leistner in 1978 and stated that "the microbial safety, stability, sensorial, and nutritional qualities of foods are based on the application of combined preservative factors (called hurdles) that microorganisms present in the food are unable to overcome". Thus, the idea of hurdle technology is the ability to combine various preservative measures (both old and new methods) that target each spoilage mechanism used by microbes, in order to inhibit their smooth growth. In order to apply this technology wisely for effective and all round preservation, a good understanding of the mechanisms of action of a wide variety of microbial growth in food. the interaction of various preservative measures and the physiologic response of microbes to each hurdle is required. Even though the use of hurdles in food preservation is not economical for food industries, it will serve as a platform for an all round improvement in the sensory and nutritional qualities of the product, while providing microbial safety and stability. Employing hurdle technology to enhance the use of natural preservatives, might make their use more cost effective by food industries and reduce the concentrations consumed for other synthetic preservatives.

VIII. CONCLUSION

Until now, approaches to seek improved food safety have relied on the search for more efficient chemical preservatives or on the application of more drastic physical treatments (e.g. high temperatures). The proven toxicity of many of the commonest chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer trends in purchasing and consumption, with demands for safe but minimally processed products without additives, have changed the tune for industries and research.

The concept of bio preservation and bio protection of food products is gaining importance in recent years. Natural preservatives (essential oils, enzymes, bacteriocins, chitosans, and organic acids) have gained momentum in food industries, a few are being utilized as preservatives in foods and could be often valid alternatives to synthetic food additives owing to their ability to prolong the shelf life of products through antioxidant and antimicrobial activity. This has opened the way for isolation, characterization and standardization of application and methods of bio preservatives to evaluate their efficacy in extending the shelf life and improve the microbial safety of pharmaceutical and food products.

Although intensive research efforts and investment have been made, very few of these new preservation methods have been implemented by the food industry to date. A number of challenges (such as intense aroma, toxicity problems, possible organoleptic changes of the food product and high cost of production) have deterred the industry from using these naturals.

Thus, it is necessary to find a balance between the effective compound dose and the risk of toxicity, whilst understanding fully the mechanism of actions of the various natural food preservative methods. This would provide solid grounds for engineering new and upgraded derivatives with optimized potency, stability and cost of production.

The application of biopreservation technology to different food systems could be considered only as an additional hurdle to good manufacturing practices. The use of combinations of different food preservation systems, is proposed as a solution to some of the economic challenges of using natural preservatives. The proper selection of bacteriocinogenic strains for each particular type of food as well as the adequate choice of bacteriocin treatment (either alone or in combination with other hurdles) may greatly reinforce the competitiveness on pathogen biocontrol in the food industry. Employment of various recent biotechnological tools such as metabolomics and bioanalytics, combinatorial biosynthesis and improved bioreactor designs by researchers would greatly enhance the development and cost-effective use of natural preservatives in the food industries.

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