

Potential use of Rosemary, Propolis and Thyme as Natural Food Preservatives

Abstract

The use of preservatives in food stuffs and beverages is essential in order to prevent spoilage due to microbial growth or undesirable chemical changes. However, the use of synthetic additives has been associated with various health problems. Therefore, consumers have turned suspicious and obverted towards ingredients from natural sources. This tendency has driven food industry in further search and development of "natural preservatives", to extend the shelf life of its products and maintain their safety. This report is focused on the current status of the natural derived preservatives and potential use of propolis, thyme and rosemary as sources for the development of effective preservatives.

Introduction

Preservatives are constituents that originate from a natural or synthetic source. Their addition to products, such as food stuffs and pharmaceuticals, targets in prevention of spoilage, which steams from microbial development or undesirable changes at molecular level. A preservative can also be a constituent with the ability of inhibition, obstruction or end of processes as these of fermentation and acidification. Preservatives lead to inhibition of microbial growth without their unavoidable destruction [1].

A preservative, in order to be entirely suitable, should have particular properties. At first, it must not be harmful for the consumers' health under normal conditions. Additionally, it should also not be decelerating for digestive enzymes and have an effective action in the same time. Furthermore, preservatives should not decompose after consumption into toxic substances. At this point, it has to be mentioned that the methods of manufacture should be appropriate, due to inconsiderate and inadequate practices leading from the preservative's usage. A final necessary property of a preservative in order to be utterly appropriate for use is easily identification and quantification that simplifies quality control of the food that contains the preservative [1].

A great amount of components has been chemically manufactured to certify low cost and stable quality (Ingredients insight, 2013). Synthetically produced preservatives have been used for hundred years for the inhibition of spoilage, due to the action of bacteria and fungi. Sodium benzoate, potassium sorbate and their mixtures are frequently used food preservatives with a broad-spectrum action against yeasts and moulds and they are recognized as generally safe and well accepted in world-wide scale [2]. For the preservation process, antioxidant enhancement can reduce the oxidative damage prompted from reactive compounds. Antioxidants can inhibit oxidation through "their reaction with free radicals, chelating catalytic metals and also by acting as reactive species scavenger". 2, 6-di-tert-butyl-p-hydroxytoluene (BHT), tert-butyl-hydroxyanisole (BHA),

propyl gallate and tert-butylhydroquinone, are the most commonly used antioxidants [3].

In the present times, however, there is a large debate about the safety of chemical preservation, as it is considered as the main cause of a large amount of teratogenicity, carcinogenicity and residual toxicity. For the above mentioned reasons, consumers have a tendency to be doubtful of the chemical substances addition, a fact that increased the demand for natural and generally more adequate preservatives [4]. Moreover, some combinations of food additives can lead to the formation of toxic and undesirable compounds. As an example, benzoic acid can react with ascorbic acid to form the carcinogenic compound benzene. Beverage companies tried to avoid the combination of benzoic acid and ascorbic acid in their formulas searching for alternatively antioxidants [5].

According to Ingredients insight (2013), there is an increased tendency of manufacturers to fast-moving consumer goods (FMCG), to request reduced manufacturing services, complete traceability and stable quality in their products. The means of handling of natural ingredients are the state of art in food industry's development technologies. The main challenge for FMCG manufacturers is the shelf life of the product and for this purpose they have industrialized many chemical components, to guarantee a stable quality. For the above mentioned reasons, there are many chances for suppliers of natural additives and preservative solutions. Natural products such as substances from plant extraction (pure compounds or specific extracts) offer good opportunities in the control of microbial growth, as a result of their chemical variety [6].

Other isolates with natural origin are the essential oils from plants rich in terpenes which also present significant antimicrobial properties. As a result, from a health and financial aspect, research focuses on the discovery of essential oils that could securely substitute chemical additives, for moderately or entirely inhibition of fungi and bacteria growth [7]. Most essential oils originating from spices and



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herbs are considered generally as safe (GRAS) and have antimicrobial properties. These properties of plant extracts, including spices and essential oils, have been reviewed [8-10].

As essential oils can be considered as a natural way of substitution of chemical preservatives, they could also substitute other natural constituents. A great amount of spices, herbs and plant extracts, demonstrate antimicrobial activities, because of its essential oil fraction [11]. Moreover, they gain ground as ingredients in the production of functional foods, beverages, toiletries and cosmetics, both for the increasing interest of consumers in natural ingredients and also because of the growing concern over chemical additives [12].

An important issue that arises from the use of natural preservatives is their multi-functional properties. Antimicrobial compounds from natural origin and especially when whole extracts or essential oils are used have a characteristic pleasant or unpleasant flavor. For example, oregano, garlic and mustard are used in certain food and beverage products to enhance their characteristic flavor profiles; control microbial growth and extend shelf life. On the other direction, when plant extracts or essential oils are used as antimicrobials, the sensorial analysis of the final product is necessary. Moreover, the interactions between plant substances such as polyphenols and terpenes with the proteins, lipids and carbohydrates have not fully characterized yet. Therefore it seems that the development of a global natural antimicrobial is very optimistic. The combination of antimicrobials with thermal treatments could be of interest for the food processing industry, since it would help to decrease the intensity of thermal treatments, while keeping the same levels of food safety and stability [13].

According to Ramirez Lo et al. it was hypothesized that the use of certain medicinal plants such as Lemongrass, Silver thicket, Geranium, Clustered frost weed, Wormseed, Creosote bush and Horehound could replace food preservatives [14]. The common in these plants is that they contain polyphenols and terpenes with known antioxidant and antimicrobial activity. Moreover, as they have been used for thousands of years to treat health disorders and prevent diseases, the possibility of being toxic is low [14].

Rosemary, thyme, and propolis are important sources of phenols and terpenes with the potential to produce extracts or essential oils with antimicrobial and antioxidant properties. The purpose of this report was to give an overview of these natural origin products, which could be used as preservatives in the food industry and substitute those which are chemically synthesized.

Rosemary (*Rosmarinus officinalis* L.)

In recent years, demand for essential oils from medicinal plants has increased, particular for rosemary oil (*Rosmarinus officinalis* L.), mainly because of its widespread use as a natural food additive, for food preservation due to its antimicrobial, antiviral, antimycotic, and antioxidant properties and, above all, because of low cost and availability. Rosemary is an evergreen shrub that grows spontaneously in Mediterranean regions; its essential oil is usually extracted by the easy-to-handle and cost-efficient, steam distillation procedure, which gives high yields of a product of appreciable quality on the basis of sensory evaluation, with remarkable functional properties and a safe alternative to synthetic antioxidants [15]. Many compounds have

been isolated from rosemary, including flavones, diterpenes, steroids, and triterpenes. Of these, the antioxidant activity of rosemary extracts has been primarily related to two phenolic diterpenes: carnosic acid and carnosol [16]. The analysis of the essential oils of *Rosmarinus officinalis* L. that grows in the wild in Northern Italy showed that its main constituents are α -pinene, borneol, 1,8-cineole and camphor [17]. It produces colorless or pale yellow oil with the characteristic flavor of rosemary and a warm camphoraceous taste. It is used in the perfume industry and as a flavor agent. Several compounds found in this oil have been reported to be inhibitory to several microorganisms. It is, however, known that variants of the same species can differ in their constituent essential oils [18].

It had been investigated [19] that *Rosmarinus officinalis* essential oil had antibacterial properties against growth of *Bacillus cereus* strains in carrot broth [19]. From another study [20], it was confirmed that its extracts, which were obtained with supercritical carbon dioxide, had shown antioxidant, antibacterial and antifungal activities [20]. The effectiveness of rosemary oil as antimicrobial agents in mozzarella cheese against *Listeria monocytogenes*, have been studied [21]. Rosemary oils inhibited *L. monocytogenes* by 0.5 log CFU/g, on day 20 at 4 °C proving the potential applications for inhibition of indigenous microorganisms in low fat food products, and for controlling growth of *L. monocytogenes* after post-processing contamination. The role of rosemary oil during the cheese-making process was also studied [22] by the addition of the oil in the sheep milk. Essential oil used at concentration of 215 mgL⁻¹ and Tween 20 was also added as an emulsifier to ensure a uniform dilution of the oil in milk. The results showed that in fortified cheeses, the use of essential oil provoked the total inhibition of *Clostridium* spp. without affecting the growth of lactic acid bacteria. Ribeiro Teodoro RA et al. studied the mycelial growth of *Penicillium* and *Aspergillus* fungi, isolated from fresh dough in presence of rosemary essential oil particles obtained by spray-drying [23]. Optimization of maximum dosage levels also studied in order to ensure the microbiological safety of foods without affecting taste. The growth inhibiting effect of *Penicillium* sp. fungus was verified at concentration of 1.0 µL/mL rosemary essential oil, and for *Aspergillus* sp., at concentration of 10.0 µL/mL oil. At 8 days of dough storage at 25 °C, a decrease of at least 0.7 log cycles of fungal growth was observed in the dough with pure oil, relative to the control.

Additionally, it had been shown [24] that *Rosmarinus Officinalis* oil, was one of the promising performing extracts in terms of both antimicrobial activity and ability to neutralize free radicals and prevent oxidation of unsaturated fatty acid [24]. Finally, a group of researchers had reported the effectiveness of its extracts in delaying the lipid oxidation on different foods, as in the case of meat fillets, with a used proportion of 200-1000 oil mg / meat kg [25].

Propolis (*Apis mellifera*)

Compounds of natural origin, such as propolis (*Apis mellifera*), have been confirmed as antimicrobial agents. It has been proved, that this property of propolis is attributed to the phenolic compounds it contains, and particularly flavonoids. These compounds derived from leaves, fruits, seeds and other plants parts and they are broadly spread and known for their antioxidant properties [26]. As a result, propolis could be established as an ideal natural preservative.

Propolis [27] is a resin, being dark green or brown in color, with a pleasant flavor of poplar buds, honey wax and vanilla but it can also have a bitter taste. When burnt, it exhibits a smell of aromatic resins of great value [27]. It is constituted by a wide variety of substances such as polyphenols, quinones, coumarins, steroids, amino acids and inorganic compounds. The majority of propolis components are of phenolic nature, mainly flavonoids. It is known that simple phenols, phenolic acids and polyphenols are active antimicrobial agents [28]. The antibacterial and antifungal activities are the most popular and among the most extensively investigated biological actions of propolis [29]. In parallel, its antioxidant activity, combined with the fact that several of its constituents are present in food and/or food additives, and is Generally Recognized as Safe (GRAS) [30] make it an ideal natural preservative.

Many studies have shown that propolis could be used as an alternative to chemical food preservative agents [31]. Propolis has shown antifungal properties, when different extracts (0.5, 1.0, 2.0, 3.0, 4.0 wt %) examined for inhibition of *Alternaria alternata*, *Aspergillus niger*, *Aspergillus parasiticus*, *Botrytis cinerea*, *Fusarium oxysporum* sp. *melonis* and *Penicillium digitatum* in culture media. Concentration of 4 wt% of propolis extract showed more than 50% inhibition against all tested microorganisms [31]. It was also reported that propolis extracts can be an effective preservative for pork meat products [32]. In the proposed methodology pork sausages treated with different extracts of propolis (0.3% ethanol extract, 0.3% water extract, 0.4% dried residue of ethanol extract) and potassium sorbate used as a reference. Stability tests carried out by storing the samples for 8 weeks at 4, 10 and 20 degrees C, Volatile basic nitrogen (VBN) measured in all samples. Results proved that propolis extracts can be used as preservatives. Moreover propolis extracts have an important contribution in promoting human health because they are naturally produced [32].

Furthermore, another example of propolis preservation properties, is a study [33] that showed its antifungal activity in four different fruit juices (apple, mandarin, white grape and orange) and concluded in the need of its further study as a natural preservative. However, they additionally concluded that due to its strong aromatic flavor, it should be added in small amounts, so as not to affect the organoleptic qualities of the product [33]. Also, it was found [34] that the addition of propolis extract in concentrations from 0.1 to 2 mg/ml inhibit the production of patulin, the common mycotoxin found in apple juice [34]. Spinelli et al. Studied the incorporation of encapsulated forms of propolis (5% of spray-dried Propolis) to a fish burger containing 9% extra virgin olive oil [35]. Results showed that that the final product possessed four times higher antioxidant activity compared to the control. The possibility of embedding the propolis as a natural ingredient in various foods, including fish to enhance the antioxidant content suggested. Ozturk used dry propolis ethanol extracts as antifungal agent on the fermented sausage and sucuk (meat product) in concentrations 50 mg/mL and found that the number of yeasts and molds can be reduced without significantly altering the color and aroma properties of the sausages [36]. Additionally, the use of the extract did not affect the number of important microorganism groups for properties such as taste, aroma and color of fermented sausage.

Finally, it is significant, that in numerous studies who have demonstrated its properties, has been used propolis from different geographic locations. Nevertheless, bee glue was always active, although it is known that in different geographic zones its chemical composition varies due to the different plant sources [37].

Thyme (*Thymus vulgaris* L.)

Thyme (*Thymus vulgaris* L.) is an ever green, small bushy herb indigenous to the Mediterranean regions. It has woody stems covered with epidermal hair. The opposite, sessile leaves are one-fourth to half an inch long, slightly rolled at the edges with a pale, hairy underside. Thyme belongs to the family of Labiatae (*Lamiaceae*), which includes Rosemary (*Rosmarinus officinalis*) and Oregano (*Origanum vulgare*). There are over 100 varieties of thyme which have been cultivated since ancient times [38]. Due to its purifying, tonic and protecting properties, mainly supported by ethno botanical sources, this essential oil is often incorporated into hygiene and skin care products such as soaps, toothpastes, shower gels, shampoos, deodorants and body lotions.

Numerous reports [39] support its antimicrobial properties and rank it among the most potent essential oils in this respect. Its efficacy has been attributed mainly to thymol and carvacrol, two phenolic compounds present in the essential oil from *Thymus vulgaris* L., as well as from other *Lamiaceae* species referred to under the common names of thyme and oregano [39]. The analysis of the essential oils of *Thymus vulgaris* L. growing in the wild in Northern Italy showed that its main constituents being p-cymene, 7-terpinene and thymol [40]. The essential oil of *Thymus vulgaris* L. is highly active as fungi toxicant and could safely be used as natural preservative to replace synthetic fungicides in the preservation and cure of some plant, human and animal fungal disease [41].

Soković et al. who had studied the antibacterial and antioxidant properties of Mediterranean aromatic plants concluded that *Thymus vulgaris* L. was between those plants, which were inhibitory to the growth of all the microorganisms [40]. Thyme was one of the most active and exhibited greatest inhibition against *Brevibacterium linens*, *Brochothrix thermosphacta*, and *Lactobacillus plantarum*. This effectiveness can be attributed to the high contents of phenols, thymol and carvacrol in the oil, which are known to be powerful antibacterial agents [40]. Some other researchers [42] reported that *Thymus vulgaris* L. essential oil at low concentrations (2, 5 and 8%) in a solution of water, propyleneglycol and an emulsifying agent, present highly effective antioxidant when used for Nile Tilapia fillets, at refrigeration temperatures and by placing the fillet immersed in the solution. The reduction of oxidative processes in tilapia fillets by using the essential oil occurred between 5.0 and 96.5%. This demonstrates its high effectiveness, even at low concentration [42]. Silva N et al. recently proved the antibacterial activity of thyme against ten food borne and food spoilage bacterial strains, *Bacillus cereus*; *Clostridium perfringens*; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; *Listeria monocytogenes*; *Pseudomonas aeruginosa*; *Salmonella enterica*; *Staphylococcus aureus*; and *Staphylococcus epidermidis* [43]. Khalili ST et al. recently used encapsulated forms of thyme oil in the preservation of tomato fruits and found that the encapsulated oil at 700 mg/l concentration was capable of preserving the quality the tomato fruit during the one month storage period [44]. Ulbin-

Figlewica et al. studied the antimicrobial activity of different concentrations (0, 1000, 2000 ppm) of thyme extracts against *Bacillus subtilis* and *Pseudomonas fluorescens* and suggest the usage of plant extracts in the development of edible films and coatings that used in order to protect foods [45]. Antimicrobial activity of the extracts offers improved food safety.

Extraction of Phenolics using Conventional and Modern Non-conventional Environmentally Friendly Techniques

Phenolic components, the main ingredients with antioxidant and antimicrobial properties, can be extracted from plants with conventional techniques such as Soxhlet extraction, Maceration and Hydro-distillation using polar solvents such as methanol ethanol, water, glycerin or their mixtures.

The major challenges of conventional extraction are [46] long extraction time, [47] requirement of costly and high purity solvent, [48] evaporation of the huge amount of solvent, [49] low extraction selectivity and [50] thermal decomposition of thermo labile compounds [51]. To overcome these limitations of conventional extraction methods, new and promising extraction techniques are introduced. These techniques are referred as non-conventional extraction techniques. Some of these techniques are:

- Enzyme-assisted extraction. The enzyme-assisted extraction of phenols can save processing time and energy, and potentially provide a more reproducible extraction process at the commercial scale [52]. An application of cell wall degrading enzymes on citrus peels has shown that enzymes have weakened or broke down the integrity of the cell walls and eased the extraction of phenols [53].
- Ultrasound assisted extraction. Ultrasonic cavitation creates shear forces that break cell walls mechanically and improve material transfer [54]. The application of ultrasound has been proven to be able to increase polyphenol extraction yields from several seed cakes [55]. Furthermore, up-scaling ultrasonic device is quite easy and cheaper than other extraction techniques [56].
- Microwave-assisted extraction. Microwave heating due to its simplicity and rapidity, was proven to be more effective in releasing antioxidant compounds from agricultural by-products such as citrus peels [57]. In another research, it was shown that with a treatment of only four minutes, higher yield of antioxidants were extracted from green tea leaves compared with reflux extraction [58].
- Supercritical fluid extraction. SFE methods are rapid, automatable, and selective, avoid the use of large amounts of toxic solvents and are particularly recommended for the extraction of thermo-labile compounds [59]. Supercritical CO₂ modified with 10% methanol was found to be a more efficient solvent than n-hexane for the isolation of phenols from olive leaves [60].
- Pressurized liquid extraction. Pressurized solvents use elevated pressures and sometimes temperatures which drastically improve the speed of the extraction process [61].

Subcritical water extraction (SWE) at 160 °C produced the highest extract yields of phenols from canola meal when compared with ethanol [62]. In a similar study, SWE was shown to efficiently recover phenolic acids (chlorogenic, caffeic, etc.) from potato peel [63]. Using 100% water is eliminating the cost of ethanol itself and the process cost of evaporating off organic solvents.

- Adsorptive extraction. Adsorption enables the separation of selected compounds from dilute solutions. Compared to alternative technologies, adsorption is attractive for its relative simplicity of design, operation and scale up, high capacity, favorable rate and low cost [64]. Weisz et al. have developed an innovative process for polyphenol removal from sunflower protein extracts by adsorption and ion exchange processes [65]. The procedure applied in this study yielded protein isolates of high quality due to almost complete polyphenol removal and considerably lightened colour.
- Pulsed electric field assisted extraction. In PEF, material located between two electrodes is exposed to a strong electrical field and subsequently pore formation occurs [66]. The use of PEF in the recovery of bioactive compounds from by-products is not well studied up to now [67].
- Assisted by molecularly imprinted polymers. Molecular imprinting technology can be used to generate specific artificial polymeric receptors like high affinity stationary phases [68]. It was applied successfully to extract and separate of three phenolic acids, including caffeic acid, from a *Salicornia herbacea* extract [69].

Conclusion

Antimicrobials from natural sources have not only proven to be effective against both spoilage and pathogens in a variety of food and beverage products, in liquid, semi-solid or solid systems but they also have a long history of being safe and, thus have the potential to replace or partly replace synthetic food additives. Antimicrobial properties attributed to their phenolic and terpen content can be extracted with several techniques. Companies should test their effectiveness in each food matrix looking for the combination of more than one natural antimicrobial ingredient from different origin, or a combination of ingredients, processes, and packaging technologies. Moreover, in the development of foods containing preservatives based on natural sources, the optimization of dosage should be considered in order to avoid undesired flavors.

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