

Original Research Article
Enhancement of Refrigerated Shelf Life of Foods Against Microbial Spoilage Using Indian Spices

Abstract

AIMS: Evaluation of the effects of treatment of aqueous extracts and powder of spices on enhancement of refrigerated shelf life of vegetables.

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.

Introduction

Spices have been used as flavouring, coloring, preservatives of foods and also as nutritional agents for human beings since early in the history (Campelo *et al.*, 2019). Aromatic plant materials including spices have been used in food preparation, preservation as well as for embalming in several countries as Hindustan and Spice Islands (Govindrajana, 1985, Sherman and Billing, 1999). The spice trade in historic

period was so crucial to economies of the countries that the rulers repeatedly mounted costly expeditions and Christopher Columbus undertook a hazardous voyage 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"(Gottardi *et al.*, 2016).

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 (Noli *et al.*, 2019). 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 (Tripathi *et al.*, 2007).

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 (Gupta, *et al.*, 2008, Roy and Garg, 2023). They can stimulate saliva secretion, promote digestion, protect against colds and influenza, and reduce nausea and vomiting (Dey and Nagababu 2022).

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 (Yang *et al.*, 2011). One of the most serious problems with food spoilage is the oxidation of lipids (He and Chen 2014). 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. Spices contain antioxidant properties, owing to the presence of phenolic substances (Batiha *et al.*, 2021) 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 phenolic compounds, flavonoids, saponins and terpenes, which are the foundation of the qualities of various of spices (Pokorny and Panek 2012; Roy and Garg, 2023). These natural compounds extracted from spices also exhibit antimicrobial activity (De

Almeida *et al.*, 2022; Dirpan and Hidayat 2023). 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 (Pop *et al.*, 2019). These properties make spice extracts a potential substitute for currently available synthetic additives and their applications in food industry is being promoted further (Vidua *et al.*, 2010).

Although natural food preservative components are viewed as an alternative to synthetic preservatives, their use is considered to be causing some health issues, particularly when used excessively and over a long period of long time (Lone *et al.*, 2015).

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.

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.

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 f 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. 1mL 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 (Bisht and Garg (2019)). These plates incubated at for 24-48 h at 28 and $37 \pm 1^{\circ}\text{C}$ 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.

$$\text{CFU/g of food} = \frac{\text{Total number of colonies} \times \text{Dilution Factor}}{\text{Volume of suspension/aliquot in the plate in ml}}$$

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 Entropathogens *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 (Roy and Garg, 2023). 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. 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. 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 coloring of foods since ages and they contain minerals, vitamins, high antioxidants, and anti inflammatory properties. They also helps in muscles cramps, tooth decay (Narayan *et al.*, 2021), boost immune system, strengthen blood vessels, reducing blood clots, and possess anti dandruff properties (Garg, 1992) and protect against skin diseases (Garg and Müller 1993).

Table 1: pH selected vegetable after treatment with aqueous extract of selected spices

Spice	Aqueous extract	Potatoes	Bottle gourd	Taro Roots	Tomatoes
Turmeric	5.9	6.38	6.8	6.14	4.9
Clove	4.6	6.02	6.8	6.06	4.7
Cumin	8.6	6.04	6.8	6.04	7.6
Cinnamon	7.4	6.08	6.7	6.30	6.7
Garlic	6.4	6.17	6.8	6.20	5.2
Black pepper	5.6	6.21	6.8	6.50	4.7
Mustard	5.4	6.25	6.5	6.5	4.9
Fenugreek	6.7	6.16	6.8	6.6	5.2

The majority of dietary pathogens are pH sensitive, low or extremely high pH values prohibit microbial development (Kolobe *et al.*, 2023). 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 (Yang *et al.*, 2022) 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. Potato and taro roots showed almost similar effect while bottle gourd were less protected and the tomatoes were highly protected with treatment of aqueous extracts of the spices (Table 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 (Fadaiji *et al.*, 2023). 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.

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 was 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).

Mostly food borne pathogens caused severe diseases like typhoid, diarrhea, and cholera etc. 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 (Wongsa *et al.*, 2023). 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 affect the human health and cause disease like diarrhea, cholera, vomiting, nausea and stomach pain (Shouket *et al.*, 2023). 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. 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 extra food is treated with dried powder of mixture of turmeric + black pepper + garlic + cumin before storage in the refrigerator, it can save the food from spoilage, can add to the flavor and aroma of the food and is safe for consumption.

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.

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Table 2: Number of bacterial colony forming units ($\times 10^4$ /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).

Vege table	Potatoes						Taro Root						Bottle gourd						Tomatoes					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 1		Day 2		Day3		Day 1		Day 2		Day3	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Turm eric	1	6	5	1	10	3	1	5	5	1	9	3	2	9	5	2	11	4	9	4	1	8	3	1
	2	7	2	7	02	2	2	9	0	1	8	3	2	8	2	2	04	3	6	3	7	6	5	1
	8	5	5	1		5	4		6	8	9	9	8		8	4		1		8		6		2
Black pepp er	1	8	5	1	10	3	1	7	5	1	9	3	2	1	5	2	11	4	9	2	1	5	3	8
	2	2	2	7	02	6	2	6	0	2	8	2	2	0	2	4	04	2	6	4	7	2	5	6
	8		5	6		8	4		6	6	9	7	8	3	8	3		4		8		6		
Fenu greek	1	9	5	1	10	3	1	8	5	1	9	3	2	1	5	2	11	4	9	5	1	8	3	1
	2	4	2	8	02	8	2	4	0	3	8	8	2	0	2	3	04	4	6	6	7	9	5	3
	8		5	9		0	4		6	4	9	9	8	2	8	6		6		8		6		0

Garlic	128	92	525	259	1002	330	124	646	504	1249	934	228	1588	214	1104	424	96	29	178	62	356	104
Mustard	128	98	525	274	1002	367	124	926	5096	9809	988	228	1588	232	1104	471	96	29	178	62	356	104
Clove	128	58	525	169	1002	315	124	566	5066	989	326	228	958	212	1104	419	96	29	178	48	356	96
Cumin	128	97	525	254	1002	344	124	686	5054	989	327	228	1588	237	1104	441	96	29	178	88	356	104
Cinnamon	128	69	525	174	1002	322	124	796	5029	989	324	228	1588	226	1104	453	96	29	178	74	356	105

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

Table 3: Number of bacterial colony forming units ($\times 10^3$ /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).

Veg etabl e	Potatoes						Taro Root						Bottle gourd						Tomatoes					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 1		Day 2		Day3		Day 1		Day 2		Day3	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Turmeric	168	458	489	120	670	289	158	548	436	136	868	312	756	490	128	836	986	086	236	234	560	866		
Black pepper	168	238	489	680	678	188	158	366	856	868	186	256	490	496	764	830	984	024	236	130	560	464		
Garlic	168	368	489	850	670	284	158	468	116	826	226	258	490	194	864	268	984	054	236	138	560	568		
Cumin	168	568	489	134	670	306	158	686	446	868	246	254	690	496	164	890	988	068	236	338	560	762		
Turmeric+ Black Pepper	168	068	489	270	678	819	157	074	436	368	628	256	078	490	484	864	190	984	014	236	056	569	092	
Turmeric + Garlic	168	088	489	374	671	180	159	083	446	496	868	866	256	490	544	863	986	036	236	038	560	160		
Turmeric + Cumin	168	098	489	455	675	156	159	088	466	588	868	964	256	490	692	868	988	058	236	092	566	168		
Tur	168	068	480	664	474	180	106	441	868	383	226	044	282	428	859	980	206	206	560	050	560	060		

meri	6	2	8	9	7	6	5	3	6	4	6	0	5	3	9	1	6	6	8	0	3	1	6	3
c +	8	2	9		0		8	4	6		8		6	8	0		4			9	6	2	0	4
Blac																								
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pepp																								
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