14. NATURAL ANTIOXIDANTS AND ANTIMICROBIALS

14.1 INTRODUCTION

The processing of food products (frozen, canning and Ready to eat dishes-RTE-) produces changes that adversely affect the color and texture of foods. These changes are minimized in some way with antioxidant additives or additives that improve or maintain texture. Throughout the last century, the food industry has used many synthetic additives to achieve the antioxidant action, due to the advantages offered in relation with the natural ones (economic, stability, compatibility with food etc.) Traditionally, to prevent food spoilage and extend its shelf life, they have been employed antioxidant additives and preservatives that have suffered some controversy regarding evidence of potential adverse health effects. Thus, the food use of the antioxidants Butylated hydroxyanisole BHA E-320 and Butylated hydroxytoluene BHT E-321, both derived from petroleum, has been prohibited in some countries like Japan. The US National Institutes of Health states that BHA is “reasonably anticipated to be a human carcinogen based on evidence of carcinogenicity in experimental animals”. Some animal studies indicate that BHT causes cancer and birth defects. Also, the food use of preservatives such as benzoic acid derivatives or biphenyl has also been questioned for health reasons, something analogous to what happened with certain colors (health alerts for detecting food colorings banned in the EU, such as Sudan 1) or emulsifiers (carrageenan, polyoxyethylenes). Therefore, due to the consumer's pressure the trend is to use natural additives.

14.2 CHARACTERISTICS AND FUNCTIONS.

Natural antioxidants

In a society increasingly aware of the importance of healthy habits, the word "antioxidant" has gained a special importance. Consumers want to include in their diet foods rich in antioxidants. These molecules are capable of retarding oxidation reactions, produced by free radicals that contribute to cellular destruction. It is for this reason that a rich in antioxidants diet, such as polyphenols or thiols, prevents oxidative stress associated with cardiovascular and neurodegenerative diseases.

In the same way, food industry sees in these substances an essential element to extend the shelf life of food products, as well as to increase their healthy properties.

In this context, antioxidants could be defined as those natural or synthetic substances that added to food prolong its freshness, maintaining its characteristic appearance, taste and smell, in normal conditions, for a long period of time, through oxidation inhibition mechanisms of some susceptible components contained in food products such as oils, fats, and essential oils.

In resume, antioxidants are the main protectors of the sensory quality (organoleptic index) and technical quality (physical-chemical index) of the food that help maintain the quality, stability and durability. Source: Spanish Association of Manufacturers and Distributors of Additives and Dietary Supplements AFCA. They consist of natural or synthetic additives and natural substances.

Antioxidants authorized for use in food

There are two large groups of antioxidants permitted for food use:

A) Antioxidants officially recognized as such:

I) Natural antioxidants

- L-Ascorbic acid (vitamin C) and ascorbate
- Alpha-tocopherol (vitamin E) and tocopherols
- Rosemary extract

II) Synthetic antioxidants

B) Alternative Antioxidants, not officially recognized as such:

I) Antioxidant Synergists

II) Bio antioxidants

Bio antioxidants are mostly formed by vitamins, colorants and flavonoids, which are the future for oxidative protection: both to reduce the levels of use of additives and to improve freshness, stability and prolong the shelf life of the food (vitamin E, carotenes, xanthophylls, anthocyanins, ...).

In the near future antioxidants bio-additives or bio antioxidants will be used. They could be defined as those natural preparations that based on its chemical composition and the presence of bioactive substances, show protection activity against oxidation of foods, both of vegetable and animal origin.

The bio antioxidants consist of a series of extracts from plants, fruits, and vegetables and derivatives, rich in natural antioxidants.

Are coming an increase of the demand of bio antioxidants and antioxidant synergists is expected in the food sector.

Natural antimicrobial: preserving food naturally

A food can be defined as "any product which, by its chemical components and organoleptic characteristics, can be part of a diet in order to satisfy hunger, satisfy your appetite and provide the nutrients that are necessary to maintain the body in health". In other words, it could be said that food is every natural or transformed product, capable of supplying the organism that ingests it with the energy and the chemical structures that the body needs to develop its biological processes (Bello, 2000).

Food preservation can be defined as the set of treatments that prolongs the shelf life, maintaining the highest possible degree of quality attributes, including color, texture, flavor and nutritional value. This definition involves a wide range of preservation: from short periods (given by domestic cooking methods and cold storage) to very long periods (given by strictly controlled industrial processes like freezing and dehydration). In any case, food preservation is based on the inactivation, delaying or preventing the growth of pathogens and saprophytes microorganisms and deals with the different factors that influence the bacterial growth or bacterial survival.

The main food preservation technologies can be classified as: (a) those which basically prevent or slow down the bacterial growth: use of low temperature, decreased aw, reduced availability of oxygen, acidification, fermentation, storage modified atmosphere, adding antimicrobials, (b) those aimed to inactivate microorganisms: pasteurization and sterilization temperatures, microwave heating, ionizing radiation, high hydrostatic pressures, pulsed electric fields and (c) those that prevent or minimize the entry of microorganisms to foods or remove them from it: and aseptic handling, centrifugation, filtration (Table 14.1) (Gould et al, 1995).

Table 14.1. Current and emerging technologies for food preservation.

Partial or total growth inhibition of microbial	inactivation of microorganisms
Low temperatures (refrigeration and freezing)	Low temperatures (refrigeration and freezing)
Water activity (aw) reduced (drying, curing)	Ionizing radiation
Acidification	Adding enzymes (lysozyme)
Fermentation	Application of high hydrostatic pressures
Adding crop products (organic acids, bacteriocins)	Electric shock from high voltage (electroporation)
Vacuum and modified atmosphere packaging	Ultrasound heat and pressure (manotermosonicación)
Addition of preservatives	
Water in oil emulsions	

With the evolution of food science now there are many chemical compounds with antimicrobial activity. The antimicrobial agent that has the oldest record is table salt, which is still used today to preserve meat products. In the twentieth century great advances in food preservation by means of chemical agents were given. That's when the study of the health problems that each agent could cause also started (Lopez-Malo, 2000). The use of chemical agents with antimicrobial activity (conservative) either by inhibiting or reducing microbial growth or by inactivating undesirable microorganisms is one of the oldest and most traditional ways for preserving food.

There is a worldwide trend toward greater consumption of fruits and vegetables, primarily motivated by a growing concern for a more balanced diet, with the lowest proportion of carbohydrates, fats and oils and with greater involvement of dietary fiber, vitamins and minerals and consume more fresh and healthy products with less preservatives and chemical origin as close to its original form. This is because it has been associated consumption of chemical preservatives such as benzoates, nitrites and nitrates and sulfur dioxide (SO2), among others, with cancer and other degenerative diseases. This creates the need to find alternatives for food preservation but always covering the same antimicrobial properties and compatibility with food (Alvarez-Parrilla, 2006).

Many foods contain natural compounds with antimicrobial activity. In nature, these compounds may play the role of prolonging the shelf life of food. Many of them have been studied for their potential as direct food antimicrobial. The use of food additives of natural origin involves the isolation, purification, stabilization and incorporation of these compounds to food with antimicrobial purposes, without adversely affecting the sensory characteristics, nutritional and guarantee their healthiness. This must be achieved while keeping costs of formulation, processing or marketing (Raybaudi-Massilia et al., 2006).

The natural antimicrobial systems can be classified by their animal, plant and microbial origin. The first group includes proteins, enzymes such as lysozyme, lipases and proteases and polysaccharides such as chitosan. The second group includes phenolic compounds from bark, stems, leaves, flowers; organic acids present in fruits and phytoalexins produced in plants, while the third group includes compounds produced by microorganisms (Beuchat, 2001). Here we will mention only those from the plant kingdom.

Plants, herbs and spices and their essential oils, are composed by a large number of substances that inhibit several metabolic activities of bacteria, yeasts and molds (Table 14.2). Antimicrobial compounds in plants are commonly contained in the fraction of the essential oil from the leaves (rosemary, sage), flowers and flower buds (cloves), bulbs (onions, garlic), rhizomes (asafoetida), fruits (pepper, cardamom) or other plant parts.

Table 14.2. Compounds with antimicrobial activity found in plants, herbs and spices

Plant, herb or spice	Main composite	Other compounds
Garlic (Allium sativum)	Diatil disulfuro, diali trisulfuro	Dietil sulfuro, alicina
Basil (Ocimum basilicum)	d-linalol, metil cavicol	Eugenol, cineol, geraniol
Cinnamon (Cinnamomum zeylanicum)	Cinnamic aldehyde	l-linalol, *p*-cimeno, eugenol
Onion (Allium cepa)	d-n-propil disulfuro	
Coriander (Coriandum sativum)	d-linalol	d-α-pineno, ß-pineno
Clove (Syzygium aromaticum)	Eugenol	Cariofileno
Cumin (Cuminum cyminum)	Cuminalhehido	*p*-cimeno
Tarragon (Artemisa dracunculus)	Metil cavicol	Anetol
Lemongrass (Cymbopogon citratus)	Citral	Geraniol
Marjoram (Origanum marjorana)	Linalo, cineol, eugenol	Metil cavicol
Mustard (Brassica hirta)	Alil isotiocianato	

Oregano (Origanum vulgara)	Timol, cravacrol	α-pineno, *p*-cimeno
Parsley (Petroselinum crispum)	α-pineno, fenol-eter-apiol	
Black pepper (Pipper nigrum)	Monoterpenes, sesquiterpenes	Oxigenates
Rosemary (Rosmarinus officinalis)	Borneol, cienol	Canfor, α-pineno
Thyme (Thymus vulgaris)	Thymol	Carvacrol, l-linalol, geraniol
Vanilla (Vanilla planifolia)	Vanillin	*p*-hidroxibenzóicos acids

Source: López-Malo et als, 2000

Some of these natural antimicrobial systems are already used for food preservation and many others are being investigated in order to obtain their approval for using them in foods. Among those currently approved are phenolic compounds that have been used as antimicrobial agents since 1867 when it began to use phenol as a sanitizer. As antimicrobials in foods, phenolic compounds can be classified as those currently approved (parabens), those approved for other uses (antioxidants) and those found in nature (polyphenolic and phenol).

Parabens (methyl, propyl and heptyl esters of p-hydroxybenzoic acid) are permitted in many countries as antimicrobials in food employing them directly. Phenolic antioxidants, namely, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) are approved as antioxidants in food to prevent rancidity of fats, oils and fatty foods. The naturally occurring phenolic compounds are widely distributed in plants and can be found in a variety of food systems, and these phenol derivatives as possessing antimicrobial activity. These phenolic compounds can be classified into the following groups:

•Simple phenols and phenolic acids: p-cresol, 3-ethylphenol, vanillic acid, ellagic acid, gallic acid, hydroquinone.

•Hydroxycinnamic acid derivatives: p-coumaric acid, ferulic, caffeic and sinapic.

•Flavonoids: catechins, proanthocyanidins, anthocyanins and flavones, flavonols and their glycosides (see Figure 1).

•Tannins: polymeric phenols from plants with the ability to precipitate proteins from aqueous solutions.

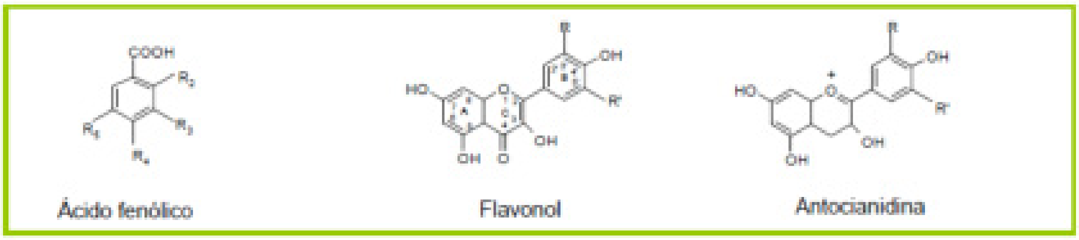

Figure 14.1. Phenolic derivates in plants

However, there are many other natural compounds that have not yet been investigated their potential uses and their possible effect on the sensory properties of foods to which they are added to. There is much to discover, in order to get healthier, nutritious and better product characteristics.

Undoubtedly, the most important aspect of the use of antimicrobials in food is the toxicological. We usually think that natural compounds that have antimicrobial activity are less toxic than the synthetic ones, but not always. For a compound with antimicrobial activity being considered appropriate for use in foods it must be shown to have no toxic effects through animal studies and that it is not responsible for changes in health when consumed regularly in foods like spices.

The problem is that the required concentrations of spices or compounds extracted from them to achieve inhibition of microbial growth are usually greater than those consumed regularly. Moreover, it is important to show that these substances are metabolized, excreted, leave no residue and do not destroy important nutrients in foods (Davidson and Zivanovic, 2003). The future of naturally occurring antimicrobial agents is determined by the current consumer attitude to the chemical preservatives. Natural antimicrobials are considered as potentially reliable sources, but their actual use in foodstuffs has been established for a few cases.

14.3 APLICATIONS IN FOOD AND EFFECTS

Blueberry extract

Scientific studies conducted in universities, have shown that blueberry extract possesses healthy and technological effects.

Its botanical name is Vaccinius myrtillus and Vaccinius corymbosum, the family of the Ericaceae, is a fruit from Asia and North America. In Spain it takes place in the Central System, apart from having many health benefits (prevention of cardiovascular disease, regulating the digestive process and dermatological protection, among others), gives technological effects as a preservative with bacteriostatic properties and it is particularly suitable for conservation from raw meats such as ground beef, and that selectively eliminates the presence of enteropathogenic bacteria such as Escherichia coli 0157 y Staphylococcus aureus.

Blueberries are rich in bioactive substances called anthocyanins, which are polyphenols with a high bacteriostatic activity. Recommended use levels range from 50-100 mg of bioactive / kg food, with the advantage that does not change the appearance and taste of the food.

Extract / juice pomegranate

Many scientific studies, developed in research centers and hospitals have shown that the extract / juice of pomegranate, provides healthy and technological benefits.

Its botanical name is Punicum granatum (meaning apple with seeds), and comes from North Africa, West Asia, Egypt, Saudi Arabia, Southern California and Spain. It has excellent health properties (high cardiovascular -the highest known- protection, reducing cholesterol and antihepatotoxic, among many other benefits), as well as giving virucidal effects and it is a powerful antioxidant. It is applicable to all food, but it is necessary to study each case before because the extract is very astringent.

The extract/juice of pomegranate is rich in tannins, being the most important bioactive substance punicalagin, whose antioxidant action is similar to rosemary extract, which owes its antioxidant effect to rosmarinic acid and carnosine. The doses of employment are in the range of 20-100 mg of bioactive substance / kg of food.

Amaranth extract

A number of scientific studies, especially in North America, have tested the health effects (nutritional) of the cereal from the Andes, called amaranth, which also has the advantage of not containing gluten, possessing at the same time good technological effects.

It comes from South American countries, where it is used for bread and other food uses. It has beneficial effects as free-of-gluten energy source with a high content of albumin, essential fatty acids and amino acid containing more lysine concentration of minerals and iron and phosphorus than other cereals such as rice, wheat, barley, etc. Moreover, it acts as a potent fungicidal preservative to baked goods, substituting propionic acid and propionates, with the advantage of improving the taste of bread and its stability versus fermentation. Amaranth, can inhibit the growth of fungi such as Penicillium roquefortii, at doses of 5 – l0 mg of bioactive / kg of bread.

Table 14.3 Antimicrobial used in model systems from fruit

Antimicrobial	Substratum	Efect	CMI (ppm)/CU	Reference
Vanillin	Agar model systems from fruit	Inhibition of radial growth rate of colonies spices Aspergillus	1000-2000	López,et. al. 1995

Eugenol, thymol, menthol and eucalyptol	Cherries	Inhibiting the growth of aerobic mesophilic bacteria, molds and yeasts	/1 ml vaporous (applied gauze moistened with essential oil)	Serrano, et. al., 2005
Carvacrol and acid Cimamico	Fresh cut melon and kiwi	Reducing counts of viable microorganisms in kiwi and extent of the lag phase of natural microbial flora in melon	1mM	Roller y Seedhar, 2002
Tangerine oil, cider, lemon and lime	Fruit salad	Increase shelf life and reduce microbial growth		Lanciotti, et. al., 2004
Methyl jasmonate	Guava	Increased tolerance against pathogen attack		Gonzalez, et. al., 2004
Methyl jasmonate and ethanol	Fresh strawberry	Fungal decay and deterioration of increased antioxidant capacity		Ayala, et. al., 1999
Hexanal	Fresh apples cut	Inhibiting the growth of aerobic mesophilic, psychrophilic, molds and yeasts	/0.15mmol/100g	Lanciotti, et. al., 1999
Hexanal and trans-2-hexanal	Fresh apples cut	Extending the shelf life of fruit by inhibiting the growth of native flora and prolonging the lag phase inoculated yeast	/ different concentrations were tested	Corbo, et. al., 2000
Hexanal, (E)-2-hexanal and hexil acetate	Fresh apples cut	Efecto bactericida contra *L. monocytogenes* y extensión de fase lag de *E.coli*, y *S. Enteriditis*	/150,150 and 20 hexane, hexyl acetate and (E) -2-hexanal respectively	Lanciotti, et. al., 2003
Glacial acetic acid vapor, hydrogen peroxide and chlorine dioxide	Whole apples	Population reduction E.coli 3.5log inoculated cfu / g using acetic acid vapor, 2 log reductions using solutions of hydrogen peroxide or chlorine dioxide and 4.5log reduction using chlorine dioxide in gaseous form	/Various	Sapers, et. al., 2003
Acetic acid vapors	Table grapes	Up to 94% reduction of the deterioration		Ayala, et. al., 2005
Hydrogen peroxide as washing solution	Whole apples	E.coli population reduction	/H2O2 (5%)	Sapers, et. al., 2002
Hydrogen peroxide as washing solution	Whole melon and cut	Population reduction of Salmonella inoculated whole melons	/H2O2(2.5 % y 5%)	Ukuku, 2004
Hydrogen peroxide Nisima, sodium lactate and citric acid applied as washing solutions	Whole melon and cut	Reduction in the transfer of E.coli O157: H7 and L. monocytogenes the whole fruit to fruit chopped	/H2O2(2.5 %) or a mixture of H2O2 (1%) + nisin 25 g / ml + sodium lactate (1%) + AC. Citric (0.5%)	Ukuku, et. al., 2005

CMI: Minimum inhibitory concentration, CU: Concentration used

Source: Raibaudi, et. Al., 2006

Olive oil

Various research centers worldwide have proven that olive oil, extracted by natural means from olives, has both health benefits to the human body, because its high level of monounsaturated fatty acid (omega-9 : oleic acid) and other polyunsaturated fatty acids, and important technological effects. Olive oil is one of the main vegetable components of the Mediterranean diet,

Olive oil is rich in a bioactive substance, hydroxytyrosol (polyphenol), which has a high antioxidant power, together with other substances: caffeic acid and oleuropein, both antioxidants also very useful for preventing oxidation in meats, especially beef and pork. The best results are found with 50-100 mg of extracts of polyphenols / kg of meat.

Marjoram oil

Several American universities and technology centers found that marjoram oil has health benefits (cardiovascular protection, reduction of cholesterol, hypolipidemic, among others) but could also be used by its technological properties as a good bacteriostatic against pathogens in meat beef, pork, etc., especially against Escherichia coli 0157 and Salmonella.

The bioactive substance is carvacrol. Recommended doses range from 50-150 mg of bioactive / kg food.

Garlic

This botanical specimen, widely used in food, very useful and helpful especially in the Latin countries, has the botanical name of Allium sativa. It smell is pungent and its consumption in cooked food is high, used mainly as a condiment. Like other species, their health benefits are widely known but due to the lasting smell that permeates the human breath and that it also produces artery hypertension it cannot be consumed in high quantities. Its technological effects are really important as a bacteriostatic preservative of wide spectrum due to bioactive substances: alliin (which becomes allicin by the alliinase enzim to) other sulfur and various sulfurous substances being also important molecules: sulfide, disulfide, trisulfide and allyl tetrasulfide.

Onion

Onion and garlic are used as a seasoning and food ingredients, although onion is used in higher doses. Onion also has a strong, pungent odor, which produces tearing. Its botanical name is Allium cepa and owns healthy properties, especially cardiovascular protector, cholesterol reducer, etc. Onion has also good bacteriostatic properties for all kinds of food.

The preservative properties of removing bacteria have been much studied, being highly active against pathogens such as strains of Escherichia coli 0157 and Staphylococcus aureus. Its active substances are: myricetin polyphenols, caffeic acid and quercetin. It also contains a sulfur substance: the thiopropionic acid. A dose of 50-120 mg of bioactive / kg food substances exerts a complete protection against bacterial growth.

Rosemary extracts (E392)

This plant extract has been one of the latest to be declared as an antioxidant additive after an exhaustive study. This bioadditive extract from the Rosmarinus officinalis plant, is rich in antioxidants bioactive substances such as rosmarinic acid and carnosine, which help prevent the oxidation of a large amount of food of animal and vegetable origin containing oxidation-labile substances. Its uses are very extensive and dose varies depending on the field of application.

Spices and herbs.

Many spices and herbs exhibit antimicrobial activity. In foods are used, for example: celery, coriander, laurel, almond, basil, coffee, angelica, leek, horseradish, peppermint, thyme, etc. The compounds in spices and herbs having antimicrobial activity are simple derivatives and complexes of phenol, which are volatile at room temperature. Spices are roots, bark, seeds, buds, leaves or fruits of herbs that are added to foods as flavoring agents. However, it is known from old times that spices and their essential oils have different degrees of antimicrobial activity. The first report of the use of spices and conservatives goes back about 1.550 years BC when the ancient Egyptians used them to store food and embalming the dead (Davidson, 2001).

Certain spices inhibit the growth of microorganisms. They are generally more effective against gram-positive organisms that against gram-negative bacteria:

- cinnamon, cloves and mustard: great preservative power.
- black / red pepper, ginger: weak inhibitors against a variety microorganisms.
- pepper, bay leaf, coriander, cumin, oregano, rosemary, sage and thyme: intermediate activity.

Table 14.4 Minimum inhibitory concentrations of essential oils tested "in vitro" against foodborne pathogens

Plant from which the EO is derived	Bacterial species	CMI approximate range (ul/ml)
Rosemary	*Escherichi coli*	4.5->10
	Salmonella typhimurium	>20
	Bacillus cereus	0.2
	Staphyloccocus aureus	0.4- 10
	Listeria monocytogenes	0.2
Oregano	*E.coli*	0.5-1.2
	S. typhimurium	1.2
	S. aureus	0.5-1.2
	E.coli	0.6
	S. typhimurium	2.5
	S. aureus	0.6
	E.coli	3.5-5
	S. typhimurium	10-20
	S. aureus	0.75-10
	L. monocytogenes	0.2
	E.coli	0.4-2.5
	S. typhimurium	>20
	S. aureus	0.4-2.5
	L. monocytogenes	0.3
	E.coli	0.45-1.25
	S. typhimurium	0.450->20
	S. aureus	0.2-2.5
	L. monocytogenes	0.156-0.45
	E.coli	>0.2
	B. cereus	0.2
	E.coli	2.5->80
	Shigella dysenteria	5->80
	S. aureus	0.6-40
	B. cereus	5-10
CMI: Minimum inhibitory concentration		

Source: Burt, 2004

14.4 ADVANTAGES AND DISADVANTAGES

Some of the advantages of using natural extracts are:

- Consumers do not associate them with artificial additives.
- Extracts have less regulation than pure chemicals.
- Some present synergies between them, the most effective extract the pure compound.

Many herbs and spices have been reported to possess antimicrobial / antioxidant properties. However there is a disadvantage in using them as antimicrobial or antioxidants: a high concentration is required to obtain an effect of preserving and therefore changes in the taste may occur. Therefore the use of herbs as antimicrobial agents is limited to foods in which the change in taste is desired.

For the application of natural antimicrobial and natural antioxidants, it is needed to check their effectiveness "in vitro" in microbiological media and also in food products. Tests "in vitro" provide valuable information about the effectiveness of a compound. Many factors have to be taken into account. For example bacterial spores are much more resistant to the effect of the antimicrobial / antioxidants than plant cells. Also the type of cell wall is an important factor. A variable associated with the effectiveness of an antimicrobial or antioxidant agent in food, is the initial number of microorganisms in the system.

We tend to think that natural compounds that have antimicrobial activity are less toxic than the synthetic ones, but not always. For a natural compound with antimicrobial activity is considered appropriate for use in foods it must be shown to have no toxic effects through animal studies and that it is not responsible for changes in health when consumed regularly in foods like spices.

The future of naturally occurring antimicrobial and antioxidant agents is determined by the current consumer attitude to the chemical preservatives. Natural antimicrobials and antioxidants are considered as potentially reliable sources, but their actual use in foodstuffs has been established for a few cases.

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