



Study of Effect Spices on *Salmonella* Contaminated food as Natural Antimicrobial Agents In Yemen's Food

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Abstract

The important of using spices as foods and flavor have been known since ancient times and as medicine and food preservatives in recent decades. This study was aimed to evaluate the effects of some spices that using in Yemen's cooking food on controlling the growth of *Salmonella* in food. The antibacterial activity of black pepper and garlic powder (1% w/w) were tested independently in red meat and chicken meat inoculated with *Salmonella*, while the cardamom and cinnamon powder (1% w/v) were tested in milk separately which inoculated with 10^3 cells of *Salmonella* per gm or mL of foods. Four treatments, that singed as a T1, T2, T3, and T4, were prepared for each test in duplicated and incubated individually at room temperature (20-25°C) and refrigerator (4°C) temperature. The results were observed that the antibacterial activity of tested spices powder have slightly effect in reducing the *Salmonella* count in foods. Also, the results of statistical analysis didn't showed significant difference ($P > 0.05$) in the antibacterial effect of all tested spices against *Salmonella* growth between treatment (T1) and (T2) in room and refrigerator temperatures. It can be concluded that the concentration (1% w/w or mL) of tested spices powder that added to tested foods that cooked according to Yemeni cooking procedure didn't showed any effects against *Salmonella*.

Keywords: Antibacterial activity, *Salmonella*, Spices, Yemeni Foods

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Introduction

Recently the growing concern about safety of food has led to the development of natural antimicrobials to control foodborne pathogens. Spices contain natural antimicrobial compounds that can be employed in stabilization and prevention of food spoilage by microbes. This could be observed when spices show initially high microbial charge and as time progresses, the microbial growth become progressively slower or it is eventually completely suppressed (Fabio et al., 2003; Kizil and Sogut, 2003).

In addition, the spice ingredients impart characteristic taste, aroma, or tastiness and color to foods. The antimicrobial properties of spices is related to its essential oil contents, which are volatile oils, obtained by different extraction methods. However, the antimicrobial activity of spices depends on microbial specie and its occurrence level, composition and concentration of spice, type of spice, and processing conditions and storage (Shelef, 1983; Farag et al., 1989).

Utilization of spices in the form of powder, extracts or essential oils to check growth of many spoilage bacteria in foods have been well documented (Meena and Sethi, 1997; Subbulakshmi and Naik, 2002). Essential oils, which have been used for decades to extend the shelf life of foods, are becoming more popular due to their antioxidant and antimicrobial properties (Goni et al., 2009). It was reported that substances in cinnamon and clove essential oils inhibited the growth of molds, yeasts, and bacteria (Matan et al., 2006). Also, a study by Gutierrez et al. (2009) who found that spices and herbs containing 0.05 to 0.1% essential oils have established activity against certain pathogens,

including *Salmonella typhimurium*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus*, and *Staphylococcus aureus* in food systems.

Spices are typically commercially used in the form of dried leaves or powder, but most of the work on spices is going on to extract their active substance in pure form and assessment of its antimicrobial activity. In the marketable form, it is important to know exactly what is the active ingredient present in a particular spice and how much of it is required for preservation of meat or other food products (Yadav and Singh, 2004).

Up to now, there is diminutive attention about the antibacterial activity of spices powder on foodborne pathogens as an alternative preservative. In Yemen, addition of spices to food in powder form

to enhance its characterization like taste, color, and flavor without known about the antimicrobial effects. Therefore, this study was aimed to evaluate the antibacterial effects of some plants in powder form against *Salmonella* bacterium contaminated red meat, chicken meat, and milk.

Materials and Methods

This study was conducted from 03 August to 05 October, 2019 in the Microbiology Laboratory, Biology department, Faculty of Sciences, Sana'a University.

Collection of plant materials

The selected spices that listed in Table 1 were purchased from a local grocery store in packaged form from Sana'a city and identified and confirmed in the Department of Biology at Sana'a University.

Common name	Botanical name	Part used
Garlic	<i>Allium sativum</i> L	Cloves
Black pepper	<i>Piper nigrum</i> L	Fruit
Cinnamon	<i>Cinnamom umburmannil</i>	Bark
Cardamom	<i>Elettaria cardamomum</i>	Seeds

Table 1: Lists of spices used in the assessment of antibacterial activities

Sample processing

The samples were grinded in a grinder. The powdered spices were used for extraction of essential oils and other extracts successively.

Test isolates and inoculum preparation

Three isolates of *Salmonella* species that subjected in this study were isolated previously from red meat, chicken meat, and milk samples (Taha et al., 2013). The bacterial concentration of isolated *Salmonella* species was adjusted to 10^3 CFU/mL by using a spread plate technique.

Antibacterial tests

In this study, four treatments were applied to evaluate the antibacterial effects of garlic, black pepper, cinnamon, cardamom powder (1% w/w or mL) against *Salmonella*. The four treatments are:

Treatment 1 (T1): one gm powder of each spices was added individually to 100 gm of sterile chicken, beef meat, and 100 mL of sterile milk that inoculated separately by *Salmonella* bacteria with concentration of 10^3 cell per mL of tested food.

Treatment 2 (T2): 100 gm each of sterile chicken meat, beef meat, and 100 mL of sterile milk contained 10^3 CFU/mL of *Salmonella*.

Treatment 3 (T3): one gm powder of spices was added separately to 100 gm of sterile chicken meat, beef meat, and 100 mL of sterile milk.

Treatment 4 (T4): 100 gm of sterile chicken meat, beef meat, and 100 mL of sterile milk was tested without addition.

Each treatment was performed in duplicate. One duplicate of four treatments was incubated at room temperature (20-25°C) and the another was incubated at refrigerator temperature (4°C) for 48 h.

Total viable count

All treatments were submitted to viable count at zero time and after 24 h and 48h. Viable counts were done by making serial dilutions in peptone water from each sample and then 0.1 mL of these dilutions were spread on surface of XLD medium, and incubated at 37°C for 24 h (Harley and Prescott, 2002). Treatments 1, 2 represent test treatments while 3, 4 treatments were a control treatments.

Statistical Analysis Data

The obtained data was subjected to statistical analysis by using IBM SPSS statistics software (version 16) to compare between the room and refrigerator incubation and used treatments. Values of $p < 0.05$ was considered statistically significant.

Results

The results of black pepper effect on red meat contaminated with 10^3 cells of *Salmonella* per gram of red meat are listed in Table (2). At room temperature, it was found that the number of viable count was increased from 6.8×10^6 cells/gm after 24 h to 1.6×10^9 cells/gm after 48 h in treatment (1). Also, the treatment (2) without black pepper powder, the number of *Salmonella* cells were increased from 1.0×10^3 cells/gm at zero time to 3.6×10^9 cells/gm after 48 h of incubation.

However, at refrigerator incubation, it was observed that the increase in *Salmonella* cells from 1.0×10^3 cells/gm at zero time to 6.1×10^5 cells/gm in (T1) and 2.4×10^6 cells/gm for (T2) after 48 h of incubation. Whereas, the sterile red meat with black pepper (T3) and sterile red meat (T4) alone showed zero number of counts after 24 h and 48 h of incubation at room and refrigerator temperatures (Table 2).

Treatments	At room temperature (20-25°C)			Refrigerator temperature (4°C)			f	P-value
	Zero time	24 h	48 h	Zero time	24 h	48 h		
T 1	1x10 ³	6.8x10 ⁶	1.6x10 ⁹	1x10 ³	3.4x10 ⁴	6.1x10 ⁵	6.712	0.09*
T 2	1x10 ³	2.3x10 ⁷	3.6x10 ⁹	1x10 ³	3.7x10 ⁴	2.4x10 ⁶		
T 3	0	0	0	0	0	0		
T 4	0	0	0	0	0	0		

*The P value is not statistically significant at the 0.05 level.

Table 2: Effects of black pepper on Salmonella in red meat

Table (3) show the results of black pepper effects on chicken meat contaminated with 10³ cells of *Salmonella* per gram of this food. It was recorded the increase in number of *Salmonella* cells from 1.0x10³ cells/gm at zero time to 5.2x10¹¹ and 1.1x10¹¹ cells/gm for T1 and T2, respectively, after 48 h of incubation at room temperature. The results of this

study were revealed that number of *Salmonella* cells were increased at refrigerator incubation from 1.0 x10³ cells/gm at zero time to 1.2x10⁵ cells/gm in T1 after 48 h of incubation. While, the T3 and T4 no showed growth after 48 h of incubation at room and refrigerator temperatures (Table 3).

Treatments	At room temperature (20-25°C)			Refrigerator temperature (4°C)			F	P-value
	Zero time	24 h	48 h	Zero time	24 h	48 h		
T 1	1x10 ³	1.4x10 ⁹	5.2x10 ¹¹	1x10 ³	3.1x10 ⁴	1.2x10 ⁵	1.026	0.397*
T 2	1x10 ³	1.2x10 ¹⁰	1.1x10 ¹¹	1x10 ³	9x10 ²	1x10 ³		
T 3	0	0	0	0	0	0		
T 4	0	0	0	0	0	0		

*The P value is not statistically significant at the 0.05 level.

Table 3: Effects of black pepper on Salmonella in chicken meat

The current study was showed the effect of garlic powder on red meat and chicken meat contaminated with 10³ cells of *Salmonella* bacteria. In the treatment T1, the *Salmonella* was increased from 10³ at zero time to 1.6x10⁹ cells/gm and 1.1x10¹¹ cells/gm in red meat and chicken meat,

respectively, after 48 h of incubation at room temperature (Table 4). Whereas, at refrigerator incubation, there are increase in viable count of *Salmonella* in red meat and no increase in chicken meat. The control treatment T3 and T4 didn't showed growth of *Salmonella* on both of red meat and chicken meat as shown in the Table (4).

Food name	Treatments	At room temperature (20-25°C)			Refrigerator temperature (4°C)			f	P-value
		Zero time	24 h	48 h	Zero time	24 h	48 h		
Red Meat	T 1	1x10 ³	1.2x10 ⁷	1.6x10 ⁹	1x10 ³	3.4x10 ⁴	9.5x10 ⁵	2.303	0.156*
	T 2	1x10 ³	2.3x10 ⁷	3.6x10 ⁹	1x10 ³	3.7x10 ⁴	2.4x10 ⁶		
	T 3	0	0	0	0	0	0		
	T 4	0	0	0	0	0	0		
Chicken	T 1	1x10 ³	1.4x10 ¹⁰	5.2x10 ¹¹	1x10 ³	1.2x10 ⁶	1.2x10 ⁸	1.008	0.403*
	T 2	1x10 ³	1.2x10 ¹⁰	1.1x10 ¹¹	1x10 ³	9x10 ²	1x10 ³		
	T 3	0	0	0	0	0	0		
	T 4	0	0	0	0	0	0		

*The P value is not statistically significant at the 0.05 level.

Table 4: Effects of garlic on Salmonella in red meat and chicken meat

The effect results of cardamom and cinnamon powder on *Salmonella* contaminated milk were listed in the Table (5). The cell number of *Salmonella* was increased from 10^3 at zero time to 2.3×10^9 and 8.8×10^8 cell/

mL in T1 treated with cardamom and cinnamon, respectively, after 48 h of incubation at room temperature. Whereas, there are very slowly in number increase at refrigerator incubation after 48 h as shown in the table (5).

Spices name	Treatments	At room temperature (20-25°C)			Refrigerator temperature (4°C)			f	p-value
		Zero time	24 h	48 h	Zero time	24 h	48 h		
Cardamom	T 1	1×10^3	1.5×10^9	2.3×10^9	1×10^3	2×10^3	7.1×10^3	1.002	0.405*
	T 2	1×10^3	2.4×10^9	1×10^{12}	1×10^3	1×10^3	6×10^2		
	T 3	0	0	0	0	0	0		
	T 4	0	0	0	0	0	0		
Cinnamon	T 1	1×10^3	1.6×10^7	8.8×10^8	1×10^3	1.3×10^3	3.1×10^4	1.459	0.283*
	T 2	1×10^3	4.7×10^7	5.4×10^9	1×10^3	1×10^3	6×10^2		
	T 3	0	0	0	0	0	0		
	T 4	0	0	0	0	0	0		

*The P value is not statistically significant at the 0.05 level.

Table 5: Effects of cardamom and cinnamon on *Salmonella* in milk.

Discussion

The worldwide demand for foods free or with low added chemical preservatives have been an increasing by consumer because synthetic preservatives could be toxic to humans. The looking for alternative or natural preservatives for extending the shelf life of foods is crucial to exclude the chemical substance as food preservative. Spices consider to be one of the most alternative natural products that have antimicrobial activity for inhibit or prevent the microbial growth in foods (Souza et al., 2005).

In this study, the effect of three spices powder on tested food contaminated with *Salmonella* bacteria were performed at temperature room and refrigerator temperature. The present results were demonstrated that the addition of black pepper powder (1% w/w) to red meat did not effectively inhibit the growth of *Salmonella* bacteria in both of T1 and T2 that incubated individually at room temperature and refrigerator temperature. The *Salmonella* count were increased significantly in T1 during incubation at room temperature from 1.0×10^3 to 1.6×10^9 cells/gm after 48 h and from 1.0×10^3 to 6.1×10^5 cells/gm after 48 h of incubation at refrigerator temperature.

In addition, similar results were obtained when added the black pepper powder (1% w/w) to chicken meat contaminated with 10^3 cells of *Salmonella* per gram. The slightly decrease of *Salmonella* cells numbers in T1 on both of incubation temperature indicated that the black pepper have the antibacterial activity in powder form even at level concentration.

The is bioactive compound in black pepper was identified a Piperine which has been reported *in vitro* to be the major contributors to the antimicrobial activity of spices (Chaudhry and Tariq, 2006). The antimicrobial activity of black pepper is due to the presence of essential oil whose aroma is dominated by monoterpenes hydrocarbons and piperine. The mechanism of antibacterial action of black pepper is not fully understood (Pundir and Jain, 2010).

The antibacterial activity of black pepper extraction has been studied Ganesh et al. (2014) against *S. typhi*, *Proteus* sp., *E. coli*, *S. aureus*, and

Pseudomonas aeruginosa. There are found different results in sensitive and resistant to spice according the extraction methods. The differences in the sensitivity between food and microorganisms may be due to the differences in spices concentrations and methods that used in this study.

The present study was revealed that the addition 1% of garlic powder independently to red and chicken meat contamination with *Salmonella* didn't showed antibacterial activity against *Salmonella* growing. Findings of the present study are similar to those reported by Hong et al. (2013) who studied the effect of garlic powder on 10^3 cell/mL of pathogenic bacteria cultivated individually on tryptic soy broth media during storage at room temperature (25°C) for 48 h. It was recorded that the garlic powders 3% did not effectively inhibit the growth of *S. typhimurium*, *E. coli* O157:H7, or *S. aureus*.

In this study, the addition garlic powder to meat increase the *Salmonella* counts and this substantiate the findings of Verluyten et al. (2004) who had been reported that the garlic can even stimulate the growth of bacteria by providing them with a carbohydrate source for growth. *Salmonella* may be able to make benefit from some of these carbohydrates, which may explain the higher final biomass obtained.

In a study, Sallam et al. (2004) documented that the antimicrobial activities of garlic materials on microorganisms in raw chicken sausage were found in such an order: fresh garlic > garlic powder > garlic oil. Another study also by Rahman et al. (2006) who examined the antimicrobial activities of dried garlic powders prepared by different drying methods against *S. aureus*, *E. coli*, *B. cereus*, *S. typhimurium* and a mixed lactic culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii*. It was recorded that the fresh garlic exhibited to be the highest activities followed by freeze-dried powder.

Aydin et al. (2007) evaluated the antimicrobial activities of different concentrations (0% to 10%) of chopped garlic on ground beef and raw meatballs. It was observed that chopped garlic delayed the growth of microorganisms in ground meat, which depended on the garlic concentrations. The addition of garlic (5% or 10%) to the raw meatball mix

reduced the microorganism counting, in terms of total aerobic mesophilic bacteria, yeast, and mold counts.

The biologically active compounds of garlic is allicin does not exist in garlic until it is crushed or cut of garlic bulb activates the enzyme alliinase which metabolizes alliin to allicin which in eventually is metabolized to vinyldithiines. This compound is very sensitive to temperature even room temperature and breakdown occurs within few hours (Kemper, 2000).

Cold extraction on the other hand may not have succeeded in bringing out the active ingredients from the bulbs. Since the efficacy of spices in vitro is often much greater than in vivo that can be explain why egarlic on meat contamination with *Salmonella* have no antimicrobial activity on *Salmonella* growing (Matthew, 2009).

In the current study, it was reported that cardamom and cinnamon powder have slight effect against *Salmonella* contaminated milk. The antimicrobial activity of cinnamon powder has been investigated in different concentration. Kuang et al. (2011) assessed the antibacterial effects of ultra-fine powder of cinnamon against some pathogenic bacteria that spoiled meat and reported that the inhibitory effect was increased with increasing powder concentrations from 0.5 to 2.5% (w/v). Also, a study by Yadav and Singh (2004) reported that the addition of cinnamon to apple juice contaminated with *E. coli* was able to kill 99.5% of the this bacterium within 3 days at room temperature.

Hong et al. (2013) recorded that the cinnamon powder (3% w/w) was the most effective botanical powder tested, and the populations of *S. typhimurium*, *E. coli* O157:H7, and *S. aureus* after 48 h of storage at room temperature (25°C). Also, Yadav et al. (2002) revealed that the cinnamon powder at 4.0% (w/w) extended the shelf life of minced chicken meat for 2 days at room (30°C) and 4 days at refrigeration.

The efficacy of spices in vitro is often much greater than in vivo, because of antimicrobial effect is concentrated and may become strongly bactericidal at high concentrations. Also, the active components can bind with food components like fats and proteins, thus decreasing their efficiency (Davidson, 1997).

However, many factors such as temperature, type of foods, pH, and water can play a role in efficacy of spices. In addition, kind of spice, composition and concentration of spice are factors that determine the antimicrobial activity (Souza, et al., 2005).

Conclusion

In conclusion, the addition of some spices such as black pepper, garlic, cardamom, and cinnamon in powder form at concentration of 1% w/w to foods that cooked according to Yemeni cooking procedure didn't showed any effects against *Salmonella*. The spices concentration that used in this study is not enough to inhibit or delay the bacterial growth in tested food. Also, spices antioxidant and antimicrobial properties destroyed or lost under the excessive warming or longer periods at higher temperatures during cooking. However, the use of these spices plants at tested concentration just impart the foods some characteristic taste, aroma, or flavor and color without protect it from microbial spoilage.

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