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# Preservative Effect of Selected Natural Flavoring Spices and Their Extracts on Microbial Quality of Meatballs During Cold Storage

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THE Spices such as paprika, cloves, piper cubeba, and their extract have been shown to possess potent antimicrobial potential against food spoilage microorganisms and pathogens. Contamination of minced meat with such pathogens may lead to foodborne poisoning. This has enforced the search for antimicrobial agents that could increase the shelf life of minced beef. This work aimed to determine the antimicrobial effect of paprika, clove, and piper cubeba powder and their extract with different concentrations against aerobic plate count, *Enterobacteriaceae* count, and *Staphylococcus aureus*. The ability of these natural additives to extend the shelf life and improve the quality of minced beef, and optimize the concentrations of these spices and their extract in minced beef. The Sensory evaluation of cooked meatballs containing added paprika, clove, and piper cubeba powders and their extracts exhibited no significant differences in the flavor, tenderness, juiciness, and overall acceptability characteristic of meatballs among all treated and control samples and revealed no negative impacts on consumer perception. Effect of natural additives and their extracts on APCs in meatballs during cold storage revealed that the studied spices and their extracts may be a source for the development of safe and new ingredients that could be used to control products spoilage and/or foodborne pathogens in foods. Treated meat samples resulted in prolonged storage life of piper cubeba, clove and paprika added samples compared with control. The antimicrobial effect these spices was found to be concentration-dependent and attributed to their phytochemical constituents. The current study concluded that there was an increase in shelf life and decrease in contamination levels of *Enterobacteriaceae* and *S. aureus* in the meatballs treated with paprika, clove, and piper cubeba.

**Keywords:** Antimicrobial, Minced meat, Sensory evaluation, Shelf life, Spices.

## Introduction

Microbial contamination caused by spoilage bacteria leads to the spoilage of meat and its various products, as this contamination causes many foodborne illnesses transmitted from food [1]. These foods may be unfitted for human consumption. The use of synthetic antimicrobials

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in food products is controlled by the regulatory laws of an individual country or international standards. There is an increasing demand for natural preservatives from some plants as safe alternatives for eliminating disease-causing pathogens, decontaminating, preserving, and covering the shelf-life of meat, and has the advantages of being more acceptable to the consumers [1].

Phytochemicals are a large group of bioactive derived from plants that have potential protective effects against diseases. The addition of herbs and spices and their extracts have been used as flavoring agents and natural food preservatives and can be improved the shelf life and thus improve the quality of meat and its products. The main effects of these compounds are enhancing color stability and inhibiting microbial growth and lipid oxidation throughout the storage of meat products [1].

Spices and their extract displayed remarkable antimicrobial potency against food spoilage organisms and pathogens [2]. The antimicrobial characteristics of spices and their extracts depended on many aspects which comprise the spices type, microbial species, and their occurrences level, spices composition, concentration, processing conditions, and storage. In this respect, the main chemical compounds were characterized in numerous spices, for instance, eugenol in clove and cumin aldehyde in cumin that were estimated the growth inhibition of pathogenic microorganisms and prevent food from spoilage [2]. However, the methods and mechanisms of action of natural antimicrobials are not completely understood [3].

Paprika a member of the Capsicum family was reported to include carotenoids and capsaicin, in which both components revealed remarkable antioxidant activity for radical scavenging [4]. Scavenging aptitude concerning diverse antioxidants might depend on the molecular structure of the antioxidants and predominantly the effective reactivity of phenolic and polyphenolic molecules that provided stable free radicals [5].

Cloves (*Syzygium aromatic* L. Myrtaceae) have been used as spices or condiments to retarding the deterioration and raise the shelf life of food products. The activity of clove and their extracts has mainly been attributed to a variety of numerous bioactive phenolic molecules, including categories of tannins, triterpenoids, sesquiterpenes, eugenol, and eugenol acetate [6]. Clove is frequently employed as a natural additive in the food industry to improve shelf-life owing to its antimicrobial significance against a variety of foodborne pathogens. The clove extract could destroy the cell walls of the microbes and permeate the cytoplasmic membranes or enter the cells of microbes, and subsequently inhibited the regular synthesis of DNA and proteins [7]. Eugenol was characterized as the main active molecule of clove extract and oil [8]. Clove can be applied correspondingly for improving sensory features, color stability, and growing the shelf life of meat and its products through the inhibition of protein and lipid oxidations [9].

*Piper cubeba* is related to the Piperaceae family and the Piper genus is one of the folkloric plants that has been utilized as a spice in many countries, including India, Indonesia, Europe, and Morocco. It is usually known in India as kababchini spice, which revealed distinctive antimicrobial, anti-inflammatory, hepatoprotective, antioxidant, and anti-allergic pharmacological characteristics [10]. Traditionally, *P. cubeba* L. has been used to marinate and season the meat. The pepper comprises valuable phytochemicals, for example, amides, alkaloids (cubebin and piperine), flavones, flavanones, lignans, neolignans, terpenes, and propenyl phenolics [11].

Minced meat is extremely delicate with a comparatively short shelf life either by kept under refrigeration owing to its biological composition, autolytic enzymes, microbial activities, and lipid oxidation. The imperative common health issue is related to the contamination of minced meat caused by pathogens, which may lead to foodborne poisoning. The shelf-life and quality of minced beef can be improved by natural additives added to the meat surface.

The current work was aimed to assess the antimicrobial effect of paprika, clove, and piper cubeba powder and their extract with different concentrations against a count of the aerobic plate, *Enterobacteriaceae*, and *S. aureus*. Correspondingly, the ability of these natural additives to cover the shelf life of refrigerated fresh meatballs, and estimate the sensory attributes of these spices and their extracts concentrations on organoleptic properties of cooked samples.

## Material and Methods

*Preparation of paprika, clove, and Piper cubeba extract*

The preparation and extraction processes of paprika, clove, and piper cubeba was done in the Food Microbiology Laboratory, Department of Food Hygiene, Safety and Technology, Faculty of Veterinary Medicine, Mansoura University, Egypt. Powder of paprika (*Capsicum Annuum* L), clove (*Syzygium aromaticum*), and Long pepper (*Piper cubeba* Linn.) were obtained from Agricultural Research Center (ARC, Giza, Egypt), while the spices of paprika, clove, and *Piper cubeba* were extracted concerning to the technique termed by Dent et al. [12]. Briefly, deionized water (1 L) was added to each of the dry plant samples (100 g) and the solution was shaken at 60°C for 90 min on a horizontal water bath shaker (Memmert WB14, Schwabach, Germany). The solution was filtered by Whatman no.1 filter papers (Whatman International Ltd., Kent, UK). The final volume of the resulted extracts was adjusted by deionized water in volumetric flasks to 500 mL, in which a Büchner funnel was used for filtration and then stored at -18°C for later usage.

*Preparation of minced beef samples*

A total of 11 kilograms of fresh minced meat were acquired from the butcher shop in Mansoura city, Egypt, and stored in the icebox during transportation to the laboratory. The meat was divided into two main parts; 2.400 kg was used for studying the antibacterial effects of both spices powder and their extracts with different concentrations; the other 8.600 kg of minced beef was separately used for sensory evaluation of meat treated with spices powder or their extracts. For bacteriological investigations, the meat was divided into nineteen groups. Eighteen, out of these nineteen groups, were treated with diverse concentrations of paprika powder (2%, 4 % and 8%), paprika extract (2%, 4% and 8%), clove powder (0.5%, 1% and 1.5%), clove extract (0.5%, 1% and 1.5%), *Piper cubeba* powder (1%, 2 % and 3%), *Piper cubeba* extract (1%, 2 % and 3%), and the last group was learned as a control group (without any treatment). The treated groups were mixed with spices powder or their extract and distributed by hand. Control and treated groups were packed individually in a sterile polyethylene bag and kept in refrigeration storage at 3±1°C and investigated for the 0, 3, 6, 9, 12, and 15, days.

*Bacteriological analysis*

For evaluation of the antibacterial effect of spices powder and their extracts, the total aerobic plate counts (APCs), total Enterobacteriaceae counts (EBCs), and total *S. aureus* count for all treated beef and the control ones were determined. Briefly, 10 g of each meat sample were homogenized in 90 mL sterile peptone water 0.1% (Oxoid CM0009) and serial dilutions were accomplished concerning the technique defined by ISO [13]. From the prepared serial dilutions, were duplicate plated on plate count agar (Oxoid CM0325), Violet Red Bile Glucose agar (VRBG), and Baird Parker agar (Merck, Germany) and then incubated at 37°C for 2 days. The bacterial colonies in countable plates were counted for all treated samples besides the control one and the total counts of each microorganism per gram were determined. To confirm the presence of coagulase enzyme for *S. aureus* colonies were conducted for both slide and tube coagulase test [14].

*Sensory analysis*

Sensory evaluation of meat samples was performed immediately on the day of meatballs preparation (0 days). The meat samples of the control and treated groups were made into meatballs weighing 30 g each. From each group, meatballs were cooked in the oven at 180 °C for 20 min and were evaluated by 15 trained panelists were rated indicators of flavor, tenderness, juiciness, and overall acceptability with the use of a 9-point hedonic scale (9 points: most desirable; 1 point: least desirable). Cooked meatballs were cooled and randomly introduced to panelists who could use redistilled water between each sample to clean their mouths [15].

*Statistical analysis*

Experiments were repeated triples for each sample and each concentration. All data were analyzed by the GLM procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC). Means of data were compared by the Tukey test. The variances were deliberated as significant values at the $P<0.05$ level.

## Results

*Sensory assessment of cooked meatballs containing added paprika, clove, and Piper cubeba powders and their extracts*

Sensory qualities of cooked meatballs treated with fresh dry powder of paprika, clove, and *Piper cubeba* and their extracts at different concentrations in addition to the control samples are shown in Table 1 & Fig. 1.

Effect of natural additives and their extracts on APCs in meatballs during cold storage at 3±1°C

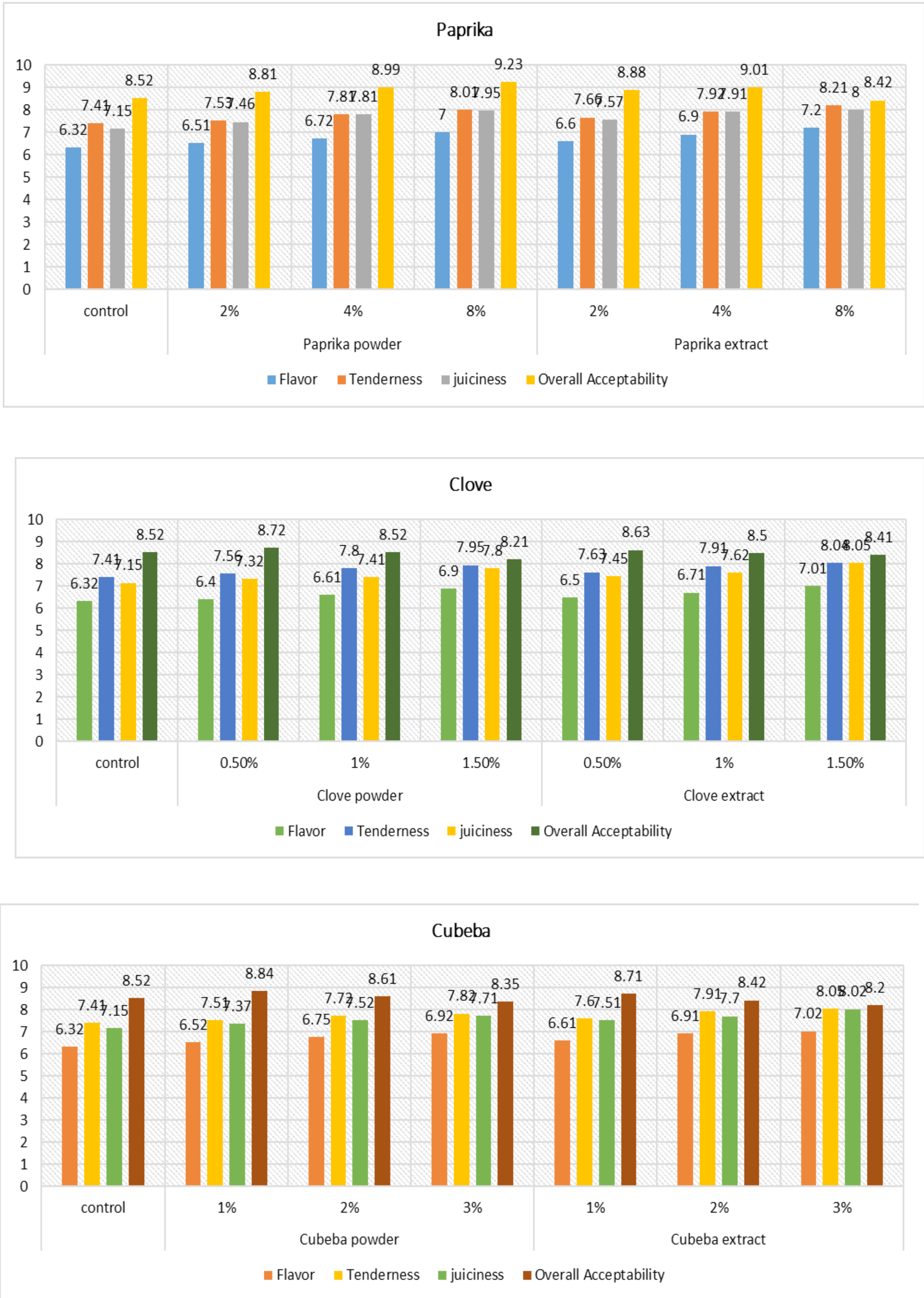


**Fig. 1. Effect of added Paprika, clove, and piper cubeba powders and their extracts on sensory characteristics of cooked meatballs.**

**TABLE 1. Sensory evaluation of cooked meatballs containing added Paprika, clove, and piper cubeba powders and their extracts.**

| Samples | | Characteristics of meatball Flavor | Tenderness | Juiciness | Overall acceptability |
|---|---|---|---|---|---|
| | **Control** | **6.32[a]** | **7.41 [a]** | **7.15 [a]** | **8.52 [a]** |
| **Paprika powder** | 2% | .51[a] | 7.53 [a] | 7.46 [a] | 8.81 [a] |
| | 4% | 6.72[a] | 7.81 [a] | 7.81 [a] | 8.99 [a] |
| | 8% | 7.00 [a] | 8.01 [a] | 7.95 [a] | 9.23 [a] |
| **Paprika extract** | 2% | 6.60 [a] | 7.66 [a] | 7.57 [a] | 8.88 [a] |
| | 4% | 6.90 [a] | 7.92 [a] | 7.91 [a] | 9.01 [a] |
| | 8% | 7.20 [a] | 8.21 [a] | 8.00 [a] | 8.42 [a] |
| **Clove powder** | 0.50% | 6.40 [a] | 7.56 a | 7.32 a | 8.72 a c |
| | 1% | 6.61 [a] | 7.80 a | 7.41 a | 8.52 a |
| | 1.50% | 6.90 [a] | 7.95 a | 7.80 a | 8.21 a |
| **Clove extract** | 0.50% | 6.50 [a] | 7.63 a | 7.45 a | 8.63 a |
| | 1% | 6.71 [a] | 7.91 a | 7.62 a | 8.50 a |
| | 1.50% | 7.01 [a] | 8.04 a | 8.05 a | 8.41 a |
| **Piper cubeba powder** | 1% | 6.52 [a] | 7.51 a | 7.37 a | 8.84 a |
| | 2% | 6.75 a | 7.72 a | 7.52 a | 8.61 a |
| | 3% | 6.92 a | 7.82 a | 7.71 a | 8.35 a |
| **Piper cubeba extract** | 1% | 6.61 a | 7.60 a | 7.51 a | 8.71 a |
| | 2% | 6.91 a | 7.91 a | 7.70 a | 8.42 a |
| | 3% | 7.02 a | 8.05 a | 8.02 a | 8.20 a |

Duncan's letters for statistical analysis: "a" is expressed as the highest values and descending to "z" which is expressed as the lowest values. Values with common letters are considered to have non-significant differences between them at the P<0.05 level.

*Paprika powders and their extracts*

The results of log10 cfu/g showed that APCs have increased over time in meatballs of control and both paprika powder and their extracts (Table 2 & Fig. 2). The initial APCs (0 days) in meatballs of control and both paprika powder and their extracts samples ranged from 4.68 to 4.01 log10 CFU/g with no substantial variance between the treated and control samples except at the concentration of 8% powder and extract (Table 2). On the other hand, on the 3rd day, meatballs treated with either paprika powder (8%) or paprika extracts (4 and 8%) displayed a significant variance (P>0.05) in APCs when relative to the control one (5.32 log10 CFU/g).

*Clove powders and their extracts*

The results of log10 cfu/g showed that APCs have increased over time in meatballs of control and both clove powders and their extracts (Table 3 & Fig. 3). The initial APCs (0 days) in meatballs of control and both clove powders and their extracts samples ranged from 4.68 to 4.19 log10 cfu/g with no substantial variance between the control and treated samples. By day 3, meatballs treated with either clove powders (1 and 1.5%) or clove extracts (0.5, 1, and 1.5%) revealed a noteworthy variance (P>0.05) in APCs in comparison to the control sample.

*Piper cubeba powders and their extracts*

Assessment of the antimicrobial effect of added piper cubeba in dry and extract form to meatballs during storage was shown in Table 4 & Fig. 4. Based on the findings, the initial total bacterial count of control samples was 4.68 log10 cfu/g which increased steadily during the storage period, reaching 9.12 log10 CFU/g on day 15. At the beginning of the experiment, bacterial counts for fresh samples at all concentrations of both piper cubeba powder and extract were declined upon application of the treatments (P<0.05).

**TABLE 2. Effect of addition of paprika powder and its extract at different concentrations on microbial quality of meatballs during cold storage at 3±1°C.**

| Paprika | Day | Control | Powder | | | Extract | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2% | 4% | 8% | 2% | 4% | 8% |
| APC | 0 | 4.68 pq | 4.51 pqr | 4.37 qrs | 4.15 rs | 4.43 qrs | 4.31 qrs | 4.01 s |
| | 3 | 5.32 no | 5.02 op | 4.93 op | 4.66 pq | 5.01 op | 4.75 pq | 4.25 qrs |
| | 6 | 6.74 hij | 6.17 klm | 5.85 m | 5.32 no | 5.99 lm | 5.51 n | 5.02 op |
| | 9 | 7.55 def | 7.14 fgh | 6.82 hi | 6.50 ijk | 6.87 hi | 6.33 jkl | 6.31 jkl |
| | 12 | 8.02 cd | 7.92 cd | 7.63 de | 7.13 fgh | 7.33 efg | 7.21 efgh | 7.03 gh |
| | 15 | 9.12 a | 9.02 a | 8.88 ab | 8.26 c | 8.72 ab | 8.59 b | 8.12 c |
| **LSD (0.05)** | | | | **0.301** | | | | |
| EBC | 0 | 3.55 opqrst | 3.52 opqrst | 3.22 rst | 3.11 st | 3.44 opqrst | 3.30 qrst | 3.00 t |
| | 3 | 4.02 mno | 3.87 mnopq | 3.52 opqrst | 3.20 rst | 3.75 nopqr | 3.41 pqrst | 3.22 rst |
| | 6 | 4.63 ijkl | 4.13 lmn | 3.98 mnop | 3.45 opqrst | 4.02 mno | 3.65 nopqrs | 3.37 qrst |
| | 9 | 5.32 efg | 5.18 fgh | 4.98 ghi | 4.37 jklm | 4.93 ghi | 4.66 ijk | 4.20 klmn |
| | 12 | 6.07 bcd | 5.82 cde | 5.66 def | 4.76 hij | 5.57 def | 5.43 efg | 4.55 ijkl |
| | 15 | 6.82 a | 6.51 ab | 5.93 cd | 5.32 efg | 6.20 bc | 5.72 cde | 4.93 ghi |
| **LSD (0.05)** | | | | **0.325** | | | | |
| ***S. aureus* count** | 0 | 4.32 lmno | 4.16 mnop | 4.00 nop | 3.77 op | 4.03 nop | 3.89 nop | 3.60 p |
| | 3 | 4.87 hijkl | 4.45 klmn | 4.16 mnop | 4.02 nop | 4.24 mno | 4.04 nop | 3.91 nop |
| | 6 | 5.32 efghi | 5.07 hij | 4.87 hijkl | 4.37 lmno | 4.88 hijkl | 4.53 jklmn | 4.20 mnop |
| | 9 | 5.92 cde | 5.78 cdefg | 5.32 efghi | 4.99 hijk | 5.26 fghi | 5.02 hijk | 4.77 ijklm |
| | 12 | 6.54 ab | 6.32 abc | 5.91 cde | 5.50 defgh | 5.99 bcd | 5.42 defghi | 5.22 fghi |
| | 15 | 6.72 a | 6.57 a | 6.22 abc | 5.82 cdef | 6.15 abc | 5.19 ghi | 5.71 cdefg |
| **LSD (0.05)** | | | | **0.355** | | | | |

Duncan’s letters for statistical analysis: “a” is expressed as the highest values and descending to “z” which is expressed as the lowest values. Values with common letters are considered to have non-significant differences between them at the P<0.05 level.

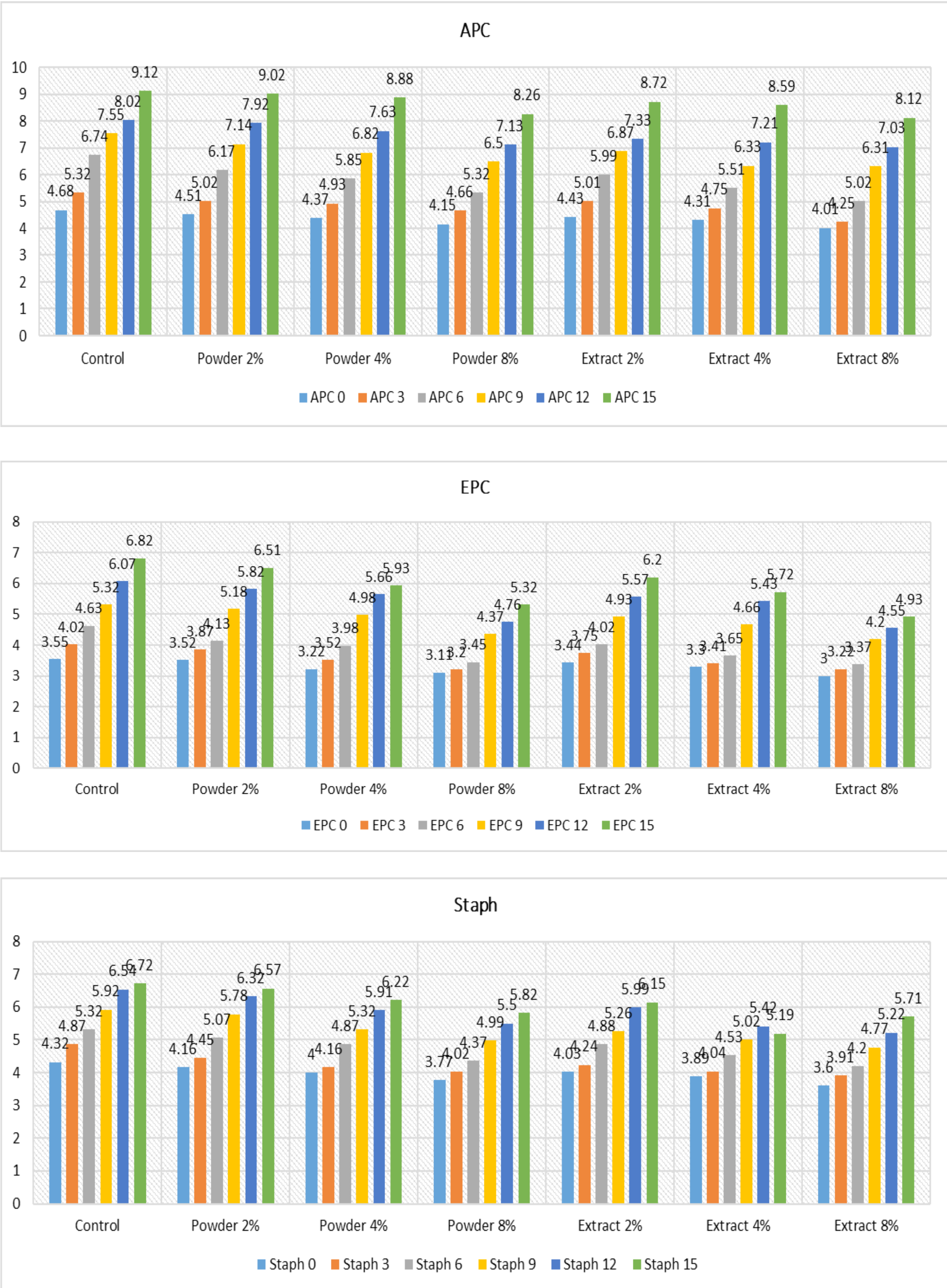


**Fig. 2. Effects of paprika powder and its extract at different concentrations on aerobic plate count (APC), Enterobacteriaceae count (EBC), and S. aureus count in meatballs during cold storage at 3±1°C.**

**TABLE 3. Effect of addition of clove powder and its extract at different concentrations on microbial quality of meatballs during cold storage at 3±1°C.**

| Clove | Day | Control | Powder | | | Extract | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0.50% | 1% | 1.50% | 0.50% | 1% | 1.50% |
| APC | 0 | 4.68 stuv | 4.43 tuvw | 4.21 vw | 4.20 vw | 4.30 uvw | 4.27 uvw | 4.19 vw |
| | 3 | 5.32 pqr | 4.88 rst | 4.47 tuvw | 4.33 uvw | 4.66 stuv | 4.42 tuvw | 4.22 vw |
| | 6 | 6.74 ijk | 5.71 op | 5.30 pqr | 4.97 rs | 5.50 opq | 5.07 qrs | 4.81 stu |
| | 9 | 7.55 fg | 6.99 hij | 6.22 lm | 5.82 no | 6.63 ijkl | 6.10 lm | 5.57 op |
| | 12 | 8.02 cdef | 7.62 efg | 7.33 gh | 6.55 jkl | 7.07 hi | 6.94 hij | 6.34 klm |
| | 15 | 9.12 a | 8.68 b | 8.44 bc | 7.86 def | 8.21 cd | 8.10 cde | 7.66 efg |
| **LSD (0.05)** | **0.3087** | | | | | | | |
| EBC | 0 | 3.55 mnopqr | 3.41 nopqr | 3.15 qr | 3.02 r | 3.37 opqr | 3.03 r | 3.01 r |
| | 3 | 4.02 ijklmno | 3.62 lmnopqr | 3.42 nopqr | 3.13 qr | 3.41 nopqr | 3.26 qr | 3.12 qr |
| | 6 | 4.63 fghi | 4.05 ijklmn | 3.77 lmnopq | 3.32 pqr | 3.91 klmnop | 3.44 nopqr | 3.22 qr |
| | 9 | 5.32 de | 4.85 efg | 4.66 fgh | 3.99 jklmno | 4.55 fghij | 4.17 hijklm | 3.73 lmnopq |
| | 12 | 6.07 bc | 5.63 cd | 5.34 de | 4.47 ghijk | 5.33 de | 5.11 def | 4.20 hijkl |
| | 15 | 6.82 a | 6.24 b | 5.70 cd | 5.10 def | 6.01 bc | 5.61 cd | 4.83 efg |
| **LSD (0.05)** | **0.357** | | | | | | | |
| ***S. aureus* count** | 0 | 4.32 klmn | 4.02 mno | 3.91 nop | 3.84 nop | 4.00 mno | 3.88 nop | 3.44 q |
| | 3 | 4.87 hij | 4.25 klmn | 4.03 mno | 3.86 nop | 4.11 lmno | 3.92 nop | 3.66 opq |
| | 6 | 5.32 fgh | 4.87 hij | 4.55 jkl | 4.13 lmno | 4.63 jk | 4.35 klmn | 3.94 nop |
| | 9 | 5.92 cde | 5.52 efg | 4.93 hij | 4.72 ijk | 4.72 ijk | 4.74 ijk | 4.46 jklm |
| | 12 | 6.54 ab | 6.01 cde | 5.64 defg | 5.22 gh | 5.81 cde | 5.20 ghi | 4.92 hij |
| | 15 | 6.72 a | 6.25 bc | 6.00 cde | 5.71 def | 6.12 cd | 5.71 def | 5.52 efg |
| **LSD (0.05)** | **0.288** | | | | | | | |

Duncan's letters for statistical analysis: "a" is expressed as the highest values and descending to "z" which is expressed as the lowest values. Values with common letters are considered to have non-significant differences between them at the P<0.05 level.

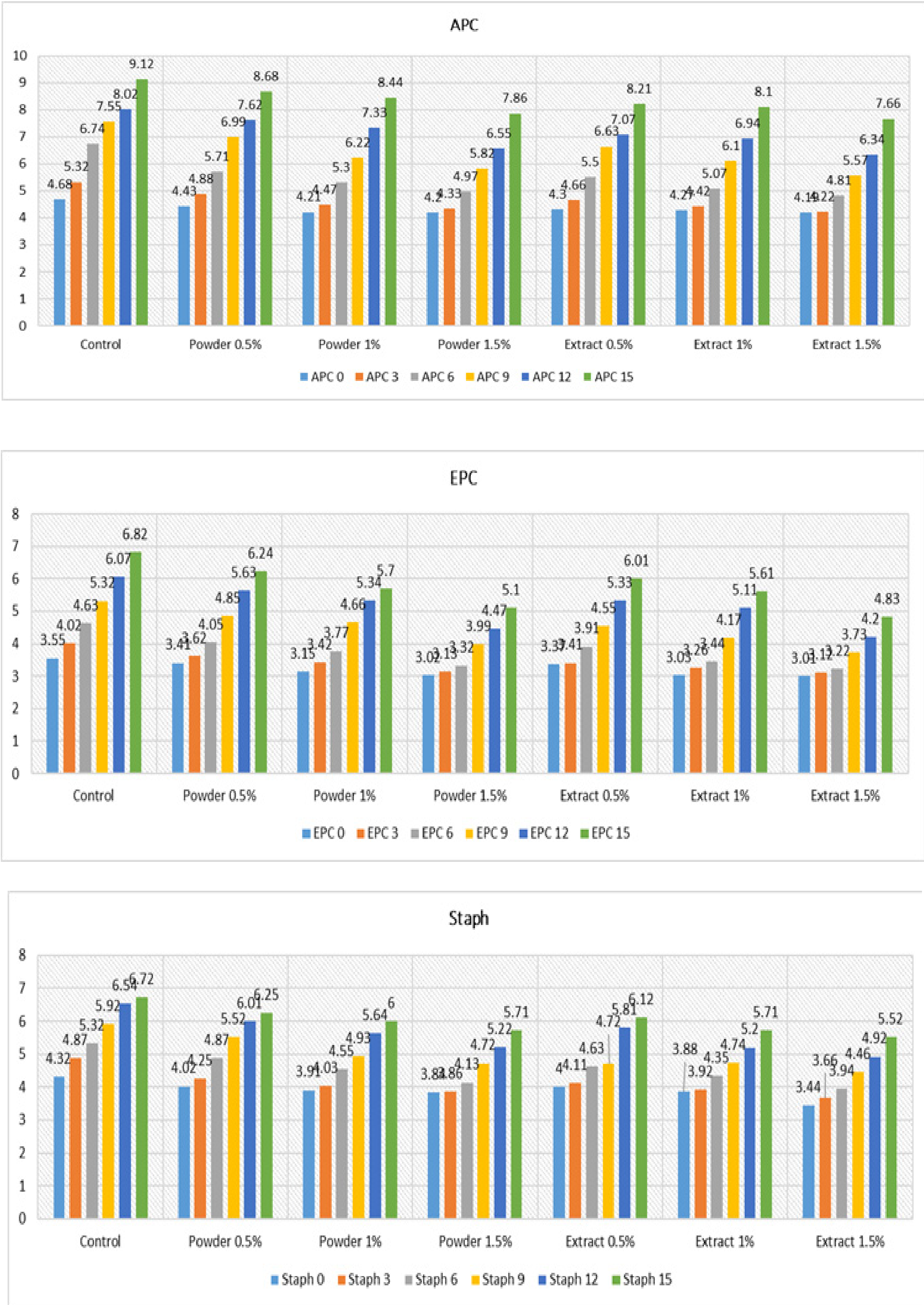


**Fig. 3. Effects of clove powder and its extract at different concentrations on aerobic plate count (APC), Enterobacteriaceae count (EBC), and *S. aureus* count in meatballs during cold storage at 3±1°C.**

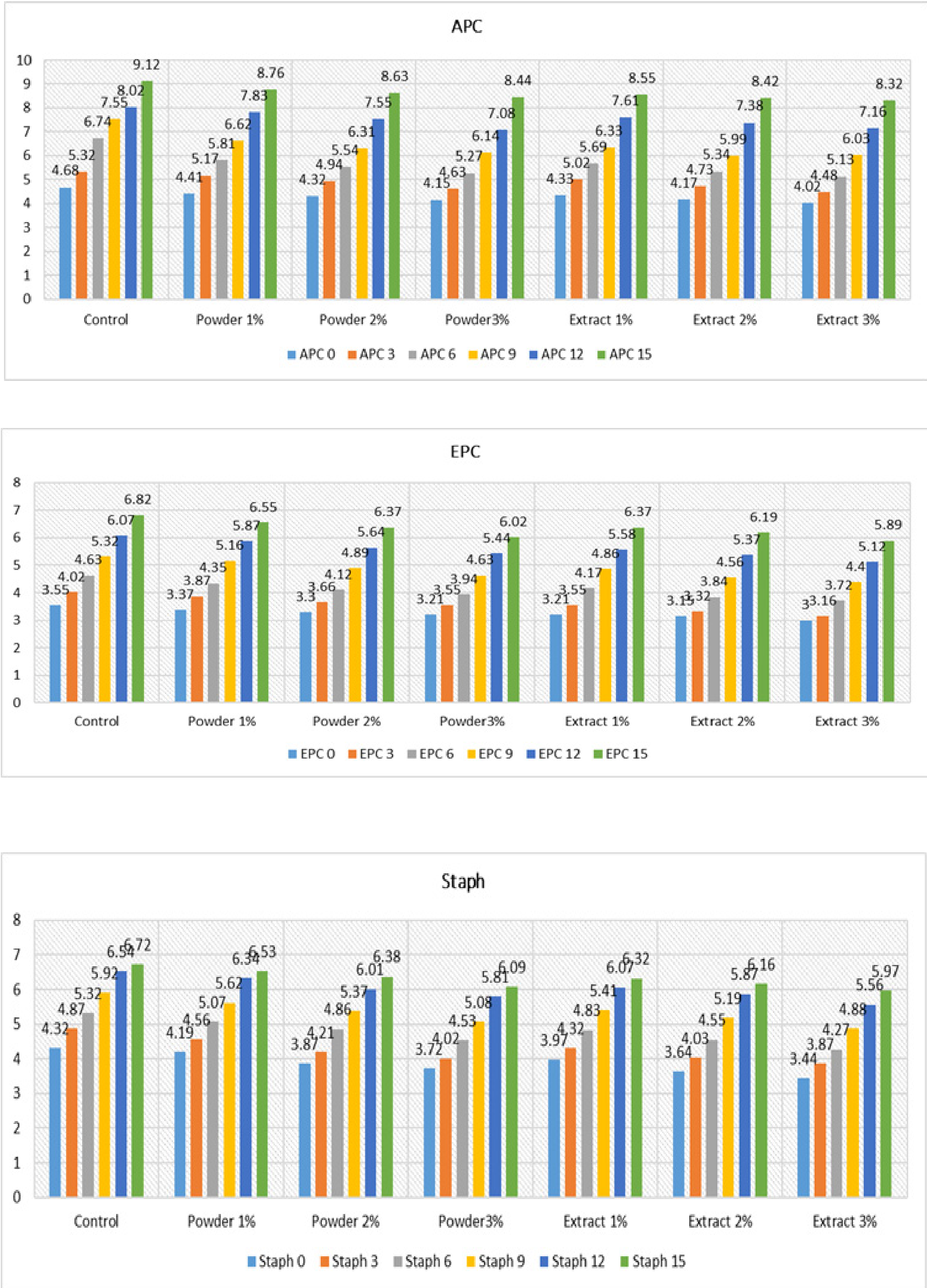


**Fig. 4. Effects of piper cubeba powder and its extract at different concentrations on aerobic plate count (APC), Enterobacteriaceae count (EBC), and *S. aureus* count in meatballs during cold storage at 3±1°C.**

**TABLE 4. Effect of addition of piper cubeba powder and its extract at different concentrations on microbial quality of meatballs during cold storage at 3±1°C.**

| Piper cubeba | Day | Control | Powder | | | Extract | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1% | 2% | 3% | 1% | 2% | 3% |
| **APC** | 0 | 4.68 q | 4.41 r | 4.32 rs | 4.15 st | 4.33 rs | 4.17 st | 4.02 t |
| | 3 | 5.32 mn | 5.17 no | 4.94 p | 4.63 q | 5.02 op | 4.73 q | 4.48 qr |
| | 6 | 6.74 h | 5.81 k | 5.54 lm | 5.27 n | 5.69 kl | 5.34 mn | 5.13 nop |
| | 9 | 7.55 f | 6.62 h | 6.31 i | 6.14 ij | 6.33 i | 5.99 j | 6.03 j |
| | 12 | 8.02 e | 7.83 e | 7.55 f | 7.08 g | 7.61 f | 7.38 f | 7.16 g |
| | 15 | 9.12 a | 8.76 b | 8.63 bc | 8.44 cd | 8.55 c | 8.42 cd | 8.32 d |
| **LSD (0.05)** | | | | **0.17** | | | | |
| **EBC** | 0 | 3.55 rs | 3.37 st | 3.30 tu | 3.21 tu | 3.21 tu | 3.15 u | 3.00 v |
| | 3 | 4.02 nop | 3.87 pq | 3.66 r | 3.55 rs | 3.55 rs | 3.32 tu | 3.16 u |
| | 6 | 4.63 l | 4.35 m | 4.12 no | 3.94 op | 4.17 n | 3.84 pq | 3.72 qr |
| | 9 | 5.32 hi | 5.16 ij | 4.89 k | 4.63 l | 4.86 k | 4.56 l | 4.40 m |
| | 12 | 6.07 de | 5.87 f | 5.64 g | 5.44 h | 5.58 g | 5.37 h | 5.12 j |
| | 15 | 6.82 a | 6.55 b | 6.37 c | 6.02 def | 6.37 c | 6.19 d | 5.89 ef |
| **LSD (0.05)** | | | | **0.128** | | | | |
| ***S. aureus* count** | 0 | 4.32 o | 4.19 o | 3.87 q | 3.72 r | 3.97 pq | 3.64 r | 3.44 s |
| | 3 | 4.87 m | 4.56 n | 4.21 o | 4.02 p | 4.32 o | 4.03 p | 3.87 q |
| | 6 | 5.32 jk | 5.07 l | 4.86 m | 4.53 n | 4.83 m | 4.55 n | 4.27 o |
| | 9 | 5.92 fgh | 5.62 i | 5.37 j | 5.08 l | 5.41 j | 5.19 kl | 4.88 m |
| | 12 | 6.54 b | 6.34 c | 6.01 efg | 5.81 h | 6.07 def | 5.87 gh | 5.56 i |
| | 15 | 6.72 a | 6.53 b | 6.38 c | 6.09 de | 6.32 c | 6.16 d | 5.97 efg |
| **LSD (0.05)** | | | | **0.1** | | | | |

Duncan's letters for statistical analysis: "a" is expressed as the highest values and descending to "z" which is expressed as the lowest values. Values with common letters are considered to have non-significant differences between them at the P<0.05 level.

*Effect of natural additives and their extracts on EBCs in meatballs during cold storage at 3±1°C*

*Paprika powders and their extracts*

Results in Table 2 indicated the effect of paprika powder and its extracts on EBCs of meatballs throughout the storage at 3±1°C. The initial EBCs (day 0) in meatballs of control and both paprika powder and their extracts and ranged from 3.55 and 3.00 log10 cfu/g with no substantial alteration between treated and control samples. By the day 3, however, meatballs treated with either paprika powder (8%) or paprika extracts (4% and 8%) revealed a noteworthy decline (P>0.05) in EBCs relative to the control (3.20, 3.41, and 3.22, respectively Vs 4.02) log 10 CFU/g.

*Clove powders and their extracts*

The effect of cloves powder and their extracts on EBCs of meatballs during storage at 3±1°C is shown in Table 3. The initial mean EBCs (day 0) in meatballs of control and both clove powders and their extracts ranged from 3.55 and 3.01 log10 cfu/g with no statistically significant difference among treated and control samples. By the day 3, meatballs treated with either clove powder (1.5%) or cloves extracts (1% and 1.5%) revealed a significant decline (P>0.05) in Enterobacteriaceae count. On days 6, 9, and 12 of storage, meatballs of treated with either cloves powder (1% and 1.5%) or cloves extracts (0.5%, 1%, and 1.5%) revealed a significant reduction (P>0.05) when compared with the control. By the end of the storage period (15 days), the meatballs of all treated samples showed a significant difference (P>0.05) when relative to the control sample.

*Piper cubeba powders and their extracts*

The initial mean Enterobacteriaceae count in control samples was high (3.55 log10 CFU/g) at the beginning of the experiment which significantly increased to 6.82 log10 CFU/g on the last day of storage. Meanwhile, the addition of piper cubeba to meat samples produced an instant lowering of the bacterial counts on day 0 which was significant at all concentrations of both powder and extract form except for 1% cubeba powder.

Effect of natural additives and their extracts on *S. aureus* count in meatballs during cold storage at 3±1°C

*Paprika powders and their extracts*

The effect of paprika powder and its extracts on *S. aureus* count of meatballs during storage at 3±1°C was presented in Table 2. The initial *S. aureus* count (day 0) in meatballs of control and both paprika powder and their extracts ranged from 4.32 and 3.60 log10 CFU/g with a noteworthy variance between the treated and control samples with 8% extract.

On days 6$^{th}$ and 9$^{th}$ of storage, meatballs of both 8% paprika powder and its extracts (4% and 8%) displayed a significant decline when compared with the control. On days 12 and 15 of storage, a considerable reduction (P>0.05) in *S. aureus* counts among samples of 8% powder and 4%, 8% extracts was observed.

*Clove powders and their extracts*

The effect of cloves powder and their extracts on the *S. aureus* count of meatballs during storage at 3±1°C is shown in Table 3. The initial *S. aureus* count (day 0) in meatballs of control and both clove powders and their extracts ranged from 4.32 to 3.44 log10 cfu/g with no statistically significant difference among treated and control samples except for clove extract (1.5%). By days 6, and 9 of storage, meatballs of both clove powder (1% and 1.5%) and cloves extracts (0.5%, 1%, and 1.5%) revealed a significant decline when compared with the control. By days 12 and 15 of storage, a significant reduction was achieved (P>0.05) in *S. aureus* count among all treated samples and the control sample.

*Piper cubeba powders and their extracts*

Results obtained in Table 4 showed that the *S. aureus* count of control samples was 4.32 log10 CFU/g initially but steadily increased with storage time, reaching 6.72 log10 CFU/g at day 15. The addition of piper cubeba significantly lowered the *S. aureus* contamination levels in powder treated samples to 6.53, 6.38, and 6.09 log10 CFU/g for 1, 2, and 3% concentrations and in extract-treated samples to 6.32, 6.16 and 5.97 log10 CFU/g, respectively, at the end of storage period.

## Discussion

Sensory traits such as flavor, tenderness, juiciness, and acceptability are the greatest significant factors to investigate the customer approval and shelf life of meat [16]. In the present study and based on the statistical analysis, no significant variances (P<0.05) were noticed in the feature's meatballs flavor, tenderness, juiciness, and overall acceptability among control and all treated samples. Also, the results revealed certain improvements in characteristics of meatballs in all treated samples when relative to the control one. These findings specified that these natural additives and their extracts can be added to meatballs without any negative effects on consumer perception. These results are matched with those of Zhang et al. [9], who observed non-significant (p > 0.05) variances in texture and flavor scores among clove extracts added sausages and the control. Moreover, Ann and Rukayadi [11] found that the addition of piper cubeba extract at different concentrations to chicken meat did not affect the odor, texture, and overall acceptability. On the other hand, Mokhtar and Youssef [16] notified that clove extracts added to beef burgers presented considerably (p<0.05) higher values of color and odor as sensory qualities than those of the control.

The aerobic plate counts estimate the total number of feasible aerobic bacteria per gram or milliliter of a food product. It is the supreme imperative information applied to assess the quality, spoilage, and safety of meat and other food [17].

The effect of natural additives and their extracts e.g. paprika powders and their extracts on APCs in meatballs at 3±1°C revealed that on the day 6th of storage, all treated samples revealed a significant reduction when compared with the control one, while by the 9th and 12th day, there is a significant difference between the control group with the concentrations of 4% and 8% of paprika powder and with the three concentrations of the extract. By the end of the storage period (day 15), control samples showed a high APCs of

9.12 log10 CFU/g, which reveals that there is a significant difference between 8%powder and the concentrations of 4% and 8% extract. The total APCs of control meatballs reached 7.55 log10 CFU/g (above the acceptable limit of 7 log10 CFU/g) [18] by the day 9th and meatballs were treated with either (2%) powder or extract while by the day 12th until the 15the day, meatballs treated with either paprika powder or extracts revealed higher bacterial counts values. Some researchers showed that paprika has the highest antibacterial activity as all the test organisms were susceptible to it at 12.5 and 25.0 mg/ml [19].

Consequently, pepper-derived products may participate in the development of safe and novel ingredients that could be utilized to control products spoilage and/or foodborne pathogens in foods, avoiding the utility of other synthetic preservers, such as sodium benzoate, nitrite or sodium metabisulfite, which have been infrequently related to potential generation of nitrosamines and allergic reactions [20]. Red pepper (Capsicum annuum) is generally utilized to supplement food flavors. Nevertheless, Red pepper comprises various phytochemical compounds, such as phenols, flavonoids, capsaicinoids, carotenoids, and vitamins that are responsible for privileged antimicrobial, anti-inflammatory, antioxidant, antiviral activities, and anticancer nutrients of pepper [21].

On the other hand, the impact of clove powders and their extracts demonstrated that by the days 6 and 9 of storage, all treated samples revealed a substantial reduction in comparison to the control sample, while by the end of the storage period (day 15), the control sample displayed a surprising APCs of 9.12 log10 CFU/g, while meatballs treated with both clove powders and their extracts demonstrated incredible decline (P>0.05) in APCs in comparison to the control sample. The total APCs of control meatballs reached 7.55 log10 CFU/g (above the acceptable limit of 7 log10 CFU/g) [18] by the day 9 of storage, while meatballs treated with either clove powders (0.5% and 1%) or clove extracts (0.5%) reached such limit by the day 12 of storage, however, meatballs treated with either clove powder (1.5%) or clove extracts (1% and 1.5%) revealed higher bacterial counts values at the day 15 of storage, which represents a noticeable growth in the shelf life of both clove powders and their extracts-treated meatballs, and shelf life extension considerably enhanced as the concentration of either clove powders or their extracts increased from 0.5% to 1.5% levels.

Similarly, Sivropoulou et al. [22] reported that the antimicrobial effect of essential oils is concentration-dependent. The control meatball samples showed a surface slime on day 8 of storage, while treated meatballs retained their feature until the day 12 for either clove powders (0.5% and 1%) or clove extract (0.5%)-treated meatballs, and the day 15 for either clove powder (1.5%) or clove extracts (1% and 1.5%)-treated meatballs. These observations agreed with APC enumeration.

The antibacterial activity of clove may be attributed to the several constituents offered by this plant such as eugenol, eugenol acetate, 2-heptanone, beta-caryophyllene, acetyl eugenoll, methyl salicylate, alpha-humulene, isoeugenol, phenyl prostanoids, methyl eugenol, dehydrodieugenol, trans-confireryl biflorin, kaempferol, aldehyde, rhamnocitrin, ellagic acid myricetin, gallic acid, and oleanolic acid [10]. The microbial activity of clove has been recorded previously by Sharma et al. [23] who established that the addition of clove essential oils expressively (P<0.05) decreased the total viable bacterial counts with an increase in concentration from 0.125% to 1% levels in the ground beef. Ali et al. [24] indicated that clove powder and its extracts exhibited significant antibacterial effects against total psychrophilic count and mesophilic count during storage of fresh sausage. Similarly, Omuero et al. [25] specified that clove extracts accessible a potent antimicrobial potency against the growth of some Gram-negative and Gram-positive bacterial and the fungal strains, and *Candida albicans* in fresh chicken meat.

Alternatively, from the impacts of piper cubeba powders and their extracts, the number of bacteria started to increase during storage reaching 8.44-8.76 log10 CFU/g for cubeba powder and 8.32-8.55 log10 CFU/g for extract-treated meat by the end of the experiment. The samples exceeded the maximum permissible level of total viable counts for minced meat (6.7 log10 CFU/ g) [26] on day 6 for control samples (6.74 log10 CFU/g) and on day 12 for both powder (7.08- 7.83 log10 CFU/g) and extract form (7.16- 7.61 log10 CFU/g) resulted in prolonged storage life of piper cubeba added samples.

The inhibitory action of piper cubeba reached its maximum effect on day 9; since, the microbial counts on spice powder-treated meat decreased significantly by 0.93, 1.24, and 1.41 logs at the concentration of 1, 2, and 3%, whereas, extract treated samples showed greater reduction of microbial load by 1.22, 1.56 and 1.52 logs, respectively, indicating that piper cubeba extract had a more inhibitory effect on bacterial populations than cubeba powder. These results were in agreement with Ann and Rukayadi [11] who found that application of piper cubeba extract with concentrations of 0.5% and 5% on chicken meat samples showed a decline in total bacterial counts by 0.48 and 1.7 logs, respectively, for samples obtained from wet markets at day 7 of refrigerated storage. This inhibitory effect may be attributed to the presence of phenols, and flavonoids in an aqueous solution of piper cubeba [27].

Furthermore, phenol compound has precipitate activity on microbial enzymes resulting in inhibiting and losing their function [28]. Also, the hydroxyl group in flavonoids can combine with cell wall proteins and break down the bacterial cell wall [29].

Enterobacteriaceae are the greatest significant indicators applied to assess the bad treatment or bad hygiene of food. The attendance of a great number might be an indication of their multiplication and suggest the multiplication of other microbes [30]. The effect of natural additives and their extracts on EBCs in meatballs during cold storage was run for paprika powders and their extracts. By the days 6th, 9th, and 12th of storage, meatballs of treated with either paprika powder (4% and 8%) or its extracts (2, 4, and 8%) revealed a substantial decrease (P>0.05) relative to the control ones. By the end of the storage period (15 days), meatballs of treated samples of paprika powder (4% and 8%) or its extracts (2, 4, and 8%) showed a substantial variance (P>0.05) when compared with the control one. The pungency of peppers is related to the accumulation of capsaicinoids, while its antimicrobial property is attributed to capsaicin that inhibits the growth of several bacteria including *Escherichia coli*, *Bacillus subtilis*, and *Salmonella* Typhimurium [31]. Also, Paprika showed the broadest antibacterial activity to the test organisms such as Salmonella and E. coli [19].

Some researchers also stated a definite antimicrobial activity of capsinosides, the derivatives of Capsinoids, against Gram-positive bacterial species, which presented disruption of biofilm formation that probable owing to their aptitude of chelating calcium, a mineral which affects biofilm architecture progress. Based on these results, Careaga et al. [32] reported a minimum inhibitory concentration of a bell pepper extract of 1.5 mL/100 gm in minced beef preventing *S.* Typhimurium growth.

For clove powders and their extracts, no studies have been reported regarding the antibacterial consequence of cloves powder or their extracts on EBCs in meat and meat products, nonetheless in vitro studies were accomplished by Tshabalala et al. [33] and Sofia et al. [34] that determined the antimicrobial activity of diverse plants' spice such as clove, thyme, black seed cumin, mustard, ginger, mint, garlic, and cinnamon against Enterobacteriaceae including *Escherichia coli* with the use of the disc diffusion technique indicating that the clove extract exerts exceptional antibacterial activity against a definite bacterial species compared to other spices and they proposed the utility of herbal spices as natural preservatives to replace partially or completely the chemical preservatives. Zengin and Baysal [35] reported that clove oil restricted the growth of native coliforms and Salmonella typhimurium artificially inoculated in the ground beef. Alternatively, Kuang et al. [36] identified that clove powder exhibited potent minimum inhibitory concentration (MIC) values against Brochothrix thermosphacta, E. coli, Pseudomonas fluorescens, and Lactobacillus rhamnosus in vitro and they recommended that traditional spice powders had extensive application prospects using essential oils owing to their low cost and amazing safety.

For piper cubeba powders and their extracts, the spice treatments were more effective in the reduction of Enterobacteriaceae on day 6 (0.16- 0.69 logs) for powder form and on day 12 (0.49-0.95 logs) for extract form. The bacterial populations at the end of the experiment ranged from 6.02 to 6.55 log10 CFU/g and from 5.89 to 6.37 log10 CFU/g in powder and extract treated samples, respectively. During the storage period, the highest reduction level was achieved by 3% piper cubeba extract concentration (0.55-0.95 logs) while, a 1% concentration of cubeba powder had the weakest effect (0.15- 0.28 logs), especially in the first 3 days of storage. Likewise, Al Rdheha et al. [37], Al-Tememy [27], and Ann and Rukayadi [11] recorded that piper cubeba extract was effective against Gram-negative bacteria, especially *Escherichia coli*.

The effect of natural additives and their extracts on *Staphylococcus aureus* count in meatballs during cold storage was assessed using paprika powders and their extracts. Our findings agreed with Salih [38] results who revealed that the oil extract of paprika influenced antimicrobial potency against *S. aureus, E. coli, P. aeruginosa*, and *K. pneumonia* owing to the attendance of capsanthin from paprika. Furthermore, oleoresins including paprika extract at concentrations above 5000 ppm had antimicrobial properties by rupturing bacterial membrane. This mechanism might affect the growth inhibition of foodborne pathogens such as *L. monocytogenes, S. aureus, B. cereus*, *Salmonella*, and *P. aeruginosa* species [39]. Ram Kumar and Pranny [40] have besides found that aqueous, alcohol and methanol extracts of pepper presented antimicrobial activities against *S. aureus* microbial species.

The results of clove powders and their extracts agreed with Nassan et al. [41] who determined the in vitro antimicrobial potency of clove water extract against *S. aureus* with the use of the agar well diffusion technique and the results recommended that clove water extract employs incredible antimicrobial potency against bacterial strains. Otherwise, Kuang et al. [36] specified that cloves powder had potent in vitro antimicrobial potency against *S. aureus*.

In the case of using piper cubeba powders and their extracts, the highest concentration of cubeba extract (3%) exhibited the greatest inhibitory effect (0.75-1.05 logs) related to control especially on day 6 and had a long storage life. These findings were supported by Al-Tememy [27] who found that the fruit extracts of piper cubeba have antibacterial action against *Staphylococcus aureus* tested strains. Also, Khan and Siddiqui [42] found that piper cubeba extracts exhibited a good antibacterial activity on Staphylococcus albus.

In general, the application of piper cubeba was more effective against *S. aureus* compared to Enterobacteriaceae and the inhibitory effect was concentration-dependent. Similar findings were observed by Rukayadi et al. [43] who found that a lower concentration of piper cubeba extract is required to kill *S. aureus* (3.2 mg/ml) than *E. coli* (6.4 mg/ml). In contrast, Al-Tememy [27] reported that aqueous extract showed higher antibacterial activity on Gram-negative *E. coli* and the lowest activity on *S. aureus*. These differences may be attributed to the difference in initial bacterial load, bacterial strains, and used concentrations which may affect the inhibitory action of added herbs.

## Conclusion

In conclusion, paprika, clove, piper cubeba, and their derived extracts can be contributed to the development of safe and new ingredients that could be used to control spoilage microbes and foodborne pathogens in foods as well as prolong their shelf life instead of the harmful synthetic preservatives.

*Ethics approval and consent to participate*

Not applicable.

*Consent for publication*

The authors have seen the draft and the final copy of this article and agree with its publication.

*Availability of data and material*

The preparation and extraction processes of paprika, clove, and piper cubeba were done in the Food Microbiology Laboratory, Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Egypt. Powder of paprika (Capsicum Annuum L), clove (Syzygium aromaticum), and cubeba piper Linn.

*Competing interests*

The authors state that they have no competing interest.

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*Author's contributions*

H. Zaher, H. El-Sherbiny, A.Y. El-Khateeb, R. Elkenany and A. Zakaria participated in the experiments, analyzing the data, drawing figures, Tables, and writing the manuscript. All authors reviewed the manuscript.

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## التأثير الوقائي لتوابل المنكهات الطبيعية المختارة ومستخلصاتها على الجودة الميكروبية لكرات اللحم أثناء التخزين البارد

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[٣] قسم الكيمياء الزراعية - كلية الزراعة - جامعة المنصورة - المنصورة - مصر.

* [١]قسم البكتريا والمناعة والفطريات - كلية الطب البيطري - جامعة المنصورة - المنصورة - مصر.

لقد ثبت أن التوابل مثل البابريكا والقرنفل وكوبيبا بايبر ومستخلصها تمتلك إمكانات قوية لمضادات الميكروبات ضد الكائنات الدقيقة المسببة لتلف الطعام ومسببات الأمراض. قد يؤدي تلوث اللحوم المفرومة بمسببات الأمراض إلى التسمم الغذائي. وقد أدى هذا إلى فرض البحث عن العوامل المضادة للميكروبات التي يمكن أن تزيد من العمر الافتراضي للحوم المفرومة. يهدف هذا العمل إلى تقييم التأثير المضاد للميكروبات للبابريكا والقرنفل ومسحوق بايبر كوببا ومستخلصهم بتركيزات مختلفة ضد عدد الصفائح الهوائية وعدد البكتيريا المعوية والمكورات العنقودية الذهبية. قدرة هذه المضافات الطبيعية على إطالة العمر الافتراضي وتحسين جودة اللحم البقري المفروم، وتحسين تراكيز هذه البهارات وخلاصتها في اللحم المفروم. لم يُظهر التقييم الحسي لكرات اللحم المطبوخة التي تحتوي على مسحوق الفلفل الحلو والقرنفل والبايبا المضافة ومستخلصاتها أي اختلافات كبيرة في نكهة كرات اللحم المميزة وطراوتها وعصيرها ومقبوليتها بشكل عام بين مجموعة المقارنة وجميع العينات المعالجة ولم تظهر أي آثار سلبية على المستهلك تصور. أظهر تأثير المضافات الطبيعية ومستخلصاتها على ناقلات المواد الغذائية في كرات اللحم أثناء التخزين البارد أن التوابل المدروسة ومستخلصاتها قد تكون مصدرًا لتطوير مكونات جديدة وآمنة يمكن استخدامها للسيطرة على مسببات الأمراض المنقولة بالغذاء في الأطعمة و / أو تلف المنتجات. أدت عينات اللحوم المعالجة إلى إطالة العمر التخزيني لعينات بايبر كوببا والقرنفل والبابريكا المضافة مقارنة بمجموعة المقارنة. وجد أن التأثير المضاد للميكروبات لهذه التوابل يعتمد على التركيز ويعزى إلى مكوناتها الكيميائية النباتية. تم الإبلاغ عن زيادة في العمر الافتراضي وانخفاض في مستويات التلوث بالبكتيريا المعوية والمكورات العنقودية الذهبية في كرات اللحم المعالجة بالفلفل الحلو والقرنفل والبايبر.

**الكلمات الدالة:** مضادات الميكروبات ، اللحم المفروم ، التقييم الحسي ، الصلاحية ، التوابل.