Enhancement of Refrigerated Shelf Life of Foods against Microbial Spoilage Using Indian Spices

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ABSTRACT


Study Design: Aqueous extract and powder of turmeric (Curcuma longa), black pepper (Piper nigrum), garlic (Allium sativum) and cumin (Cuminum cyminum) were used to enhance refrigerated shelf life of potatoes (Solanum tuberosum), taro roots (Colocasia esculenta), bottle gourd (Lagennaria siceraria) and tomatoes (Solanum lycopersicum).

Place and Duration: The study was made at Shobhit Institute of Engineering & Technology, Meerut during the period of January to December, 2022.

Methodology: Microbial counts technique was used to evaluate the impact of spice treatment on boiled foods stored in the refrigerator.

Results: We found that aqueous treatment of spices was not as effective as treatment with powdered spices. Black pepper treatment enhanced the storage life of all four vegetables followed
by turmeric, garlic and cumin. The mixed treatment with turmeric, black pepper, garlic and cumin was very effective and safe for consumption for up to 3 days when stored in refrigerator.

**Conclusion:** Unconsumed extra foods are commonly stored in refrigerator throughout the world and it is suggested that if the boiled vegetables are treated with a mixture of powder of turmeric + black pepper + garlic + cumin, they may be stored for longer time.

**Keywords:** Vegetables; spices; turmeric; black pepper; cumin; garlic; microbial counts; refrigerated shelf life.

1. **INTRODUCTION**

Spices have been used as flavouring, colouring, and preservatives of foods and also as nutritional agents for human beings since early in the history [1]. Aromatic plan materials including spices have been used in food preparation, preservation as well as for embalming in several countries as Hindustan and Spice Islands [2,3]. The spice trade in historic period was so crucial to economies of the countries that the rulers repeated mounted costly expeditions and Christopher Columbus undertook a hazardous voyages to establish routes to spice growing countries-India. As per United States Food and Drug Administration (FDA), spice is an "aromatic vegetable compound in whole, broken, or crushed form, the main purpose of which in food is seasonings rather than nutrition" and from which "no component of any volatile oil or other flavoring method has been removed" [4].

Around the world, more than 100 different types of spices are produced. Asia is the primary producer of spices mainly cinnamon, pepper, nutmeg, cloves, ginger, turmeric, black pepper, cumin, fenugreek etc. while Europe generally grows basil, bay leaves, celery leaves, chives, coriander, dill tips, thyme, and watercress. Instead, pepper, nutmeg, ginger, allspice, and sesame seed are the most commonly grown spices in the United States [5]. While spices (primarily dried seed, fruit, root, bark, or plant products) have traditionally been used for rituals, skincare products, and fragrance, flavoring, coloring, and preservation characteristics have widespread applications in the traditional food preparations and the food industry [6].

Most spices contain dozens of chemicals as secondary metabolites which evolved in these plants to protect them against herbivorous insects and vertebrates, fungal, bacterial, viral pathogens and parasites as antimicrobial agents and repellents. Many chemicals extracted from spices have been demonstrated to exhibit antibacterial activity against food borne and Entropathogens [7,8]. They can stimulate saliva secretion, promote digestion, protect against colds and influenza, and reduce nausea and vomiting [9].

Food spoilage is a common phenomenon where food is permanently deteriorated and becomes tasteless or destroys its quality. These changes can be caused by a wide range of reasons, including physical (oxygen, temperature, and light) and biological (enzymatic activity and microbial growth and development). Despite current production-chain technology (such as storage in the freezer, pasteurization, drying, and preservatives), it appears difficult to totally eliminate the risk of food spoilage [10]. One of the most serious problems with food spoilage is the oxidation of lipids [11]. To prevent spoiling, food companies use antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), whereas their safety is being challenged, and customers are developing more interest in natural products [12]. Spices contain antioxidant properties, owing to the presence of phenolic substances [13] and possess antimicrobial properties with no side effects on our system rather they enhance the digestibility, improve taste and flavor including the pleasant colour. The primary constituents of all spices include phenolics compounds, flavonoids, saponins and terpenes, which are the foundation of the qualities of various of spices [8, 14]. These natural compounds extracted from spices also exhibit antimicrobial activity [15,16]. Further these natural bio-preservatives can play an important role in overcoming challenges connected with microbial resistance while also keeping food products protected and safe without causing side effects that are harmful to customers [17]. These properties make spice extracts a potential substitute for currently available synthetic additives and their applications in food industry is being promoted further [18].

Although natural food preservative components are viewed as an alternative to synthetic preservatives, their use is considered to be...
2. MATERIALS AND METHODS

In this study, we designed the experiments to evaluate the microbial counts of foods treated with and without treatment of aqueous extracts and powdered spices separately. All selected spices turmeric (Curcuma longa), black pepper (Piper nigrum), garlic (Allium sativum), and cumin (Cuminum cyminum) were purchased from the local market of Meerut (U.P.), India and sorted out for any impurities. These were thoroughly washed under running water, wiped with tidy and clean dry cloths, then dried under sunlight, grounded into a fine powder and stored in an airtight container. All selected vegetables potatoes (Solanum tuberosum), taro root (Colocasia esculenta) and bottle gourd (Lagennaria siceraria) and tomatoes (Solanum lycopersicum) were also purchased from local market of Meerut; U.P. These vegetables were thoroughly rinsed under running water. These vegetables were boiled, peeled, and sliced into medium-sized pieces.

2.1 Treatment of Vegetables with Spices

5 g of each of the above 4 boiled vegetable was placed in 50 mL of aqueous extract of each of the test 4 spices individually as well as in combinations and their pH was determined using a glass electrode with a digital pH meter (Systronic µ pH system 362). 100 g of each of the boiled vegetable was also treated with 200 mg of the dried powder of each of test spice - turmeric, black pepper, garlic and cumin separately and in combinations. To prepare the combination equal amounts of the spice were mixed and only 200 mg of the mixture was used for each of the 100 g of boiled vegetable. The powder of the spice was sprinkled over the surface of the vegetable uniformly so as to cover the whole surface. The control included untreated boiled vegetables. Twelve samples for each of the treatment and control were prepared and incubated glass jar in the refrigerator in the kitchen (where the temperature varied from 4-10°C) and the samples were drawn after every 24 h and processed for microbial counts.

2.2 Microbial Counts from Spice Treated and Untreated Samples

Three samples of each treatment and control were collected aseptically for microbial examination immediately after treatment on zero day and after every 24 for 3 days from the refrigerated samples. Total bacterial counts as colony forming units were assessed from each of the three samples of each treatment and control separately by preparing a suspension of 1 g of sample in 10 mL of sterile distilled water from which the dilution series of 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ were prepared. 1 mL aliquot of each of the suspension was poured into sterile 10 cm diameter Petri dish to which 20 mL of sterile nutrient agar medium was poured aseptically when it was cool but still molten [21]. These plates incubated at for 24-48 h at 28 and 37 ± 1°C and were examined for colony counts. The highest dilution which showed non-overlapping countable number of colonies was considered for calculations of microbial counts in terms of colony forming units. CFU/g of food = Total number of colonies × Dilution Factor / Volume of suspension / aliquot in the plate in mL

3. RESULTS AND DISCUSSION

The antimicrobial activities of aqueous extracts of common kitchen spices - turmeric, black pepper, clove, cumin, cinnamon, garlic and fenugreek against the common food borne and Entopathogens Pseudomonas aeruginosa (ATCC 9027), Bacillus subtilis (ATCC 6633), Shigella flexneri (ATCC 12022), Cronobacter sakazakii (ATCC 29544), Escherichia coli (ATCC 8739), Staphylococcus aureus (ATCC 6539), Salmonella enterica (ATCC 14028) and Vibrio cholerae (ATCC 3906) have earlier been demonstrated in our laboratories [8].

During the present study, only turmeric, garlic, black pepper and cumin were evaluated for their potential to increase shelf life of stored vegetables in refrigerator as these are the most common spices used in all food preparations [22]. The pH of the aqueous extract of selected spices and the foods after treatment with species are shown in Table 1 which shows that except cumin extract all spices produced acidic values. Tomato paste was basically acidic in nature but after treatment with spices, its acid value decreased and cumin brought drastic change to make it alkaline [23, 24]. The alkaline property of cumin helps in digestion of food and reduces acidic waste in the body, provide antioxidant property and helps in prevention diseases like diabetes, liver cirrhosis, high blood pressure and cancer. Spices have been used in flavoring and
Table 1. pH selected vegetable after treatment with aqueous extract of selected spices

<table>
<thead>
<tr>
<th>Spice</th>
<th>Aqueous extract</th>
<th>Potatoes</th>
<th>Bottle gourd</th>
<th>Taro Roots</th>
<th>Tomatoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turmeric</td>
<td>5.9</td>
<td>6.38</td>
<td>6.8</td>
<td>6.14</td>
<td>4.9</td>
</tr>
<tr>
<td>Clove</td>
<td>4.6</td>
<td>6.02</td>
<td>6.8</td>
<td>6.06</td>
<td>4.7</td>
</tr>
<tr>
<td>Cumin</td>
<td>8.6</td>
<td>6.04</td>
<td>6.8</td>
<td>6.04</td>
<td>7.6</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>7.4</td>
<td>6.08</td>
<td>6.7</td>
<td>6.30</td>
<td>6.7</td>
</tr>
<tr>
<td>Garlic</td>
<td>6.4</td>
<td>6.17</td>
<td>6.8</td>
<td>6.20</td>
<td>5.2</td>
</tr>
<tr>
<td>Blackpepper</td>
<td>5.6</td>
<td>6.21</td>
<td>6.8</td>
<td>6.50</td>
<td>4.7</td>
</tr>
<tr>
<td>Mustard</td>
<td>5.4</td>
<td>6.25</td>
<td>6.5</td>
<td>6.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>6.7</td>
<td>6.16</td>
<td>6.8</td>
<td>6.6</td>
<td>5.2</td>
</tr>
</tbody>
</table>

The majority of dietary pathogens are pH sensitive, low or extremely high pH values prohibit microbial development [28]. Food is digested by our intestines with the help of pancreatic organs that release digestive enzymes. The acidity and alkalinity of the original food is important when the digested food reaches the bloodstream through the gastrointestinal tract walls [29] and alkaline foods have numerous advantages.

Scientists are investigating the innovative methods to save the wastage of food by increasing better storage conditions and by increasing shelf life of stored food materials in the refrigerator. With this view point the antimicrobial activities of common Indian spices was used as a strategy to pre-treat the foods with selected spices before storage in the refrigerator and Table 2 shows that when aqueous extract turmeric, black pepper, fenugreek, garlic, mustard, clove, cumin and cinnamon were used to treat the boiled potatoes, taro roots, bottle gourd and tomatoes, it was found that all spices reduced the microbial counts of test foods, however, the highest reduction was observed by clove followed by cinnamon, turmeric and garlic (Figs. 1, 2, 3). Potato and taro roots showed almost similar effect while bottle gourds were less protected and the tomatoes were highly protected with treatment of aqueous extracts of the spices (Table 2, Figs. 1 and 2). Food spoiling is a mechanism that occurs when a product appears undesirable or unhealthy for human consumption. The identification of spoiled food can be critical in preventing food borne infections, and different technologies have been developed to detect spoilage of food including the foul smell and visibility of softness of food tissues [30]. The protective value of aqueous extract of spices remained active till 3 days of incubation in the refrigerator, thereafter the food started giving foul smell.

The microbial colonies produced during storage with and without spice treatment were counted and the results are presented in Tables 2, 3 and Fig. 3. The treatment and untreated materials were also tested for foul smell and visible symptoms of spoilage and it was noticed that the powdered spice treated foods did not show any foul smell till 3 days while the untreated (control) exhibited the foul smell in 24-36 h. Hence, it is suggested that powdered spices may be used to enhance shelf life of foods in the refrigerator.

In second set of experiment the treatment was given with dried powder of the four most commonly used spices-turmeric, black pepper, garlic and cumin and their mixtures. Surprisingly, it was observed that the number of microbial counts in control as well as treated foods dropped down 10 fold when compared with aqueous extracts. Black pepper was highly active in control of microbial counts in all four vegetables - potatoes, taro roots, bottle gourd and tomatoes followed by garlic turmeric and cumin powder (Table 3). When the mixture of turmeric + black pepper; turmeric + garlic, turmeric + cumin and the mixture of turmeric + black pepper + garlic + cumin were used it was found that the mixture of all four spices was very effective in protection of foods against spoilage and from the microbial counts (Table 3).
Table 2. Number of bacterial colony forming units (× 10⁴/g fresh weight) isolated from aqueous extract spices-treated and untreated foods stored in refrigerator at 4 to 10°C temperature over a period of 3 days (each figure is an average of 3 independent replicate)

<table>
<thead>
<tr>
<th>Vegetable Spices</th>
<th>Potatoes</th>
<th>Taro Root</th>
<th>Bottle gourd</th>
<th>Tomatoes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 1</td>
</tr>
<tr>
<td>C T C T C T C T C T C T C T C T C T C T C T C T C T C T C T C T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td>128</td>
<td>67</td>
<td>525</td>
<td>171</td>
</tr>
<tr>
<td>Black pepper</td>
<td>128</td>
<td>82</td>
<td>525</td>
<td>176</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>128</td>
<td>94</td>
<td>525</td>
<td>189</td>
</tr>
<tr>
<td>Garlic</td>
<td>128</td>
<td>92</td>
<td>525</td>
<td>259</td>
</tr>
<tr>
<td>Mustard</td>
<td>128</td>
<td>98</td>
<td>525</td>
<td>274</td>
</tr>
<tr>
<td>Clove</td>
<td>128</td>
<td>58</td>
<td>525</td>
<td>169</td>
</tr>
<tr>
<td>Cumin</td>
<td>128</td>
<td>97</td>
<td>525</td>
<td>254</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>128</td>
<td>69</td>
<td>525</td>
<td>174</td>
</tr>
</tbody>
</table>

Abbreviation used: C = Control; T = treated with spice;

Table 3. Number of bacterial colony forming units (× 10⁵/g fresh weight) isolated from powdered spices-treated and untreated foods stored in refrigerator at 4 to 10°C temperature over a period of 3 days (each figure is an average of 3 independent replicates)

<table>
<thead>
<tr>
<th>Vegetable Spices</th>
<th>Potatoes</th>
<th>Taro Root</th>
<th>Bottle gourd</th>
<th>Tomatoes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 1</td>
</tr>
<tr>
<td>C T C T C T C T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turmeric</td>
<td>168</td>
<td>45</td>
<td>489</td>
<td>120</td>
</tr>
<tr>
<td>Black pepper</td>
<td>168</td>
<td>23</td>
<td>489</td>
<td>68</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>168</td>
<td>36</td>
<td>489</td>
<td>85</td>
</tr>
<tr>
<td>Garlic</td>
<td>168</td>
<td>56</td>
<td>489</td>
<td>134</td>
</tr>
<tr>
<td>Cumin</td>
<td>168</td>
<td>0.65</td>
<td>489</td>
<td>20</td>
</tr>
<tr>
<td>Turmeric + Black Pepper</td>
<td>168</td>
<td>0.86</td>
<td>489</td>
<td>34</td>
</tr>
<tr>
<td>Turmeric + Garlic</td>
<td>168</td>
<td>0.96</td>
<td>489</td>
<td>45</td>
</tr>
<tr>
<td>Turmeric + Cumin</td>
<td>168</td>
<td>0.22</td>
<td>489</td>
<td>09</td>
</tr>
</tbody>
</table>
Fig. 1. Aqueous extract spices-treated and untreated foods (A). Potatoes (B). Taro root (C). Bottle gourd and (D). Tomatoes

(A) Potatoes

(B) Taro root

(C) Bottle gourd

(D) Tomatoes

(A) Potatoes with turmeric

(B) Taro root with turmeric
(C) Bottle gourd with turmeric  
(D) Tomatoes with turmeric  
(E) Potatoes with black pepper  
(F) Taro root with black pepper  
(G) Bottle gourd with black pepper  
(H) Tomatoes with black pepper
(I) Potatoes with garlic

(J) Taro root with garlic

(K) Bottle gourd with garlic

(L) Tomatoes with garlic

(M) Potatoes with cumin

(N) Taro root with cumin
Fig. 3. Bacterial colonies formed on spices-treated and untreated foods stored in refrigerator at 4 to 10°C temperature over a period of 3 days (A) Day 0, (B) Day 1, (C) Day 2 and (D) Day 3

Mostly food borne pathogens caused severe diseases like typhoid, diarrhea, and cholera etc [31]. Normally cooked food eliminates microorganisms on food before it reaches our plate while uncooked foods are typical sources of food illness since they do not pass through high temperature [32,33]. Microbes can contaminate food in a variety of ways, including growth, nutrient consumption, enzymatic alterations, unpleasant flavors, chemical breakdown, and the production of chemicals. Food deterioration affects the human health and cause disease like diarrhea, cholera, vomiting, nausea and stomach pain [34,35,36]. An increasing percentage of consumers especially after Covid-19 pandemic prefer minimal processing of foods, without artificial preservatives. Many of these processed foods and novel food types represented innovative food system in terms to health hazard and nutritional risk [37,38]. In considering this, and depending on greater awareness of the complex nature of microbial interactions, recent strategies focus increasingly on the potential that biological preservation offers. The antimicrobial properties of common spices were used in this study to evaluate their potential to increase shelf life of refrigerated foods without any chemical preservatives. It is a common practice in Indian, Asian and western kitchen including Nordic countries the extra foods are stored in the refrigerator for further use but many times it develop foul smell and get deteriorated due to microbial growth. Our studies shows that if the
Our study showed that Indian spices may be used to increase the shelf life of refrigerated foods and the treatment of boiled vegetable with dried powder of turmeric + black pepper + garlic + cumin within eatable limits (200 mg/100 g) can enhance shelf life of refrigerated food by protecting them from microbial growth and contamination. Additionally, they add flavor, aroma and color to the food. The spices are safe to be used for human consumption. They have numerous health benefits.

4. CONCLUSIONS

Our study showed that Indian spices may be used to increase the shelf life of refrigerated foods and the treatment of boiled vegetable with dried powder of turmeric + black pepper + garlic + cumin within eatable limits (200 mg/100 g) can enhance shelf life of refrigerated food by protecting them from microbial growth and contamination. Additionally, they add flavor, aroma and color to the food. The spices are safe to be used for human consumption. They have numerous health benefits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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