Potential Effectiveness of Essential Oil as Natural Food Preservatives Compared with Chemical

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Abstract

In this study, investigations were carried out to assess the efficiency of two plant essential oils; rosemary and thyme as natural food preservatives. The effect of the plant essential oils at concentrations of 1% and 2% was studied in the soft cheese against *Staphylococcus aureus* and *Escherichia coli* at fridge temperature over a 5 day period. The essential oils performed well in the inhibition of *Staphylococcus aureus* and *E. coli* it is concluded that selected plant essential oils can act as potent inhibitors of both microorganisms in a food product, Thyme was found to be very active against *E. coli* in 2% and 1% (2.00, 2.66) respectively and *S. aureus* in 2% and 1% (7.66, 14.66) respectively followed by Rosemary in *E. coli* at 1% and 2% (4.33, 7.00) respectively also in *S. aureus* it effect in 2% more than 1% (5.00, 47.66) respectively. At the same time we compared their effect with weak acid as chemical preservatives in citric acid was found to be more effective than thyme and rosemary in 1% and 2% in *E.coli* and *S. aureus* (2.33, 1.00), (2.33, 1.33) respectively both essential oil and weak organic acid decreased growth of bacteria significantly when compared with control. Therefore, essential oils could be regarded as a source of food preservatives instead of chemical compounds.

Introduction

In spite of the modern improvement in food hygiene and food production techniques, food safety is an increasingly important public health issue (1, 2). It has been estimated that as many as 30% people in the industrialized countries suffer from the foodborne disease each year, and in 2000 at least two million people died of diarrheal diseases world wide (3). Therefore, are still needed new methods for the reducing or inhibiting foodborne pathogens, possibly in combination with the existing methods (the hurdle principle, packaging under the modified atmosphere, heating, refrigeration, the addition of antimicrobial compounds) (3; 4, 5).

For this reason there, is scope for new methods of producing safe foods that have a natural or green image. Another problem is the use of animal wastes as organic fertilizers, whether in organic or non-organic agriculture, that gives rise to concerns about the possible contamination of agricultural produce with pathogens and the possible contamination of ground and surface water (6). One of such possibilities is the use of essential oils for different reasons (7, 8, 9, and 5). Essential (volatile) oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, notably antibacterial, antifungal, and antioxidant properties (10, 11, and 12). The biological activity of essential oils depends on their chemical composition, which is determined by the plant genotype and is greatly influenced by several factors such as geographic origin and environmental and organic conditions (13). Many species and herbs exert antimicrobial activity due to their essential oils factors. Some scientists reported the antibacterial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic, and onion against both bacteria and molds.

The composition, structure, as well as functional groups of the oils play an important role in determining their antimicrobial activity (14, 15). The compounds containing phenolic groups are usually most effective (16, 17). The components present in essential oils like these have been known to possess antimicrobial activity and some are classified as generally recognized as Safe (GRAS) substances and therefore can be used to
prevent the post-harvest growth of native and contaminant bacteria. The essential oil fractions sensitize the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, as well as the impairment of bacterial enzyme system and cell respiration (18, 6). Rosemary (Rosmarinus officinalis L.) is a spice and medicinal herb widely used around the world. Of the natural antioxidants, rosemary has been widely accepted as one of the spices with the highest antioxidant activity (19). Rosemary essential oil is also used as an antibacterial, antifungal (20) and anticancer agent (21) (22).

Investigated the antibacterial activity of selected essential oils against some food spoilage organisms. They concluded that the essential oils of cinnamon, clove and rosemary were the most active. Similar results were obtained by (23) for the antibacterial activity of rosemary essential oil against Bacillus cereus strains grown in carrot broth. 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 (24).

The main compounds responsible for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1,8-cineole (25,26, 27). Thyme (Thymus vulgaris L.) belongs to the family of Labiatae (Lamiaceae), which include (Rosmarinus officinalis) and Oregano (Origanum vulgare) (28). The essential oil of Thymus vulgaris L. is highly active as fungi toxicant and could safely be used as a natural preservative to replace synthetic fungicides in the preservation and cure of some plant, human and animal fungal disease (29) (29).

Who had studied the antibacterial and antioxidant properties Mediterranean aromatic plants concluded that Thymus vulgaris L. was among those plants, which were inhibitory to the growth of all the microorganisms (30). Thyme was one of the most active and exhibited the 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 (30). The problem for the food industry is to fulfill the demands of minimum changes in food quality and maximum security (31). Chemical additives have generally been used to combat specific microorganisms. Beth et al. (32) reported that organic acids, such as lactic and citric acid, also can enhance or contribute to the flavor of acidified or fermented food, such as sausage, cheese and pickles. A large number of chemicals have been described that show potential as food preservatives, only a relatively small number are allowed in food products, due in large part to the strict rules of safety adhered to by the food and Drug Administration food (FDA) and to a lesser extent to the fact that not all compounds that show antimicrobial activity in vitro do so when added to certain foods (33, 34).

To enhance the shelf life of foods, several chemical preservatives have been employed (35). Currently, limited information is available on the activity of chemical food preservative on the growth of food borne pathogens in food products. The aim of the present work was to evaluate the antimicrobial effect of some essential oils; however, little quantitative data are available on the antimicrobial activity against food borne pathogens of essential oils isolated from the plants cultured in different geographical areas.

Material and Method

Essential Oil Material

Rosemary and thyme oil were purchased from Baghdad markets and used for study their antimicrobial effect as natural food preservatives. While the citric acid was powder and used for the comparison with the essential oil as chemical food preservatives.

Microorganisms and Media

The bacterial isolates E. coli, S. aureus, isolated from patients with food poisoning and wound infection; the bacteria were obtained, as clinical isolates, from Al–Yarmook Teaching Hospital, Baghdad, Iraq. Bacterial cultures were maintained on nutrient agar (NA). Subcultures were made monthly and stored at 4 °C until required for use.

Antimicrobial Activity Test

The soft cheese was purchased from a market. The packaging showed the absence of artificial preservatives. Preliminary
experiments in which nutrient agar plates were inoculated with cheese at 1/10 dilution and incubated at 37°C for 48 h, exhibited no microbial contamination of this product. The used procedure was based on that of (36), 10 g of cheese was added to 90 ml of phosphate buffered saline (PBS) which was prepared in the laboratory, and homogenized for 2 min.

Plant essential oils of Rosmarinus officinal, Thymus vulgaris L and citric acid were added to the cheese mixture to achieve final concentrations of 1 % and 2% PBS was used as a control. The mixture of plant essential oil with the cheese was homogenized for further 30 s to ensure even mixing. The cheese mixture was inoculated with 100µl of staphylococcus aureus or E. coli cultures, then mixed thoroughly with the cheese mixture by gently squeezing the bags by hand and the concentration of both organisms in the cheese determined at 0 hours and 1, 2, 3 and 4 days using the serial dilutions and spread plate technique(37).

Statistical Analysis
The Statistical Analysis System (38) program was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

Result and Discussion
Antimicrobial Activity
The antimicrobial activity of EOs has been extensively studied and demonstrated against a number of microorganisms, usually using a direct-contact antimicrobial assay, such as different types of diffusion (39). The studied essential oils showed various degrees of inhibition against the two bacterial strains using the drop diffusion method (Figs. 1, 2).

The present data exhibited the potential effect of plant essential oils as natural food preservatives against staphylococcus aureus and Escherichia coli in cheese in compared with chemical preservatives and control. It should be taken into consideration that the inhibition reported here was for 1/5 diluted cheese and therefore may not be a true reflection of inhibition achieved in cheese, but was necessary due to practical considerations. Despite this, the research showed the potential application of plant essential oils as natural food preservatives. With respect to food contamination with food borne pathogens, both rosemary and thyme oils revealed their suitability in this corner. The preservative properties of weak organic acids have been exploited by mankind for thousands of years. The antimicrobial activities of many different weak acid food.
preservatives have been well documented (40). Organic acids such as acetic, lactic, and citric acids have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food systems including poultry (41) and fish (42). In the concentration, 1 % performed well in the inhibition of the two organisms as we see in (Table 1, 2).

Table 1: Effect of Active ingredient in tow concentration 1%, 2% on E.coli growth for five days as a food preservatives

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Const. %</td>
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<td>Const. %</td>
<td>Const. %</td>
<td>Const. %</td>
</tr>
<tr>
<td>Th.</td>
<td>78.66 ± 3.17</td>
<td>33.0 ± 7.5</td>
<td>90.33 ± 5.2</td>
<td>38.66 ± 10.9</td>
<td>43.0 ± 6.92</td>
</tr>
<tr>
<td>R.</td>
<td>57.3 ± 1.45</td>
<td>80.0 ± 11.5</td>
<td>57.33 ± 5.6</td>
<td>67.33 ± 4.3</td>
<td>55.33 ± 18.2</td>
</tr>
<tr>
<td>Cit.</td>
<td>77.66 ± 1.2</td>
<td>78.33 ± 4.41</td>
<td>22.66 ± 1.45</td>
<td>15.33 ± 8.3</td>
<td>2.33 ± 0.88</td>
</tr>
<tr>
<td>Cont.</td>
<td>79.0 ± 3.6</td>
<td>82.66 ± 13.8</td>
<td>245.0 ± 2.8</td>
<td>245.0 ± 8.6</td>
<td>234.66 ± 4.3</td>
</tr>
<tr>
<td>LSD value</td>
<td>8.42 * 32.66 * 13.59 * 27.49 * 32.68 * 17.05 * 7.91 * 10.88 * 5.48 * 5.67 *</td>
<td>8 (P&lt;0.05).</td>
<td></td>
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</table>

Table 2: Effect of Active ingredient in tow concentration 1%, 2% on staphylococcus aureus for five days as food preservatives

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
<th>5th day</th>
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<tr>
<td></td>
<td>Const. %</td>
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<td>Const. %</td>
<td>Const. %</td>
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<tr>
<td>Th.</td>
<td>49.0 ± 7.5</td>
<td>70.0 ± 10.4</td>
<td>150.0 ± 5.7</td>
<td>79.66 ± 10.6</td>
<td>117.66 ± 4.6</td>
</tr>
<tr>
<td>R.</td>
<td>63.66 ± 3.6</td>
<td>58.66 ± 12.3</td>
<td>140.3 ± 2.6</td>
<td>273.0 ± 5.1</td>
<td>163.3 ± 7.2</td>
</tr>
<tr>
<td>Cit.</td>
<td>51.66 ± 3.6</td>
<td>80.66 ± 13.7</td>
<td>33.33 ± 5.4</td>
<td>45.33 ± 10.1</td>
<td>1.33 ± 0.33</td>
</tr>
<tr>
<td>Cont.</td>
<td>66.66 ± 4.4</td>
<td>75.0 ± 12.9</td>
<td>246.66 ± 14.5</td>
<td>226.66 ± 14.5</td>
<td>253.33 ± 12.0</td>
</tr>
<tr>
<td>LSD value</td>
<td>15.75 * 24.56 * 27.35 * 34.70 * 24.11 * 24.81 * 37.41 * 50.53 * 20.73 *</td>
<td>(P&lt;0.05).</td>
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Thyme oil reduced the growth of E. coli after 3 days to 43.0 while in staphylococcus urease show well performed in 5 th day to 14.66 In comparison, rosemary oil reduced the growth of E. coli in the 5th day to 4.33 while in staphylococcus aureus show well performed in the 4th day to 10.33. In citric acid the tow organisms growth inhibition in the 2nd day, 22.66 in E. coli and 33.33 in staphelococcus aureus as we see in (Fig. 3, 4) At the concentration of 2% Rosemary oil proved to be more effective against Staphylococcus urease and E. coli in soft cheese than 1% concentration. The oil reduced the growth to 46.00 in E.coli after three days and 19. 66 in Staphylococcus urease after four days in the other hand thyme show well effect against E. coli in the 4th day 21.00 while in staphylococcus aureus show well performed on the 4th day 10.66In case of citric acid the growth of staph.aureus was reduced to 45.33 and the growth of E. coli reduce to 15.33 in the 2nd day as we see in (Fig. 5, 6). All results showed significant growth inhibition of both organisms in the soft cheese when used rosemary, thyme and citric acid compared with the control group (p > 0.05).

Figure 3: Effects of preservatives on growth of E col. during 5 days / 1% concentration
This results mainly because of its antimicrobial, antiviral, antimitotic, and antioxidant properties so we can use it instead of chemical preservatives 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. 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 (43). The effectiveness of rosemary oil as antimicrobial agents in mozzarella cheese against Listeria monocytogenes, have been studied (44). The role of rosemary oil during the cheese-making process was also studied (45) this study agreed with the addition of the oil in the sheep milk. Essential oil used at the 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. Additionally, it had been shown that Rosmarinus Officinal is 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 (46). 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 (47). For all of that the use of essential oils as inhibitors of the growth of spoilage and pathogenic microorganisms is a good choice for replacement of chemical additives in foods.

The essential oil of thyme and its constituent, thymol, were commercially acquired (Sigma-Aldrich). Escherichia coli is the predominant species among facultatively anaerobic intestinal bacteria. The ability to ferment glucose and lactose and to produce acid is amongst the characteristics of this gram-negative bacillus of the family Enterobacteriaceae (48). In this study, the essential oil of T. vulgaris and its major compound thymol showed bacteriostatic and bactericidal activities against E. coli strains \textit{in vitro}.

Nonetheless, the activity of the essential oil was superior to the compound alone. Such finding is explained by the fact that the high antimicrobial activity showed by some essential oils results from the synergism of the major components (49). The antimicrobial activity of the thyme essential oil and of thymol has been evaluated in other studies. (50) Reported significant activity of the extract and essential oil of thyme against E. coli and Salmonella strains, with the MIC of 640 μg mL\(^{-1}\). Such activity was attributed to the high concentration of thymol in the extract (39.7%) and in the essential oil (48.49%). It was also reported antimicrobial activity of the essential oil of thyme against E. coli 5% (V/V) and other food borne bacteria (51). An important role of bacteriostatic and bactericidal activity of the essential oils of thyme and oregano against E. coli O157: H7 isolated from bovine feces has also been observed (52). The phenolic compounds carvacrol and thymol are responsible for the activity of these oils. In solution, weak acid preservatives exist in a pH-dependent equilibrium between the undissociated and dissociated state.

Preservatives have optimal inhibitory activity at low pH because this favors the uncharged, UN dissociated state of the molecule which is freely permeable across the plasma membrane and is thus able to enter the cell. Therefore, the inhibitory action is classically believed to be due to the compound crossing the plasma membrane in the UN dissociated state. In conclusion, the essential oils revealed a significant antibacterial activity against both Gram positive and Gram negative bacteria and may be regarded as one of the alternative sources for food preservation.

**Conclusion**

The results of the present study showed that the essential oil extracts could be used as a natural antibiotic in low concentrations against gram positive and gram-negative bacteria. Therefore, further studies are recommended for the isolation and purification the Essential oil extract from other plants and herb which have a great antibacterial activity to use it as preservatives instead of chemical antibiotic and organic acid preservatives.

**References**
