

Full Length Research Paper

Gum acacia coating with garlic and cinnamon as an alternate, natural preservative for meat and fish

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Meat and fish, the most perishable foods need to be preserved preferably with natural preservatives. In our study, we have proven that spices like garlic and cinnamon that are used as regular ingredients in cooking can act as preservative due to their rich antibacterial and antioxidant profile. The minimum inhibitory concentration (MIC) determined by macrobroth dilution method against five spoilage and disease causing bacteria (*Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus cereus*) was found to be between 0.03 to 0.2884 mg/ml and 0.061 to 0.24 mg/ml for garlic and cinnamon, respectively. Activity index (0.90) was maximum for garlic extract (GE) against *E. coli*. The antibacterial activity of both extract was maximum at 60°C for all bacteria. In the case of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, the IC₅₀ value for cinnamon extract (CE) (0.527 µg/mL) was significantly ($p \leq 0.05$) lower than GE (2.60 µg/mL). The antibacterial activity of gum arabic coating with garlic and cinnamon (GAGC) was dose dependent. At 0.125 g/g of meat, there was three fold decrease in log colony forming units (cfu) of viable cells/ml than the control (without coating), whereas for turmeric coating, there was exponential increase of viable cells. The shelf life of coated meat and fish was increased by three weeks at 5°C.

Key words: Gum acacia coating, garlic, cinnamon, antioxidant, antimicrobial, meat, fish.

INTRODUCTION

Meat and fish are most perishable food due to their ideal nutrient composition. Spoilage is caused due to infection and subsequent decomposition of meat mostly by microorganisms (Gram, 2002) which are a part of the animal or added during handling or processing. Fish (Almudena et al., 2007) and meat (Givens, 2005) contain significant amount of polyunsaturated fatty acids and lipids, for which they readily go rancid on storage (Harris and Tall, 1994). Numerous studies have reported that lipid oxidation in meat products is controlled or reduced by the addition of antioxidants (Gray et al., 1996; Nissen et al., 2004). With the increased demand of high quality, safety, extended shelf life of meat and fish, numerous preservation technologies such as super chilling, high hydrostatic

pressure, radiation, chemical active and modified atmosphere packaging are done or proposed (Panagiotis et al., 2002; Hugas et al., 2002; Zhou, 2010).

Nowadays, extensive research is being done in bio edible coating or film to delay or prevent the spoilage of most perishable food. These coatings acts as an envelope, prevents the exchange of transfer of gasses (O₂ and CO₂) (Bourtoom, 2008) and acts like a barrier for aromatic compounds, thus preventing quality changes in food (Miller and Krochta, 1997). Gum arabic (GA, E-Number 414) also known as acacia gum, is a mixture of polysaccharides and glycoprotein obtained from the stems and branches of *Acacia Senegal* and *A.seyal*. It is widely used in food industry as a stabilizer, thickening agent and as an emulsifier in soft drinks, syrup, gummy candies, textile, pottery, cosmetics and pharmaceutical industries. In folk medicine, it has been used to treat inflammation of the intestinal mucosa and externally to cover inflamed sur-

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faces. Aqueous solution of GA inhibited growth of *Porphyromonas gingivalis*, and *Prevotella intermedia* (Clark et al., 1993). Polysaccharide matrices are able to encapsulate aroma compounds and entrap active ingredients, thereby enhancing safety and nutritional and sensory attributes (Falguera et al., 2011).

Indian food comprises of addition of lots of spices in order to enhance taste. Spices are often added while cooking meat and fish in order to add taste, aroma, color and flavour. Many reports are there on the antioxidant and antimicrobial potential of herbs and spices like basil, rosemary, clove, pepper, mustard (Sebranek et al., 2005; Tipsrisukond et al., 1998). Garlic (*Alium sativum* L.) holds an important value due to its prophylactic and therapeutic actions. Sulphur and polyphenols present in garlic respond to the antibacterial, antifungal and antioxidant activity (Yara Queiroz et al., 2009). Garlic has been described to exhibit antimicrobial activity (Fleischauer et al., 2000), antitumor activity (Karasaki et al., 2001; Sundaram et al., 1996), as well as antithrombotic, antiarthritic, hypo-lipidemic, and hypoglycaemic activities (Duraka et al., 2002). *Cinnamomum zeylanicum* L., commonly known as cinnamon, is rich in cinnamaldehyde as well as b-caryophyllene, linalool and other terpenes. Cinnamaldehyde is the major constituent of cinnamon leaf oil and provides the distinctive odour and flavour associated with cinnamon. It is used worldwide as a food additive and flavouring agent (Nikos, 2009). It has been found that cinnamaldehyde and eugenol inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall of bacteria (Helander et al., 1995). Turmeric (*Curcuma longa*) has long been used as a colouring and flavouring agent for foods. Curcuminoids are the main component of turmeric and have a range of pharmacological activities (Santosh et al., 2011).

Due to the consumer awareness of chemical preservatives, extensive studies are been made on natural preservatives for preservation of meat and fish products. Sung-Jin et al. (2013) used leafy vegetables for preservation of beef patties. Thus, our objectives for this study was to extend the shelf life of meat and fish coated with spice extracts incorporated coating which would not alter the taste and flavour of the food product or add a new undesirable taste, and a comparative study of antibacterial activity between turmeric and garlic-cinnamon mixture to prove that age old usage of turmeric as antimicrobial agent in meat and fish is not ideal. Turmeric, cinnamon and garlic were selected because they are often used while cooking meat and fish. A coating was made with the spice mixtures-garlic and cinnamon (GAGC) that had both antibacterial and antioxidant activity because these two properties are important to increase the shelf life of food.

MATERIALS AND METHODS

Chemicals

Gum Arabic in powder form, was obtained from Rankem, RFCL

limited, (New Delhi). 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, ferrozine, and ferrous chloride were purchased from Sigma Chemical Company, nutrient agar, Macconkey agar, peptone, disodium ethylene diamine tetra acetic acid (Na₂EDTA) was purchased from Himedia (India). All other chemicals were obtained from Sisco Research Lab (Mumbai, India).

Bacteria cultures

Escherichia coli, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis* and *Proteus mirabilis* were stored at 2°C. Bacteria were grown on nutrient agar, and subcultured in peptone saline water, respectively before the start of the experiment.

Preparation of aqueous extract and coating

Garlic, turmeric and cinnamon aqueous extract was made as reported by Madhumita and Ramalingam (2010). Garlic (GS) and cinnamon (CS) solution was added at a range of 6.25 to 12.5% of gum acacia; same way coating was prepared with turmeric aqueous extract. Both aqueous extract and coatings were used for antibacterial activity whereas the extracts alone were used for antioxidant assays.

DPPH radical scavenging activity

The DPPH radical scavenging was performed based on the method of Yen and Chen (1995). Various concentrations of GS and CS (1 ml) were used for the assay. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration as:

$$\text{RSA (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of extract}) / \text{Absorbance of control}] \times 100}{1}$$

The standard used was ascorbic acid (Nooman et al., 2008).

Reducing power activity

The reducing power of the extracts was evaluated by the method of Oyaizu (1986). Various concentrations of the extracts (250 to 1000 µg/mL) were used for the assay. The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (250 to 1000 µg/mL) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Metal chelating assay

Ferrous ions chelating activity was determined based on modified method of Dinis et al. (1994). Into tubes containing 60 and 40 µL of cinnamon extract, garlic extract and disodium ethylene diamine tetra acetic acid (disodium EDTA) was added, respectively. The chelating efficiency was compared with the chelating activity of disodium EDTA. The inhibition percentage of ferrozine -Fe²⁺ complex was calculated by the formula:

$$\text{Inhibition of ferrozine (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of Extract}) / \text{Absorbance of control}] \times 100}{1}$$

Antibacterial activity of garlic and cinnamon at different temperatures

The antibacterial activity of garlic aqueous extract and cinnamon

aqueous extract was tested against all five bacteria. The spices were surface sterilised and then made an aqueous solution with distilled water with concentration of 0.2145 g soluble solids per g of garlic and 0.04125 g total soluble solids/g of cinnamon. The *in vitro* antibacterial activity of the aqueous extracts was carried out by well diffusion method. Sterile nutrient agar plates were swabbed with actively growing log phase culture of respective organism. Wells around 6 mm were bored on the agar and 100 μ L of extract were added, respectively. The plates were incubated upright for 24 h at 37°C and the zones of inhibition were measured. Gentamicin was used as positive control. Similarly, the same was done with spices heated at temperature at, 60, 80, 100 and 120°C. The activity index (AI) was calculated as compared with those of their respective standard reference drugs (AI = Inhibition Zone of test sample/Inhibition zone of standard). Gentamicin (10 mcg) was used as standard drug against five bacterial cultures.

Minimum inhibitory concentration (MIC)

MIC of the extract against each of the tested organism was determined by macro both dilution method. Extract solutions at concentrations of 0 to 0.4884 mg/mL by serial two fold dilution was prepared in peptone saline water. The tubes were inoculated with five test organism and incubated for 24 h. The O.D value was observed at 600 nm. The concentration at which there is no visible growth of bacteria was the MIC.

Study on the growth pattern of bacteria with garlic and cinnamon extract

Garlic and cinnamon solution (each 2.5 mL) was added to 100 ml of sterile nutrient broth to which 1 ml of log phase *S. aureus* and *E. coli* was separately added. The control was free from extract. A 10 h study was done in which 100 μ L of inoculums was used for lawn culture over nutrient agar plates. The total viable counts of the control and treated sample were noted down.

Effect of GAGC on the bacterial contaminants over packaged raw fish and meat

The fresh raw fish, sardine and chicken pieces were coated with GAGC (with extract of 0.0625 and 0.125 g/g of fish and meat, respectively) and GA as control. Then it was kept for drying for 20 min at 50°C. The pieces were then stored at room temperature in sealed packets which had no vapour permeability and the gases inside it was not modified or made vacuum. The total viable count of the control and treated samples was determined immediately and up to 18th hour for fish and 9th hour for meat.

Effect of GAGC on raw meat and fish at 5°C

The above coated meat and fish and control (without coating) were kept at 5°C for three weeks. Each week, the sample was serially diluted in sterile distilled water and 100 μ L of the final diluted sample was seeded on the sterile nutrient plates (for aerobic total count) and Mac conkey plates (for coliform bacteria and non lactose fermenting enteric bacteria). The plates were kept at 37°C for incubation for 24 h and after that, the cfu/mL were noted down.

Statistical analysis

All experiments were carried in triplicates. The experimental results are expressed as means \pm SD of three parallel measurements. The results were processed using Microsoft Excel 2007 and the data

were subjected to one way analysis of variance (ANOVA). Significant differences between groups were determined at $P < 0.05$.

RESULTS

DPPH radical scavenging activity

The ability of the various extracts to scavenge the DPPH free radical (by donation of hydrogen atom) is shown in Figure 1. Cinnamon showed good antioxidant activity when compared to garlic. Antioxidant activity was dose dependent. Cinnamon showed more antioxidant activity than garlic. The IC₅₀ value for cinnamon was 0.527 μ g/mL which was significantly lower than 2.60 μ g/mL of garlic extract.

Reducing power activity

Extracts having antioxidant potential is said to have reducing power capacity. In this study, it was seen that over the various concentration, cinnamon and ascorbic acid had almost similar reducing powers (Figure 2).

Metal chelation activity

The dark purple colour formed due to the ferrozine - Fe²⁺ complex is decreased by the metal chelator compounds that exist in the reaction mixture. The absorbance at 562 nm is indirectly proportional to the concentration of the extract. As in Figure 2, aqueous extract of cinnamon had 35% less metal chelation activity compared to Na₂EDTA; garlic extract did not have a dose dependent metal chelation activity. It had only 32% metal chelation activity at both concentration of 0.1 and 0.6 mg/mL.

Antibacterial activity

The survival of common spoilage and pathogenic bacteria encountered in meat and fish product was studied in the presence of GAGC, garlic and cinnamon extract in nutrient agar plates. The counts of all the test organism were significantly ($p \leq 0.05$) affected by the coating and extracts and it was dose dependent (Table 1). The MIC for garlic and cinnamon by macro broth dilution was found to be between 0.03 to 0.2884 mg/ml and 0.061 to 0.24 mg/ml, respectively. Garlic extract had more antibacterial activity than cinnamon. The antibacterial activity was highest at 60°C (Table 2).

Study on the growth pattern of bacteria with garlic and cinnamon paste

The two extracts had good antibacterial activity in combination. It was tested over Gram positive *S. aureus*

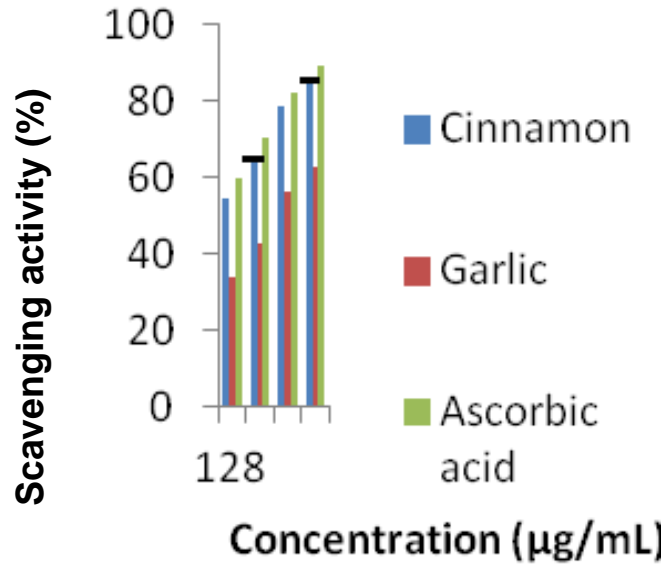


Figure 1. Antioxidant activity of cinnamon, garlic, measured as percent scavenging of DPPH radical. Each value represents mean \pm standard deviation of three experiments.

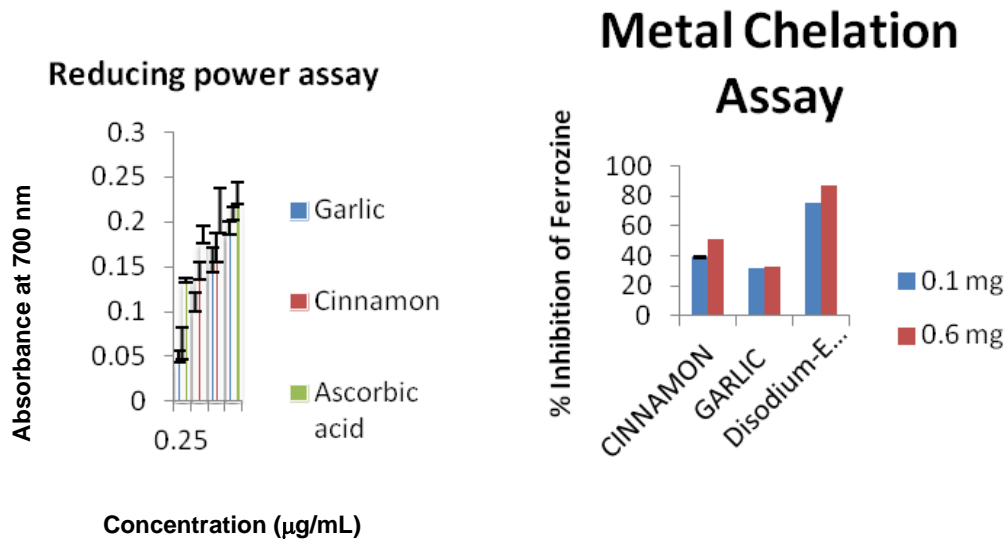


Figure 2. Reducing power and metal chelation activity of extracts. Values are the mean \pm standard deviation of three replicate experiments.

and gram negative *E. coli*. In presence of the extract from the 3rd hour, *S. aureus* started to enter in the death phase and there was a very short log phase of 1 h from 2nd to 3rd hour and for *E. coli*, the growth of bacterium started to reduce hour wise. From the 5th to 8th hour, it was in stationary phase with size of 5.06 ± 0.032 cfu/mL after which it further declined. But in the case of control, it had undergone all the four phases of growth curve as shown in Figure 3.

Effect of GAGC on the bacterial contaminants over packaged raw fish and meat

The coating along with extract less than 0.015 g/g of meat or fish was not able to control the growth of spoilage bacteria. At higher concentrations (0.0625 and 0.125 g/g), the antibacterial activity was enhanced. At 0.0625 g/g of fish, total log cfu of viable cells were 8.66/ml at the 18th hour whereas it was 10.026/ml for

Table 1. The MIC values and activity index of cinnamon and garlic extract against 5 different test cultures which are found to grow on meat and fish.

| Test organism | Concentration of cinnamon extract (mg/mL) | Concentration of garlic extract (mg/mL) | Activity index with cinnamon extract | Activity index with garlic extract |
|---------------------|---|---|--------------------------------------|------------------------------------|
| <i>S. aureus</i> | 0.2466 | 0.0902 | 0.38 | 0.83 |
| <i>E. coli</i> | 0.06165 | 0.036 | 0.80 | 0.90 |
| <i>B. cereus</i> | 0.06165 | 0.0721 | 0.71 | 0.89 |
| <i>P. mirabilis</i> | 0.1233 | 0.2884 | 0.80 | 0.76 |
| <i>E. faecalis</i> | 0.1233 | 0.1442 | 0.70 | 0.79 |

Table 2. The mean values \pm standard error of zone of inhibition at different temperature.

| Name of organism | Extract | 40°C | 60°C | 80°C | 100°C | 120°C |
|------------------------------|----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | | Zone of inhibition (cm) | Zone of inhibition (cm) | Zone of inhibition (cm) | Zone of inhibition (cm) | Zone of inhibition (cm) |
| <i>Proteus mirabilis</i> | Garlic | 1.2 \pm 0.012 | 1.5 \pm 0.023 | 1.3 \pm 0.04 | 1.32 \pm 0.09 | 1.1 \pm 0.03 |
| | Cinnamon | 1.5 \pm 0.02 | 1.6 \pm 0.091 | 1.2 \pm 0.14 | 1.1 \pm 0.041 | 1.1 \pm 0.064 |
| <i>Enterococcus faecalis</i> | Garlic | 2.0 \pm 0.04 | 2.5 \pm 0.052 | 1.8 \pm 0.089 | 1.8 \pm 0.14 | 1.7 \pm 0.034 |
| | Cinnamon | 1.9 \pm 0.09 | 2.1 \pm 0.041 | 1.4 \pm 0.012 | 1.2 \pm 0.098 | 1.2 \pm 0.091 |
| <i>Bacillus cereus</i> | Garlic | 1.2 \pm 0.03 | 2.0 \pm 0.067 | 1.5 \pm 0.041 | 1.4 \pm 0.034 | 1.4 \pm 0.041 |
| | Cinnamon | 1.2 \pm 0.041 | 1.2 \pm 0.091 | 1.6 \pm 0.057 | 1.6 \pm 0.087 | 1.6 \pm 0.012 |
| <i>E. Coli</i> | Garlic | 1.2 \pm 0.05 | 2.2 \pm 0.012 | 1.4 \pm 0.094 | 1.4 \pm 0.015 | 1.3 \pm 0.089 |
| | Cinnamon | 1.1 \pm 0.09 | 1.5 \pm 0.041 | 1.4 \pm 0.045 | 1.3 \pm 0.056 | 1.2 \pm 0.053 |
| <i>Staphylococcus aureus</i> | Garlic | 1.5 \pm 0.04 | 2.2 \pm 0.089 | 1.5 \pm 0.056 | 1.4 \pm 0.030 | 1.2 \pm 0.067 |
| | Cinnamon | 1.1 \pm 0.06 | 1.8 \pm 0.053 | 1.1 \pm 0.019 | 1.3 \pm 0.068 | 1.2 \pm 0.052 |

fish without coating. Interestingly, the coating with turmeric was less efficient than GEAC in controlling the bacterial growth. With increased concentration of garlic and cinnamon extract in coating (0.125 g/g of meat), there was three fold decrease in log cfu of viable cells/ml. There was exponential growth of bacteria in the case of uncoated and coated meat with turmeric (Figure 4).

Shelf life study of meat and fish (at 5°C) with garlic cinnamon coating (GAGC)

The shelf life of fish and meat (at 5°C) with GAGC was extended to three weeks while the microbial load decreased week wise. Coliform bacterial load of coated meat was eradicated at 2nd week whereas non lactose fermenting bacterial load was eliminated at 1st week only. For fish with coating and extract, the coliform and non lactose fermenting colonies were absent at first week only. The aerobic total bacterial count for fish (Table 3) came down to 10000 times at the third week.

DISCUSSION

There are extensive reports on antibacterial activity of garlic juice, lyophilised powder, aqueous and alcoholic extract against bacteria, and multiple drug resistant bacteria (Srinivasan, 2009; Harris et al., 2001). The antibacterial activity of cinnamon oil has been mostly studied (Charu et al., 2008; El Baroty et al., 2010) against fungi, food borne pathogens and spoilage bacteria. Cinnamon bark is rich in cinnamaldehyde which is highly electronegative and inhibits amino acid decarboxylase activity (Sakaguchi, 1995), interferes with the electron transfer and reacts with nucleic acid and proteins, therefore inhibiting the growth of microorganisms. The methanol, ethanol extract of turmeric and turmeric oil had antibacterial activity against *Bacillus subtilis*, *B. macerans*, *B. lichineformis* and *Azotobacter* (Shagufta, 2010) which was not in accordance with our study.

There are extensive reports on *in vitro* antimicrobial activity of these spices but no report on its application.

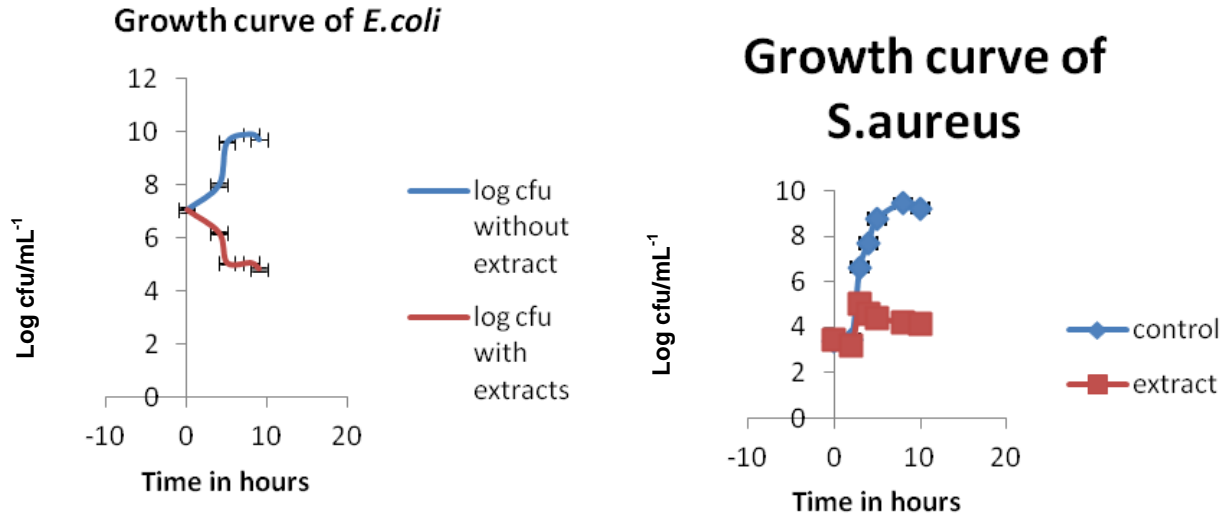


Figure 3. Growth pattern of *S. aureus* and *E. coli* in the presence of both extract and control without extract. All experiments were done in triplicates.

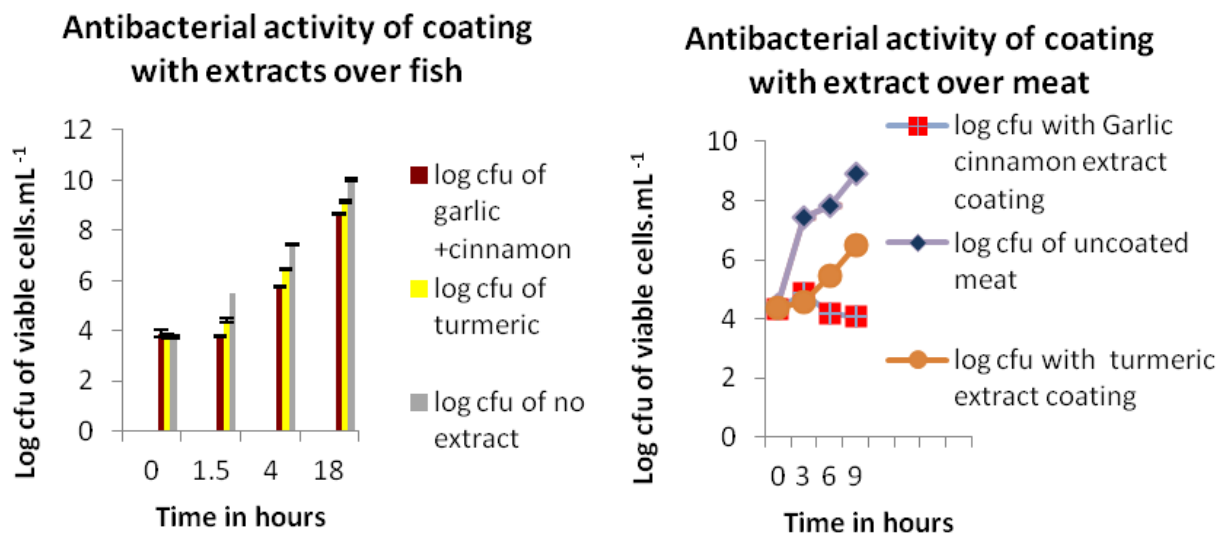


Figure 4. Total bacterial count of meat and fish coated with GAGC during closed packaged storage. Values are the mean \pm standard deviation of three replicate experiments.

During cooking meat and fish, raw garlic or/and cinnamon is often used. Thus, to make use of the same condition, aqueous extract of spices were only used for antibacterial study and was added in the bio edible coating to preserve meat and fish. From ancient time in India, the common practise is to marinate raw meat and fish with turmeric and salt prior to cooking but in our study, we found that turmeric itself is not much effective in inhibiting the growth of food borne bacteria but garlic and cinnamon extract was more potent in controlling the bacterial growth. Both the extract retained their antibacterial activity even at 120°C, but antibacterial activity was maximum at 60°C. Thus, the same coating can be used for a wide range of temperature treated food products.

The viscosity of gum Arabic makes coating and binding of the extract easier. The common usage of turmeric for preservation of fish and meat was not found much efficient. Meat and fish was able to be preserved for three weeks with GAGC.

Free radicals are involved for lipid peroxidation (Halliwell and Gutteridge, 2000), thus, these results in rancidity of foods that are rich in fat content. Cinnamon had good dose dependent antioxidant property and it was almost the same as standard ascorbic acid. The potency of antioxidant activity of cinnamon essential oil was same as synthetic antioxidants butylated hydroxy toluene (BHT) and greater than butylated hydroxy anisole (BHA) (El-Baroty et al., 2010). Cinnamedehyde and eugenol were

Table 3. The mean values \pm standard deviation value of total microbes load on 1 g of fish and meat with GAGC and without coating at 5°C. All experiments were done in triplicates.

| Period | Without coating | Aerobic plate count | Coliform | Non lactose fermenting bacteria | With coating and extract | Aerobic plate count | Coliform | Non lactose fermenting bacteria |
|----------|-----------------|-----------------------|--------------------|---------------------------------|--------------------------|---------------------|-----------------|---------------------------------|
| 0th week | Meat | 57400 \pm 0.094 | 200 \pm 0.019 | 100 \pm 0.023 | Meat | 57321 \pm 0.067 | 200 \pm 0.01 | 100 \pm 0.0209 |
| | Fish | 3400 \pm 0.023 | 200 \pm 0.02 | 600 \pm 0.078 | Fish | 3300 \pm 0.0598 | 70 \pm 0.094 | 14 \pm 0.030 |
| 1st week | Meat | 589000 \pm 0.055 | 780 \pm 0.019 | 560 \pm 0.039 | Meat | 34800 \pm 0.058 | 120 \pm 0.076 | 0 |
| | Fish | 56900 \pm 0.029 | 430 \pm 0.011 | 780 \pm 0.081 | Fish | 1450 \pm 0.089 | 0 | 0 |
| 2nd week | Meat | 45200000 \pm 0.045 | 5700 \pm 0.052 | 4500 \pm 0.083 | Meat | 2300 \pm 0.024 | 0 | 0 |
| | Fish | 358000 \pm 0.084 | 7600 \pm 0.040 | 8790 \pm 0.019 | Fish | 560 \pm 0.019 | 0 | 0 |
| 3rd week | Meat | 680000 \pm 0.094 | 656800 \pm 0.049 | 62500 \pm 0.030 | Meat | 190 \pm 0.056 | 0 | 0 |
| | Fish | 249000000 \pm 0.084 | 798000 \pm 0.076 | 970000 \pm 0.0498 | Fish | 10 \pm 0.057 | 0 | 0 |

the most active components in cinnamon oil for antioxidant activity. The presence of phenolic compounds in the cinnamon extract proved to be the active component responsible for antioxidant activity in ethanol and water extracts (Manchini et al., 1998).

The antioxidant activity of garlic was comparable to the reports of Rasul et al. (2012) where it exhibited more free radical scavenging activity than ferric reducing power. With the concern of food safety with the synthetic preservatives, fish had been coated with gelatine chitosan film incorporated with clove oil, which reduced the growth of gram negative bacteria but could not control the growth of *Lactobacillus* (Gomez et al., 2010). But in our study, GAGC reduced the growth of both gram positive bacteria (aerobic plate count) and gram negative coliforms

and non lactose fermenting bacteria in both food samples meat and fish.

Conclusion

The problem of natural means of preserving the most perishable group of products, meat and fish, can be addressed by the development of antimicrobial and antioxidant blend preservative. The present study demonstrates the efficacy of gum Arabic along with garlic, cinnamon extract as a potent antibacterial and antioxidant agent that can be used for the preservation and shelf life extension of meat and fish products. While cooking meat and fish, the protein undergoes denaturation and the membrane undergoes structural damage which makes it sensitive to lipid

oxidation (Gray et al., 1996). Thus, it is always better to add antioxidants before the cooking process to minimise oxidation rancidity in meat and flesh products. Further in this study, it is demonstrated that instead of age old use of turmeric as preservative, garlic and cinnamon can be the best alternate for preserving perishable food.

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