ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(2): 583-599

REVIEW ARTICLE

Preservatives in Food Products – Review

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Received 22 Jan 2011; Revised 17 Feb 2011; Accepted 13 Mar 2011

ABSTRACT

The objective of this review is to examine the available safety/toxicity literature on preservatives like olive polyphenols, microbial fermented tea, essential oil, parabens, and the micro organisms. Antimicrobial activities of microbial fermented tea are much less known than its health beneficial properties. These antimicrobial activities are generated by fermentation process with tea leaves as substrates. The essential oil of *Thymus vulgaris* L. showed highest antifungal efficacy, significant anti-aflatoxigenic efficacy, fungi toxicant and synthetic fungicidal effect. Parabens are readily absorbed through the gastrointestinal tract and are rapidly excreted in the urine. The Enterococcus genus, a member of the lactic acid bacteria (LAB) is found in the intestines of humans and other animals. Although sometimes associated with pathogenicity, these bacteria are used as probiotic cultures and nowadays extensively used for the production of bacteriocins the enterocins. This study gives a proof of using preservatives and its efficacy in food and food products.

Key Words: Toxins, Food, Safety, Preservative

INTRODUCTION:

Food is an essential thing for human survival. Except our own garden plants, all the food used today has some preservatives. Preservatives are the substances, which are used to prevent food spoilage from microorganism. Food preservation is used from the ancient times. This will inhibit the growth of microorganisms like bacteria and fungi.

Food preservatives becomes an essential thing nowadays, this plays an important role during food transportation. This will preserve the food for a long duration from the spoilage. Each and every packaged food items has some preservatives, without them the food has no longer survive. Radioactive materials like cobalt-30 are used as food preservative. Modern packaging techniques includes vacuum and hypobaric packaging also acts as preservation technique. Food preservatives aim to preserve the appearance of food, preserve the food characteristics like odor, taste and food is preserved for a long time.

The olive is the fruit of an evergreen olive tree that grows in the temperate climate of the Mediterranean region [1]. The content of phenolic compounds in olives and olive oil depends on the cultivars and the ripeness of the fruit at the time of harvest. The oil contained in olives is normally extracted by a multi-stage process, which involves crushing the whole olive (including the pits), kneading the resulting paste, collection of the free flow oil, pressure separation and collection of residual oil in the grinds, and separation of solids and vegetation water from the olive oil. Generally virgin olive oil has a free acidity, expressed as oleic acid, of not more than 2% as well as the other characteristics that correspond to those fixed for this category in this standard. Because of the strong antioxidant properties of the olive phenols, several investigators have attempted to isolate phenols from the vegetation water, but because the entire olive is macerated, the vegetation water

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contains some undesirable substances from the crushed pits.

Recently, several microbial fermented teas got noticed in the Western world, probably not only because of trade expansions between china and the west, but also because of several health beneficial claims associated with microbial fermented tea. Recently, some higher plant products viz. azadirachtin from *Azadirachta indica* [2], carvone from *Carum carvi* [3] and allylisothiocyanate from mustard [4] have attracted the attention of microbiologists to search for some newer phyto chemicals for their exploitation as antimicrobials. Such plant products would be biodegradable and safe to human health [5]. A few studies reveal that extracts from microbial fermented teas contain natural antimicrobial components that have inhibitive effect on several food-borne pathogen and spoilage bacteria [6]. Application of natural antibacterial agents has been increasingly noticed as a novel trend in biological preservation of foods in recent years. Essential oils (EOs) of some angiosperms have been investigated for their fungitoxicity against the toxigenic strain of *A. flavus* (*Navjot 4NSt*), a potent post harvest storage fungus of deteriorating cereals and pulses.

Parabens, alkyl esters of PABA, are a class of antimicrobial agents used singly or in combination to exert the intended antimicrobial affects against molds and yeasts. These substances can have multiple biological effects, but it is generally considered that their inhibitory effects on membrane transport and mitochondrial function processes are key for their actions. The parabens meet several of the criteria of an ideal preservative, in that they have a broad spectrum of antimicrobial activity, are safe to use (i.e. relatively non-irritating, non-sensitizing, and of low toxicity), are stable over the pH range, and are sufficiently soluble in water to produce the effective concentration in aqueous phase. Antimicrobial activity of paraben increases as the chain length of the ester group increases, but since solubility decreases with increasing chain length, the lower esters (methyl and propyl) are the practical choices for use in foods. Methyl and propyl parabens has been used as an antimicrobial preservative in foods, drugs and cosmetics for over 50 years. There have been several previous safety assessments undertaken on this substance by several agencies, including FAO/WHO, FDA and FEMA.

Lactic Acid Bacteria (LAB) is a diverse group of beneficial bacteria which have been inadvertently used by mankind for thousands of years. They have been used as starter cultures for preparation of an array of fermented dairy products which include yoghurt, cheese, buttermilk, kefir and many products indigenous to various regions of the world. The preparation of these products has been documented in archaic texts of various regions such as ancient Iraq [7]. The LAB has been divided into many genera, and those important in food include *Lactobacillus, Lactococcus, Enterococcus, Pediococcus, Leuconostoc and Streptococcus.* Among them the genus *Enterococcus* is of particular interest.

The members of the genus *Enterococcus* are found in many food products. They are most frequently present in many traditional European cheeses prepared mostly from raw ewes' or goats' milk, where they are thought to have initially made their way as contaminants from the feces of animals, water or milking equipment and storage tanks, and thereafter became an important component of artisanal cultures [8]. These bacteria now play a fundamental role in the ripening of these cheeses, probably by proteolysis, lipolysis and citrate breakdown, hence adding a unique taste and flavour to these products [9]. In addition to providing the peculiar taste and flavour, the *enterococci* also act as protective agents against various pathogens, such as *Listeria monocytogenes*, a common pathogen found in meat and dairy products.

Table 1: Utilization of enterococcal strains for in-situ bacteriocin production

Producing strain	Enterocin produced	Product tested	Typical target organisms	Reference
E. faecum 7C5	Undefined bacteriocin	Taleggio (Italian soft smear cheese)	*L. monocytogenes*	(Giraffa and Carminati, 1997)
E. faecium 7C5	Undefined bacteriocin	Milk	*Listeria innovua*	(Giraffe et al., 1995)
Lactococcus lactis MG 1614	Enterocin A	Cottage cheese	*L. monocytogenes*	(Liu et al., 2008)
E. faecium WHE 81	Enterocins A and B	Munster cheese	*L. monocytogenes*	(lzquierdo et al., 2009)
E. *faecum* F58	Enterocins L50A and B	Goat's milk and Jben (Moroccan goat milk's cheese)	*L. monocytogenes*	(Achemchem et al., 2006)
E. faecalis A-48-32	Enterocin AS – 48	Non-fat hard cheese	*Bacillus cereus*	(Munoz et al., 2004)
E. faecalis A-48-32	Enterocin AS – 48	Skimmed milk and Non-fat unripened soft cheese	*Staphylococcus aureus*	(Munoz et al., 2007)
E. faecium CCM 4231, *E. Faecium* RZS C13 *and Lactobacillus sakei* CTC494	Ebterocin CCM 4231, enterocin 13 and sakacin K	Spanish style dry fermented sausages	*Listeria spp.*	(Callewaert et al., 2000)
Enterococcus casseliflavus IM 416KI	Enterocin 416K1	Cacciatore (Italian sausages)	*L. monocytogenes*	(Sabia et al., 2003)
E. faecium CTC492	Enterocins A and B	Dry fermented sausages	*L. innocua*	(aymerich et al., 2000a)
E. faecium CTC492 *and L. sakei* CTC 494	Enterocins A and B and Sakacin K	Cooked pork	*L.sakei* CTC746	(Aymerich et al., 20020

IJPBA, Mar - Apr, 2011, Vol. 2, Issue, 2

2. TYPES OF FOOD PRESERVATIVES

Food preservatives may be classified as Natural, Artificial, and Microbial preservatives.

2.1. Natural Preservatives:

Natural food preservatives are good to our health. They do not harm our health. Natural preservatives are sugar, salts, vinegar and rosemary extracts. Well known preservative techniques are refrigerating, boiling, pickling, and dehydrating are used in kitchens

2.2. Artificial Preservatives:

Artificial preservatives are the chemical substance that stops the growth and activities of the microorganisms and helps to preserve the foods for a longer time without affecting its natural characteristics. It includes Antimicrobial agents and Antioxidants. Antimicrobial agents are used to prevent the action of micro organisms. Certain antimicrobial agents are benzoates, nitrites, calcium propionate, sorbates, EDTA and sodium benzoates. Antioxidants are the agents which are used to prevent the oxidation caused in the food material. Certain antioxidants are BHT, BHA, formaldehyde and ethanol.

2.3. Microbial preservatives:

Microbial preservatives are the preservatives which inhibit the growth of bacteria and fungi, or anti-oxidants such as oxygen absorbers, which inhibit the oxidation of food constituents.

3.DESCRIPTION, SPECIFICATIONS, OCCURRENCE AND SOURCE

3.1. Natural preservatives:

An aqueous olive pulp extract, is a standardized freeze-dried powder prepared as a byproduct during the processing of the pulp of olives (*Olea europaea L.*) for oil extraction. The powder has an odor of processed olives and a characteristic aromatic sour/olive flavor. The powder is composed of 98–99% dry solids, including 1–2% citric acid and 6% poly phenols. Other constituents of the extract include protein, fat and carbohydrates. The biologically important constituents of olive pulp extract are polyphenols. Among the phenolics, the major constituent of the pulp extract is hydroxytyrosol (50–70%), while other polyphenols present include oleuropein (5–10%), tyrosol (0.3%), oleuropein aglycone and gallicacid.

3.1.1. Phenols in olives:

Olive fruit is known to contain simple, as well as complex phenolic substances. These phenolics are responsible for the stability of the oil from oxidation and for the organoleptic properties [10]. In olive oil, phenols are present at levels up to 1% by weight, both as simple and complex compounds. Hydroxytyrosol and tyrosol, as well as the lipid soluble oleuropein and ligstroside aglycones, are partially released (5–10% of the total in olives) from olives into the oil during production (crushing), while the substantial proportion remains in the water phase (vegetation water).

Vegetation water is a good source of phenolic antioxidants (1–1.8% w/v), as about 90% of the phenols in olives are transferred to the water phase during the pressing of the drupes. Approximately 10–20% of the total phenol content can be recovered; the only bioactive catechol recovered is hydroxytyrosol [11]. In another study, Fernandez-Bolanos et al., [12] reported extraction of 3 kg of hydroxytyrosol (90–95% purity) from 1000 kg of olives during liquid–solid waste of two-phase (conventional) olive oil processing. Recently, CreAgri Inc., has been granted two patents for the recovery of hydroxytyrosol from olive mill water (US Patent numbers 6,416,808 and 6,197,308).

3.1.1.1. Hydroxytyrosol:

Hydroxytyrosolol, also known as 3,4-dihydroxytyrosol or 3,4-dihydroxyphenylethanol, is the major component of the phenolic fraction of olive extract and olive oil; the presence of hydroxytyrosol has also been identified and quantified in wines [13]. Hydroxytyrosol is present in olive oil either as simple phenol or esterified with elenolic acid to form oleuropein aglycone. Pure hydroxytyrosol is a clear, colorless, tasteless liquid and can be hydro soluble or lipo soluble. It is considered as the most potent (as measured by ORAC) phenolic antioxidant of olive oil.

3.1.1.2. Oleuropein:

Oleuropein is a phenolic secoiridoid glycoside found in the bark, leaves and fruit of the olive tree, as well as in some other genera of the *Oleaceae*. The most abundant phenolic substance in the drupe is a bitter glycoside that constitutes up to 14% of the fruit's dry weight.

3.1.1.3. Tyrosol:

Tyrosol , a minor component of olive oil has a faint sweet fruity-floral odor and a sweet but very weak taste.

3.1.2. Tea extract:

Both Puer tea and Fuzhuan brick-tea are microbial fermented black teas, where as Kombucha is a fermented drink of tea extract supplemented with sucrose and fermented with yeasts and acetic-acid bacteria. All three tea(s) have shown obvious antibacterial effects.

Puer tea has not only a unique flavour but also several health beneficial properties, such as suppressing fatty acid synthase expression [14], acting as an inhibitor of lipid and non lipid oxidative damage and also exhibiting metal-binding ability, reducing power, and scavenging effect [15]. Recently, anti mutagenic and antimicrobial activities of Puer tea were also reported [16]. Fuzhuan brick-tea is another kind of microbial fermented tea uniquely found in China [17].

Kombucha is a slightly sweet-sour flavoured tea beverage, obtained by fermentation of sweetened boiled tea with a mixed culture of yeasts and acetic-acid bacteria [18]. Kombucha is also frequently called ''tea fungus'' in the literature, although there is actually no fungus involved in the fermentation [19]. This beverage reportedly has a number of health benefits, against metabolic disease, arthritis, psoriasis, constipation, indigestion, and hypertension, but there are few solid scientific evidences available yet for its efficacy [20].

3.2. Artificial preservatives:

Methyl paraben is a colorless crystalline or white powder. It is odorless or has a faint characteristic odor and a slight burning taste. Methyl paraben is resistant to hydrolysis in hot and cold water but hydrolyzes in alkaline solutions. It is stable in air. Aqueous solutions of methyl paraben buffered at pH 3 and 6 showed no decomposition when heated for 2 h at 100 °C or for 30min at 120°C. Methyl paraben is an ester of p-hydroxy benzoic acid. It is produced by the methanol esterification of p-hydroxybenzoic acid in the presence of sulfuric acid. Aqueous solutions of methyl paraben, at pH 36, may be sterilized by autoclaving at 120°C for 20 min, without decomposition [21]. Aqueous solutions at pH 3–6 are stable (less than 10%decomposition) for up to about 4 years at room temperature. Aqueous solutions at pH 8 or above are subject to rapid hydrolysis (10% or more after about 60 days of storage at room temperature). Natural occurrence of methyl paraben has been reported in

cloudberry, yellow passion fruit juice, white wine, botrytised wine and Bourbon vanilla. The amount of methyl paraben detected in cloudberry is around 0.15 ppm [22].

Propyl paraben is a stable colorless crystalline or white powder with practically no odor or taste. Propyl paraben is an ester of PABA and is produced by esterifying PABA with n-propanol, using an acid catalyst such as sulfuric acid and an excess of propanol. It is stable in air and is resistant to hydrolysis in hot and cold water, as well as in acidic solutions. Above pH 7, appreciable hydrolysis occurs.

Table 2: FDA approved uses of methyl and propyl parabens in food (21 CFR, 2000)

Source	Citation number	Food category	Permitted functionality	Use limits
FDA	21 CFR 150.141 Food Standards. Artificially sweetened fruit jelly, Optional ingredient	Artificially sweetened	Preservative	Not to exceed 0,1%
FDA	21 CFR 150.161 Food Standards. Artificially sweetened fruit jams. Optional ingredient	Artificially sweetened fruit preserves and jams	Preservative	Not to exceed 0.1%
FDA	21 CFR 172.515 Food additives. Permitted for direct addition to agents and food for human consumption. Subpart F-Flavoring agents and related substances. Synthetic flavoring substances and adjuvants	No restriction	(12) Flavoring agents and adjuvants	CGMP[a]
FDA	21CFR 181.23 Prior sanctioned ingredients. Certain substances employed in the manufacture of food – packaging materials. Antimycotics	No restriction	(12) Antimicrobial	CGMP[a]
FDA	21 CFR 184.1490 Direct food substances affirmed GRAS.	No restriction	(2) Antimicrobial agent	CGMP[b] 0.1%
FEMA	FEMA GRAS No. 2710	No restriction	Preservative	0.1%

3.3. Microbial preservatives:

With the discovery of bacteriocins, the use of *enterococci* as starter cultures or co-cultures has been studied by various researchers not only for their organoleptic properties, but also for their negative effect on food pathogens by production of enterocins [23], as shown in the table 1. If the bacteriocin producing *enterococcal* strain has properties that make it suitable for use as starter culture such as high acid and flavour production, then it offers a double advantage of being used as sole culture for both fermentation and food preservation However, if the enterocin producing strain is being used along with another starter culture, then it should not interfere with the acid and flavour producing properties of the starter strain, otherwise the preservative effect of the enterocin will be nullified by a low quality product.

4. APPLICATIONS

Preservatives are natural, artificial and microbial are used to maintain the shelf life and characteristics of various food items. Methods of preserving foods have been used for centuries and include natural techniques such as smoking fish and meat as well as adding salts. Refrigerating and freezing food items also falls under natural ways of preserving foods. Food preservatives can also enhance the appearance of food items as well as add nutritional value.

4.1. Economical:

There is no such thing as a broad spectrum natural preservative. However, there are natural items that can have preservative qualities. These can reduce the microbial activity (such as essential oils).

The olive fruit, its oil and the leaves of the olive tree have a myriad of medicinal and other uses. Primarily, olives are used for their oil or as table olives and are an important part of the Mediterranean diet. Because of their organoleptic characteristics, olives require processing prior to consumption [24]. In both fruit and oil, the phenolics constitute a complex mixture, although there are some notable differences in composition between the two that are attributed to a series of chemical or enzymatic alterations of some phenols during oil extraction. These modifications include hydrolysis of glycosides by glucosidases [25], oxidation of phenolic compounds by polyphenol oxidases and, the polymerization of free phenols [26]. The quality of virgin oil is affected by the presence of phenolic compounds in olive fruits, as these compounds are partly responsible for the stability and sensory characteristics.

Parabens is widely used as an antimicrobial preservative in cosmetics, food products and pharmaceutical formulations. It may be used either alone, in combination with other parabens, or with other antimicrobial agents. In cosmetics, methyl paraben is the most frequently used antimicrobial preservative.

4.2. Cosmetics:

The cosmetic products are basically the derivatives of either suspensions or emulsions. Both the natural and microbial preservatives have not ability to survive in the either suspensions or emulsions. Hence both the natural and microbial preservatives are not preferred in the cosmetic products. Parabens are widely used cosmetic preservatives present in a large variety of products, including face, body and hand creams, lotions and moisturizers; eye makeup products; foundation and other makeup products; night creams and lotions; cleansing products; hair conditioners; bubble baths; shampoos; mud packs; underarm deodorants; skin lighteners; and sachets [27]. Methyl and propyl parabens are the most commonly used preservatives in cosmetics [28]. Parabens are found in all types of formulations and have a use in over 13,200 formulations [29]. Concentrations of parabens are usually less than 0.3%, with the most common preservative system containing 0.3% methyl paraben and 0.1% propyl paraben but may range up to 1%. Parabens formulate well because they have no perceptible odor or taste, are practically neutral, do not produce discoloration, and do not cause hardening or ‘‘muddying’’ [30].

The popular use of paraben preservatives in cosmetics and toiletries arises from their low toxicity, broad spectrum of activity, worldwide regulatory acceptance, biodegradability, and low cost. Other advantages of parabens are found in their low tendency towards absorption in commonly used plastics of primary packaging material.

Taking advantage of each paraben’s solubility characteristics, various concentrations of methyl paraben and propyl paraben can be added to the base’s water and oil phases, respectively. These favorable characteristics make the combination of methyl paraben and propyl paraben the most frequently used preservative system [31].

According to the industry’s voluntary submissions to the FDA in 1981, the number of product formulations and maximum use concentrations for methyl, propyl parabens were 6606, 5868 and 25%, respectively [32]. Commonly, formulations contain parabens in concentrations up to 1%. Methyl paraben individually or with other parabens is used in all 13 product formulation categories. Products containing these ingredients may contact the skin, hair and scalp, lips, mucosa (oral, ocular and vaginal), axillae and nails. Products containing parabens may be used on an occasional or daily basis and their use may extend over a period of years. Frequency of application and duration of exposure may be continuous. Methyl paraben is the most used preservative in cosmetics [33]. In an investigation of 215 cosmetic products, a maximum of 0.32% methyl paraben and 0.32% propyl paraben were present in paraben positive cosmetics. Of all paraben-containing cosmetics tested, 98% contained methyl paraben.

4.3. Food and food products:

Because of artificial microbial preservatives have low toxicity and effective antimicrobial activity, parabens, including methyl paraben, have been used in food for more than 50 years. Under FDA regulation, methyl and propyl parabens are generally recognize as safe (GRAS) when used as chemical preservatives in foods, with a use limit of 0.1% (21 CFR 184.1490). Types of food that may contain parabens include alcoholic beverages, frozen dairy products gelatins, grain products, jams, jellies, marmalades, mincemeat, olives, pickles, relishes, preserves, processed fruits and vegetables, tomato pulp, tomato puree, catsup, fruit juices, soft drinks, puddings, seasonings, soft candy, sugar substitutes, syrups and sweet sauces [34]. Parabens are used in coffee extracts, fruit juices, pickles, sauces, soft drinks, processed vegetables, baked goods, seasonings, sugar substitutes and frozen dairy products at concentrations of between 450 and 2000 ppm [35]. In a LSRO/FASEB [36] report it is stated that methyl paraben is not reported to be used in fats and oils, processed fruits, or alcoholic beverages. Higuera-Ciapara and Nieblas [37] reported the use of hydrogen peroxide- methyl paraben for preservation and stability of corn tortillas held at room temperature.

Methyl paraben is also permitted for use in certain standardized foods including artificially sweetened fruit jelly (21 CFR 150.141) and artificially sweetened fruit preserves and jams (21 CFR 150.161) at a level not to exceed 0.1% by

weight of the finished food and are mentioned in table 2. Methyl paraben, propyl paraben and butyl paraben are permitted as direct food additives for use in synthetic flavoring substances and adjuvants in the minimum quantities required to produce their intended effects (21 CFR 172.515). As indirect food additives, methyl paraben and propyl paraben are permitted by prior sanction as antimycotics in food packaging materials with no limit or restriction (21 CFR 181.23).

In addition to the in-situ production, purified or semi purified forms of enterocins have also been explored as preservatives in various foods, especially in non fermented products. *Enterocin* AS-48 appears to be the most promising candidate in this regard as evident. It is the first enterocin which was purified to homogeneity and characterized, and is the most extensively studied enterocins. Similarly enterocins A and B have been tested as food preservatives especially in meat products due to their strong anti-listerial activity.

There is an increasing trend nowadays to consume ready-to-eat fruit and vegetable products, with minimal processing and preservatives. However, the consumption of such products is not without risk, especially the products which have high pH are more prone to contamination by pathogens. A number of disease outbreaks have been reported due to the consumption of such products [38]. Use of *enterocin* AS-48, as a safe, natural antimicrobial offers a good alternative to conventional preservatives to prevent the risk of food borne outbreaks [39].

A series of experiments by Grande et al. [40] investigated the stability of *enterocin* AS-48 in fruit and vegetable juices. The results indicated that a variable loss in antimicrobial activity occurred when a concentrate of *enterocin* AS-48 was diluted 10 times in selected fruit and vegetable juices. Among the vegetables, no loss in residual activity occurred in cabbage juice, whereas some loss of activity occurred in celery juices (85.71% of control treatment). This was followed by pumpkin, egg plant, lettuce, spinach, leek, green bean, and avocado juices (from 40.05 to 57.14% of control treatment), with endive and asparagus juices showing the lowest activity. However, doubling the bacteriocin concentration revealed some bacteriocin activity (9.16 to 26.66%) in broccoli, tender onion, and broad bean, cucumber, and red pepper juices.

The genus *Bacillus* and other related genera such as *Paenibacillus* and *Alicyclobacillus* consist of heat resistant endospore forming bacteria. Though not pathogenic have often been involved in food spoilage and, therefore, need to be controlled. *Enterocin* AS-48 has been found not only to successfully control the vegetative cells of *Bacillus spp*. but also greatly increases the heat sensitivity of endospores, thereby reducing the time and temperature of heat treatment during processing. This can also improve the quality of the food products which may be affected due to intense heat treatment.

The endospores of *Alicyclobacillus* spp. are thermo acidophilic and normal heat treatments applied during processing of fruit and vegetable products are insufficient for their complete destruction. They, therefore, grow during storage and are responsible for spoilage causing off-flavours in the product [41]. The potential of *enterocin* AS-48 to control the vegetative cells and spores of *A. acidoterrestris* DSMZ 2498 strain was tested in freshly prepared or commercial fruit juices. The enterocin was added at a rate of 2.5 µg/ml to freshly made apple and orange juices.

In addition to the spore forming bacteria, the efficacy of broad range enterocin AS-48 has been investigated against many other *Gram-positive* and *Gram-negative* bacteria in different vegetable and fruit products. *L. monocytogenes* has been an organism of concern found as a contaminant in many vegetable products, causing food poisoning. To explore the effect of *enterocin* AS-48 on this pathogen, fresh alfalfa sprouts, soybean sprouts, and green asparagus were artificially inoculated with *L. monocytogenes* CECT 4032 before treatment with different concentrations (5, 12.5 or 25 µg/ml) of *enterocin* AS-48. The samples were stored at 6, 15 or 22 °C. The results revealed that at 6 and 15 °C, the pathogen remained below detection levels for 5 days at all concentrations of bacteriocin tested in contrast to the control where the counts were always above detection limits. At 22°C *L. monocytogenes* was not completely inhibited, however, the counts were still significantly lower (Pb: 0.05) as compared with the control. The studies on the synergistic effect of *enterocin* AS-48 along with other treatments have

been further extended to control Gram-negative bacteria in vegetable products. This effect was investigated in soybean sprouts treated with selected species of *enterobacteria* and tested for synergism. When tested against *S. enterica*, no significant reduction (Pb: 0.05) was found in samples treated with AS-48 alone. In contrast the combined treatment of heating at 65°C, 25µg/ml enterocin and alkaline pH (9.0), significantly (Pb: 0.05) reduced the viable counts during storage at 15 °C for 48h. Similarly combined treatment of *enterocin* AS-48 with of the selected preservatives (lactic, polyphosphoric and peracetic acids, sodium hypochlorite, hexadecyl pyridinium chloride and hydrocinnamic acid) significantly reduced (Pb: 0.05) the *S. enterica* counts during storage at 15 °C for at least 48h.

Milk is considered as a complete food and an excellent medium for the growth of various types of microorganisms as well. Therefore, during production and transport many microbes find their way as contaminants into milk, and these microbes often include pathogens such as staphylococci and *Listeria spp*. These micro-organisms may survive due to poor processing or during preparation of such products as raw milk cheese, and therefore can cause food poisoning outbreaks [42]. The use of enterocins in dairy products can provide a natural hurdle in the growth of microorganisms. The enterocins can either be produced in-situ by the addition of enterocin producing cultures in the dairy products, or by the addition of crude or purified preparations.

In addition to dairy products, other products of animal origin i.e., meat, poultry and sea foods, are also highly prone to microbial growth because of pH and nutrients. The microbes may range from spoilage organisms to food pathogens such as *E.coli*, *Salmonella* spp., *Campylobacter* spp. and *L. monocytogenes* [43]. Due to the presence of such organisms in meat products, many food borne outbreaks have occurred [44]. Therefore, the use of enterocins in the preservation of meat, poultry and fish products has potential especially as nisin is not effective as a preservative in meat products and it also has reduced activity against *L.monocytogenes* [45]. The use of broad-spectrum enterocins such as AS-48, or the strongly anti-listerial enterocins such as A and B have potential in meat preservation. The effectiveness of *enterocin AS-48* has been tested against *L. monocytogenes* and *S. aureus* which were artificially inoculated (103 cfu/g) in model sausages in independent trials. The enterocin was either added in a semi purified form or the producer strains were inoculated for in-situ enterocin production during preparation of model sausages. The results indicated that approximately 40 µg/ml of crude bacteriocin preparation was required for complete inhibition of the pathogens during storage period of 9 days. The addition of producer strains (107 cfu/g) during preparation of model sausages also helped in controlling the growth but complete inhibition could not be achieved [46].

4.4. Pharmaceutical applications:

Parabens were first employed as preservatives in pharmaceutical products in the mid-1920s [47]. Parabens have been incorporated as preservatives in a variety of drug formulations. Combinations of parabens are more active than individual parabens [48]. Parabens are or have been used in suppositories, anesthetics, eyewashes, pills, syrups, weight gaining solutions, injectable solutions, and contraceptives. Use concentration varies from product to product but seldom exceeds 1%. Methyl paraben is used in injections (0.065–0.25%), ophthalmic preparations (0.015–0.05%), oral solutions and suspensions (0.015–0.2%), topical preparations (0.02–0.3%) and vaginal preparations (0.1–0.18%) [49]. Golomb and Shipigelman [50] described the advantages of using parabens, which make them suitable for imparting antibacterial properties to implanted biomaterials.

Methyl and propyl parabens are used in a number of over-the-counter (OTC) drugs (21 CFR310.545). The Ophthalmic Drug Panel of FDA's Bureau of Drugs has determined that these two ingredients, if used alone, are unsuitable as preservatives in OTC ophthalmic products because they are irritating to eyes if used at concentrations effective against micro-organisms. Other OTC panels have concluded that methyl paraben is a safe and effective preservative in concentrations of 0.1–0.2% in products for anorectal application and other antimicrobial uses [51].

5. DISCUSSION

5.1. Estimation methods of food preservatives:

A range of different procedures are available for estimating the dietary intake of food preservatives [52]. The various methods are summerised in (**Table 3**).

Table 3: Comparison of different methods for estimating intake

Method	Cost	All dietary sources of exposure covered	Reaction with food matrix allowed for	Analysis of samples required	Intake data on individuals provided
Per-capita	Low	No	No	No	No
Food diary	Medium	Possibly	Yes	No (literature data)	yes
Food frequency	Medium	Possibly	Yes	No (literature data)	Yes
Total diet	High	No	Yes	Yes	No
Duplicate diet	High	Yes	Yes	Yes	Yes
Biomarker	High	Yes	Yes	Yes	Yes

5.1.1. Per-capita method:

In this approach, information is obtained from the food preservative and food manufacturing industries on yearly production or usage of the preservative. This value is then divided by the number of people in the population. The main advantage of the per-capita technique is that it is a very cost-effective means of obtaining estimates of average intake arising from preservative usage.

5.1.2. Food diary records

In this technique, individuals keep a record of all the food items eaten during a study period, which is typically up to 10 days. This food consumption data is then combined with the expected concentrations of the preservative in each foodstuff and the resulting daily intake calculated. A number of variations on this theme are possible. The concentration of the preservative in each food item may be estimated on the basis of information provided by the food manufacturer or from the results of analytical surveys in the literature.

5.1.3. Dietary recall and food frequency

Dietary recall studies are retrospective and involve establishing preservative intakes on the basis of individuals' recollection of intake of specific food items. As with the food diary record approach, these data are then combined with expected concentrations of the preservative to estimate daily intake. This again is combined with analytical data on the concentration of the preservative in the target food stuffs and dietary intake calculated.

5.1.4. Total diet study:

In this approach the types and quantities of food that make up the average British diet are calculated from the National Food Survey (Peattie *et al.*, 1983). The major items are prepared as if for consumption and then amalgamated into 20 separate food groups, e.g. bread and cereals, fish, milk, etc. Each group is chemically analysed and the average daily intake of the preservative calculated. The technique has the advantage that the information produced is derived from real food stuffs prepared in a manner reflecting consumer practice.

5.1.5. Targeted surveys:

In this method, analytical surveys are conducted on those food items which are known to contain the preservative of interest. This information may then be combined with data on national food consumption figures to provide an estimate of the average intake of the preservative. Alternatively, the analytical data may be utilized in combination with more detailed information, available from recent comprehensive studies on dietary habits of individual adults and children to provide an indication of actual intake.

5.1.6. Duplicate diet studies:

In this approach duplicate portions of each component (or occasionally just particular items) of an individual's diet are purchased and prepared as for consumption. The duplicate samples are then analyzed to provide and has the particular advantage of reflecting actual intake but is necessarily very expensive.

5.1.7. Biomarker-based methods:

The application of biomarkers to the intake estimation of preservatives, and other food chemicals, is an area of considerable current interest. The principle of the technique involves measuring the compound of interest, or one of its metabolites, in a body fluid such as blood or 24 h urine samples. Initially, developmental work is required to establish the quantitative relationship between the daily dietary intake of the compound and its biomarker, e.g. the amount of the biomarker excreted in urine in the following 24 h. Once this is known the concentration in a 24 h urine sample may be used to estimate the dietary intake of the compound in the preceding 24 h.

This approach may then be employed in surveillance studies by collecting 24 h urine samples from the target population, measuring the biomarker concentration in each individual's sample and thence calculating dietary intake. Biomarker-based methods have been widely used to assess occupational exposure to potentially harmful chemicals in the work place [53].

5.2. Analytical methods:

Many analytical methods have been reported for the determination of preservatives, including spectrophotometric methods [54], gas chromatography [55], HPLC [56], ion chromatography [57], HPTLC-UV, Fourier Transform Near-Infrared (FT-NIR), TLC and micellar electro kinetic capillary chromatography. The chemometric methods are an effective way to analyze simultaneously several analytes [58].

There is sufficient qualitative and quantitative scientific evidence to determine the safety-in-use, i.e., the acceptable daily intake (ADI) for aqueous pulp extract. Ordinarily, ADIs are derived by applying an uncertainty factor to the no effect level in an animal study. Based on the NOAEL of 2000 mg/kg/day from a 13-week study in rats and also from a maternal and developmental toxicity study, and applying uncertainty factors of 10 for interspecies differences and 10 for interspecies differences, an ADI for ingestion of olive pulp extract by humans can be determined. The overall uncertainty factor of 100 was judged appropriate, based on the considerations of animal studies and the fact that constituents of the extract are frequently consumed from food. Application of the uncertainty factor of 100 to the NOAEL of 2000 mg/kg/day yields a safe intake for humans of 20 mg of olive pulp extract /kg per day or 1200 mg/day (for an adult weighing 60 kg).

In summary, based on a critical evaluation of the available human, animal, analytical, and other scientific studies, and a history of exposure and use of components of aqueous olive pulp extract through table olives, olive products and olive oil, the consumption of the extract is considered safe at levels up to 1200 mg/day.

The tea catechins or polyphones have played the role of inhibition of microbial growth. This doubt can be excluded by the fact that the antibacterial activity increases with the fermentation time; namely, the longer the fermentation is, the more antibacterial activity the sample has. Nevertheless, Chou, Lin, and Chung reported that conventional fermentative processed teas have decreased antimicrobial activity with the fermentation time and this implies that the originally present tea catechins or polyphenols lose their antimicrobial activity during the enzymatic oxidation. In their study, *B. subtilis*, *E. coli*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Salmonella spp.* and *S. aureus* were used to test the antimicrobial activity of various tea extracts. Among the six test microorganisms, *P. fluorescens* was the most sensitive to the extracts, whereas *B. subtilis* was the least sensitive. Extract of green tea, the unfermented or non-oxidized tea, showed the strongest antimicrobial activity followed by the partially fermented tea products such as black tea, the completely fermented (oxidized) tea, showed the least antimicrobial activity. It was also noted that extracts of oolong tea prepared in summer exhibited the strongest antimicrobial activity, followed by those prepared in spring, winter and fall [59].

Mo et al. [60] have done a microbiological analysis on samples from the indigenously fermented Puer tea. Microbial counting and identification revealed that A. *niger* was the dominating microorganism during the fermentation. Antimicrobial activity of fermentation samples shows inhibitory effect on several food-borne bacteria, including spore-forming bacteria *Bacillus cereus*, *Bacillus subtilis*, *Clostridium perfringens* and *Clostridium sporogenes.*

In a similar manner to studying Puer tea, we analyzed the microbiological composition and tested the antimicrobial activity of extracts from the indigenously fermented Fuzhuan brick-tea (unpublished data). Microbial counting and identification revealed that *Aspergillus* spp., *Penicillium* spp. and *Eurotium* spp. were the main microorganisms isolated from the samples during fermentation and *Eurotium spp*. was the dominating fungus during the fermentation. Antibacterial tests of extracts of fermented tea showed inhibitory effect on several food-borne bacteria, including spore-forming bacteria *B. cereus, B. subtilis, C. perfringens and C. sporogenes*.

The antimicrobial activity of Kombucha was tested against a number of pathogenic microorganisms. *Staphylococcus aureus, Shigella sonnei, Escherichia coli, Aeromonas hydrophila,*

Yersinia enterolitica, Pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus epidermis, Campylobacter jejuni, Salmonella enteritidis, Salmonella typhimurium, B. cereus, Helicobacter pylori, and *Listeria monocytogenes* were found to be sensitive to Kombucha. According to the literature on Kombucha, acetic acid is considered to be responsible for the inhibitory effect towards a number of microbes tested. However, kombucha exerts antimicrobial activities against *E. coli, S. sonnei, S. typhimurium, S. enteritidis,* and *C. jejuni*, even at neutral pH and after thermal denaturation of Kombucha. This finding suggests the presence of antimicrobial compounds other than acetic acid or large proteins in Kombucha.

Among the tested Bacillus bacteria, tea polyphenols show antibacterial effects towards *Bacillus stearothermophilus,* which is a thermophilic spore-forming bacterium. The heat resistance of *B. stearothermophilus* spores is reduced by the addition of tea polyphenols. *Clostridium thermoaceticum*, an anaerobic spore-forming bacterium, also exhibits reduced heat resistance of its spores in the presence of tea polyphenols. Epigallocatechin gallate, a main component of tea polyphenols, shows strong activity against both *B. stearothermophilus and C. thermoaceticum.*

The fungitoxicity of isolated Essential Oils (EO) was tested against the toxigenic strain of *A. flavus* following poisoned food technique [61] using potato dextrose agar (Himedia Ltd., Mumbai) as nutrient medium are summerised in table 8. Requisite amounts of the oil dissolved separately in 0.5 ml of 5% tween-20 were pipette aseptically to different pre-sterilised Petri plates (100 mm diameter) containing 9.5 ml of PDA medium so as to procure the requisite concentration of 1.0 and 1.5 µl ml−1 . For control set requisite amount of sterilized distilled water in place of oil was added to the medium. A fungal disc (5 mm diameter) of the toxigenic strain of *A. flavus*, cut from the periphery of seven days old culture with the help of a cork borer, was inoculated aseptically to the centre of the poured Petri plates of treatment and control sets. The plates were incubated at 27±2 °C for seven days. Diameter of fungal colonies of treatment and control sets was measured. The percentage mycelial inhibitions were calculated by the mean value of colony diameters by the following formula [62] and were presented in(**Ttable 4**).

Percentage of mycelial inhibition = $\frac{dc-dt}{dc} \times 100$

Where, dc = average diameter of fungal colony in control set

dt = average diameter of fungal colony in treatment set

Table 4: Antifungal screening of some higher plant essential oils of different family against the toxigenic strain of *A. flavus*.

Plant name	Family	Antifungal activity	
		1.0µl ml^{-1}	1.5µl ml^{-1}
Aegle marmelos	*Rutaceae*	70.53±2.56	83.36±2.11
Ageratum conyzoides	*Asteraceae*	83.93±3.05	95.30±2.02
Artemisia vulgaris	*Asteraceae*	25.33±1.61	30.73±1.44
Callistemon lanceolatus	*Myrtaceae*	66.83±2.84	81.66±2.54
Citrus reticulate	*Rutaceae*	22.90±2.02	28.63±2.22
Commiphora mukul	*Useraceae*	60.40±2.34	73.43±2.60
Curcuma longa	*Zingiberaceae*	81.63±2.86	91.53±1.69
Eucalyptus citriodora	*Myrtaceae*	67.30±2.82	81.732.28
Eupatorium connabinumlamiaceae	*Asteraceae*	62.00±3.43	81.86±2.33
Hyptis suaveolens	*Lamiaceae*	91.63±2.39	100.00±0.00
Murraya koenigii	*Rutaceae*	35.06±1.92	52.36±2.65
Thumus vulgaris	*Lamiaceae*	10.00±0.00	100.00±0.00
Ocimum gratissimum	*Lamiaceae*	90.43±1.62	95.30±1.80
Zingiber officinalis	*Zingiberaceae*	87.63±2.07	93.70±3.40

To find out the minimum inhibitory concentration (MIC) at which the thyme oil showed absolute fungi toxicity, experiments were carried out by the usual poisoned food technique [63]. Different concentrations of the oil viz. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 µl ml were prepared by dissolving separately its requisite amount in 0.5 ml 5% tween- 20 and then admixing with 9.5 ml of PDA. The inoculated Petri plates were incubated for seven days at 27± 2 °C. The nature of toxicity (fungi static/fungicidal) of the essential oil was determined following Kumar et al. [64]. The inhibited fungal discs of oil treated sets were re-inoculated on fresh medium after washing with distilled water and revival of their growth was observed.

The spectrum of fungi toxicity of thyme oil was evaluated against eight fungi *viz., F. oxysporum, C. herbarum, C. lunata, A. terreus, A. niger, A. fumigatus, A. alternata and B. theobromae* at its MIC i.e. 0.7 µl ml-1 by the usual poisoned food technique on PDA as nutrient medium.

The safety limit of thyme EO was determined by recording LC50 value on mice following Srivastva and Saxena [65]. Mice (*Mus musculus L.*) with an average weight and age (35 g, 3months) were selected as test animal for the mammalian toxicity experiments. Requisite amount of thyme EO was mixed properly with tween-80 and distilled water (2:1) to prepare different solutions containing desired dose of oil from 100–300µl. 0.5 ml of each solution of EO was orally administered separately through a syringe with catheter to each set containing 12mice. In control sets equal volume of stock of tween-80 and distilled water (2:1) was given to mice. After 72 h, the mortality of the animals was recorded and LC50 was calculated in terms of per kg body weight of mice.

Methyl paraben and propyl paraben have been affirmed by the FDA as GRAS for direct addition to food (21 CFR 184.1490) in concentrations up to 0.1% and by prior sanction as indirect addition via packaging materials (21 CFR 181.23). Both compounds are used to inhibit molds and yeasts in various foods. They are active against Gram positive and a few Gram negative organisms. In addition to FDA, the Joint AO/WHO Expert Committee on Food Additives (JECFA) and the Flavor and Extract Manufacturer's Association (FEMA) has also approved the use of methyl and propyl parabens in foods mentioned in (**Table 5**) with a possible average daily intake of 0.237mg. JECFA in 1974 recommended that the group ADI for the methyl, ethyl and propyl esters of p-hydroxybenzoic acid was 0–10 mg/kg body weight/day. Usual and maximum use levels of methyl paraben as FEMA food flavor ingredients are given in Table 5. The FEMA reported possible average daily intake (PADI) of methyl paraben is 0.22 mg.

Table 5: FEMA use levels for methyl and propyl paraben

Food category	Usual use level (ppm)		Maximum use level (ppm) Methyl Paraben	
	Methyl Paraben	Propyl Paraben		Propyl Paraben
Baked goods	0.99	0.97	0.99	0.97
Fats and oils	-	0.32	-	1.00
Frozen airy	0.00	0.06	0.01	0.08
Fruit juice	-	0.10	-	0.10
Processed vegetables	1.00	1.00	1.00	1.00
Soft candy	0.00	0.06	0.01	0.08
Gelatin and pudding	0.00	-	0.00	0.01
Non-alcoholic beverages	-	-	0.01	0.01
Alcoholic beverage	0.00	-	-	0.02
Milk Products	0.50	-	0.00	-
Cheese	0.00	-	0.00	-
Meat Products	0.00	-	0.00	-
Sugar substitutes	0.00	0.25	0.50	0.025

Use of paraben, including methyl paraben, is covered by an EU Directive, the ''European Parliament and Council Directive No. 95/2/EC of February 20, 1995 on food additives other than colors and sweeteners". Preservation of cosmetic products with methyl-, ethyl-, propyl-, butyl- and benzyl paraben, with a maximum concentration of 0.8% (w/w), calculated as p-hydroxybenzoic acid is permitted by the Danish and EEC regulations [66]. Parabens may be present in some cosmetics labeled as ''hypoallergenic''. Several other countries, including Canada, Japan, Norway, the Philippines, Sweden and Switzerland, have also approved the use of parabens as antimicrobial food additives. In Italy, methyl paraben may be used as a direct food additive.

The parabens are effective in acid, neutral and slightly alkaline solutions. Beyond pH 8, hydrolysis can occur and this reduces preservative efficiency [67]. Parabens are known for their inhibitory action on microbial growth [68]. Spore germination is much more susceptible to parabens than vegetative growth in fungi or bacteria [69].

Parabens, including methyl paraben, have been found effective in low concentrations against fungi and bacteria. Parabens are more active against fungi than bacteria and are more active against gram-positive bacteria than gram-negative bacteria. The antifungal and antibacterial properties of methyl paraben against a number of strains are summarized in the Final Report on the Safety Assessment of Methyl, Ethyl, Propyl and Butyl Parabens. Microbiological activity of methyl paraben against different microbes is summerised in (**Table 6**).

Table 6: Inhibitory concentrations of parabens

Organisms	Concentration
MIC Value a	
E. coli	2000
S. aureus	2000
P. aeruginosa	4000
MIC value b	
E. coli	800
P. aeruginosa	1000
MIC (giving 50% inhibition of growth and uptake process) Concn (mM)	
E. coli ML308-225	5.5
P. aeruginosa ATCC 9027	3.6
B.sublitis ATC6633	4.3
Inhibitory concentrations (µg/ml)	
C. albicans	1000
S. cerevisiae	1000
Trichophyton spp.	160
Penicillium spp.	500
Aspergillus spp.	600
Lethal concentrations (µg/ml)	
C. albicans	5000
P.Chrysogenum	5000
A. Niger	5000
MIC value (µg/ml)	
A. oryzae	600
Trichoderma lignorum	250
ACTT 8678	
Sarcena hutea	4000
Enterobacter cloacae ATCC 23355	1000
Proteus vulgaris ATCC 8427	2000
MIC values[c] (µg/ml)	
E.coli DC0	1400
E. coli DC2 (envelop defective)	1000
P. aeruginosa 799	1800
P. aeruginosa 799/61 (envelop defective muatant)	1000

Enterocins A and B can effectively check the growth of *L. monocytogenes* when incorporated in biodegradable films (alginate, zein and polyvinyl alcohol). When incorporated at a concentration of 2000 arbitrary units/cm2 (AU/cm2) with air packaging they effectively checked the growth of *L. monocytogenes* in cooked ham for 8 days. With vacuum packaging the effectiveness was further increased showing no increase from inoculated levels of *L. monocytogenes* during15 days of refrigerated storage [70]. Enterocin 416K1, produced by E. *casseliflavus IM 416K1* when coated to a LDPE (low density polyethylene) film effectively controlled a listerial strain, *L. monocytogenes NCTC 10888*. The enterocin coated film effectively decreased the listerial counts during first 24h, and the trend was maintained for two weeks at refrigerated storage and for longer times at higher temperatures. A different trend was observed when fresh soft white cheese was packed in film coated with enterocin 416K1. The pathogen counts were 1 log unit lower than the control for up to 28 days under refrigeration, but only about a week at 22°C, with the control treatment decreasing by 0.5 log units after 28 days [71].

6. SUMMARY:

Based on a critical evaluation of the available human, animal, analytical, and other scientific studies, and a history of exposure and use of components of aqueous olive pulp extract through table olives, olive products and olive oil, the consumption of the extract is considered safe at levels up to 1200 mg/day. With the trend of increasing use of natural and biological preservatives in food products, natural antimicrobial agents from microbial fermented tea may offer an innovative and interesting measure for such applications. However, a breakthrough in this field can only be realized after several critical aspects are clarified for the application of these potential, novel and natural antimicrobial substances from microbial fermented tea. The present review describes some unique microbial fermentation of tea and the antimicrobial activities formed during the fermentation process.. On the basis of antifungal as well as anti-aflatoxigenic activity, broad spectrum fungitoxicity, superiority over some prevalent synthetic fungicides and non-mammalian toxicity, the thyme essential oil would thus be recommended as a plant based ideal preservative for enhancement of shelf life of stored food commodities. A thorough examination of the scientific literature indicates that parabens are of a low order of both acute and chronic toxicity. Other parameters of safety assessment are also negative. Parabens have been approved for use in food over the past three decades by the US Food and Drug Administration (FDA), several foreign governments, the European Community and the joint FAO/WHO Expert Committee on food additives. It has also been generally recognized as safe GRAS) by the flavour and extract manufacturers association (FEMA). As a result of this nearly universal acceptance of the safety of parabens, they are used in a variety of foods and in a range of concentrations, but overall consumption of methyl paraben is 55 mg/kg/day and propyl paraben is 1.3 mg/kg/day. Use of enterocin producing cultures does not provide adequate preservation or is not feasible as in non-fermented products, then the alternative option is the production of enterocins ex-situ and the inclusion of their purified or semi purified forms in the food products to act as food preservative. The enterocins can be added either directly in foods or incorporated into edible or non-edible antimicrobial films. However, the use of purified or semi purified preparations of bacteriocins as food preservatives have legal implications. Despite being produced by LAB, any new bacteriocin intended to be used as food preservative is considered as an additive and needs prior approval by the regulatory authorities, requiring detailed safety information supported by toxicological data, proof of efficacy in foods, description of manufacturing process, as well as the safe maximum levels.

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