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Studies on the Effect of Essential Oils of Spices on Physico-Chemical, Microbial and Organoleptic Properties OF Chicken Meat Patties during Refrigerated Storage

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Abstract

At the storage temperature of 5+1^{0}C samples treated with essential oil of garlic with 1:250 concentration, up to day 8 and 1:500 and 1:1000 concentrations resulted in lower pH values up to day 6 of storage. Clove with 1:250 and 1:500 concentrations resulted in lower pH values up to day 12 of storage and 1:1000 concentration decreased the pH values up to day 10 of storage, cinnamon with 1:250, 1:500 and 1:1000 concentrations resulted in lower pH values up to day 8 of storage compared to the control. A significant ($p<0.05$) reduction in 2–thiobarbituric acid values on day 6 of storage compared to the control in the samples treated with essential oils of garlic, clove and cinnamon at 1:250 and 1:500 concentrations, whereas in samples with 1:1000 concentration showed a significant ($p<0.05$) reduction on day 4 of storage. Total plate count of chicken meat patties with essential oil of garlic at 1:250 and 1:500 concentrations resulted in decreased counts on day 2 of storage, then there was a gradual increase, and the counts were well within the prescribed standards up to day 10, day 6 and day 4 in patties with garlic at 1:250, 1:500 and 1:1000 concentrations respectively, with clove at 1:250 and 1:500 concentrations the counts were significantly ($P<0.05$) reduced up to day 6 and day 4 respectively, afterwards gradual increase was observed and they were well within the prescribed standards up to day 12 of storage for all the three concentrations, cinnamon at 1:250 and 1:500 concentrations the counts were significantly ($P<0.05$) reduced up to day 4 of storage, then gradual increase was observed and they were well within the prescribed standards up to day 14, day 12 and day 8 for 1:250, 1:500 and 1:1000 concentrations respectively. Yeast and mold count of patties with essential oil of garlic, at 1:250, 1:500 and 1:1000 concentrations significantly ($P<0.05$) reduced up to day 12, 10 and day 4 of storage respectively, with clove the counts were significantly ($P<0.05$) reduced up to day 14 of storage at 1:250 concentration whereas at 1:500 and 1:1000 concentrations the counts were significantly ($P<0.05$) reduced up to day 10 and day 4 respectively. Essential oil of cinnamon at 1:250, 1:500 and 1:1000 concentration significantly ($P<0.05$) reduced the counts up to day 12, day 10 and day 4 of storage respectively. With regard to the colour and flavour scores of samples with the mixture of essential oils of garlic, clove and cinnamon at 1:250, 1:500 and 1:1000 concentrations were found to have higher colour and flavour scores compared to the control through out the storage period but the samples with the mixture of essential oils of spices at 1:500 concentration had higher flavour scores when compared to the rest of the treatments. Hence clove at 1:250 concentration is considered to be the best one to increase the shelf life of chicken meat patties.

Keywords:Spices; pH; 2-TBARS values; microbial load; organoleptic qualities

Introduction

Recently there has been an increasing interest in discovering new natural antimicrobial substances or bio preservatives (Sagdic *et al*., 2003a) for the preservation of meat and meat products. Bio preservatives include a range of natural products from plants, animals and microorganisms which can be used to improve the keeping quality of foods, reduce or eliminate the pathogenic microorganisms and improve the overall quality of foods.

Being plant natural food stuffs, spices appear to be an alternative for the chemical antimicrobials to the consumers who tend to question their safety (Farag *et al*., 1989; Sagdic *et al*., 2003b). Although, spices have been well known for their medicinal, preservative and antioxidant properties, currently they have been used with primary purpose of enhancing the flavour of foods rather than extending shelf-life (Aktug and Karapinar, 1986; Ristori *et al*., 2002). In recent years antimicrobial properties of spices have been documented and interest con-

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tinued to the present

Since a long time Garlic clove and cinnamon have been used in the traditional Indian food preparations and further they are among the ten most inhibitory oils of spices (Deans and Ritchie, 1987). The essential oil (Quinta essentia) fraction of spices is often the most inhibitory to growth and survival of microorganisms (Draughon, 2004). Hence the essential oils of spices or bio preservatives have the greatest advantage to cater to the demands of the consumer for the natural preservatives. As the awareness about the bio preservatives is increasing reliable information is required for the scientific community and to the consumers about their antimicrobial activity and public health significance.

Materials and methods

The birds were procured from the local market, slaughtered and dressed conventionally in the mini slaughter house of the department of Livestock Products Technology by adopting all the hygienic precautions. Chicken meat mince was prepared by following all the aseptic precautions. To test for a particular essential oil of spices, meat mince was divided into four groups, one for the control and the remaining three for the essential oil of particular spice at three different concentrations. The essential oils of garlic, clove and cinnamon were tested at a concentration of 1:250, 1:500 and 1:1000 and were added to meat at 2% level. For estimation of sensory attributes the meat mince was mixed with the mixture of all the three essential oils of spices at 1:250, 1:500 and 1:1000 concentrations. For estimation of pH, 2–thiobarbituric acid (2-TBARS) values and for microbiological analysis the meat mince was mixed with essential oil of garlic, clove and cinnamon separately at three different concentrations viz., 1:250, 1:500 and 1:1000. Samples drawn from these groups were stored at refrigeration temperature ($5 + 1^0$C) and analyzed at 2 days interval till spoilage for physico-chemical characteristics, microbial counts and sensory evaluation.

Percent cooking loss was estimated by recording the difference between the pre and post cooking weights and expressed in percentages.

The emulsion stability of the samples was determined by taking 25 grams of patty mix and placed in a polyethylene bag and heated at 80^0C for 30 minutes in a water bath. Cookout was drained and cooked mass was cooled and weighed and loss in weight was expressed as percentage (Baliga and Madaiah 1970).

The water holding capacity of the sample was determined by taking 25 grams of patties mix and it was blended by addition of 75ml of distilled water for 90 seconds in high speed blender. 35ml of meat slurry centrifuged at room temperature at 1000 rpm for 15 minutes. After centrifugation the volume of supernatant liquid was collected in a graduated cylinder. The percent of swelling determined by the following formula (Wierbicki *et al*., 1962)

$$\text{Water holding capacity} = \frac{300 - 11.43}{100} \times S/100$$

S = Amount of supernatant collected

The pH of the sample was determined by taking meat sample weighing 25g, and it was blended with 100ml of distilled water for one minute in a mechanical blender. From the total homogenate a 50ml aliquot portion was immediately used for determination of pH. The pH was recorded by immersing the combined glass electrode of digital pH meter (Model: Deep Vision, Deluxe pH meter 101) in the homogenate (Jay, 1964). The pH meter was calibrated with standard buffer solutions of pH 4.00 and 9.00 prior to measurement.

The 2–thiobarbituric acid reactive substance value of the samples was determined by taking 10 grams of chicken meat patty sample and it was blended with 50ml of distilled water. Another 48ml of distilled water was used to wash the blender thoroughly into the homogenate and 2ml of Hydrochloric acid (1 part of concentrated Hydrochloric acid in 2 parts of distilled water) was added. Then a pinch of paraffin and glass beads were added. The homogenate was distilled by using Kjeldahl flask. First 50ml of distillate was collected in a measuring cylinder. Then 5ml of the distillate was mixed with 5ml of 2–thiobarbituric acid reagent in a stoppard flask and boiled for 35 minutes in a boiling water bath. A blank was also prepared by heating 5ml of 2–thiobarbituric acid reactive reagent with 5ml of distilled water in boiling water bath. The sample was cooled and absorbance was measured at 538nm in Spectrophotometer (Spectronic–21, Bausch and Lomb). The optical density was multiplied by 78 to get milligrams of malonaldehyde/kg of sample

(Tarladgis *et al*., 1960).

The total plate count per gram of chicken meat patties at refrigeration temperature was estimated by taking 11 grams of sample and it was thoroughly blended with 99 ml of sterile phosphate buffer diluent for 2 minutes. 1 ml sample was aseptically pipetted out into tubes containing 9 ml of phosphate buffer diluent. Serial dilutions of the sample were prepared and the samples in duplicate were inoculated by pour plate method. On Standard Plate Count agar for enumeration of total microbial load, 1ml of the inoculum was transferred into Petri dish to which molten media i.e. Standard Plate Count agar maintained at 45^0C was poured and mixed with inoculum by gentle rotating movements and allowed to solidify. The plates for each dilution was incubated at 37^0C . After an incubation period of 24 hours, the plates revealing 30 to 300 colonies were selected and the plate counts were expressed as log10 cfu/gram of meat after multiplying the total number of colonies with dilution factor (Chestnut *et al*., 1977). For estimation of yeast and molds the procedure used for estimation of total plate count was adopted except that Sabourads Dextrose agar was used in place of Standard Plate Count agar.

The chicken meat patties prepared were shallow pan fried and subjected to a five member taste panel to evaluate colour, and flavour.

Statistical Analysis

Statistical analysis of the data was carried out as per the SPSS (Version 10.0) software.

Results

The analysis of variance revealed that the overall mean ± SE values of pH of chicken meat patties as influenced by incorporation of essential oil of garlic and clove at 1:250,1:500 and 1:1000 concentrations and cinnamon at 1:250 and 1:500 concentrations were significantly (P< 0.05) different from the control (Table 1). Among the treatments chicken meat patties with essential oil of garlic and clove at 1:1000 was recorded a higher pH values. As the storage period increased, the pH of the chicken meat patties was increased gradually and a sharp increase in pH was observed between day 6 and day 8 of storage with garlic and with clove a gradual increase in pH was observed from day 4 of storage and no significant difference (p<0.05) was observed on day 4 and day 6 again on day 12 and day 14 of storage. Essential oil of cinnamon showed that there was an increase in pH up to day 14 of storage but it was not significant (P< 0.05) up to day 4 of storage, afterwards a significant (P< 0.05) increase in pH was observed up to day 12 of storage.

Value of 2-TBARS of chicken meat patties as influenced by incorporation of essential oil of garlic and clove at 1:250, 1:500 and 1:1000, cinnamon at 1:250 and 1:500 concentrations were differed significantly (P< 0.05) from the control (Table 2). Among the treatments chicken meat patties with essential oil of garlic, clove and cinnamon at 1:1000 concentration were recorded higher 2-TBARS values. As the storage period increases the

Table 1. Effect of essential oils of spices on pH during refrigerated storage

Storage period in days	Control	Treatment with essential oil of garlic at a concentration of			Overall mean	Treatment with essential oil of clove at a concentration of			Overall mean	Treatment with essential oil of cinnamon at a concentration of			Overall mean
		1:250	1:500	1:1000		1:250	1:500	1:1000		1:250	1:500	1:1000	
0	5.73 ± 0.29^{a3}	5.59 ± 0.11^{a1}	5.67 ± 0.09^{a2}	5.70 ± 0.09^{a3}	5.67 ± 0.08ab	5.51 ± 0.08^{a1}	5.60 ± 0.05^{ab2}	5.65 ± 0.05^{ab2}	5.62 ± 0.07^{a}	5.59 ± 0.09^{a1}	5.62 ± 0.03^{a1}	5.69 ± 0.07^{a1}	5.66 ± 0.07^{a}
2	5.71 ± 0.38^{a2}	5.61 ± 0.10^{a1}	5.64 ± 0.09^{a1}	5.69 ± 0.12^{a1}	5.66 ± 0.10^{a}	5.54 ± 0.06^{a1}	5.52 ± 0.07^{a1}	5.58 ± 0.06^{a2}	5.59 ± 0.09^{a}	5.66 ± 0.08^{a1}	5.68 ± 0.07^{a1}	5.74 ± 0.05^{b2}	5.70 ± 0.09^{a}
4	5.94 ± 0.17^{b2}	5.85 ± 0.16^{b1}	5.87 ± 0.11^{b1}	5.81 ± 0.11^{ab1}	5.87 ± 0.07^{bc}	5.62 ± 0.07^{b1}	5.66 ± 0.05^{b1}	5.67 ± 0.06^{b1}	5.72 ± 0.05^{ab}	5.74 ± 0.11^{ab1}	5.79 ± 0.06^{b1}	5.81 ± 0.06^{c1}	5.82 ± 0.05^{a}
6	6.02 ± 0.04^{bc2}	6.05 ± 0.12^{c2}	5.95 ± 0.08^{c1}	5.93 ± 0.18^{b1}	5.99 ± 0.05^{c}	5.71 ± 0.04^{c1}	5.73 ± 0.05^{b1}	5.79 ± 0.05^{c2}	5.81 ± 0.03^{b}	5.89 ± 0.12^{b1}	5.98 ± 0.06^{c2}	5.96 ± 0.05^{d2}	5.96 ± 0.04^{b}
8	6.60 ± 0.16^{c3}	6.28 ± 0.09^{d1}	6.47 ± 0.12^{d2}	6.48 ± 0.15^{c2}	6.46 ± 0.07^{d}	5.93 ± 0.03^{d1}	5.91 ± 0.05^{c1}	5.91 ± 0.07^{d1}	6.09 ± 0.07^{c}	6.09 ± 0.08^{c1}	6.19 ± 0.07^{d2}	6.22 ± 0.04^{e2}	6.28 ± 0.06^{c}
10	6.86 ± 0.10^{c4}	6.64 ± 0.17^{e2}	5.97 ± 0.03^{e1}	6.98 ± 0.03^{d3}	6.61 ± 0.09^{de}	6.04 ± 0.05^{e1}	6.14 ± 0.06^{d2}	6.38 ± 0.08^{e3}	6.34 ± 0.07^{d}	6.46 ± 0.05^{d2}	6.38 ± 0.04^{e1}	6.54 ± 0.07^{f3}	6.54 ± 0.05^{d}
12	6.78 ± 0.13^{cd2}	6.55 ± 0.12^{f1}	6.73 ± 0.08^{f2}	6.77 ± 0.15^{e2}	6.71 ± 0.06^{ef}	6.35 ± 0.05^{f1}	6.32 ± 0.05^{e1}	6.49 ± 0.05^{f2}	6.49 ± 0.05^{e}	6.71 ± 0.04^{e1}	6.83 ± 0.03^{f2}	6.79 ± 0.05^{g2}	6.79 ± 0.03^{e}
14	6.81 ± 0.08^{d2}	6.68 ± 0.14^{f1}	6.98 ± 0.16^{g3}	6.93 ± 0.11^{e3}	6.85 ± 0.06^{f}	6.46 ± 0.05^{g1}	6.45 ± 0.05^{f1}	6.51 ± 0.05^{e2}	6.57 ± 0.05^{e}	6.89 ± 0.07^{f1}	6.94 ± 0.03^{g2}	$6.96\,0.04^{h2}$	6.91 ± 0.03^{e}
Overall mean	6.31 ± 0.09^{B}	6.16 ± 0.07^{A}	6.16 ± 0.08^{A}	6.29 ± 0.09^{AB}		5.90 ± 0.09^{A}	5.92 ± 0.05^{B}	6.00 ± 0.06^{C}		6.13 ± 0.07^{A}	6.18 ± 0.07^{A}	6.21 ± 0.07^{AB}	

2-TBARS values of the chicken meat patties were increased gradually and with garlic a sharp increase in 2-TBARS values from day 6 of storage and in treatment with clove and cinamon increase in 2-TBARS values from day 0 to day 14 of storage were observed.

Table 2. Effect of essential oils of spices on 2-TBARS values during refrigerated storage.

Storage period in days	Control	Treatment with essential oil of garlic at a concentration of			Overall mean	Treatment with essential oil of clove at a concentration of			Overall mean	Treatment with essential oil of cinnamon at a concentration of			Overall mean
		1:250	1:500	1:1000		1:250	1:500	1:1000		1:250	1:500	1:1000	
0	0.41 ± 0.10^{a2}	0.34 ± 0.01^{a1}	0.31 ± 0.01^{a1}	0.32 ± 0.02^{a1}	0.34 ± 0.02^{a}	0.36 ± 0.06^{a1}	0.35 ± 0.08^{a1}	0.31 ± 0.05^{a1}	0.36 ± 0.03^{a}	0.34 ± 0.05^{a1}	0.36 ± 0.03^{a1}	0.33 ± 0.06^{a1}	0.36 ± 0.03^{a}
2	0.54 ± 0.10^{b4}	0.39 ± 0.02^{b1}	0.43 ± 0.02^{b2}	0.49 ± 0.03^{b3}	0.46 ± 0.03^{ab}	0.39 ± 0.07^{b1}	0.40 ± 0.06^{a1}	0.51 ± 0.05^{b2}	0.46 ± 0.04^{b}	0.36 ± 0.03^{a1}	0.38 ± 0.04^{a1}	0.40 ± 0.04^{b1}	0.42 ± 0.03^{a}
4	0.83 ± 0.04^{c3}	0.53 ± 0.02^{c1}	0.59 ± 0.02^{c1}	0.73 ± 0.02^{c2}	0.67 ± 0.03^{b}	0.44 ± 0.05^{b1}	0.51 ± 0.07^{b2}	0.59 ± 0.04^{c3}	0.59 ± 0.04^{c}	0.40 ± 0.04^{b1}	0.44 ± 0.04^{b1}	0.48 ± 0.03^{c2}	0.54 ± 0.04^{ab}
6	1.51 ± 0.06^{d4}	0.61 ± 0.05^{d1}	0.78 ± 0.03^{d2}	1.34 ± 0.07^{d3}	1.06 ± 0.08^{c}	0.52 ± 0.06^{c1}	0.58 ± 0.07^{b2}	0.90 ± 0.07^{d3}	0.88 ± 0.09^{d}	0.48 ± 0.03^{c1}	0.52 ± 0.04^{c2}	0.66 ± 0.05^{d3}	0.79 ± 0.09^{bc}
8	2.01 ± 0.06^{e4}	0.73 ± 0.07^{e1}	0.91 ± 0.05^{e2}	1.59 ± 0.08^{e3}	1.31 ± 0.11^{d}	0.55 ± 0.06^{c1}	0.67 ± 0.04^{c2}	0.98 ± 0.06^{d3}	1.05 ± 0.12^{e}	0.53 ± 0.03^{d1}	0.56 ± 0.04^{d1}	0.81 ± 0.06^{e2}	0.98 ± 0.13^{cd}
10	2.84 ± 0.03^{f4}	1.11 ± 0.10^{f1}	1.48 ± 0.07^{f2}	1.76 ± 0.04^{f3}	1.80 ± 0.14^{e}	0.68 ± 0.07^{d1}	0.81 ± 0.04^{d2}	1.54 ± 0.09^{e3}	1.47 ± 0.18^{f}	0.58 ± 0.05^{e1}	0.61 ± 0.04^{e2}	0.96 ± 0.04^{f3}	1.25 ± 0.20^{d}
12	3.71 ± 0.03^{g4}	1.37 ± 0.11^{g1}	1.78 ± 0.11^{g2}	1.92 ± 0.03^{g3}	2.20 ± 0.19^{f}	1.19 ± 0.10^{e2}	1.09 ± 0.10^{e1}	1.63 ± 0.03^{f3}	1.91 ± 0.22^{g}	0.69 ± 0.04^{f1}	0.79 ± 0.05^{f2}	1.33 ± 0.03^{g3}	1.63 ± 0.26^{e}
14	4.18 ± 0.04^{h2}	1.47 ± 0.11^{h1}	1.96 ± 0.03^{h2}	1.99 ± 0.04^{g2}	2.40 ± 0.22^{f}	1.41 ± 0.15^{f1}	1.47 ± 0.12^{f1}	1.71 ± 0.05^{g2}	2.19 ± 0.24^{h}	1.01 ± 0.03^{g1}	1.16 ± 0.05^{g2}	1.46 ± 0.04^{g3}	1.95 ± 0.27^{f}
Overall mean	0.41 ± 0.10^{a2}	0.34 ± 0.01^{a1}	0.31 ± 0.01^{a1}	0.32 ± 0.02^{a1}		0.69 ± 0.06^{A}	0.74 ± 0.06^{A}	1.02 ± 0.08^{B}		0.55 ± 0.03^{A}	0.60 ± 0.04^{AB}	0.80 ± 0.06^{B}	

essential oils of garlic, clove and cinamon at 1:250, 1:500 and 1:1000 were significantly ($P< 0.05$) different from the control (Table 3). Among the treatments also significant ($P< 0.05$) difference was observed and high total plate count of chicken meat patties was observed with essential oil of garlic, clove and cinnamon at 1:1000 concentrations. The analysis of variance further revealed that there was a decrease in total plate count in all the treatments on day 2 of storage from there a gradual increase in total plate count was observed in garlic and cinnamon treatments where as with clove the counts were started increasing from day 6 of storage.

Table 3. Effect of essential oils of spices on total plate count during refrigerated storage.

Storage period in days	Control	Treatment with essential oil of garlic at a concentration of			Overall mean	Treatment with essential oil of clove at a concentration of			Overall mean	Treatment with essential oil of cinnamon at a concentration of			Overall mean
		1:250	1:500	1:1000		1:250	1:500	1:1000		1:250	1:500	1:1000	
0	5.72 ± 0.31^{a2}	4.84 ± 0.31^{a1}	4.75 ± 0.27^{a1}	4.79 ± 0.17^{a1}	5.03 ± 0.15^{a}	4.51 ± 0.09^{a1}	4.77 ± 0.05^{a2}	4.8 ± 0.06^{a2}	4.96 ± 0.12^{a}	4.12 ± 0.06^{a1}	4.43 ± 0.05^{a1}	4.62 ± 0.06^{a2}	4.72 ± 0.15^{a}
2	6.30 ± 0.16^{b2}	3.49 ± 0.22^{b1}	3.49 ± 0.25^{b1}	3.51 ± 0.13^{b1}	4.20 ± 0.27^{b}	3.84 ± 0.08^{b1}	4.31 ± 0.09^{b2}	4.96 ± 0.07^{b3}	4.85 ± 0.20^{b}	3.53 ± 0.07^{b1}	3.71 ± 0.07^{b1}	4.34 ± 0.05^{b2}	4.47 ± 0.23^{a}
4	6.58 ± 0.13^{b3}	4.87 ± 0.37^{b1}	5.61 ± 0.29^{b2}	5.68 ± 0.20^{b2}	5.68 ± 0.18^{c}	3.71 ± 0.07^{a1}	4.56 ± 0.07^{a2}	5.13 ± 0.10^{a3}	5.00 ± 0.22^{a}	3.79 ± 0.07^{a1}	4.06 ± 0.05^{a2}	4.89 ± 0.08^{a3}	4.83 ± 0.23^{b}
6	7.41 ± 0.10^{c4}	4.88 ± 0.33^{b1}	5.52 ± 0.20^{b2}	6.49 ± 0.17^{b3}	6.08 ± 0.22^{d}	4.25 ± 0.07^{a1}	4.91 ± 0.06^{a2}	5.39 ± 0.07^{a3}	5.49 ± 0.25^{b}	4.01 ± 0.09^{a1}	4.29 ± 0.06^{a1}	5.12 ± 0.05^{a2}	5.21 ± 0.28^{c}
8	8.73 ± 0.15^{d4}	4.85 ± 0.33^{b1}	6.10 ± 0.11^{c2}	6.81 ± 0.17^{c3}	6.62 ± 0.31^{e}	4.86 ± 0.04^{a1}	5.16 ± 0.07^{a2}	5.56 ± 0.05^{a3}	6.08 ± 0.33^{c}	4.52 ± 0.07^{a1}	4.95 ± 0.06^{a1}	5.93 ± 0.05^{a2}	$6.03\pm0.34d$
10	8.80 ± 0.04^{e4}	5.38 ± 0.30^{c1}	6.43 ± 0.17^{c2}	7.54 ± 0.07^{b3}	7.04 ± 0.28^{f}	5.05 ± 0.06^{b1}	5.59 ± 0.06^{b2}	5.78 ± 0.09^{b2}	6.31 ± 0.31^{d}	4.78 ± 0.14^{a1}	5.46 ± 0.05^{a2}	6.42 ± 0.05^{a3}	6.37 ± 0.32^{c}
12	8.86 ± 0.05^{e3}	6.68 ± 0.33^{d1}	7.28 ± 0.15^{d2}	7.62 ± 0.04^{b2}	7.61 ± 0.19^{g}	5.59 ± 0.05^{b1}	5.78 ± 0.06^{b2}	$5.94\pm0.06g1$	6.54 ± 0.28^{e}	5.22 ± 0.07^{b1}	5.79 ± 0.06^{b2}	6.68 ± 0.05^{a3}	6.64 ± 0.29^{d}
14	8.93 ± 0.03^{e3}	6.94 ± 0.19^{e1}	7.34 ± 0.03^{b2}	7.73 ± 0.04^{b2}	7.73 ± 0.16^{g}	6.01 ± 0.05^{c1}	5.86 ± 0.04^{b1}	6.44 ± 0.07^{b2}	6.81 ± 0.26^{e}	5.58 ± 0.10^{b1}	6.16 ± 0.06^{a2}	6.92 ± 0.05^{a3}	6.9 ± 0.27^{g}
Overall mean	7.67 ± 0.19^{D}	5.24 ± 0.18^{A}	5.82 ± 0.19^{B}	6.27 ± 0.21^{C}		4.73 ± 0.11^{A}	5.12 ± 0.08^{B}	5.51 ± 0.08^{C}		4.44 ± 0.10^{A}	4.86 ± 0.12^{B}	5.62 ± 0.14^{C}	

Total plate count of chicken meat patties with Yeast and molds of chicken meat patties with essential oil of garlic, clove and cinamon at 1:250, 1:500 and 1:1000 concentration were significantly (P< 0.05) different from control (Table 4). Among the treatments also significant (P< 0.05) difference was observed and high yeast and mold count was observed in all the treatments at 1:1000 concentration. The analysis of variance further revealed that there was a decrease in yeast and mold count up to day 6 of storage with garlic and cinnamon, with clove decrease in yeast and mold count on day 2 of storage was observed from there a gradual increase in was observed.

Colour scores of chicken meat patties as influenced by incorporation of essential oils of garlic, clove and cinnamon mixture at 1:250, 1:500 and 1:1000, flavour scores at 1:250 and 1:500 concentrations were significantly (P< 0.05) different from the control (Table 5). Among the treatments

Table 4. Effect of essential oils of spices on yeast and moulds count during refrigerated storage.

Storage period in days	Control	Treatment with essential oil of garlic at a concentration of			Overall mean	Treatment with essential oil of clove at a concentration of			Overall mean	Treatment with essential oil of cinnamon at a concentration of			Overall mean
		1:250	1:500	1:1000		1:250	1:500	1:1000		1:250	1:500	1:1000	
0	2.41 ± 0.08^{a4}	2.33 ± 0.03^{a3}	2.38 ± 0.03^{a3}	2.45 ± 0.03^{a3}	2.39 ± 0.02^{a}	2.43 ± 0.06^{a3}	2.52 ± 0.05^{a}	2.64 ± 0.05^{a4}	$2.50\pm0.03b^{a}$	2.59 ± 0.07^{a3}	2.63 ± 0.07^{a3}	2.76 ± 0.04^{a4}	2.60 ± 0.04^{a4}
2	2.63 ± 0.05^{a4}	1.49 ± 0.05^{a}	1.63 ± 0.02^{a}	2.21 ± 0.04^{a2}	1.99 ± 0.10^{a}	1.51 ± 0.06^{a}	1.72 ± 0.04^{a1}	2.31 ± 0.04^{a2}	2.04 ± 0.10^{a}	1.66 ± 0.04^{a1}	1.93 ± 0.03^{a1}	2.46 ± 0.04^{a2}	2.17 ± 0.08^{a}
4	2.85 ± 0.03^{a4}	1.64 ± 0.05^{a1}	1.75 ± 0.03^{a1}	2.38 ± 0.03^{a2}	2.16 ± 0.10^{a}	1.73 ± 0.08^{a1}	1.81 ± 0.05^{a1}	2.43 ± 0.07^{a2}	2.21 ± 0.10^{a}	1.83 ± 0.04^{a1}	1.98 ± 0.05^{a1}	2.49 ± 0.04^{a2}	2.29 ± 0.09^{a}
6	3.10 ± 0.03^{a4}	1.79 ± 0.05^{a1}	1.86 ± 0.03^{a1}	2.71 ± 0.02^{a3}	2.36 ± 0.12^{a}	1.79 ± 0.08^{a1}	1.94 ± 0.08^{a1}	2.79 ± 0.08^{a3}	2.41 ± 0.12^{a}	1.93 ± 0.03^{a1}	2.01 ± 0.05^{a1}	2.84 ± 0.04^{a3}	2.47 ± 0.11^{a}
8	3.35 ± 0.04^{a4}	1.88 ± 0.07^{a1}	2.15 ± 0.03^{a2}	2.96 ± 0.03^{a3}	2.59 ± 0.13^{a}	1.88 ± 0.05^{a1}	2.13 ± 0.06^{a2}	3.08 ± 0.05^{a3}	2.61 ± 0.13^{a3}	2.11 ± 0.06^{a2}	2.20 ± 0.06^{a2}	3.16 ± 0.05^{a4}	2.71 ± 0.12^{a}
10	3.44 ± 0.03^{a4}	2.07 ± 0.06^{a1}	2.34 ± 0.02^{a2}	3.14 ± 0.03^{a3}	2.75 ± 0.12^{a}	2.08 ± 0.06^{a1}	2.42 ± 0.04^{a2}	2.85 ± 0.14^{a3}	2.70 ± 0.11^{a}	2.32 ± 0.03^{a2}	2.37 ± 0.05^{a2}	3.41 ± 0.04^{a3}	2.89 ± 0.11^{a}
12	4.56 ± 0.04^{a4}	2.28 ± 0.11^{a2}	2.75 ± 0.03^{a3}	3.25 ± 0.03^{a3}	3.21 ± 0.18^{a}	2.21 ± 0.08^{a2}	2.76 ± 0.04^{a3}	3.29 ± 0.09^{a3}	3.21 ± 0.18^{a}	2.48 ± 0.05^{a2}	2.84 ± 0.04^{a3}	3.59 ± 0.05^{a4}	3.37 ± 0.17^{a}
14	4.74 ± 0.03^{a4}	2.37 ± 0.15^{a2}	3.01 ± 0.03^{a3}	3.82 ± 0.05^{a4}	3.49 ± 0.19^{a}	2.32 ± 0.09^{a2}	3.19 ± 0.06^{a3}	3.94 ± 0.05^{a4}	3.55 ± 0.19^{a}	2.67 ± 0.05^{a2}	3.29 ± 0.05^{a3}	3.68 ± 0.05^{a4}	3.60 ± 0.16^{a}
Overall mean	3.39 ± 0.12^{D}	1.98 ± 0.05^{A}	2.23 ± 0.07^{B}	2.87 ± 0.07^{C}		1.99 ± 0.05^{A}	2.31 ± 0.07^{B}	2.92 ± 0.08^{C}		2.20 ± 0.05^{A}	2.41 ± 0.07^{B}	3.05 ± 0.07^{C}	

Table 5. Effect of essential oils of spices on colour and flavour of the product during refrigerated storage.

Storage period in days	Colour					Flavour				
	Control	Treatment with essential oil of spices at a concentration of			Overall mean	Control	Treatment with essential oil of spices at a concentration of			Overall mean
		1:250	1:500	1:1000			1:250	1:500	1:1000	
0	8.43 ± 0.05^{b4}	7.71 ± 0.04^{a1}	7.48 ± 0.03^{a2}	7.55 ± 0.06^{b3}	7.79 ± 0.29^{a}	8.73 ± 0.03^{b3}	7.93 ± 0.04^{a2}	7.98 ± 0.04^{f2}	7.83 ± 0.03^{f1}	8.12 ± 0.08^{f}
2	7.32 ± 0.03^{a4}	6.81 ± 0.05^{d1}	6.93 ± 0.04^{a2}	6.92 ± 0.04^{a3}	6.99 ± 0.22^{f}	8.64 ± 0.03^{a4}	7.76 ± 0.03^{f2}	7.96 ± 0.05^{f3}	7.64 ± 0.05^{e1}	8.00 ± 0.08^{f}
4	6.17 ± 0.05^{f4}	6.74 ± 0.07^{d1}	6.66 ± 0.05^{f2}	6.54 ± 0.06^{f3}	6.52 ± 0.18^{e}	8.48 ± 0.04^{f4}	7.43 ± 0.03^{e1}	7.84 ± 0.03^{e3}	7.58 ± 0.05^{e2}	7.83 ± 0.09^{ef}
6	5.54 ± 0.05^{e4}	6.63 ± 0.05^{c1}	6.06 ± 0.06^{e2}	6.41 ± 0.06^{e3}	6.16 ± 0.15^{d}	8.21 ± 0.04^{e4}	6.84 ± 0.03^{d1}	7.81 ± 0.04^{de3}	7.40 ± 0.05^{d2}	7.57 ± 0.11^{e}
8	5.01 ± 0.04^{d4}	6.41 ± 0.06^{b1}	5.93 ± 0.04^{d2}	6.23 ± 0.06^{d3}	5.89 ± 0.18^{c}	6.44 ± 0.03^{d2}	6.53 ± 0.05^{c3}	7.78 ± 0.03^{d4}	$6.23\pm0.03c1$	6.75 ± 0.13^{d}
10	4.82 ± 0.04^{c3}	5.78 ± 0.06^{a1}	5.88 ± 0.04^{c2}	5.86 ± 0.03^{c4}	5.50 ± 0.20^{b}	5.31 ± 0.03^{c1}	6.24 ± 0.05^{b3}	6.96 ± 0.04^{c4}	6.01 ± 0.04^{b2}	6.13 ± 0.12^{c}
12	3.13 ± 0.05^{b1}	5.62 ± 0.04^{a1}	5.79 ± 0.06^{b1}	5.80 ± 0.06^{b2}	5.08 ± 0.20^{a}	3.18 ± 0.06^{b1}	6.10 ± 0.05^{a3}	6.24 ± 0.05^{b4}	5.92 ± 0.06^{b2}	5.36 ± 0.26^{b}
14	2.83 ± 0.03^{a1}	5.54 ± 0.05^{a2}	5.70 ± 0.04^{a1}	5.71 ± 0.05^{a3}	4.94 ± 0.19^{a}	2.94 ± 0.03^{a1}	5.93 ± 0.03^{a4}	6.18 ± 0.06^{a2}	5.16 ± 0.04^{a3}	5.05 ± 0.23^{a}
Overall mean	5.41 ± 0.26^{C}	6.40 ± 0.11^{A}	6.30 ± 0.15^{B}	6.37 ± 0.12^{D}		6.49 ± 0.33^{A}	6.85 ± 0.11^{AB}	7.09 ± 0.18^{BC}	6.72 ± 0.14^{C}	

no significant (P> 0.05) difference was observed and high colour and flavour scores were observed at 1:250 and 1:500 concentrations respectively. The analysis of variance further revealed that there was a significant (P<0.05) decrease in colour and flavour scores was observed as the storage period increases.

Discussion

Chicken meat patties treated with essential oil of garlic at 1:250 concentration resulted in lower pH value (within the range of 5.5 to 6.5) up to day 8 of storage, where as at 1:500 and 1:1000 concentrations resulted in lower pH values up to day 6 of storage compared to the control. The decreased pH may be attributed to differential inhibitory activity of garlic on bacteria, lactic acid bacteria being the least sensitive microorganism (Rees *et al*., 1993; Gonzalez-Fandos *et al*., 1999). Garlic can even stimulate the growth of lactic acid bacteria by providing them with a carbohydrate source of growth (Paludan-Miller *et al*., 1999) and stimulate lactic acid production. The results of this study were in accordance with Yadav *et al*. (2005) in minced chicken meat, Hasan Yetim *et al*. (2006) in pastrim treated with cemen mix where garlic was one ingredient, Ockerman and Sun (2007) in Chinese style sausage.

Essential oil of clove at 1:250 and 1:500 concentrations resulted in lower pH value up to day 12 of storage, where as at 1:1000 concentration resulted in lower pH values up to day 10 of storage compared to the control. The decrease in pH might be due to eugenol which stimulates the lactic acid accumulation, which in turn causes the pH to drop more rapidly further inhibiting microbial activity (Varel and Muller, 2004). The results of this study were in agreement with Yadav *et al.* (2005) in minced chicken during 10 days of refrigerated storage and Inderjith Singh *et al*. (2005) in chicken meat treated with alcoholic extract of clove.

Lower pH value up to day 8 of storage compared to the control were observed in chicken meat patties treated with essential oil of cinnamon at 1:250, 1:500 and 1:1000 concentrations. The decrease in pH might be due to cinnamaldehyde which stimulates the lactic acid accumulation, which in turn causes the pH to drop more rapidly further inhibiting microbial activity (Varel and Muller, 2004). The results of this study were in accordance with Inderjith Singh *et al*. (2005) in chicken meat treated with alcoholic extract of cinnamon.

Irrespective of the concentration of essential oil of garlic, clove and cinnamon used, pH of the chicken meat patties was increased with an increase in storage period. The significant (P<0.05) increase in mean pH values might be due to presence of flour and a concomitant increase in bacteria which release metabolites during their metabolism (Jay, 1996) and also oxidation of fatty acids during storage. The differences in pH value between the control and treatments might be due to the differential antimicrobial activity of the essential oil of garlic on bacterial multiplication.

Chicken meat patties treated with essential oil of garlic at 1:250 and 1:500 concentrations resulted in significantly (P<0.05) lower TBARS values up to day 6 and day 4 of storage respectively where as a significantly (P<0.05) lower TBARS values could be found up to day 2 of storage at 1:1000 concentration. The reduction in TBARS values may be due to garlic, which is believed to contain up to 5 percent dry weight of non protein sulfur amino acid secondary metabolites such as S-allyl-L-cysteine S oxide or allin. Allin is found predominantly in garlic, is cleaved by allinase enzyme upon the homogenization of garlic to form ammonium pyruvate and 2-propenesulfenic acid. The later compound undergoes self condensation to yield the diallyl thiosulfinate allicin which provides the garlic with its odour or flavour and is often ascribed to its antioxidant activity (Vipraja Vaidya *et al*., 2008).

The results of this study were in agreement with Abdalla (1999) in both cooked and stored breast and thigh muscles. Furthermore Mei - Chin Yin and Wen-Shen Cheng (2002) in ground beef and Yadav *et al*. (2005) in minced chicken meat.

Chicken meat patties treated with essential oil of clove at 1:250 and 1:500 concentrations significantly (P<0.05) registered lower TBARS values up to day 8 and day 6 of storage, respectively, where as a significantly (P<0.05) lower TBARS values could be found up to day 4 of storage at 1:1000 concentration. The inhibition mechanism of eugenol to lipid peroxidation is due to its interference with the chain reaction by trapping the active oxygen and further it is metabolized to dimmer and the dimeric compound (dieugenol), which inhibits lipid peroxidation at the level of propagation of free

radical chain reaction like α-tocopherol (Masahiro Ogata *et al*., 2000).

Essential oil of cinnamon at 1:250 and 1:500 concentrations registered significantly (P<0.05) lower TBARS values up to day 10 and day 8 of storage, where as a significantly (P<0.05) lower TBARS values could be found up to day 4 of storage at 1:1000 concentration. The antioxidant activity of cinnamon may be attributed to the scavenging capacity of cinnamaldehyde which can scavenge 2,2-diphenyl 1 picryl hydrazyl radical (DPPH) and the hydroxyl radical (OH radical). The scavenging of DPPH and OH radicals by cinnamon oil is attributed to the hydrogen donating capacity of the phenolic component of cinnamic oil (Erich Schmidt, 2006).

The results of this study were in agreement with. Yadav *et al*. (2005) in minced chicken meat during 10 days refrigerated storage, Naveena *et al*. (2006) in buffalo meat and Saumya Dwivedi et al. (2006) in cooked ground beef. However there was a progressive increase in TBARS values in all the treatments with an increase in storage period.

Chicken meat patties with essential oil of garlic at 1:250 concentration resulted in 3 log reduction in total plate count compared to the control on day 10 of storage, whereas at 1:500 concentration a 2 log reduction and at 1:1000 concentration a 1 log reduction was found on day 10 of storage. The decreased total plate count of chicken meat patties might be due to allicin, the antimicrobial compound of garlic. Meat treated with essential oil of garlic at 1:250 concentration met the standards for fresh chilled meat of 106 cfu/gm (Agarwal and Bhilegaonkar, 2003) on day 10 of storage but exceeded the same on day 12 and day 14, whereas at 1:500 and 1:1000 concentrations the standards were met up to day 6 and day 4 of refrigerated storage, respectively. Irrespective of concentrations used the total plate count was increased with an increase in storage period.

The results of this study were in agreement with Al Deilaimy and Barakat (1970) in camel meat, Mei-Chin Yin and Wen-Shen Cheng (2002) in ground beef, Yadav *et al*. (2002b) in dressed chicken, Sallam *et al.* (2004) in raw chicken sausages. Similarly Ali Aydin *et al*. (2007) observed a decrease in total plate count of ground beef and raw meat balls after treatment with chopped garlic.

Chicken meat patties treated with essential oil of clove at 1:250, 1:500 and 1:1000 concentrations were resulted in 3 log reduction in total plate count compared to the control on day 12 of refrigerated storage. The decreased total plate count of chicken meat patties might be due to eugenol, the antimicrobial compound of clove. Chicken meat patties treated with essential oil of clove at 1:250 and 1:1000 concentrations met the standards for fresh chilled meat of 106 cfu /gm (Agarwal and Bhilegaonkar, 2003) up to day 12 of storage, whereas the counts in samples treated with 1:500 concentration were within the standards limit even on day 14 of storage. Irrespective of concentrations used, the total plate count was increased gradually with an increase in storage period. These results were in accordance with Naveena *et al*. (2006) in buffalo meat steaks., Yadav *et al*. (2005) in minced chicken meat, Angella Melissa A Carlos and Mark A Harrison (1999) in chicken meat stored at 4^0C.

Chicken meat patties treated with essential oil of cinnamon at 1:250 and 1:500 concentrations were resulted in 3 log reduction in total plate count compared to the control on day 14 and day 12 of storage, whereas samples treated at 1:1000 concentration resulted in 2 log reduction on day 8 of storage. The decreased total plate count of chicken meat patties might be due to cinnamaldehyde, the antimicrobial compound of cinnamon. Meat treated with essential oil of cinnamon at 1:250 and 1:500 concentrations met the standards for fresh chilled meat of 106 cfu /gm (Agarwal and Bhilegaonkar, 2003) up to day 14 and day 12 of storage, respectively, whereas the counts in samples treated with 1:1000 concentration were within the standards limit up to day 8 of storage. Irrespective of concentrations used, the total plate count was increased gradually with an increase in storage period.

The results were in agreement with Quattara *et al*. (2001) in pre cooked shrimp had synergistic effect in reducing total counts with at least a 12 day extension of shelf life. Yadav *et al*. (2002a) had treated minced chicken meat with cinnamon powder and reported about 1 log less aerobic bacterial counts after 24 hours of storage.

Chicken meat patties with essential oil of garlic at 1:250 concentration resulted in 2 log reduction in yeast and mold count compared to the control on day 14 of storage, at 1:500 concentration a 2 log reduction and at 1:1000 concentration less than 1 log reduction was observed on day 12 and day 8 of storage, respectively. The decreased yeast and mold

count of chicken meat patties might be due to the allicin, the antimicrobial compound of garlic. These results were in agreement with Conner and Beuchat (2006) who revealed that garlic at 25 ppm acts as a potent inhibitor of yeast, Ali Aydin *et al*. (2007), who observed complete inhibition of yeast and mold in raw meat balls with 10 percent garlic.

Essential oil of clove at 1:250 concentration resulted in 2 log reduction in yeast and mold count compared to the control on day 14 of storage, however at 1:500 and 1:1000 concentrations there was 1 log reduction compared to the control on day 14 and day 12 of storage, respectively. The decreased yeast and mold count of chicken meat patties might be due to the eugenol, the antimicrobial compound of clove.

These results were in agreement with Bullerman *et al*. (1977) who indicated that eugenol at 200 ppm completely inhibited the mold growth, Angella Melissa A Carlos and Mark A Harrison (1999) who observed that 0.5 percent clove oleoresin significantly inhibited the growth of yeast and molds at 4^0C, Juglar *et al*. (2002) who found that commonly occurring mycotoxigenic fungi can be controlled with clove oil, Hamly and Mahmoud (2005) who revealed that clove at 20 percent concentration exerted a potential anti yeast effect.

Cinnamon at 1:250 concentration resulted in 2 log reduction in yeast and mold count compared to the control on day 14 of storage, however at 1:500 and 1:1000 concentrations there was 1 log reduction and less than 1 log reduction compared to the control on day 14 and day 8 of storage, respectively. The decreased yeast and mold count of chicken meat patties might be due to the cinnamaldehyde, the antimicrobial compound of cinnamon.

The results of this experiment were in accordance with Bullerman *et al*. (1977) who indicated that cinnamon at 200 ppm completely inhibited the mold growth, Mawson *et al*. (2006) who observed inhibition of mold with cinnamon oil at 1000 µl, Hamly and Mahmoud (2005), who revealed that cinnamon at 20 percent concentration exerted a moderate inhibitory activity on yeast, Jonathan Mosqueda-Melga *et al*. (2008) who observed inactivation of yeast and mold with cinnamon bark oil at 0.05 to 0.30 percent concentration. Irrespective of the concentration of essential oil of garlic, clove and cinnamon, the yeast and mold count was significantly ($P<0.05$) increased as the storage period increased, this might be due to the relative availability of conducive temperature and moisture for the growth of yeast and molds.

Chicken meat patties treated with mixture of essential oils of garlic, clove and cinnamon at 1:250, 1:500 and 1:1000 concentrations were found to have higher mean colour scores compared to control throughout the storage period and there was no significant ($P>0.05$) difference between the treatments. The increased mean colour scores of chicken meat patties with the mixture of essential oils of spices may be due to the fact that garlic and clove could effectively reduce metmyoglobin content of meat, which is an undesirable meat pigment (Reema and Sahoo, 2004). Further Souza *et al*. (2005) had also reported a stabilizing effect of spice mixture including clove on colour of fresh pork. Irrespective of the concentration of the mixture of essential oils of garlic, clove and cinnamon in chicken meat patties, the mean colour scores were decreasing with increasing refrigerated storage period. This might be due to oxidative fading of pigment and lipid oxidation resulting in non enzymatic browning of product. The results of this study were in association with Al-Delaimy and Barakat (1970) who found that treatment with 15 and 25 percent garlic resulted in improved colour of camel meat, Mei-Chin Yin and Wen-shen Cheng (2002) who revealed that exogenous addition of garlic derived organosulfur compounds significantly delayed the oxidation of metmyoglobin of ground beef and Yadav *et al*. (2005) who revealed that there was an improvement in colour scores of chicken meat which has been treated with 0.15 percent clove with 0.08 percent lactic acid.

A significant ($P<0.05$) difference was noticed in overall mean flavour scores between the treatments and also between the storage periods. In all the treatments no detectable off flavour could be noticed through out the storage period. Chicken meat patties with mixture of essential oils of garlic, clove and cinnamon at 1:500 concentration had higher flavour scores through out the storage period compared to the rest of the treatments. The acceptable flavour scores through out the storage period were may be due to volatile compounds present in essential oils of garlic, clove and cinnamon. Even though there was a decrease in flavour scores as the storage period increased, they were well within the acceptable range. The decreased flavour scores may be due to fat oxidation during storage.The re-

sults of this study were in agreement with Yaseman Yanar and Hasan Fenercioglu (1999) who revealed that meat balls with garlic were found to be preferable in flavour, Yadav *et al*. (2005) who reported that aqueous extract of garlic at 4 percent in minced chicken exerted a potential increase in flavour scores and Saumya Dwivedi *et al*. (2006) who reported that clove at 1 percent and cinnamon at 0.5 percent level had improved the flavour in cooked ground beef.

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