

Case Report

Application of Bioactive Cotton Packages for Packaging and Storage of Grains Using Aromatic Components

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Abstract

In recent years, studies on the utility of essential oils and their components in the preservation of food commodities in order to enhance shelf-life has been successfully carried out. These essential oils and their components (Carvacrol, Eugenol and Cinnamaldehyde) can be used as antimicrobials and food preservative agents; however, their use raises concerns because of several reported side effects of synthetic oils. Due to their antimicrobial potential, essential oil constituents could be used as food preservatives for grains, cereals, vegetables and fruits. The aim of the treatments was carried out on the treated cotton packaging to maximize the benefit from its antimicrobial activity to extend grain Storage periods. The cotton fabrics treated in three steps: Carboxymethylation of cotton fabric (CMC) Cationization of cotton fabric 3-Treatment of the fabrics by reactive-cyclodextrine (RCD). Modified and unmodified cotton fabrics were treated with antibacterial agent by dipping them at room temperature for 2 hours under stirring in dimethyl sulfoxide (DMSO) solution containing 200, 250, 250 ppm of Eugenol, carvacrol and cinnamaldehyde respectively the samples were then roll-squeezed at pick up 100% and dried at room temperature. All treated and untreated fabrics were subjected to antimicrobial tests. Cotton fabric composites treated with cinnamaldehyde had the highest impact on reducing microbial preparation during storage period followed by cotton fabric composite treated by Carvacrol followed by cotton fabric composite treated by Eugenol which is less impactful to reduce microbial count during the storage period.

Introduction

Antimicrobial packaging is considered a tool of active packaging. Antimicrobial packaging is defined as a system that can kill or inhibit pathogenic and spoilage microorganisms responsible for food contamination. The new trend in antimicrobial packaging is by adding an antimicrobial agent or by using polymers that have antimicrobial properties that satisfy traditional packaging requirements. Antimicrobial packaging substances must increase the lag phase and reduce the rate of microbial growth of microorganisms to develop the shelf life and maintain the quality and safety of foods [1].

Natural antimicrobials have shown sufficient capacity to reduce microbial contamination inside food packages, as reported in several studies [2,3]. Therefore, the adding of efficient antimicrobials to packaging materials food

will improve foodstuff shelf life by removing undesirable pathogens and/or delaying microbial spoilage [4,5]. Different antimicrobials have been added to different packaging materials.

In particular, the bioactive molecules of essential oils, herbs, and spices have been tested due to their antiviral, antifungal, and insecticidal properties [4,6]. Carvacrol is one of the major components of the Labiatae family, including, Satureja, Origanum, Thymus, Thymbra and Coridothymus [7,8], and has been shown to have broad-spectrum antimicrobial activity, and it has been scanned by a large number of researchers worldwide. Carvacrol is consider a monoterpenic phenol.

Carvacrol is mentioned to have a big set of biological properties, including antifungal, phytotoxic, insecticidal, antioxidant, antitumor, antimutagenic, antiparasitic, and

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antimicrobial activities [7,9,10]. Furthermore, carvacrol in the United States and Europe has been confirmed as a safe food additive due to the “generally recognized as safe” status, and it is also used as a flavoring agent in sweets, chewing gum and beverages [2,11].

Based on the presiding reasons, the use of carvacrol in active packaging systems is an emerging area with potential to be applied in the food industry [12,13]. Cinnamaldehyde is an effective inhibitor of the development of yeasts, bacteria, and molds as well as toxin production by microorganisms. The inhabitation of the accession of a number of bacteria such as *Bacillus* spp., *Enterobacter sakazakii*, *Enterobacter* spp., *E. coli* O1587:H7, *Escherichia coli*, *Micrococcus* spp., *Listeria innocua*, and *Staphylococcus* spp. [14-16] and postharvest pathogenic molds including *Penicillium digitatum*. As one of the phenols, eugenol (2-methoxy-4-allylphenol), $C_{10}H_{12}O_2$, is a principal component of clove bud oil (ca. 72% - 80%). The physical properties of eugenol include a clove-like odor; it is a colorless to slightly yellow liquid with a spicy aroma [17]. It is also classified as safe material by the FDA.

Eugenol exhibits an inhibitory effect on the growth of *B. cereus*, *Campylobacter jejuni*, *E. coli*, *E. coli* O1587:H7, *P. digitatum*, *L. monocytogenes*, *Pseudomonas fluorescens*, *Salmonella enterica*, *S. enteritidis*, and *S. aureus* [4,18]. Dorman and Deans [19] reported that eugenol shows the widest spectrum of activity against 24 out of 25 bacteria, except for *Leuconostoc cremoris*.

The aim of the present study was to use types of cotton fabric packaging composite treated by Eugenol, carvacrol and cinnamaldehyde respectively to maximize the benefit from its antimicrobial activity to extend grain storage periods.

Materials and methods

Materials

Chemicals: Essential oils (Eo) main compounds (Carvacrol, Eugenol and Cinnamaldehyde) used in this study were obtained from Sigma-Aldrich and their purity was greater than 98%.

Monochlorotrizinyl- β -cyclodextrin, referred here as reactive R-cyclodextrin (RCD), was provided by Wacker Chemie GmbH, Germany. Sodium carbonate, sodium hydroxide, acetic acid, monochloroacetic acid were of laboratory grade chemicals. 3-chloro-2-hydroxypropyl trimethyl ammonium chloride (69%) of technical grade chemical was kindly supplied under the commercial name [Quat188] by Aldrich and Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich, with a purity greater than 99%.

Fabrics: Cotton fabric mill de-sized, scoured or bleached, plain weave was supplied by Misr Company for spinning and weaving Mehalla El-Kobra, Egypt. The fabric was further purified in the laboratory by washing at 100 °C for 60 minutes

using a solution containing 2g/L sodium carbonate and nonionic wetting agent (Egypsol). The fabric was washed several times with boiling water, followed by running water, and finally dried under ambient conditions.

Methods

In the presented study the cotton fabrics treated in three steps: Carboxymethylation of cotton fabric (CMC) Cationization of cotton fabric 3-Treatment of the fabrics by reactive-cyclodextrine (RCD), Modified and unmodified cotton fabrics were treated with antibacterial agent by dipping them at room temperature for 2 hours under stirring in dimethyl sulfoxide (DMSO) solution containing 200, 250, 250 ppm of Eugenol, carvacrol and cinnamaldehyde respectively the samples were then roll-squeezed at pick up 100% and dried at room temperature. All treated and untreated fabrics were treated against antimicrobial tests.

Cat-ionization of cotton fabric

Chemical modification of the cotton fabric through cationization was carried out as per the pad-dry-cure method [20]. The experimental procedures adopted were as follows: 3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (Quat-188) was mixed with sodium hydroxide solution at a NaOH/Quat-188 molar ratio of 2:1. The cotton fabric was padded in this mixture in two dips and two nips, and then squeezed to a wet pick-up of about 100%. The cotton fabric was dried at 40 °C for 10 min and cured at 120 °C for 3min. Thus, treated cotton gauze was washed with cold water and 1% acetic acid, followed by several washing cycles and finally dried under the normal laboratory conditions [21] (Figure 1).

Partial Carboxymethylation of cotton gauze (PCMC)

Cotton fabric was partially carboxymethylated to yield (PCMC) by a method similar to those previously reported [22]. PCMC was produced in a two-stage process. The first stage refers to a mercerization process in which, cotton fabric was impregnated with 15 wt. % aqueous NaOH for 5min at room temperature, squeezing to a wet pick up of 100% then dried at 60 °C for 5min. Etherification is the second stage in which the alkali treated samples were steeped in aqueous solution of sodium salt of monochloroacetic acid (3mol) for 5min at room temperature. These samples were then squeezed to 100

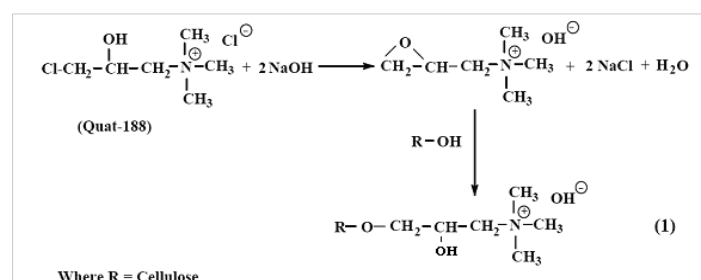


Figure 1: Chemical modification of the cotton fabric through cationization.



% wet pick up, sealed in plastic bags and heated at 80 °C for 1h then washed and dried at room temperature.

Treatment of the fabrics by reactive-cyclodextrin (RCD) followed by antibacterial agents

Treatment of cotton fabric, CMC and Cat-ionized cotton fabric with reactive-cyclodextrin (RCD) was carried out by pad-dry-cure method according to the following conditions: cotton fabric, CMC and Cat-ionized cotton fabric were steeped separately in an aqueous solution containing RCD (100 g/l) and 20 g/l sodium hydroxide putting in the water bath for 1h at 60 °C, at the end the samples were squeezed to a wet pick up 100% by padding in two dips and two nips. The treated fabrics were then dried at 50 °C for 5 min and then cured at 120 °C for 3 min. The fabric was washed with cold water containing 1% acetic acid, followed by several washing cycles and finally dried under the normal ambient conditions. Then modified cotton, CMC and Cat-ionized fabrics previously prepared were treated with antibacterial agents by dipping them separately under stirring in dimethylsulfoxide (DMSO) solution containing 250ppm of Eugenol, Carvacrol and Cinnamaldehyde respectively at room temperature for 2h. The samples were then roll-squeezed at pick up 100% and dried at room temperature [14].

Testing and analysis

Mechanical properties test: The mechanical properties were evaluated by strip method according to ASTM D 5035:2006 using a universal testing machine (INSTRON 4201) at room temperature with crosshead speed of 20 mm/min. The tensile strength and elongation at auto break were measured for both untreated cotton fabrics and those treated fabrics. The samples were cut into strips of 5 cm width and 20 cm length, and each data point represents the average of three measurements [23].

Antibacterial activity test: The antibacterial properties of the treated cotton fabrics were evaluated according to an American Association of Textile Chemist and Colorists (AATCC) test method 100–2004 [24]. Two bacteria, Gram-positive bacteria, *Staphylococcus aureus*, abbreviated as *S. aureus*, and Gram-negative bacteria, *Escherichia coli*, abbreviated as *E. coli*, were used. A total of 10µg of the treated cotton fabrics was added to a tube containing 5 mL of freshly prepared brain heart infusion broth BHIB (HiMedia, India), which is inoculated with the nominated bacteria (1.6×10^5 /mL). The tubes were incubated at 37 °C for 24 h in the presence of light source. The turbidity of the test tubes was compared visually to the control BHIB tube. Each tube was diluted, and fractions were plated on Nutrient Agar plates and incubated at 37 °C for 24 h. Colony forming units/mL was calculated by multiplying the number of colonies by the dilution factor. Antibacterial activities were expressed in terms of the percentage reduction of the microorganisms and calculated by eq 1.

$$\text{Bacterial Reduction (R)\%} = \frac{(A - B)}{A} \times 100 \quad (1)$$

Where A and B are the number of microorganism colonies on untreated and treated cotton fabrics, respectively.

Microbial count test

Microbiological examination: The microbiological examinations of samples included the determination of total aerobic and anaerobic counts, mould counts, Coliform, and *Bacillus cereus*. Twenty-five grams of each sample were homogenized in 225 mL peptone water (0.1%) using a stomacher model 400 (Seward Laboratory, London, UK) for 1-2 minutes to give a final dilution of 1:10. Samples were then serially diluted and plated using the appropriate medium.

The microbiological experiments were not replicated; therefore, no statistical analysis was performed. The results presented are descriptive in nature.

Total bacteria

Were placed on PCA medium using pour plate technique according to [25]. The inoculated plates were incubated at 35 °C for 3 days. The developing colonies were counted, and the Total Aerobic Bacterial counts (TABC) were expressed as colony forming units (CFU) per gram of samples.

Escherichia coli: *Escherichia coli* was counted according to the method described by [26] using MCA medium. The plates were incubated at 44 °C for 24-48 hr and the suspected colonies were streaked on EMBA and incubated at 44 °C for 24 h.

Total coliform: Plate count agar was poured into the plates [26], evenly distributed and incubated at 37°C for 48 h. Colonies were counted using an illuminated magnifying colony counter. To evaluate any difference between the use of water and saline, this procedure was repeated using sterile saline.

Bacillus cereus

Bacillus cereus was enumerated on MEYPA [27]. The plates were incubated for 16- 24 h at 37 °C. Confirmation tests of suspected colonies were biochemically performed by testing acid formation from different sugars.

Total mold count

Per gram sample were counted on CDYEA medium according to [28] using pour plate technique. Samples were serially diluted, plated and the inoculated plates were incubated at 25 °C for 3-5 days and then counted.

Results and discussions

Scoured cotton, CMC and Cat-ionized cotton fabric were reacted separately with reactive-cyclodextrin (RCD) by pad-dry-cure method by using an aqueous solution containing RCD (100 g/l) and 20 g/l sodium hydroxide. Then modified cotton, CMC and Cat-ionized fabrics were treated separately



with antibacterial agents including Eugenol, Carvacrol and Cinnamaldehyde as previously described in the experimental part. Mechanical properties of treated and untreated fabrics were evaluated, and all samples were monitored for antibacterial, and Microbial count test. The results obtained, along with appropriate discussion, are as follows.

Mechanical properties

Modification of scoured cotton in different conditions for obtaining CMC as well as Cat-ionized fabrics in addition to other treatments with RCD and antimicrobial agents in specific conditions may lead to loss of the tensile strength of the treated fabrics, so mechanical properties of the treated and untreated samples were evaluated due to its importance in storage package properties. Measured properties were the elongation at auto break and tensile strength for the fabrics before and after treatments.

Table 1 shows the mechanical properties of untreated and treated samples with different treatments with RCD or with RCD followed by different antimicrobial agents (Eugenol, Carvacrol and Cinnamaldehyde).

All values are means of triplicates $\pm$ SD

It is observed that the untreated sample has elongation around 11.5mm and tensile strength of 81.85 kgF. After treatment with RCD or with RCD followed by different antimicrobial agents (Eugenol, Carvacrol and Cinnamaldehyde), followed by drying and curing, the elongation and tensile strength marginally decrease and reach 10.8 mm and 73.95 kgF, respectively indicating that the treatment causes a loss in the elongation at break by 6% and tensile strength by about 9%, for CMC treated fabrics the elongation and tensile strength decreased marginally, reaching 10.2 mm and 72.65 kgF, respectively, indicating that the treatment caused a loss in the elongation at break by 11% and tensile strength by about 11%, for Cat-ionized treated fabrics the elongation and tensile strength marginally decrease and reach 9.80 mm and 71.0

kgF, respectively indicating that the treatment causes a loss in the elongation at break by 15% and tensile strength by about 14% and these losses percentage are practically acceptable for all treated samples and proved that such treatments does not significantly damage the strength properties of cotton [5,14,20].

Antibacterial properties

Antibacterial activities of the treated and untreated cotton fabric were determined against two kinds of bacteria, namely *Staphylococcus aureus* (*S. aureus*) (as gram-positive bacteria) and *Escherichia coli* (*E. coli*) (as gram-negative bacteria) according to Agar Diffusion Method (AATCC Test Method 100-2004) [24]. Inhibition zone diameter formed around the test samples were taken as a measure for antimicrobial activity. Results obtained are set out in Table 2.

- Untreated scoured fabrics did not show any antimicrobial properties towards *S. aureus* or *E. Coli*.
- All cotton substrates CMC or cat-ionized cotton shows antimicrobial properties towards *S. aureus* or *E. Coli*.
- Different treatments of cotton fabrics with RCD or with RCD and oils extract enhances its antibacterial activity towards both *S. aureus* and *E. Coli* where the inhibition zone RCD treated cotton fabric shows 19 and 21 mm for *E. Coli* and *S. aureus* respectively, presence of oils with RCD lead to increase the inhibition zone which increases to 26 and 28 mm for Cinnamaldehyde oil and for Carvacrol oil increases to 25 and 26 mm while increases with Eugenol oil to 24 and 26 for *E. Coli* and *S. aureus* respectively demonstrating the improvement of antibacterial properties for the treated cotton fabrics.
- Carboxymethylated cotton (CMC) show inhibition zone 15, 17 towards *E. Coli* and *S. aureus* respectively, the treatments of (CMC) with RCD or with RCD and oils extract enhances its antibacterial activity towards both

Table 1: Effect of treatment type on the Mechanical properties of cotton fabrics.

Substrate No	Treatment	Mechanical Properties	
		Tensile strength (KgF)	Elongation at auto break (mm)
Blank	Scoured Cotton	81.85 $\pm$ 6	11.5 $\pm$ 3
1	Cotton treated with RCD	75.65 $\pm$ 5	11.0 $\pm$ 3
2	Cotton treated with RCD followed by Cinnamaldehyde	74.70 $\pm$ 5	10.8 $\pm$ 3
3	Cotton treated with RCD followed by Carvacrol	73.95 $\pm$ 5	10.8 $\pm$ 3
4	Cotton treated with RCD followed by Eugenol	74.15 $\pm$ 5	10.9 $\pm$ 3
5	CMC	75.50 $\pm$ 4	10.6 $\pm$ 3
6	CMC treated with CD	73.75 $\pm$ 4	10.5 $\pm$ 3
7	CMC treated with CD followed by Cinnamaldehyde	73.90 $\pm$ 4	10.6 $\pm$ 3
8	CMC treated with CD followed by Carvacrol	72.65 $\pm$ 4	10.2 $\pm$ 2
9	CMC treated with CD followed by Eugenol	74.10 $\pm$ 4	10.5 $\pm$ 2
10	Cat-ionized Cotton	72.80 $\pm$ 4	10.25 $\pm$ 2
11	Cat-ionized Cotton treated with CD	72.15 $\pm$ 4	10.25 $\pm$ 2
12	Cat-ionized Cotton treated with CD followed by Cinnamaldehyde	71.85 $\pm$ 4	10.00 $\pm$ 2
13	Cat-ionized Cotton treated with CD followed by Carvacrol	71.25 $\pm$ 4	9.80 $\pm$ 2
14	Cat-ionized Cotton treated with CD followed by Eugenol	71.0 $\pm$ 4	9.80 $\pm$ 2

All values are means of triplicates $\pm$ SD

**Table 2:** Effect of treatment type on the antibacterial properties of cotton fabrics.

Substrate No	Treatment	Inhibition zone (mm)	
		<i>E. coli</i> (G ^{ve})	<i>S. aureus</i> (G ^{ve})
Blank	Scoured Cotton	0	0
1	Cotton treated with RCD	19	21
2	Cotton treated with RCD followed by Cinnamaldehyde	26	28
3	Cotton treated with RCD followed by Carvacrol	25	26
4	Cotton treated with RCD followed by Eugenol	24	26
5	CMC	15	17
6	CMC treated with CD	19	21
7	CMC treated with CD followed by Cinnamaldehyde	24	27
8	CMC treated with CD followed by Carvacrol	24	25
9	CMC treated with CD followed by Eugenol	22	24
10	Cat-ionized Cotton	19	21
11	Cat-ionized Cotton treated with CD	24	26
12	Cat-ionized Cotton treated with CD followed by Cinnamaldehyde	29	30
13	Cat-ionized Cotton treated with CD followed by Carvacrol	26	28
14	Cat-ionized Cotton treated with CD followed by Eugenol	26	27

S. aureus and *E. Coli* where the inhibition zone RCD treated CMC fabric shows 19 and 21mm for *E. Coli* and *S. aureus* respectively, presence of oils with RCD lead to increase the inhibition zone which increases to 24 and 27 mm for Cinnamaldehyde oil and for Carvacrol oil increases to 24 and 25 mm while increases with Eugenol oil to 22 and 24 for *E. Coli* and *S. aureus* respectively proving the improvements of antibacterial properties for the treated CMC fabrics.

- v. Cat-ionized cotton show inhibition zone 19, 21 towards *E. Coli* and *S. aureus* respectively, the treatments of Cat-ionized cotton with RCD or with RCD and oils extract enhances its antibacterial activity towards both *S. aureus* and *E. Coli* where the inhibition zone RCD treated Cat-ionized fabric shows 24 and 26 mm for *E. Coli* and *S. aureus* respectively, presence of oils with RCD lead to increase the inhibition zone which increases to 29 and 30 mm for Cinnamaldehyde oil and for Carvacrol oil increases to 26 and 28 mm while increases with Eugenol oil to 26 and 27 for *E. Coli* and *S. aureus* respectively demonstrating the improvement of antibacterial properties in the treated cat ionized fabrics.
- vi. All the treated fabrics indicates enhancement in its antibacterial activity towards both *S. aureus* and *E. Coli* and the inhibition zone of treated cat-ionized cotton with RCD followed by Cinnamaldehyde is the best between all the treated fabrics. These results were similar to those obtained by Hashem, et al. [29].

Results of microbial count test

A known weight from Egyptian wheat were stored in a package made from treated cotton package for 9 months and the microbial count was monitored at 0, 1, 3, 6, and 9 months. In this study, we present the data obtained over a 9-month period. The package contents were monitored for total anaerobic bacteria count, total mold count, coliform, and *Bacillus cereus*.

Microbial count results for wheat storage period from one month to nine months in various treatments of cotton fabric which has been treated in the three volatile active compounds (Carvacrol, Eugenol and Cinnamaldehyde) showed that the cotton fabric treatment which named (Cat-ionized + cyclodextrin with three volatile active compounds) had the greatest impact in reducing microbial count during storage period followed by cotton fabric (CMC + cyclodextrin with three volatile active compounds) followed by (Cotton + cyclodextrin with three volatile active compounds) which was less effective in reducing microbial count. Cotton fabric composites treated with cinnamaldehyde had the highest impact on reducing microbial growth during the storage period followed by cotton fabric composite treated by Carvacrol followed by cotton fabric composite treated by Eugenol which is less impactful to reduce microbial count during the storage period (Tables 3-5). Also, all treatments were the results of counting at zero time. These results are supported by the findings of Sanla-Ead, et al. [30] who reported that the cinnamaldehyde and eugenol had 'moderate-strong inhibitory' and 'strong-highly solid inhibitory' qualities, individually. Additionally, this shows the potential of cinnamaldehyde and eugenol for application in antimicrobial packaging films or coatings. In this examination cinnamaldehyde and eugenol were explored for their antimicrobial movement against 10 pathogenic and decay microbes and three strains of yeast. Cinnamaldehyde-consolidated and eugenol-joined methyl cellulose films were set up to acquire dynamic antimicrobial bundling materials.

Also, Bryan et al. [24] showed that the inhibitory effect of carvacrol in various grouping of bacterial development was a significant barrier to the growth of all bacterial isolates studied, except *Pseudomonas aeruginosa*.

Various investigators have used essential oils and their components, either in pure or formulation forms, to enhance the shelf-life of food commodities in different storage containers such as those made of cardboard, tin, glass, polyethylene, or natural fabrics and have observed significant enhancement of

**Table 3:** Results of antimicrobial count test of cotton substrates in which Egyptian Wheat stored for different periods.

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
All Treatments	0	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
Cotton treated with CD	1	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
	3	9×10^4	8×10^8	8×10^4	6×10^5	9×10^6
	6	8×10^4	9×10^8	7×10^4	5×10^5	8×10^6
	9	8×10^4	1×10^9	7×10^4	5×10^5	8×10^6
	1	6×10^4	5×10^8	5×10^4	4×10^5	6×10^6
Cotton treated with CD followed by Cinnamaldehyde	3	5×10^4	4×10^8	3×10^4	3×10^5	5×10^6
	6	2×10^4	2×10^8	2×10^4	2×10^5	3×10^6
	9	1×10^4	1×10^8	1×10^4	1×10^5	2×10^6
	1	7×10^4	6×10^8	6×10^4	5×10^5	7×10^6
Cotton treated with CD followed by Carvacrol	3	6×10^4	5×10^8	4×10^4	4×10^5	6×10^6
	6	4×10^4	3×10^8	3×10^4	3×10^5	5×10^6
	9	3×10^4	2×10^8	2×10^4	2×10^5	4×10^6
	1	8×10^4	7×10^8	7×10^4	6×10^5	8×10^6
Cotton treated with CD followed by Eugenol	3	7×10^4	6×10^8	5×10^4	5×10^5	7×10^6
	6	5×10^4	4×10^8	4×10^4	4×10^5	6×10^6
	9	3×10^4	3×10^8	3×10^4	3×10^5	5×10^6

Table 4: Results of antimicrobial count test of Carboxymethylated cotton substrates in which Egyptian Wheat stored for different periods.

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
All Treatments	0	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
Carboxymethylated Cotton (CMC) treated with CD	1	9×10^4	8×10^8	8×10^4	6×10^5	9×10^6
	3	9×10^4	9×10^8	8×10^4	6×10^5	9×10^6
	6	8×10^4	9×10^8	7×10^4	5×10^5	8×10^6
	9	8×10^4	1×10^9	7×10^4	5×10^5	8×10^6
	1	5×10^4	4×10^8	4×10^4	3×10^5	5×10^6
Carboxymethylated Cotton (CMC) treated with CD followed by Cinnamaldehyde	3	4×10^4	3×10^8	2×10^4	2×10^5	4×10^6
	6	1×10^4	1×10^8	1×10^4	1×10^5	2×10^6
	9	9×10^3	8×10^7	9×10^3	8×10^4	1×10^6
	1	6×10^4	5×10^8	5×10^4	4×10^5	6×10^6
Carboxymethylated Cotton (CMC) treated with CD followed by Carvacrol	3	5×10^4	4×10^8	3×10^4	3×10^5	5×10^6
	6	3×10^4	2×10^8	2×10^4	2×10^5	4×10^6
	9	2×10^4	1×10^8	1×10^4	1×10^5	3×10^6
	1	7×10^4	6×10^8	6×10^4	5×10^5	7×10^6
Carboxymethylated Cotton (CMC) treated with CD followed by Eugenol	3	6×10^4	5×10^8	5×10^4	4×10^5	6×10^6
	6	4×10^4	3×10^8	3×10^4	3×10^5	5×10^6
	9	2×10^4	2×10^8	2×10^4	2×10^5	4×10^6

Table 5: Results of antimicrobial count test of Cat-ionized cotton substrates in which Egyptian Wheat stored for different periods.

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
All Treatments	0	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
Cat-ionized Cotton treated with CD	1	9×10^4	8×10^8	8×10^4	6×10^5	9×10^6
	3	9×10^4	9×10^8	8×10^4	6×10^5	9×10^6
	6	8×10^4	9×10^8	7×10^4	5×10^5	8×10^6
	9	8×10^4	1×10^9	7×10^4	5×10^5	8×10^6
	1	1×10^4	1×10^8	2×10^4	1×10^5	3×10^6
Cat-ionized Cotton treated with CD followed by Cinnamaldehyde	3	7×10^3	6×10^7	8×10^3	8×10^4	1×10^6
	6	6×10^3	3×10^7	5×10^3	7×10^4	6×10^5
	9	3×10^3	6×10^6	1×10^3	3×10^4	2×10^5
	1	3×10^4	3×10^8	3×10^4	3×10^5	4×10^6
Cat-ionized Cotton treated with CD followed by Carvacrol	3	2×10^4	2×10^8	2×10^4	2×10^5	3×10^6
	6	1×10^4	1×10^8	8×10^3	9×10^4	1×10^6
	9	8×10^3	8×10^7	5×10^3	7×10^4	8×10^5
	1	4×10^4	4×10^8	4×10^4	4×10^5	6×10^6
Cat-ionized Cotton treated with CD followed by Eugenol	3	3×10^4	3×10^8	3×10^4	3×10^5	5×10^6
	6	2×10^4	2×10^8	1×10^4	2×10^5	3×10^6
	9	1×10^4	1×10^8	9×10^3	8×10^4	1×10^6

shelf-life [20]. An earlier study reported that some essential oil constituents such as carvacrol, eugenol, cinnamaldehyde, citronellol, farnesol, and nerol capable of protecting chili fruits and seeds from fungal infection for up to 6 months [21,31]. The use of *Cymbopogon pendulous* essential oil as a fumigant increased groundnut shelf-life by 6–12 months [32], which proves to be more effective than *P. roxburghii* essential oil. These differences in efficacy of essential oils components may be related to the use of oils from different plant species, as

well as to their chemical composition, dose level, and storage container type.

The bioactivity may be associated with the high cinnamaldehyde content in the composition of essential oils. Therefore, these major components may be considered as possible sources for the development of new antimicrobial agents and may be used in synergy with currently available synthetic antibiotics or antimicrobials. In addition,

Table 6: Comparison between all substrates in the presence of RCD and RCD+Carvacrol, Eugenol and Cinnamaldehyde.

Treatment	Time (Month)	Total anaerobic bacteria count	Total mould count	Coliform	Bacillus cereus	Total bacterial count
All Treatments	0	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
Cotton treated with RCD	1	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
	3	9×10^4	8×10^8	8×10^4	6×10^5	9×10^6
	6	8×10^4	9×10^8	7×10^4	5×10^5	8×10^6
	9	8×10^4	1×10^9	7×10^4	5×10^5	8×10^6
CMC treated with RCD	1	9×10^4	8×10^8	8×10^4	6×10^5	9×10^6
	3	9×10^4	9×10^8	8×10^4	6×10^5	9×10^6
	6	8×10^4	9×10^8	7×10^4	5×10^5	8×10^6
	9	8×10^4	1×10^9	7×10^4	5×10^5	8×10^6
Cationic treated with RCD	1	9×10^4	8×10^8	8×10^4	6×10^5	9×10^6
	3	9×10^4	9×10^8	8×10^4	6×10^5	9×10^6
	6	8×10^4	9×10^8	7×10^4	5×10^5	8×10^6
	9	8×10^4	1×10^9	7×10^4	5×10^5	8×10^6
Cotton treated with RCD followed by Carvacrol	1	7×10^4	6×10^8	6×10^4	5×10^5	7×10^6
	3	6×10^4	5×10^8	4×10^4	4×10^5	6×10^6
	6	4×10^4	3×10^8	3×10^4	3×10^5	5×10^6
	9	3×10^4	2×10^8	2×10^4	2×10^5	4×10^6
CMC treated with RCD followed by Carvacrol	1	6×10^4	5×10^8	5×10^4	4×10^5	6×10^6
	3	5×10^4	4×10^8	3×10^4	3×10^5	5×10^6
	6	3×10^4	2×10^8	2×10^4	2×10^5	4×10^6
	9	2×10^4	1×10^8	1×10^4	1×10^5	3×10^6
Cat-ionized Cotton treated with RCD followed by Carvacrol	1	3×10^4	3×10^8	3×10^4	3×10^5	4×10^6
	3	2×10^4	2×10^8	2×10^4	2×10^5	3×10^6
	6	1×10^4	1×10^8	8×10^3	9×10^4	1×10^6
	9	8×10^3	8×10^7	5×10^3	7×10^4	8×10^5
All Treatments	0	9×10^4	7×10^8	8×10^4	6×10^5	9×10^6
Cotton treated with RCD followed by Eugenol	1	8×10^4	7×10^8	7×10^4	6×10^5	8×10^6
	3	7×10^4	6×10^8	5×10^4	5×10^5	7×10^6
	6	5×10^4	4×10^8	4×10^4	4×10^5	6×10^6
	9	3×10^4	3×10^8	3×10^4	3×10^5	5×10^6
CMC treated with RCD followed by Eugenol	1	7×10^4	6×10^8	6×10^4	5×10^5	7×10^6
	3	6×10^4	5×10^8	5×10^4	4×10^5	6×10^6
	6	4×10^4	3×10^8	3×10^4	3×10^5	5×10^6
	9	2×10^4	2×10^8	2×10^4	2×10^5	4×10^6
Cationic fabric treated with RCD followed by Eugenol	1	4×10^4	4×10^8	4×10^4	4×10^5	6×10^6
	3	3×10^4	3×10^8	3×10^4	3×10^5	5×10^6
	6	2×10^4	2×10^8	1×10^4	2×10^5	3×10^6
	9	1×10^4	1×10^8	9×10^3	8×10^4	1×10^6
Cotton treated with CD followed by Cinnamaldehyde	1	6×10^4	5×10^8	5×10^4	4×10^5	6×10^6
	3	5×10^4	4×10^8	3×10^4	3×10^5	5×10^6
	6	2×10^4	2×10^8	2×10^4	2×10^5	3×10^6
	9	1×10^4	1×10^8	1×10^4	1×10^5	2×10^6
CMC treated with RCD followed by Cinnamaldehyde oil	1	5×10^4	4×10^8	4×10^4	3×10^5	5×10^6
	3	4×10^4	3×10^8	2×10^4	2×10^5	4×10^6
	6	1×10^4	1×10^8	1×10^4	1×10^5	2×10^6
	9	9×10^3	8×10^7	9×10^3	8×10^4	1×10^6
Cat-ionized Cotton treated with RCD followed by Cinnamaldehyde	1	1×10^4	1×10^8	2×10^4	1×10^5	3×10^6
	3	7×10^3	6×10^7	8×10^3	8×10^4	1×10^6
	6	6×10^3	3×10^7	5×10^3	7×10^4	6×10^5
	9	3×10^3	6×10^6	1×10^3	3×10^4	2×10^5

cinnamaldehyde is promising as a prototype for derivatives with antibacterial properties and as an enhancer of antibiotic efficacy [33].

All results are presented to illustrate and compare the effects of different treatments and arranged from highest to lowest impact effect on the microbial count in Table 6.

Limitations

A limitation of this study is that the microbiological experiments were not replicated, and statistical validation was not performed. Accordingly, the findings should be interpreted as descriptive observations.

Conclusion

Carvacrol, Eugenol and Cinnamaldehyde as essential oil constituents have pronounced antimicrobial and food preservative properties that have great significance in the food industry. Therefore, the various properties of essential oil constituents provide an opportunity to use natural, safe, eco-friendly, practical, renewable, and biodegradable antimicrobials for food product protection sooner rather than later. Cotton fabric composites treated with cinnamaldehyde had the highest impact on reducing microbial preparation during storage period followed by cotton fabric composite treated by Carvacrol followed by cotton fabric composite treated by Eugenol which is less impactful to reduce microbial count during the storage period.

Authors' contributions

All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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