ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 45(4): 1541-1554,.(2007).

APPLICATION OF SOME SPICES AS NATURAL ANTIMICROBIAL AGENTS IN PAN BREAD.

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ABSTRACT

The antimicrobial activity of seven tested spices at different concentration 1%, 1.5% and 2% and mixed ratios of cinnamon and clove was evaluated against five strains of bacteria, three strains of fungi and one strain of yeast .Results showed that all individual spices showed strong or moderate antibacterial activity against the tested bacterial strains, and it was increased with increasing the concentration of all tested spices. Laurel leaves, cardamom, basil and ginger showed strong inhibitory effects against the *Staphylococcus aureus*. Cardamom and laurel leaves reduced the counts of *E. coli* from $6x10^9$ to $1.0x10^2$and $1.2 x10^2$ cfu/ml, respectively. Both clove and basil reduced the counts of *Salmonella* from $6x10^9$ to $1.1 x10^3$ cfu/ml at concentration of 2 %. About 2 % of cardamom and cinnamon reduced the count of *Saccharomyces cervisiae* from $1x10^8$ to $2.2 x 10^4$ and $2.1 x10^5$ cfu/ml. All tested spices reduced the growth of *A. flavus* higher than *A. niger*. A 2% of clove and ginger reduced the growth of *A. niger* from $3x10^7$ to $2.1x10^3$and $2.8x10^3$ cfu /ml, respectively. At concentration of 2% of cinnamon and basil reduced the growth of *Penicillium sp.* Fungal and yeast strains were relatively sensitive to mixed ratios of cinnamon and clove than the individual spices at the same concentration. Addition of 1% of laurel leaves, basil, cinnamon and clove,2% of fennel, cardamom and ginger and 1% of mixed ratios of cinnamon: clove were chosen to test their effectiveness against microorganism in pan bread. In conclusion addition of 1% of cardamom, cinnamon and clove increased shelf- life of pan bread by three or four days more than the control sample while addition of 1% of mixed ratios of cinnamon and clove increased shelf-life of pan bread by two days than control sample during storage at room temperature.

Key words: Fennel, Cardamom, Cinnamon, Basil, Ginger, Clove, Laurel leaves Antimicrobial, pan bread.

INTRODUCTION

Spices have traditionally been used to preserve foods, as well as to enhance flavor and odor (Gill and Holley, 2004). In the past decade interest on the topic of antimicrobial plant extracts have been growing. Various spices and herbs extracts have been used for the purpose of food preservation, (Sheng-Hsien *et al.*, 2007).

Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation. Common culinary herbs, spices and

aromatic plant that exhibit antimicrobial activity could provide sources of acceptable natural alternative. (Gould, 1996 and Pascal *et al.*, 2002).

The use of chemicals to enhance the safety of many foods is of great interest to the food industry. Chemical preservatives vary in their ability to kill microorganisms. Effectiveness depends on the types of microorganisms and the physical and chemical characteristic of food. (Mau *et al.*, 2001).

The exploration of naturally occurring antimicrobials for food preservation receives increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives, (Schuenzel and Harrison, 2002)

There is strong interest in the use of naturally occurring compounds which have antibacterial activity for preservation of minimally processed foods. *Bacillus cereus* is a spore – forming pathogen often associated with two kinds of food- borne illnesses, a diarrheal and animnt syndrome, caused by two distend toxins. This microorganism is widely distributed in the natural environment and it easily spread to many types of foods, especially those of plant origin. (Valero and Giner, 2006).

The stability of some food against attack by microorganisms is due to the fact they contain naturally occurring substances with antimicrobial activity. Some spices are known to contain essential oils that possess antimicrobial activity, such as eugenol in clove, allicin in garlic, and cinnamic aldehyed and eugenol in cinnamon. (Mau *et al.*, 2001).

Cinnamon (*Cinnamomum verum*) is the oldest spicy reddish-brown and as a warm, spicy and very pleasing. Cinnamon may be used in tomato,cake and cookies. **Clove** (*Eugenia cryphlla.*) oleoresin clove prepared from clove buds is brownish-green in color and viscous. Whole ground form of the spice may be used with fruit and some meat. **Ginger** (*Zingiber officinale*) was one of the first spices. The pungency of ginger is attributed to zingerome, shoshgol and ginerogl. **Laurel leaves** (*laurus nobilis*),the oleoresin is a dark green extremely viscous extract. Laurel leave is essential in mixed pickling spices and used in seasoning for meat products. **Fennel** seed (*Foeniculum vulgare*), the oleoresin is brownish – green liquid and used commercially to soup, baked goods, ice cream, and seasoning for prepared meats. **Cardamom** (*Elettaria cardamomum)* is used as cooking spices. The major organic flavoring substances of cardamom oil are cineole, methylheptanone, geraniol. The oleores in prepared from ground cardamom seeds is dark green liquid with a minimum volatile oil.**Basil** (*Ocimum basilicum*) is a fragrant, low growing annual herb and one of the most delicate when fresh. I t has a pleasantly sweet, spicy flavor, warm. Basil is a natural accompaniment to tomatoes and tomato product. The green leaves may be used in salads and on pizzas (Farrell, 1985).

Bakery products are an important part of a balanced diet and today, a wide variety of such products can be found on the supermarket shelves. (Abdulrahman *et al.*, 2007).

Bakery products are important staple foods in most countries and cultures. However, mold growth and staling are tow problems that limit the shelf life of both

high and intermediate – moisture bakery products. Fungi contaminants have been isolated from bakery products and identified as species of *Eurotium*, *Aspergillus* and *Penicillum*. (Guynot *et al.*, 2003).

In unpreserved bread a shelf – life of 3-4 days may be expected especially if the hygiene in the factory is not sufficiently high. Besides the repelling sight of visible growth, fungi are responsible for off-flavor formation and the production of mycotoxins and allergenic compound. Also, the yeast commonly known as chalk moulds are important spoilers of bread. Since industrialization, urbanization and change of life style started to put demands for longer shelf-life of bread, the use of sorbet and propionic acid has been the main choice. (Nielsen and Rios, 2000).

The objective of this work was to study in vitro antimicrobial activity of some individual spices (fennel, cardamom, basil, cinnamon, ginger, clove and laurel leaves) and mixed of cinnamon and clove at different ratios against nine microbial strains. The potential application of using them for extending the shelf life of pan bread as a real system was also evaluated.

MATERIALS AND METHODS

1.Materials: -

1.1 spices: -

Cinnamon (*Cinnamomum verum*), Clove (*Eugenia cryphlla.*), Ginger (*Zingiber officinale*), Laurel leaves (*laurus nobilis*), Fennel (*Foeniculum vulgare*), Cardamom (*Elettaria cardamomum*)and Basil (*Ocimum basilicum*)were purchased from local market at Cairo. All spices were ground into fine powder.

1.2 Microbial strains:-

All spices were individually tested against microorganisms including five strains of bacteria, *Staphylococcus aureus* (ATCC 29213), *Salmonella s p* (ATCC 14028) *Escherichia coli* (DSM 498*) Bacillus cereus* (NRRL 374) and *Bacillus subtilis* (NRRL1020) and three strains of fungi, *Aspergillus niger* (EMCC 104), *Aspergillus flavus* (EMCC 125) and *Penicillium sp* (ATCC 2887) and one strain of yeast, *Saccharomyces cervisiae* (NRRL 1095). These strains were obtained from Department of Microbiology and the Microbiological Resources, Center, (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

1.3 Culture media:-

Nutrient agar or broth medium was used for culturing of all tested bacterial strains. Universal medium was used for culturing of *Saccharomyces cerevisiae*. Malt extract, broth and agar, was used for the culturing of fungus strains (Oxoid, 1990).

2. Methods:-

2.1 Evaluation of antimicrobial activity of tested spices:-

Antimicrobial activity of tested spices were tested individually using agar dilution method)Galindo-Cuspinera *et al.*, 2003) against five bacterial, three fungal and one yeast strains.

2.1.1 Bacteria and yeast:-

Mother cultures of bacterial and yeast strains were set up 24 hr in a suitable medium before the assay in order to reach the stationary phase of growth. A positive blank medium containing approx. $6x10^9$ and $1x10^8$ cfu /ml of bacterial and yeast strains, respectively, was prepared. One ml of the adjusted suspensions was cultured in a 9 ml of a suitable broth medium in test tubes containing different concentrations of the tested spices. Tubes were incubated for 24 hr at 30 °C and serial decimal dilutions were prepared. Standard plate counts technique was used to determine the total microbial counts after incubation at 30°C for 48 hr and expressed it as cfu /ml. At least three replicates were performed for each microorganism.

2.1.2 Fungi:-

Spores suspension of fungi were prepared by shaking 10 ml of malt extract broth medium with an active sporulating (5-days old fungal culture). Ten ml of the spore suspension was added to 90 ml of malt extract broth containing different concentration of tested spices A positive blank medium containing approx. $3x10^7$ cfu /ml of fungi was prepared. At least three replicates were performed for each microorganism.Tubes were incubated for 4 days at 25-28°C and serial decimal dilutions were prepared and standard plate technique counts was used to determine the counts of fungi.

2.2 Pan bread manufacture:-

Pan bread was produced using the modified formula according to Kent-Jones and Amos (1967).

Flour	**100 g**
Fresh compressed yeast	2g
Sugar	3g
Oil	3g
Water	According to farinograph results (60 ml)

The ingredients were gently mixed together and the mixing was continued until the dough had a smooth appearance. The dough was left for fermentation for about 60 min. Dough was scaled at 200 gm pieces, rounded and left for a rest period of 10 min. The pieces were molded into cylindrical shape to fit greased bread pans. The pan were incubated in a fermentation cabinet to proof at 30°C and 85 % relative humidity for about 45 min. Then baked at 240°C for 25 min. Bread loaves were allowed to cool on racks for about 1 hr and packaged in polyethylene pages before evaluation. For antimicrobial application the tested spices were added to pan bread formula to replace 1, 1.5 and 2 % of wheat flour.

3. Microbiological analysis:-

Different pan bread samples were examined for total bacterial counts, spore forming bacteria counts and yeast and fungi counts, according to (ICMSF, 1986)

4 Sensory evaluation:-

A10 panelists from the staff members of the Food Science Department. Faculty of Agriculture, Ain Shams., University were asked to evaluate appearance,

texture, crumb color, crust color, odor, taste and overall acceptability of the processed pan bread according to (Faridi and Rubenthaler, 1984)

5. Statistical analysis:-

The experimental data analyzed using analysis of variance and Duncan' s multplie range test at ($P < 0.05$).The data were analyzed according to User' s Guide of Statistical Center of Faculty of Agriculture, Ain Shams, University (SAS,1996).

RESULTS AND DISCUSSION

1. Antimicrobial activity of individual and mixed ratios of tested spices:-

1.1 Antibacterial activity:-

Agar dilution method was used to investigate the antimicrobial activities of seven different spices and mixed ratios of cinnamon and clove against five bacterial strains. The inhibitory effects of different concentration of the tested spices on the bacterial viable counts are represented in Table (1). *Staphylococcus aureus* is one of the most common of the gram- positive bacteria causing food poisoning. All individual spices showed strong or moderate antibacterial activity against the tested microorganisms and, it was increased with increasing the concentration of all tested spices from 1 to 2%. In addition, laurel leaves, cardamom, basil and ginger showed strong inhibitory effects against the *Staphylococcus aureus*, followed by cinnamon but fennel and clove showed low inhibitory effect at concentration of 2%.Results in Table (1) also revealed that cardamom, laurel leaves showed higher reduction in count of *E. coli* counts from $6x10^9$ to $1.0x10^2$ and $1.2 x10^2$ cuf /ml, respectively, followed by cinnamon and clove at concentration of 2 %. On the other hand, 2% of basil, ginger and clove are able to drastically reduce the growth of *B. cereus* and *B. subtilis*. Sandri *et al.* (2007) stated that, spores of *Bacillus* species were resistant to the antimicrobial action of the essential oil. However the growth and viability of their vegetative cells drastically inhibited by low concentration of this oil.

Salmonella is a gram-negative bacterium which caused serious food poisoning and many preservatives are used to eliminate its growth. Concentration of 2% for both clove and basil was able to reduce the growth of *Salmonella* sp. to 1.1 $x10^3$ cfu/ml. It can be concluded that most of the evaluated spices has stronger antibacterial activity and broader spectrum against the tested organisms.

As can be seen in Table (1) mixed ratios of cinnamon and clove (1: 1, 1: 3 and 1:5) exhibited stronger antibacterial activity than the individual spices at the same concentration. For example, a 2% concentration of 1:5 of cinnamon and cloves totally inhibited the growth of different tested bacterial strains (except of *Staphylococcus aureus*), whereas, a ratio of 1:3 only inhibited the growth of *E. coli* and *Bacillus subtilis*. Also, mixed of cinnamon and clove exhibited stronger antibacterial activity against *Staphylococcus aureus* than individual spices at low concentration. Similar results were obtained by Gulcin *et al.* (2003), who stated that all extracts of anise showed strong antibacterial activity against *Staphylococcus aureus*.

1.2 Antifungal activity:-

During the last years there has been growing interest in testing natural compounds of different origins as defense for cultivated plants against phytopathogenic fungi (Bowers and Locke, 2000)

Table (1): Antibacterial activity of different concentration of tested spices and mixed ratios of cinnamon and clove.

Spices Conc. (%)	Total viable count (cfu/ml)				
	Staphyloc--occus aureus	*Salmonella sp.*	*E. coli*	*Bacillus cereus*	*Bacillus subtilis*
Fennel					
1	2.8×10^{6}	4.4×10^{6}	1.1×10^{6}	1.8×10^{6}	6.0×10^{5}
1.5	2.6×10^{4}	2.9×10^{6}	2.1×10^{5}	1.3×10^{6}	4.0×10^{5}
2	2.5×10^{4}	3.2×10^{5}	3.1×10^{3}	2.0×10^{4}	4.0×10^{5}
Cardamom					
1	3.0×10^{6}	4.0×10^{6}	2.2×10^{3}	1.8×10^{7}	3.9×10^{6}
1.5	4.0×10^{5}	2.0×10^{5}	2.1×10^{3}	2.4×10^{5}	2.8×10^{5}
2	3.0×10^{3}	2.0×10^{5}	1.0×10^{2}	2.0×10^{4}	1.2×10^{3}
Laurel leaves					
1	2.9×10^{5}	2.8×10^{5}	1.4×10^{7}	2.5×10^{6}	1.2×10^{7}
1.5	2.2×10^{5}	2.2×10^{5}	2.2×10^{5}	2.3×10^{6}	6.0×10^{6}
2	2.4×10^{3}	2.1×10^{4}	1.2×10^{2}	1.2×10^{3}	1.2×10^{3}
Basil					
1	9.2×10^{6}	2.8×10^{6}	1.1×10^{3}	3.3×10^{4}	3.9×10^{5}
1.5	4.5×10^{3}	4.0×10^{5}	3.3×10^{2}	3.3×10^{4}	2.1×10^{4}
2	3.3×10^{3}	1.1×10^{3}	3.3×10^{2}	2.0×10^{2}	2.2×10^{2}
Cinnamon					
1	2.2×10^{5}	3.1×10^{6}	2.2×10^{5}	1.9×10^{6}	2.3×10^{5}
1.5	1.1×10^{5}	2.0×10^{6}	3.2×10^{3}	2.0×10^{6}	3.3×10^{4}
2	2.0×10^{4}	8.0×10^{3}	2.1×10^{2}	1.3×10^{3}	1.1×10^{4}
Ginger					
1	1.2×10^{5}	2.2×10^{7}	1.3×10^{6}	2.2×10^{3}	2.1×10^{5}
1.5	2.1×10^{4}	2.1×10^{5}	1.2×10^{3}	1.1×10^{3}	3.4×10^{4}
2	3.1×10^{3}	2.1×10^{5}	1.2×10^{3}	2.1×10^{2}	1.2×10^{2}
Clove					
1	1.2×10^{6}	1.8×10^{5}	3.0×10^{6}	2.0×10^{5}	3.3×10^{6}
1.5	2.0×10^{5}	1.2×10^{5}	1.4×10^{4}	3.0×10^{4}	2.3×10^{5}
2	2.0×10^{5}	1.1×10^{3}	2.2×10^{2}	1.1×10^{2}	1.2×10^{3}
Cinnamon: Clove (1:1)					
1	3.3×10^{3}	2.2×10^{4}	1.1×10^{3}	2.1×10^{3}	4.0×10^{3}
1.5	3.2×10^{3}	3.0×10^{2}	1.2×10^{3}	2.0×10^{2}	2.0×10^{2}
2	2.0×10^{2}	2.1×10^{2}	1.1×10^{2}	2.0×10^{2}	2.0×10^{2}
Cinnamon: Clove (1:3)					
1	3.2×10^{4}	2.3×10^{4}	1.5×10^{3}	2.2×10^{4}	2.8×10^{3}
1.5	2×10^{3}	2.1×10^{2}	1.5×10^{3}	2.1×10^{3}	2.2×10^{3}
2	2×10^{3}	2.0×10^{2}	0	2.0×10^{2}	0
Cinnamon: Clove (1:5)					
1	2.8×10^{4}	2.2×10^{3}	2.2×10^{4}	2.8×10^{3}	2.0×10^{3}
1.5	2.7×10^{2}	2.1×10^{2}	2.1×10^{2}	2.7×10^{2}	2.0×10^{2}
2	2.7×10^{2}	0	0	0	0

(cfu/ml) Initial viable count of bacteria 6×10^{9}

Antifungal activity of tested spices at different concentration and mixed ratios of cinnamon and clove was studied against three strains of fungi and one strain of yeast and the results are given in Table (2). Concentration of 2% for cardamom reduced the growth of *Saccharomyces cervisiae* from initial inoculums of $1x10^8$ to 2.2 $x10^4$ cfu /ml compared to approximately 2.1 to $7.7x10^5$ cfu /ml for the other tested spices. Most of the tested spices showed stronger or moderate inhibitory effects against *A. flavus* than the other tested fungal strains. Concentration of 2% for clove and ginger reduced the growth of *A. niger* from $3x10^7$ to 2.1 and $2.8x10^3$ cfu /ml respectively, whereas cardamom showed little inhibition effect on the same strain. On the other hand, *A. flavus* was more sensitive to basil and laurel leaves than other tested spices at different concentration. For instance, 1%, 1.5 % and 2% of basil reduced the growth of *A. flavus* from $3x10^7$ to $1.5x10^4$, $2.1x10^2$ and 1.1 $x10^2$ cfu /ml respectively. At concentration of 2% cinnamon and basil reduced the growth of *Penicillium sp* from $3x10^7$ to about $1x10^3$ and $1.3x10^3$ cfu /ml, respectively

Results in Table (2) also revealed that fungal and yeast strains were relatively sensitive to mixed ratios of cinnamon and clove than the individual spices at the same concentration. For example, a 2% of 1:3 and 1:5 cinnamon and clove completely inhibited the growth of *A. niger*, *A. flavus* and *penicillium sp* compared to individual spices, whereas, the same mixture show moderate activity against *Saccharomyces cervisiae*.

In general, all the tested spices exhibited antifungal activity. The most susceptible strain was *A. flavus* for basil and laurel leaves while it was *A. niger* for clove and ginger. In particular clove, basil, laurel leaves and cinnamon were the most active spices in inhibiting the growth of all fungal strains tested while fennel was the least active. Similar results were obtained by Omidbeygi *et al.* (2007). They found that complete inhibition of growth of *A. flavus* was observed by thyme and summer savory. Also Sheng-Hsien *et al.* (2007) found that the hot extracts of cinnamon and cassia inhibited significantly the growth of *A. niger*.

2. Application of some spices in pan bread:-

Partial studies are recommended on the use of selected spices and their derivative during production of foods. Food product requires a very low initial microbial load and inhibition during production period for adequate shelf life (Boyraz and Ozcan, 2006). Therefore, different concentrations of individual and mixed ratios of the tested spices were used as natural antimicrobial and flavoring agents in pan bread.

2.1 Selection of best concentration of the tested spices:-

Statistical analysis of panelist scores for sensory properties of pan bread containing different tested spices were evaluated to choice the best concentration of spices which achieved high overall acceptability concentration of spices. The received scores of pan bread containing different concentration of tested spices are given in Table (3). No significant differences were observed in appearance, texture and crumb color of pan bread containing 1%, 1.5% and 2% of fennel, cardamom and ginger. However, addition of the other tested spices significantly reduce all sensory properties of pan bread. Therefore addition of 1% of laurel leaves, basil, cinnamon and clove and 2% of fennel, cardamom and ginger was chosen to test their effectiveness against spoilage microorganism in pan bread. Panelists scores for sensory properties of pan

bread containing mixed ratios of cinnamon and clove were given in Table (4). It noticed that increasing the concentration of mixed ratios of cinnamon to clove more than 1% significantly reduced overall acceptability of pan bread, therefore, a 1% of all mixed ratios was chosen to test their effectiveness against microorganism.

Table (2): Antifungal activity of different concentration of tested spices and mixed ratios of cinnamon and clove.

Spices Conc. (%)	Total viable count (cfu/ml)			
	Saccharomyces cervisiae	*A. niger*	*A. flavus*	*Penicillium Sp.*
Fennel				
1	3.2×10^{7}	3.3×10^{6}	2.1×10^{6}	2.1×10^{6}
1.5	3.3×10^{6}	2.2×10^{6}	2.1×10^{6}	2.2×10^{5}
2	2.2×10^{5}	1.2×10^{5}	1.1×10^{5}	2.8×10^{4}
Cardamom				
1	9.0×10^{7}	2.2×10^{7}	2.2×10^{6}	2.4×10^{7}
1.5	2.8×10^{5}	2.4×10^{6}	2.9×10^{7}	2.2×10^{7}
2	2.2×10^{4}	2.1×10^{6}	2.2×10^{5}	2.0×10^{5}
Laurel leaves				
1	4.4×10^{6}	4.4×10^{6}	2.3×10^{6}	2.2×10^{6}
1.5	5.5×10^{5}	6.6×10^{5}	2.2×10^{3}	4.5×10^{5}
2	2.2×10^{5}	5.5×10^{5}	1.2×10^{2}	4.5×10^{5}
Basil				
1	6.6×10^{6}	3.0×10^{5}	1.5×10^{4}	2.9×10^{5}
1.5	7.7×10^{5}	3.0×10^{5}	2.1×10^{2}	2.9×10^{5}
2	7.7×10^{5}	4.0×10^{4}	1.1×10^{2}	1.3×10^{3}
Cinnamon				
1	2.1×10^{6}	2.2×10^{6}	1.0×10^{6}	2.1×10^{5}
1.5	2.0×10^{6}	2.1×10^{4}	2.1×10^{5}	2.1×10^{5}
2	2.1×10^{5}	2.0×10^{4}	2.2×10^{2}	1.0×10^{3}
Ginger				
1	2.2×10^{7}	2.1×10^{5}	2.5×10^{5}	2.8×10^{7}
1.5	2.2×10^{6}	2.1×10^{4}	2.2×10^{5}	2.1×10^{5}
2	3.1×10^{5}	2.8×10^{5}	2.0×10^{5}	2.1×10^{5}
Clove				
1	2.2×10^{6}	2.1×10^{5}	2.0×10^{7}	1.2×10^{5}
1.5	1.2×10^{6}	1.1×10^{4}	3.0×10^{6}	2.1×10^{4}
2	2.1×10^{5}	2.1×10^{5}	1.2×10^{4}	2.1×10^{4}
Cinnamon: Clove (1:1)				
1	4.4×10^{5}	4.4×10^{5}	2.1×10^{5}	4.1×10^{4}
1.5	2.0×10^{5}	2.2×10^{5}	1.1×10^{5}	2.1×10^{5}
2	6.0×10^{2}	2.1×10^{2}	2.0×10^{2}	2.2×10^{2}
Cinnamon: Clove (1:3)				
1	2.2×10^{5}	2.2×10^{5}	2.0×10^{5}	2.2×10^{2}
1.5	2.0×10^{5}	2.0×10^{2}	3.2×10^{2}	1.1×10^{2}
2	2.0×10^{2}	0	1.1×10^{2}	0
Cinnamon: Clove (1:5)				
1	2.8×10^{5}	2.8×10^{5}	2.6×10^{4}	2.2×10^{5}
1.5	2.3×10^{2}	2.7×10^{2}	2.3×10^{2}	2.1×10^{2}
2	2.1×10^{2}	0	0	0

Initial viable count of yeast 1×10^{8} Initial viable count of fungi 3×10^{7}

Table (3): Sensory properties of pan bread containing different concentration of tested spices.

Spices conc. (%)	Appearance (20)	Texture (20)	*Crumb* color (20)	Crust color (10)	Taste (20)	Odor (10)	Overall acceptability (100)
Fennel							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.2^{ab}	9.3^{ab}	95.0^{a}
1	19.1^{a}	18.9^{a}	19.4^{a}	9.1^{a}	18.2^{b}	8.4^{b}	93.1^{a}
1.5	19.1^{a}	19.5^{a}	19.0^{a}	9.3^{a}	18.9^{ab}	9.0^{ab}	94.8^{a}
2	18.7^{a}	19.3^{a}	19.1^{a}	9.0^{a}	19.5^{a}	9.4^{a}	95.0^{a}
Cardamom							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.2^{a}	9.3^{a}	95.0^{a}
1	18.6^{a}	18.5^{a}	18.6^{a}	6.4^{ab}	18.2^{bc}	8.2^{b}	90.5^{bc}
1.5	18.2^{a}	18.4^{a}	18.3^{a}	8.2^{b}	17.8^{c}	8.3^{b}	89.2^{c}
2	19.1^{a}	18.9^{a}	19.0^{a}	9.0^{a}	18.8^{ab}	9.2^{a}	94.0^{ab}
Laurel leaves							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.2^{a}	9.3^{a}	95.0^{a}
1	17.5^{b}	17.5^{ab}	17.3^{b}	7.0^{b}	16.6^{b}	6.8^{b}	82.7^{b}
1.5	16.5^{b}	16.4^{bc}	16.5^{b}	6.1^{bc}	14.3^{c}	5.8^{c}	75.6^{c}
2	14.6^{c}	15.8^{c}	14.6^{c}	5.2^{c}	12.8^{c}	4.8^{d}	67.8^{d}
Basil							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.2^{a}	9.3^{a}	95.0^{a}
1	17.0^{b}	17.6^{ab}	16.8^{b}	7.4^{b}	17.6^{b}	8.3^{ab}	84.7^{b}
1.5	15.2^{c}	17.2^{bc}	15.2^{c}	6.2^{c}	16.4^{c}	7.3^{b}	77.5^{c}
2	13.0^{d}	15.9^{c}	14.1^{c}	4.9^{d}	15.1^{d}	5.8^{c}	68.0^{d}
Cinnamon							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.1^{a}	9.3^{a}	95.0^{a}
1	17.9^{ab}	17.7^{b}	17.6^{bc}	7.9^{b}	17.9^{b}	9.0^{b}	87.5^{bc}
1.5	17.5^{b}	17.7^{b}	16.7^{cd}	7.4^{bc}	17.9^{b}	8.5^{b}	84.6^{c}
2	16.5^{b}	17.2^{b}	16.2^{d}	6.5^{c}	17.3^{b}	8.2^{b}	84.4^{c}
Ginger							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.2^{a}	9.3^{a}	95.0^{a}
1	19.4^{a}	19.2^{a}	18.8^{a}	8.9^{a}	18.4^{a}	9.1^{a}	93.8^{a}
1.5	19.4^{a}	19.2^{a}	18.6^{a}	8.7^{a}	18.4^{a}	8.9^{a}	93.2^{a}
2	19.4^{a}	19.4^{a}	18.7^{a}	8.9^{a}	18.8^{a}	9.1^{a}	94.3^{a}
Clove							
0	19.1^{a}	18.9^{a}	19.1^{a}	9.4^{a}	19.2^{a}	9.3^{a}	95.0^{a}
1	17.5^{b}	17.6^{b}	16.9^{b}	7.5^{b}	17.1^{b}	7.9^{b}	84.5^{b}
1.5	16.3^{b}	17.4^{b}	15.8^{c}	6.5^{c}	15.1^{c}	6.8^{c}	77.9^{c}
2	16.4^{b}	16.7^{b}	15.7^{c}	6.1^{c}	12.4^{d}	5.8^{d}	73.1^{d}

a, b, ... There is no significant difference between any two means, with the same column for the same spice, have the same letter ($p > 0.05$).

2.2 Antimicrobial activity of individual and mixed spices in pan bread:-

Microbiological analysis of pan bread containing the chosen concentration of each tested spices during storage period at room temperature are presented in Table

(5). It could notice that the control sample had higher total microbial counts than pan bread contained different tested spices during storage. For example, total bacterial counts reached $2x10^2$ and $1.3x10^3$ cfu/g after 7and 5 days of storage of samples contained 1% of clove and 2% of fennel compared to $1.5x10^6$ cfu/g in the third day of storage for control sample. It can observed that addition of 1% of, cinnamon, clove and 2% of cardamom increased shelf- life of pan bread by three or four days than the control sample. The counts of spores was lower for all pan bread containing tested spices than the control while pan bread containing clove, ginger, cardamom and laurel leaves reduced the growth of yeast and fungi than the other tested spices during storage period. No microbial counts were detected in pan bread contained individual tested spices until the third days of storage.

Table (4): Sensory properties of pan bread containing mixed ratios of cinnamon and clove.

Spices conc. (%)	Appearance (20)	Texture (20)	Crumb color (20)	Crust color (10)	Taste (20)	Odor (10)	Overall acceptability (100)
Cinnamon: Clove (1:1)							
0	17.9^a	18.2^a	18.3^a	8.6^a	18.8^a	9.2^a	89.3^a
1	17.6^a	17.9^a	17.8^a	8.6^a	18.3^a	9.0^a	89.2^a
1.5	17.5^a	17.7^a	17.3^a	8.0^{ab}	18.2^a	8.7^{ab}	87.4^{ab}
2	17.2^a	17.4^a	16.9^a	7.8^b	17.3^a	8.3^b	84.9^b
Cinnamon: Clove (1:3)							
0	17.9^a	18.2^a	18.3^a	8.6^a	18.8^a	9.2^a	89.3^a
1	17.4^a	17.4^a	17.0^a	7.9^a	16.5^a	7.9^a	84.1^a
1.5	17.3^a	17.3^a	16.4^{ab}	7.2^{ab}	15.2^{ab}	7.6^{ab}	81.0^a
2	16.7^a	16.7^a	15.8^b	6.8^b	13.6^b	6.9^b	76.5^b
Cinnamon: Clove (1:5)							
0	17.9^a	18.2^a	18.3^a	8.6^a	18.8^a	9.2^a	89.3^a
1	17.2^a	17.3^a	16.8^a	7.5^a	15.7^a	7.6^a	82.1^a
1.5	16.7^{ab}	16.7^a	16.1^{ab}	7.1^{ab}	13.6^{ab}	7.3^a	77.5^b
2	16.1^b	16.7^a	15.5^b	6.4^b	12.0^b	6.6^a	73.3^c

a, b, ... There is no significant difference between any two means, with the same column for the same spice, have the same letter ($p > 0.05$).

Antimicrobial effect of mixed ratios spices on pan bread are presented in Table (6) Results showed that bacterial, spores and yeast & fungi counts were not detected in pan bread contained mixed ratios of spices until the third days of storage. Also, pan bread containing mixed ratios of cinnamon and clove recorded low level of microbial counts than control sample during storage. These means that addition of 1% of mixed ratios of cinnamon and clove increased shelf- life of pan bread by two days more than control sample. Hirasa and Takemasa, (1998) mentioned that a synergistic taste effect is when the taste of one feed component is enhanced by association with other food component but, a suppressive effect is when a certain taste is decreased in its strength by combine with other components (also knew as the offset effect) that explained the result in this study.

Table (5): Antimicrobial effect of tested spices on microbiological analysis of pan bread during storage at room temperature.

Spices Conc (%)	Total Counts (cfu/ g)	Storage periods (days)							
		zero	1	2	3	4	5	6	7
Control (0%)	Bacteria	1×10^{2}	3×10^{2}	3×10^{3}	1.5×10^{6}	——	——	——	——
	Spores	0.9×10^{2}	2×10^{2}	2×10^{2}	1.2×10^{3}	——	——	——	——
	Yeast &Fungi	ND	1×10^{2}	4×10^{2}	1.9×10^{4}	——	——	——	——
Fennel (2%)	Bacteria	ND	ND	ND	ND	4×10^{2}	1.3×10^{3}	——	——
	Spores	ND	ND	ND	ND	1.2×10^{2}	1.9×10^{2}	——	——
	Yeast &Fungi	ND	ND	ND	ND	1.2×10^{2}	1.3×10^{2}	——	——
Cardamom (2%)	Bacteria	ND	ND	ND	ND	3×10^{2}	3×10^{2}	1.3×10^{3}	——
	Spores	ND	ND	ND	ND	9×10	9×10	1.8×10^{2}	——
	Yeast &Fungi	ND	ND	ND	ND	1.4×10^{2}	2×10	1.2×10^{3}	——
Laurel leaves (1%)	Bacteria	ND	ND	ND	ND	3×10^{2}	6×10^{2}	——	——
	Spores	ND	ND	ND	ND	1.4×10^{3}	1.5×10^{3}	——	——
	Yeast &Fungi	ND	ND	ND	ND	2×10	3×10	——	——
Basil (1%)	Bacteria	ND	ND	ND	ND	9×10^{2}	1.1×10^{3}	——	——
	Spores	ND	ND	ND	ND	1.2×10^{2}	1.3×10^{2}	——	——
	Yeast &Fungi	ND	ND	ND	ND	1.5×10^{2}	1.5×10^{2}	——	——
Cinnamon (1%)	Bacteria	ND	ND	ND	ND	16×10^{2}	16×10^{2}	2.8×10^{3}	——
	Spores	ND	ND	ND	ND	1.2×10^{2}	14×10^{2}	1.6×10^{2}	——
	Yeast &Fungi	ND	ND	ND	ND	1.2×10^{2}	8×10	1.2×10^{3}	——
Ginger (2%)	Bacteria	ND	ND	ND	ND	14×10^{2}	14×10^{2}	——	——
	Spores	ND	ND	ND	ND	1.2×10^{2}	1.2×10^{2}	——	——
	Yeast &Fungi	ND	ND	ND	ND	1×10^{2}	2×10	——	——
Clove (1%)	Bacteria	ND	ND	ND	ND	3×10	3×10	2×10^{2}	2×10^{2}
	Spores	ND	ND	ND	ND	2×10	2×10	2×10	1×10^{2}
	Yeast &Fungi	ND	ND	ND	ND	3×10	3×10	3×10	1×10^{2}

ND:not detected (——): Beginning of visible growth of fungi

Table (6): Antimicrobial effect of 1% of tested mixed spices on microbial analysis of pan bread during storage at room temperature.

Spices Conc. (%)	Total Counts (cfu/g)	Storage periods (days)					
		Zero	1	2	3	4	5
Control (0%)	Bacteria	1×10^{2}	3×10^{2}	3×10^{3}	1.5×10^{6}	-------	-------
	Spores	0.9×10^{2}	2×10^{2}	2×10^{2}	1.2×10^{3}	-------	-------
	Yeast &Fungi	ND	1×10^{2}	4×10^{2}	1.9×10^{4}	-------	-------
Cinnamon: Clove (1:1)	Bacteria	ND	ND	ND	ND	1.4×10^{3}	1.8×10^{3}
	Spores	ND	ND	ND	ND	8.0×10	9.0×10
	Yeast &Fungi	ND	ND	ND	ND	9.0×10	1.6×10^{2}
Cinnamon: Clove (1:3)	Bacteria	ND	ND	ND	ND	2.2×10^{3}	2.4×10^{3}
	Spores	ND	ND	ND	ND	5.0×10	1.2×10^{2}
	Yeast &Fungi	ND	ND	ND	ND	4.0×10	1.8×10^{3}
Cinnamon: Clove (1:5)	Bacteria	ND	ND	ND	ND	1.4×10^{3}	1.4×10^{3}
	Spores	ND	ND	ND	ND	1.3×10^{2}	1.4×10^{2}
	Yeast &Fungi	ND	ND	ND	ND	1.6×10^{2}	1.2×10^{3}

ND:not detected (----): Beginning of visible growth of fungi

In conclusion, addition of 1% of individual cinnamon and clove was more effectiveness for extending the shelf life of pan bread than mixed ratios of them. These results support the possibility of the use of some spices as potential alternative antimicrobial agent to be applied in some foods. (Mau *et al.*, 2001) mentioned that further studies are needed to examine how the mixed extract exhibits its antimicrobial activity in practical food systems and to identify the compounds and their structures in each purified fraction. In addition, sensory evaluation studies on the food with the mixed extract added areas of investigation.

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استخدام بعض التوابل كمواد طبيعية مضادة للميكروبات فى خبز التوست

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تم تقييم النشاط المضاد للميكروبات لسبع توابل بتركيزات مختلفة ١٪، ١,٥٪ و٢ ٪ ونسب خلط من القرفة والقرنفل ضد خمس سلالات من البكتريا، وثلاثة سلالات من الفطر و سلالة واحدة من الخميرة. واظهرت النتائج ان التوابل المختبرة المفردة ذات نشاط مضاد للبكتريا قوى ومتوسط ضد سلالات البكتريا المختبرة.ووجد ان النشاط المضاد للبكتريا يزداد بزيادة تركيز التوابل المختبرة. اعطي ورق اللورا، والحبهان والريحان والزنجبيل نشاط مثبط قوى ضد ميكروب *Staphylococcus aureus* في حين سجل الحبهان وورق اللورا اختزال كبير لاعداد *E. coli* من ٦×١٠^{٩} الى ١ ×١٠^{٢} و١,٢ ×١٠^{٢} وحدة تكوين الخلية / مل على التوالى. اختزل كلا من القرنفل والريحان اعداد السالمونيلا من

٦×١٠^{٩} الى ١,١×١٠^{٢}وحدة تكوين الخلية/مل عند تركيز ٢٪. ان تركيز ٢٪ من الحبهان يختزل اعداد *Saccharomyces cervisiae* من ١×١٠^{٨} الى ٢,٢ × ١٠^{٤} و ٢,١ × ١٠^{٥} وحدة تكوين الخلية / مل واظهرت النتائج انخفاض اعداد *A. flavus* بنسبة عالية عن *A. niger* بواسطة كل من ا لتوابل المختبرة. ادى استخدام ٢٪ من القرنفل و الجنزبيل الى اختزال اعداد *A. niger* من ٣× ١٠^{٢} الى ٢,١× ١٠^{٢} و ٢,٨× ١٠^{٢}وحدة تكوين الخلية/مل.كما تم خفض اعداد *Penicillium sp* بواسطة القرفة و الريحان عند تركيز ٢ ٪. كما اظهرت النتائج ان سلالات الخميرة والفطرذات حساسية نسبية لنسب خلط القرفة مع القرنفل عن التوابل المفردة عند نفس التركيز.وتم اختيار تركيز ١ ٪ لكل من ورق اللورا،الريحان،القرفة، القرنفل و الجنزبيل، تركيز ٢٪ لكل من الشمر، الحبهان والجنزبيل و تركيز ١ ٪ لجميع نسب الخلط لدراسة تاثيرهم المضاد للميكروبات فى خبز التوست. اظهرت خلاصة النتائج ان اضافة ١ ٪ من الحبهان والقرفة والقرنفل ادى الى زيادة فترة صلاحية خبز التوست من ثلاثة الى اربعة ايام عن العينة الكنترول بينما ادى اضافة ١ ٪ من نسب خلط القرفة والقرنفل الى زيادة فترة صلاحية خبز التوست يومان عن العينة الكنترول عند التخزين على درجة حرارة الغرفة.