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Effect of different stabilizers on the antibacterial activity of "ginger garlic paste"

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ABSTRACT. The effect of five stabilizers i.e. citric acid, sodium metabisulfite, sodium benzoate, olive oil and ascorbic acid mixed in the ginger - garlic paste were evaluated against five pathogens (*E.coli, Staphylococcus aureus, Salmonella typhi, Proteus mirabilis* and *Enterobacter aerogenes*). Activity of the control paste decreased during storage. Antimicrobial activity of the paste was stabilized by various stabilizers when incorporated. Sodium metabisulfite, olive oil and ascorbic acid were found to be effective to stabilize the antibacterial activity of the paste considerably. *E.coli* and *Salmonella typhi* showed more resistance in case of citric acid and sodium benzoate provided in the paste. @ JASEM

Through ages spices have served human being in many areas such as food, flavours, preservatives, antioxidants and drugs. There has been a great shift from the prescription of antibiotics to the use of medicinal plants (Ekwenye and Elegalam 2005). Bacterial food borne pathogens are major threat to become a serious kind of infections. Fresh spices are perishable in nature and the causes of spoilage are improper handling, growth of spoilage microorganisms, and action of naturally occurring enzymes, chemical reactions and structural changes during the storage (Ahmad 2004). Various stabilizers have been used for a very long time to enhance the shelf life and maintain the aroma, flavor and other qualities of foods. More and more women entering the work force demand a change in the way of daily cooking. Therefore ready to use spices are in demand. Ginger (*Zingiber officinale*) and garlic (*Allium sativum* L.) have been used as spices and an ingredient in folk medicine in many Asian foods especially in Indian cuisine since ancient times. There have been a number of studies showing the antimicrobial activity of garlic essential oil, garlic and ginger extracts (Friedman et al., 2002; Ekwenye and Elegalam, 2005). Both ginger and garlic possess volatile oils and chemical compounds responsible for pungent flavours, especially gingerols and allicin respectively. Allicin (diallyl thiosulfinate) has antioxidant, antibacterial antibiotic properties (Augusti, 1996). Flavones, flavonoids and Flavonols are chemical compounds in these spices, active against microorganisms. Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring, they are synthesized by plants in response to microbial infections (Dixon et al., 1983).

Several compounds like ascorbic acids, citric acids, sodium metabisulfite etc have been used to extend the shelf life of various fruits and vegetables (Saper and Miller, 1995; Santerre et al., 1991). Many studies have been reported for the use of such compounds in garlic or ginger products to stabilize their physical properties like color and flavour (Son et al., 1996; Bae and Lee, 1990; Kim et al., 1981). It is commonly observed that organic and inorganic stabilizers are frequently used in the commercial production of fruits and vegetables based products. However, there is limited information in the literature regarding the antibacterial properties of the ginger/garlic paste stabilized with different stabilizer in different food products. The object of present investigation was to circumspect the effect of stabilizers on the antimicrobial activity of the "ginger garlic paste".

MATERIALS AND METHODS

Preparation of extract: Fresh ginger rhizomes and garlic bulbs were procured from local supermarket. Ginger rhizomes were washed with sterile water and then peeled with a blunt knife. Garlic bulbs were de-scaled manually and washed with sterile water and a fine paste of ginger and garlic (1:1 w/w) was prepared thereafter in a food processor ground for 15 min. Five stabilizers at various levels were prepared independently as well as added in the paste to control the antimicrobial properties of the paste and themselves. Stabilizers with their respective percentages (w/w) used in the study are as given below:

Citric acid: 0.1%, 0.2%, 0.3%
Sodium metabisulfite: 0.1%, 0.15%, 0.2%
Sodium benzoate: 0.1%, 0.15%, 0.2%
Olive oil: 5.0%, 10.0%, 20.0%
Ascorbic acid: 1%, 2%, 4%
Control paste (fresh / stored): no stabilizer

The representative samples for evaluation of antimicrobial activity were obtained from the pastes dispensed in pre-labeled glass jars and stored for two weeks at 4ºC in the refrigerator. 5% aqueous solution of ginger garlic paste was prepared from each sample including control (fresh / stored paste) for the antibacterial test. All samples solutions were placed

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overnight at ambient temperature for maximum extraction.

Bacterial pathogens: Pure cultures of bacterial species (*Escherichia coli, Staphylococcus aureus, Salmonella typhi, Proteus mirabilis, Enterobacter aerogenes*) obtained from PCSIR laboratories complex, Lahore and from pathological laboratories of local hospitals were used in the present study. Prior to inoculation, all bacterial strains were sub cultured thrice onto fresh nutrient agar media respectively to obtain a more vigorous population. The stock cultures were incubated at 37 ºC for 24 h.

Test for antibacterial activity: Bacterial cultures were serially diluted in normal saline solution. From 10-3 dilution, 1ml was dispensed to the sterile nutrient agar media culture plates in a clean environment and plates were rotated clock wise and then anticlockwise 7 times each for equal distribution of bacterial inoculum. Placed the extract or stabilizers impregnated saturated discs (5mm diameter) of Whatman filter paper #1 on pre-inoculated culture media and inoculated at 37 ºC for 24h. Recorded the zones of inhibition in each case measured as the diameter of the clear growth inhibited zones. Three categories of solutions were used for experiments as listed below:

1. Extract of ginger-garlic paste fresh or stored.
2. Solutions of stabilizers at various levels.
3. Extract of ginger-garlic paste provided with different levels of above mentioned stabilizers.

The data obtained in present study was statistically analyzed with one-way analysis of variance (ANOVA) in completely randomized design. The means were separated by Duncan multiple range test at 5% level of significance as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Results of 5% aqueous solutions of ginger garlic paste containing different percentage levels of stabilizers as well as without stabilizers (fresh/stored) subjected to observe its activity against test microbes (Table i, ii and iii). Though both fresh as well as stored ginger and garlic paste have strong antimicrobial activity against *E. coli*, *S. aureus*, *P. mirabilis* and *E. aerogenes* (Table 1). *S. typhi* was not checked by the stored paste whereas it was controlled by the fresh paste effectively. Present results indicate that antimicrobial activity of the paste was reduced during storage as depicted in table 1.

These findings are supported by the results reported by Uhart et al. (2006) who found that the aqueous extract of garlic only, posed potent bacteriostatic principle against many bacteria (*E.coli, B. cereus and S. aureus*) at varying concentrations. While ginger showed very mild inhibitory action against these three pathogenic bacteria and was unable to show complete growth inhibition. Table 2 shows the antimicrobial activity, if any, of various stabilizers only at different levels used in the study. Citric acid and ascorbic acid were effective against all the five microbes used in the study. Sodium meta-bisufite was effective against *E.coli* and sodium benzoate was ineffective against *S. typhi* only. Olive oil was found to be ineffective in case of *S. typhi* and *P. mirabilis* as shown in Table 2. Table 3 shows the effect of different stabilizers like citric acid, sodium metabisulfite, sodium benzoate, olive oil and ascorbic acids, added to the ginger garlic paste in order to stabilize the antimicrobial activity as well as to see the effect of fore cited stabilizers on the shelf life of ginger garlic paste. With the addition of these stabilizers the pH of the paste is lowered due to which growth of microbes is inhibited as compared to control. Gupta and Ravishankar (2005) also gave the similar statement while discussing their results.

Table 1: Antibacterial activity of fresh and stored ginger garlic paste against common pathogens

Ginger+Garlic Paste	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi*	*Proteus mirabilis*	*Enterobacter aerogenes*
Fresh Control	13.00^{a}±1.41	12.5^{a} ± 0.71	12.00^{a}±1.41	13.2^{a}±3.25	15.08^{a}±1.87
Control After Storage	8.4^{a} ± 0.85	9.25^{b} ± 0.78	0.00^{d}	5.5^{c}±0.71	8.5^{b}±0.71

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Table 2: Antibacterial activity of various stabilizers against common pathogens

Stabilizers	*Escherichia coli*	Staphylococcus aureus	*Salmonella typhi*	*Proteus mirabilis*	*Enterobacter aerogenes*
Citric acid (% w/v)					
0.10	11.00^{de}±1.41	11.75^{cd}±1.06	6.5^{c}±0.71	3.00^{de}±1.41	11.5^{def}±2.12
0.20	11.00^{de}±1.41	17.5^{b}±0.71	12.00^{b}±4.24	3.5^{de}±1.41	13.00^{cde}±2.83
0.30	12.5^{cd}±0.71	14.00^{bc}1.41	14.00^{b}±1.41	7.5^{cd}±0.71	17.75^{bc}±1.06
Sodium metabisulfite (% w/v)					
0.10	10.5^{def}±0.71	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{g}
0.15	9.5^{efg}±3.53	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{g}
0.20	7.75^{fg}±0.35	0.00^{e}	0.00^{d}	0.00^{e}	0.00^{g}
Sodium benzoate (% w/v)					
0.10	7.5^{fg}±0.71	11.5^{cd}±0.71	0.00^{d}	6.5^{cd}±0.71	8.00^{ef}±2.83
0.15	8.00^{fg}±1.41	9.5^{d}±0.71	0.00^{d}	3.00^{de}±1.41	7.5^{ef}±2.12
0.20	6.5^{g}±0.71	11.75^{cd}±1.06	0.00^{d}	7.5^{cd}±0.71	6.00^{fg}±0.71
Olive oil (%v/v)					
5.00	6.5^{g}±0.71	9.00^{d}±2.83	0.00^{d}	0.00^{e}	0.00^{g}
10.00	6.5^{g}±0.71	3.5^{e}±1.41	0.00^{d}	0.00^{e}	13.00^{cde}±1.41
20.00	7.00^{g}±1.41	8.5^{d}±2.1	0.00^{d}	0.00^{e}	18.00^{bc}±1.41
Ascorbic acid (% w/v)					
1.00	14.25^{c}±0.35	24.75^{a}±1.06	11.5^{b}±2.12	10.75^{bc}±0.35	16.25^{bcd}±1.06
2.00	19.25^{b}±0.35	16.5^{b}±2.12	14.00^{b}±1.41	14.5^{b}±0.71	21.25^{b}±1.06
4.00	22.00^{a}±0.00	27.00^{a}±1.41	21.00^{a}±1.41	19.5^{a}±0.71	28.00^{a}±0.85

Table 3: Effect of different stabilizers on antibacterial activity of stored ginger garlic paste against common pathogens

Stabilizer	*Escherichia coli*	Staphylococcus aureus	*Salmonella typhi*	*Proteus mirabilis*	*Enterobacter aerogenes*
Citric acid (% w/w)					
0.10	0.00^{d}	12.0^{abc} ± 1.41	0.00^{d}	13.5^{abc} ± 0.77	15.0^{abc} ± 0.71
0.20	0.00^{d}	15.5^{a} ± 3.54	0.00^{d}	13.5^{abc}±0.85	10.5^{c}± 2.12
0.30	0.00^{d}	10.5^{bc} ± 2.12	0.00^{d}	12.75^{abcd}±0.35	12.75^{bc}± 1.77
Sodium metabisulfite (% w/w)					
0.10	3.0^{c} ± 0.14	9.5^{c} ± 2.12	0.00^{d}	16.25^{a} ± 1.77	12.75^{bc} ±1.06
0.15	10.5^{a}± 0.71	11.00^{abc} ± 2.83	11.5^{ab} ± 0.71	13.0^{abcd} ± 2.83	13.25^{bc} ±1.20
0.20	7.5^{b} ± 0.71	8.00^{c} ± 0.35	10.5^{b} ± 0.71	14.5^{ab} ± 1.41	11.75^{bc} ±1.06
Sodium benzoate (% w/w)					
0.10	0.00^{d}	9.5^{c} ± 2.12	0.00^{d}	11.0^{bcd}± 0.14	15.0^{abc} ± 2.83
0.15	0.00^{d}	12.5^{abc} ± 0.42	0.00^{d}	12.0^{abcd} ± 2.83	18.0^{ab} ± 4.24
0.20	0.00^{d}	9.5^{c} ± 0.85	0.00^{d}	9.5^{cd}± 0.37	20.0^{a} ± 7.07
Olive oil (% v/w)					
5.00	11.0^{a} ± 2.83	12.0^{abc} ± 1.41	0.00^{d}	12.25^{abcd} ± 1.06	12.75^{bc} ± 4.60
10.00	12.5^{a} ± 0.42	0.00^{d}	7.5^{c} ± 0.71	9.75^{cd} ± 3.18	12.0^{bc} ± 0.57
20.00	10.0^{a} ± 2.83	11.0^{abc} ± 0.35	0.00^{d}	8.75^{d} ± 1.06	11.5^{bc} ± 0.57
Ascorbic acid (% w/w)					
1.00	0.00^{d}	10.5^{bc} ± 0.57	11.0^{b} ± 1.41	12.75^{abcd} ± 2.47	11.75^{bc} ± 1.06
2.00	0.00^{d}	12.5^{abc} ± 0.42	12.5^{a} ± 0.71	13.25^{abcd} ± 3.18	16.0^{abc} ± 2.83
4.00	11.0^{a} ±1.41	15.0^{ab} ± 4.42	10.5^{b} ± 0.57	14.5^{ab} ± 0.78	14.75^{abc} ± 1.06

Citric acid was found to be a weak stabilizer in case of *E.coli* and *Salmonella a*nd both microorganisms showed a great resistance at its each level. Whereas, the activity of *Staphylococcus* was inhibited and the highest zone of inhibition (15.5mm) was formed on 0.20% (w/v) concentration of citric acid in ginger garlic solution. The activity of *Proteus* and *Enterobacter* was inhibited on all concentrations of citric acid in paste and highest zones of inhibition were 13.5mm and 15mm on 0.1% of citric acid respectively. These results do not corroborate with the findings of Gupta and Ravishankar (2005) who reported strong antimicrobial activity in commercial ginger paste due to the addition of weak organic acids as preservatives. While comparing the activity of sodium metabisulfite alone (Table 2) with that of mixed with the paste (Table 3). It was observed that sodium metabisulfite stabilized the antibacterial activity of ginger garlic paste considerably even when this stabilizer had effectiveness only in case of *E.coli* as shown in table-2. The minimum inhibitory concentration of sodium metabisulfite mixed in the paste in case of *E.coli, Staphylococcus, Salmonella and Enterobacter* was 0.15 %w/w and the diameter of zone was 10.5, 11, 11.5 and 13.25mm respectively. While the activity of *Proteus* was greatly inhibited at 0.20%w/w with 14.5mm diameter of zone of inhibition. Present study is supported by the fact that the stabilizers may enhance or stabilize

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the flavonoids that are responsible to disrupt the microbial membrane (Tsuchiya et al., 1996).

Sodium benzoate could not enhance the antibacterial activity of ginger garlic paste in case of *E.coli* and *Salmonella* and both microorganisms exhibited a great resistance. The activity of other three bacterial species (*Staphylococcus, Proteus* and *Enterobacter*) was inhibited on all three concentrations (0.10, 0.15 and 0.20% w/w). The MIC in case of *Staphylococcus* and *Proteus* was 0.15% w/v with diameter zone of inhibition 12.5 and 12mm respectively. The highest zone of inhibition (20mm) was formed in case of *Enterobacter* at 0.20% w/w of sodium benzoate in ginger garlic paste (Table 3). Present results showed that olive oil is a strong stabilizer for antibacterial activity of ginger garlic paste. Olive oil is effective to stabilize the activity of the paste against *E.coli*, the best level of olive oil in this case was 10% w/v and the diameter of zone of inhibition was 12.5mm. In case of *Staphylococcus, Proteus* and *Enterobacter,* the lowest level of oil was 5% w/v, which gave the highest zone of inhibition 12, 12.25 and 12.75mm respectively. Only 10% w/v of olive oil in the ginger garlic paste inhibited the activity of *Salmonella*. This attribute could be due to the oil present in the paste as Farbood et al. (1976) also mentioned in their studies that a potential decrease in the penetration of the spice into the microbial cell could be due to the formation of a fat coat around the cell.

Ascorbic acid itself had efficient antimicrobial properties against all the five microbes used (Table 2). While mixed with the paste it was found that the efficiency of the paste was reduced indicating that it had no beneficial effect on antimicrobial activity in case of *E.coli* and only high level of concentration (4% w/w) gave the 11mm diameter zone of inhibition. The growth inhibition of *Staphylococcus* and *Proteus* was high at higher concentration of ascorbic acid (4% w/w) in ginger garlic paste while *Salmonella* and *Enterobacter* gave 12.5 and 16mm diameter zone of inhibition at 2% w/w of ascorbic acid. The antibacterial activities of extract with stabilizer were found to be dependent on the concentration of the stabilizers. From these results it can be concluded that the ginger garlic paste itself has antimicrobial activity which decreases with the passage of time during storage without any stabilizer added. Different stabilizers with/without inherent antimicrobial activity are able to stabilize/ enhance this property of the paste under consideration. Sodium metabisulfite, ascorbic acid and olive oil are found to be good at marinating the antimicrobial activity of the paste.

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