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# CHEMICAL AND MICROBIOLOGICAL EVALUATION OF BLACK PEPPER AS A NATURAL FOOD PRESERVATIVE

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**ABSTRACT:** Now, in industrial food production usually use of preservatives is common practice. Hence, there is growing concern among consumers among the harmful effects of various chemical preservatives. As a result, there is an increasing demand for foods that has undergone a few or no processing. Recently, there are considerable interest in finding alternatives to chemical preservatives for use in such industries in the natural forms. Possible solutions include the use of natural ingredient from plant extracts as antioxidant and antimicrobial agents. This paper provides a practice solution for the replacement of artificial preservatives by natural traditional chemical compounds in some meat products. While concentrated piperine approximately of 95% extracted from black pepper species (*Piper nigrum*) was investigated as a natural preservative against the foodborne bacteria associated with two types of protein products (meat and chicken luncheon) collected from local market. The black pepper antimicrobial characteristics on some spoilage microorganisms was studied in two famous Egyptian companies. The chemical composition of crude luncheons was quite significantly different in moisture and pH, while total fats and proteins had high significant differences at ($p<0.05$). The total bacterial counts in the first days after black pepper addition were decreased and showed high significant differences ($p<0.001$) for the two types of luncheons (chicken and beef) in the two companies, and the addition of piperine was more effective from the grinded or complete black pepper seeds on the total bacterial counts which decreased the limits of studied product from $3.4\times10^2$ to $8.5\times10^2$ cfu after 96 hours. Moreover, a significant negative correlation with inverse relationship between the piperine addition and the total bacterial counting during the time taken for the luncheon to spoil. In addition, black pepper as spices help to preserve as well as improve the shelf life of luncheons items. The static contact and shelf life measurements were performed as a function of time in order to investigate the biochemical properties of the luncheons samples especially those treated with the piperine formula to be more effective as a preservative due to its greater degree of spicy in the Scoville scale (higher pungency content) and better penetration into the luncheon manufactures.

**Keywords:** Black pepper, Piperine, Luncheon, Natural preservatives.

## INTRODUCTION

Nowadays, there has been an increase in food consumer awareness not only of the calorific value, but also of the food content of individual nutrients and components together with their risks and health benefits (Özvural *et al*., 2016). The adverse potentials of common chemical preservatives are leading producers to look for new alternatives in food processing. One such possibility is the use of ingredient from plant origins, in the form of fresh powder or aqueous extracts rich in antimicrobial active compounds. Other options are returning to traditional production extraction methods for using the specific ingredient products that have antimicrobial properties (Marta Sośnicka, 2019). In developed countries, the augmentation rate of foodporn diseases in civilization caused by lifestyle and excessive food consumption is encouraging many people to turn to traditional and poor hygienic raw materials, which are perceived to be more wholesome. A healthy lifestyle involves not only different physical

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activity habits, but also purchasing natural, less processed products, without artificial additives and preservatives. Therefore, food manufactures and technologists face the challenge of supplying products without chemical additives or dyes, but at the same time of high quality and with a long shelf life. Although there is no formal definition of what constitutes a 'clean label', leading to a variety of approaches by manufacturers and different interpretations by consumers, the common denominator is the lack of or low processing, a short list of ingredients, a lack of chemical additives (Karwowska and Kononiuk, 2020). In the same trend, black pepper (*Piper nigrum L*) is known as the king of spices. It is widely used as a spice worldwide and has various applications, including medicinal and nutritional uses. Piperine (flavonoid compound), its active compound in black pepper, shows an important physiological effects, such as antioxidant and antibacterial properties, additionally to it has been enhances digestion. It also enhances drug bioavailability and exhibits non-genotoxic, anti-mutagenic, and antitumor properties (Scotter and Castle 2004). Black pepper was found to be rich in other active aromatic compounds, lignans amides, polyphenols, alkaloids and specific flavonoids (Carocho *et al.,* 2014). The primary concentrated compound of black pepper is piperine (Comi *et al.,* 2015). The antibacterial activities of piperine were investigated against a group of Gram-negative bacteria, including *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumonia* and group of Gram-positive bacteria such as *Bacillus subtilis*, *Micrococcus albus*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*. Also, piperine exhibited significant antibacterial activity against the most common industrial bacterial strains, with a minimum inhibitory concentration of 60 mg/L (Sakkas *et al*., 2016). The most important that, black pepper is proposed use as the most successful natural food preservative due to its highly antimicrobial and antioxidant properties. Notably, its antioxidant capacity rivals that of synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used in food preservation. Consequently, naturally occurring antioxidants may surpass BHT and BHA in terms of their capability to neutralize mutagens in food (Solórzano-Santos and Miranda-Novales, 2012). Considering the antimicrobial and antioxidant attributes found in black-pepper-derived products, investigations have achieved their certain utility for food preservation as antimicrobial agents especially the meat products, with the aim of managing foodborne pathogens, increasing shelf life and mitigating product spoilage.

## MATERIALS AND METHODS

### 1. Plant extracts preparation.

The black pepper seeds were purchased from a big store in the local market, air-dried plant samples at 50°C were used as source of piperine. The mild grinding seeds were finely homogenized. About 0.5 g powder sample was weighed and transferred to a 250 ml round bottom flask fitted with a reflux condenser. Pure ethanol (50 ml) was added, swirl and mixture were refluxed for 4 hours then it was re-cooled to the room temperature. The solution was filtered and collected in a 100 ml volumetric flask. The solvent was evaporated by a rotary evaporator to obtain the crude extract (AOAC, 2000).

### 2. Chemical composition for black pepper

The major chemical composition (ash, moisture, crude fats, total carbohydrates, and crude protein) for black pepper were determined according to the protocols of Association of Official Analytical Chemists (AOAC, 2000).

### 3. Fractionation of phenolic compounds for black pepper extract by using HPLC.

The quantitative analysis of phenolic compounds in black pepper ethanolic extract determined by using high performance liquid chromatography (HPLC) analysis at the Faculty of Agriculture Research park, Cairo University according to the method of Goupy *et al*. (1999).

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Kromasil $C_{18}$ column (4.6 mm x 250 mm inner dimeter, 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 343 nm. The injection volume was 0.45 µm for each of the sample solutions. The column temperature was maintained at 40°C and the gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1 $ml.min^{-1}$, standards were dissolved in a mobile phase and injected into HPLC, the retention time and peaks area were used to calculate phenolic compounds concentration by using the data of Agilent software according to the protocol of (Gauthami *et al*., 2015).

## 4. Microbial strain and culture media

The identified foodborne microbial strains were isolated from chicken and meat luncheon. The common cocci form bacterial strains ($G^{+ve}$ cocci), and *Bacilli* forms ($G^{-ve}$ short rods and $G^{+ve}$ sporulated long rods) were morphological identified. Bacterial strains were cultivated aerobically in Luria-Bertani [LB] medium, the pre-cultures were performed during 24 h of fermentation (log phase) at 35±2 °C, while Mueller-Hinton [MH] agar was used for the disc diffusion method according to the protocol described by the clinical and laboratory standards institute guidelines (CLSI, 2021).

## 5. Determination of antimicrobial effective concentration

The modified micro-double dilution method was used, according to the method of Saginur *et al*., 2006 as follows: Add 1 ml of Mueller-Hinton broth to two groups of 15 test tubes. 1 ml of pre-prepared concentrations of 200 mg of extract piperine at broth media, 1000 µl was added to tube No.1. The materials were mixed by using a micropipette by withdrawing the culture 10 times to obtain a concentration of 10 mg/ml for piperine. Progressive dilutions were transferred form tube No.1 to other tubes, the process was repeated up to tube No.15. and mix in the same previous way to obtain a concentration of 0.5 mg/ml for piperine. After that, 100 µl were added of various bacterial suspension incubated for 18 hours at a concentration of $1.5x10^5$ cells/ml compared to the third of McFarland tubes. Add to tube No.16 the Mueller-Hinton broth and bacterial suspension only as positive control. Tube No.17 contains only the Mueller-Hinton broth as negative control. The tubes were incubated at 35±2 °C for 24 hours. Examination of bacterial growth is visible and the minimal inhibitory concentrations (MICs) $mg.ml^{-1}$ of the piperine was observed (no visible growth). While the next dilution with no growth in the culture plate was considered the minimal bactericidal concentrations (MBCs) $mg.ml^{-1}$. Each experiment was carried out in triplicate. Stock solutions of the plant extract carried out by adding equivalents balances to 1 ml of dimethyl-sulph-oxide (DMSO) at each serial dilution, further progressive dilutions to obtain the final concentrations of zero, 5, 10, 15, 30 and 60 %, the growth control consisting of clear media and growth culture (negative and positive control) (CLSI, 2021).

## 6. Statistical analysis

Each experiment was carried out in triplicate and the mean values were recorded with its standard divisions. However, the final data were subjected to statistical analysis using the SPSS (Software version no.20). Differences between extracts were tested by one-way analysis of variance (ANOVA). Probability value for the statistical test was 0.5%. Also, Duncan test were used to compare the differences of the inhibition zones between control group with plants extracts (Fahim and Hussein, 2017).

# RESULTS AND DISCUSIONS

## 1. The chemical compositions

The obtained results in Table (1) indicated that black pepper seeds contain moisture 9.14%, total ash 5.42%, total lipids 8.57 %, crude protein

25.52%, and total carbohydrates 51.53 %. The results of this study are similar to those obtained by Govindarajan (1977), when studying the chemical composition of black pepper seeds.

**Table 1: The major chemical composition of black pepper.**

| Major composites | % |
|---|---|
| Moisture | 9.14 % |
| Ash | 5.42 % |
| Total carbohydrates | 51.35 % |
| Total protein | 25.52 % |
| Total lipids | 8.57 % |

## 2. Phenolic compounds fractionation by HPLC

Plants are successfully defended themselves with a complex set of preformed structures and inducible reactions. These type of defense response is often associated with a localized hypersensitive cell reaction (Singleton, *et al.,* 1999). Phenolic compounds in plants are mainly responsible in the interaction between the invader's microorganisms and the plant. They are toxic to these pathogeneses and their post-infection production and accumulation are more intense in resistant plant cultivars than in susceptible ones. Chlorogenic acid, caffeic acid, and *p*-coumaric acid are examples of such phenolic compounds. Although some of the individual phenolics may reach concentrations toxic to the pathogens, several of these compounds often accumulate simultaneously in the infected tissue, and it is possible that their combined toxic effect, rather than the fungitoxic impact of each one separately, is responsible for the inhibition of infection in resistant cultivars (Giordano *et al.,* 2023).

Twelve phenolic compounds of peppers extract could be identified and quantified by HPLC (Table 2 and Figure 1). The results indicate the presence of five phenolic compounds that are present in a large proportion compared to the rest of the isolated phenolic compounds, which are, in order, hesperidin (376.82 mg/Kg), quercetin (222.61 mg/Kg), resveratrol (144.26 mg/Kg), caffeic acid (93 mg/Kg), and rosemarinic acid (81.64 mg/Kg).

Accumulated polyphenol components in various pepper types is essentially found to be influenced with ripening stage and genotype Shaha *et al.* (2013). So, obtained data relating the identification of phenolic constituents of peppers extracts were not easy to compare with literature data. However, our data slightly in agreement with those of Shaimaa *et al.* (2016), who stated that hesperidin, quercetrin, rutin, catechol, oleuropein, chlorogenic, pyrogallol and 3-hydroxy tyrosol, were presented at higher levels than other phenolics in of pepper types. While Nagy *et al.* (2015) summarized that the major phenolic compounds in New Hybrids of black peppers were naringenin-di-glucoside, catechin, vanillic acid-derivative and luteolin-glucoside.

A few papers have shown that black pepper extracts obtained with the different solvent systems show good antioxidant activity (Nahak and Sahu, 2011; Sapam *et al.,* 2018). In a study of Nahak and Sahu, (2011), it was demonstrated that black pepper ethanolic extract had the best antioxidant activity in all analyzed concentrations compared to aqueous or methanolic extracts.

This indicates that not only phenolic compounds are responsible for the antioxidant and antibacterial activity of black pepper, but also other bioactive compounds that have been extracted, such as some of the alkaloids. It cannot be ruled out that the antioxidant activity of black pepper ethanolic extract may be a consequence of the synergistic effect of phenolic compounds with other biomolecules isolated from the plant material (Nahak and Sahu, 2011).

**Table 2: The minor chemical composition of black pepper (mg/Kg).**

| Minor composites | Retinal Time | Specific Area | Amount |
|---|---|---|---|
| Vanillic acid | 6.67 | 649.70 | 33.02 |
| Caffeic acid | 7.28 | 906.78 | 93.00 |
| P Coumaric | 9.13 | 479.82 | 2.92 |
| Rutin | 9.75 | 130.57 | 16.12 |
| O Coumaric | 10.31 | 374.57 | 32.79 |
| Myricetin | 10.90 | 334.31 | 66.15 |
| Resveratrol | 11.14 | 469.50 | 144.26 |
| Hesperidin | 12.46 | 2960.40 | 376.82 |
| Quercetin | 13.34 | 1203.08 | 222.61 |
| Rosemarinic acid | 13.86 | 254.27 | 81.64 |
| Apigenin | 14.99 | 250.88 | 35.25 |
| Kaempferol | 16.62 | 137.35 | 17.92 |
| Max concentrations | 12 components were detected | | 376.82 |

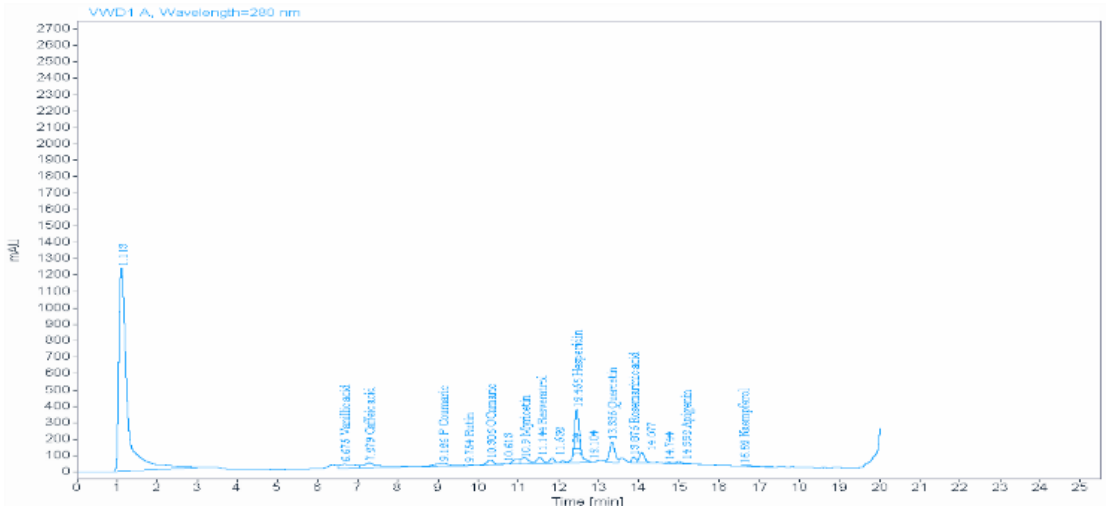



**Figure (1): HPLC profile analysis chart for phenolic components in the black pepper**

## 3. Bacterial investigation of meat and chicken luncheon products.

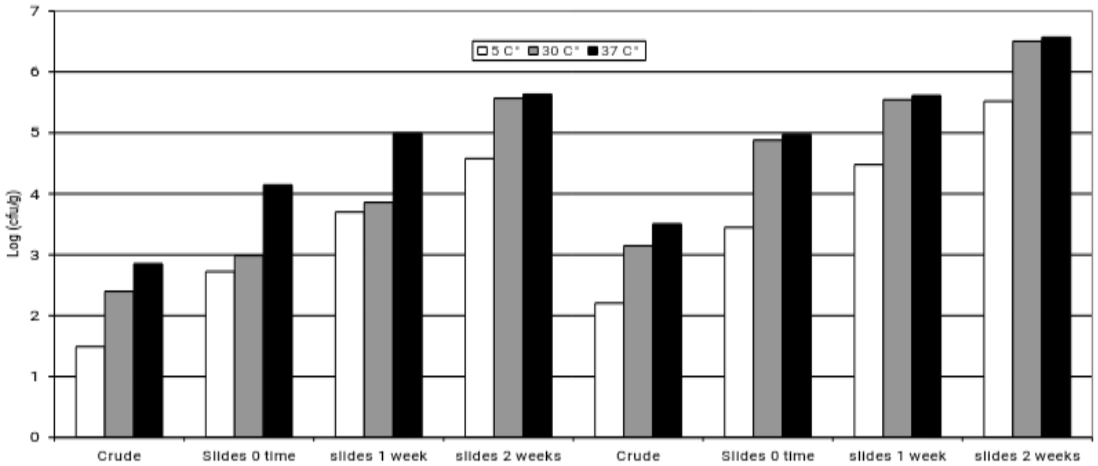
Seven different bacterial counts from two national meat and chicken luncheon products sold and consumed in Egypt. These meat and chicken products were (product 1) and (product 2) collected and stored during winter, spring and summer seasons. The bacterial isolates were determined and classified by microbiological technique. In addition, content of protein, fat, moisture and pH-value were also determined in samples under investigation. Also, the antibacterial activity of four plant extract oils were examined against the three bacterial genus isolated from the different luncheon products. For the Product (1) – Chicken luncheon: the data in Table (3) and Fig. (3) show the different bacterial counts in meat and chicken luncheon samples collected during winter season (1st samples group). The data mean of three different bacterial counts for chicken luncheon with its standard divisions, winter samples were calculated, i.e.,

The total bacterial counts ranged from 3.1 x 10 to 7.1 x $10^2$ cfu/g for crude luncheon and the total coliform ranged from 0.3 x 10 to 2.1 x 10 with absence of faecal coliform which were in normal ranges according to the Egyptian Standards (ES). While the total bacterial counts were increased by storage after sliding, since recorded 5.0 x $10^2$ to 1.4 x $10^4$, 5.0 x $10^3$ to 9.8 x $10^4$ and 3.8 x $10^4$ to 4.3 x $10^5$ cfu/g for the stored slides after 2 days, 1 week and 2 weeks from sliding, respect. Which were reached to higher than those recommended by (ES). These counts should be no more than$10^4$ cfu/g for total counts with the absence of faecal coliform and pathogenic bacteria and only $10^2$ cfu/g in case total coliform. While the results presented in Table (3) and Fig. (3) Also show the mean of the bacterial counts in chicken luncheon samples collected during spring season of the same company product. The (2nd samples group) It conspicuous that the mean number of total bacterial counts ranged from 4.5 x 10 to 6.9 x $10^2$ cfu/g for crud luncheon and the total coliform ranged from 0.7 x 10 to 2.5 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded 6.2 x $10^2$ to 2.7 x $10^4$, 4.2 x $10^3$ to 2.5 x $10^5$ and 5.3 x $10^4$ to 8.8 x $10^5$ cfu/g for the stored slides after 2 days, 1 week and 2 week from sliding, respectively.

**Table 3: Product-1 microbiological survey average by (cfu/g) during the year seasons.**

| Bacterial counts | | Chicken luncheon | | | | Meat luncheon | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Crude luncheon | Slides Zero time | slides first week | slides second week | Crude luncheon | Slides Zero time | slides first week | slides second week |
| Total counts | 5 C° | 3.1 x 10 | 5.3 x $10^2$ | 5.0 x $10^3$ | 3.8 x $10^4$ | 1.6 x $10^2$ | 2.8 x $10^3$ | 3.0 x $10^4$ | 3.3 x $10^5$ |
| | 30 C° | 2.5 x $10^2$ | 9.7 x $10^3$ | 7.2 x $10^4$ | 3.7 x $10^5$ | 1.4 x $10^3$ | 7.6 x $10^4$ | 3.5 x $10^5$ | 3.2 x $10^6$ |
| | 37 C° | 7.1 x $10^2$ | 1.4 x $10^4$ | 9.8 x $10^4$ | 4.3 x $10^5$ | 3.2 x $10^3$ | 9.4 x $10^4$ | 4.1 x $10^5$ | 3.7 x $10^6$ |
| Coliform counts | T coliform | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | F coliform | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Salmonella | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Shigella | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

*Samples were taken in three replicates.




**Chicken luncheon** **Meat luncheon**

**Figure 3 : product (1) - Log Colony forming units/ gram log (cfu/g).**

Also, the results presented in Table (3) and Fig. (3) show the mean of the bacterial counts in chicken luncheon samples collected during summer season of the same company product. The (3rd samples group). It conspicuous that the mean number of total bacterial counts ranged from 2.6 x 10 to 9.7 x $10^2$ cfu/g for crud luncheon and the total coliform ranged from 0.5 x 10 to 2.7 x 10 with the absence of faecal coliform and pathogenic bacteria which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded 7.4 x $10^2$ to 4.1 x $10^4$, 9.9 x $10^3$ to 5.2 x $10^5$ and 2.2 x $10^4$ to 7.7 x $10^5$ cfu/g for the stored slides after 2 days, 1 week and 2 week from sliding, respectively. Which also were reached to higher than those recommended by (ES). In the same concept, the product (1) -meat luncheon: from the data in Table (3) and Fig. (3) show the different bacterial counts in meat luncheon samples collected during winter season (1st samples group). The mean of three different bacterial counts, winter samples were calculated, i.e., The total bacterial counts ranged from 1.6 x $10^2$ to 3.2 x $10^2$ cfu/g for crud luncheon and the total coliform ranged from 0.5 x 10 to 2.5 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were increased by storage after sliding, since recorded 2.8 x $10^3$ to 3.4 x $10^5$, 7.6 x $10^4$ to 3.2 x $10^6$ and 9.4 x $10^4$ to 3.7 x $10^6$ cfu/g for the stored slides after 2 days, 1 week and 2 week from sliding, respectively. Which were reached to higher than those recommended by (ES). These counts should be no more than $10^4$ cfu/g for total counts with absence of faecal coliform and only $10^2$ cfu/g in case total coliform. While presented results show the mean of the bacterial counts in meat luncheon samples collected during spring season. The (2nd samples group) conspicuous that the mean number of total bacterial counts ranged from 1.1 x $10^2$ to 3.8 x $10^3$ cfu/g for crud luncheon and the total coliform ranged from 0.8 x 10 to 2.8 x 10 with absence of faecal coliform which were in normal ranges according to the (ES). And the total bacterial counts were also increased by storage after sliding, since recorded 3.7 x $10^3$ to 1.8 x $10^5$, 6.2 x $10^4$ to 4.3 x $10^6$ and 1.5 x $10^5$ to 5.9 x $10^6$ cfu/g for the stored slides after 2 days, 1 week and 2 weeks from sliding, respect. Which also were reached to higher than those recommended by (ES). The results show the mean of the bacterial counts in meat luncheon samples collected during summer season of the same company product. The (3rd samples group) It conspicuous that the mean number of total bacterial counts ranged from 4.5 x 10 to 6.9 x $10^2$ cfu/g for crude luncheon and the total coliform ranged from 0.7 x 10 to 2.5 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded 6.2 x $10^2$ to 2.7 x $10^4$, 4.2 x $10^3$ to 2.5 x $10^5$ and 5.3 x $10^4$ to 8.8 x $10^5$ cfu/g for the stored slides after 2 days, 1st week and 2nd week from sliding, respectively. Which also were reached to higher than those recommended by Egyptian Standards, these results are in harmony with (Beals, 2004 and Nabil *et al*., 2014).

**However, Product (2) – Chicken luncheon:** Concerning the results presented in Table (4) and Fig. (4) show the mean of the bacterial counts in chicken luncheon samples collected during winter season (1st samples group). The winter samples conspicuous that the mean number of total bacterial counts ranged from 2.6 x 10 to 9.7 x $10^2$ cfu/g for crud luncheon and the total coliform ranged from 0.6 x 10 to 3.4 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were increased by storage after sliding, since recorded 7.4 x $10^2$ to 2.2 x $10^4$, 4.7 x $10^3$ to 1.6 x $10^5$ and 4.1 x $10^4$ to 7.7 x $10^5$ cfu/g for the stored slides after 2 days, 1 week and 2 weeks from sliding, respect. Which were reached to higher than those recommended by (ES). While the 2nd samples group (spring samples) show that, the mean number of total bacterial counts ranged from 2.6 x 10 to 9.7 x $10^2$ cfu/g for crude luncheon and the total coliform ranged from 0.8 x 10 to 3.5 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded

7.4 x $10^2$ to 4.1 x $10^4$, 9.9 x $10^3$ to 5.2 x $10^5$ and 2.2 x $10^4$ to 7.7 x $10^5$ cfu/g for the stored slides after 2 days, 1 week and 2 weeks from sliding, respect. Which also were reached to higher than those recommended by (ES). Whoever, the results show the mean of the bacterial counts in chicken luncheon samples collected during summer season of the same company product. The (3rd samples group) It conspicuous that the mean number of total bacterial counts ranged from 2.6 x 10 to 9.7 x $10^2$ cfu/g for crude luncheon and the total coliform ranged from 0.6 x 10 to 3.4 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded 2.7 x $10^3$ to 6.5 x $10^4$, 8.2 x $10^3$ to 3.2 x $10^5$ and 6.4 x $10^4$ to 1.1 x $10^6$ cfu/g for the stored slides after 2 days, 1 week and 2 weeks from sliding, respectively., also were reached to higher than those recommended by (ES).

In the same concept, **the product (2) meat luncheon:** Concerning the presented results show the mean of the bacterial counts in meat luncheon samples collected during winter season. The 1st samples group conspicuous that the mean number of total bacterial counts ranged from 1.2 x $10^2$ to 5.8 x $10^3$ cfu/g for crude luncheon and the total coliform ranged from 0.9 x 10 to 3.8 x 10 with absence of faecal coliform which were in normal ranges according to (ES). While the total bacterial counts were increased by storage after sliding, since recorded 4.8 x $10^3$ to 2.9 x $10^5$, 6.9 x $10^4$ to 5.3 x $10^6$ and 1.6 x $10^5$ to 5.1 x $10^6$ cfu/g for the stored slides after 2 days, 1 week and 2 week from sliding, respectively. Which were reached to higher than those recommended by (ES). While the 2nd samples group (spring samples) show that, the mean number of total bacterial counts ranged from 2.1 x $10^2$ to 3.9 x $10^3$ cfu/g for crud luncheon and the total coliform ranged from 1.4 x 10 to 4.4 x 10 with absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded 8.1 x $10^3$ to 4.2 x $10^5$, 7.3 x $10^4$ to 9.1 x $10^6$ and 3.3 x $10^5$ to 9.8 x $10^6$ cfu/g for the stored slides after 2 days, 1 week and 2 week from sliding, respectively. Which also were reached to higher than those recommended by (ES).

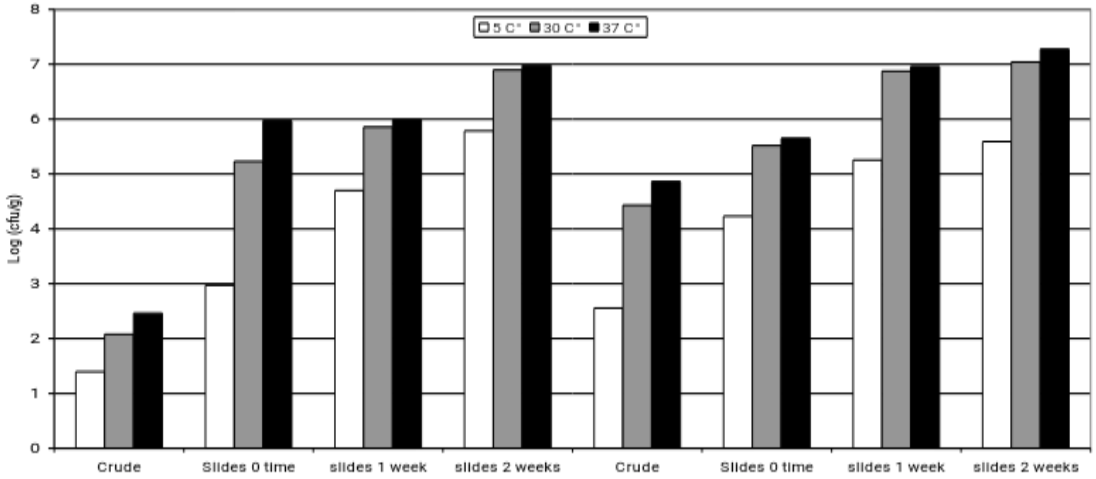
**Table 4: product-2 microbiological survey average by (cfu/g) during the year seasons.**

| Bacterial Counts | | Chicken luncheon | | | | Meat luncheon | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Crude luncheon | Slides Zero time | slides first week | slides second week | Crude luncheon | Slides Zero time | slides first week | slides second week |
| Total counts | 5 C° | 2.5 x $10^2$ | 9.4 x $10^2$ | 5.0 x $10^4$ | 6.1 x $10^5$ | 3.6 x $10^2$ | 1.7 x $10^4$ | 1.8 x $10^5$ | 3.9 x $10^5$ |
| | 30 C° | 1.2 x $10^3$ | 1.7 x $10^5$ | 7.2 x $10^5$ | 7.9 x $10^6$ | 2.7 x $10^4$ | 3.3 x $10^5$ | 7.5 x $10^6$ | 1.1 x $10^7$ |
| | 37 C° | 2.9 x $10^3$ | 9.4 x $10^5$ | 9.8 x $10^5$ | 9.5 x $10^6$ | 7.3 x $10^4$ | 4.5 x $10^5$ | 9.1 x $10^6$ | 1.9 x $10^7$ |
| Coliform counts | T coliform | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | F coliform | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Salmonella | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | Shigella | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

*Samples were taken in three replicates.

However, the results show the mean of the bacterial counts in meat luncheon samples collected during the summer season of the same company product. The (3rd samples group) conspicuous that the mean number of total bacterial counts ranged from 1.9 x $10^2$ to 4.9 x $10^3$ cfu/g for crude luncheon and the total coliform ranged from 1.9 x 10 to 5.1 x 10 with the absence of faecal coliform which were in normal ranges according to the (ES). While the total bacterial counts were also increased by storage after sliding, since recorded 1.5 x $10^4$ to 5.8 x $10^5$, 5.9 x $10^4$ to 1.4 x $10^7$ and 5.2 x $10^5$ to 1.8 x $10^7$ cfu/g

for the stored slides after 2 days, 1 week and 2 week from sliding, respectively. Which also were reached to higher than those recommended by Egyptian Standards and these results accord with (Nabil *et al*., 2014).




**Chicken luncheon** **Meat luncheon**

**Figure (2): Product (2) - Log Colony forming units/ gram log (cfu/g).**

## 4. Effects of piperine and capsaicin extracts on antimicrobial activities.

The addition of piperine extraction or complete and grained black pepper seeds indicated to the inhibitory effects of black pepper and piperine extract versus all studied microbial species groups with various concentrations. It is clear from the data that the inhibitions activities of the addition of piperine extract was more effective from the grinded or complete black pepper seeds on the total bacterial counts which decreased the limits of studied products from $3.4\times10^2$ to $8.5\times10^2$ cfu after 96 hours. Moreover, a significant correlation showed an inverse relationship between the piperine addition and the total bacterial growth during the time taken for the luncheon to spoil. The pure piperine was more antimicrobial efficiency by comparing with the other addition at all concentrations, the inhibition zones were dramatically increased by increasing the extract concentrations with recorded the lowest levels of minimal inhibitory concentrations (MICs) by (black pepper) with 95% piperine form riches to 12 µg/mL for Grame positive bacteria ($G^{+Ve}$) and by 60 µg/mL Grame negative bacteria ($G^{-Ve}$), when the minimal bactericidal concentrations (MBCs) were about 25 µg/mL against Gram-positive bacteria, 125 µg/mL against Gram-negative bacteria and other organisms and the amount of 12 - 60 ml/kg (piperine sufficient for every kilogram of luncheon). While the maximum inhibition zones ranged from 17.4 to 21.3 mm as maximum, these results are in accordance with those reported by Carocho, (2014) and Nabil *et al*. (2014) which confirmed the effects of piperine as antimicrobial agent due to the ability of this compounds to bind with microbial cell walls and prevent cell division and growth. These results are in accordance with Nabil *et al*. (2014), and no evidence was recorded for the presence of $G^{-ve}$ bacterial strains from coliform group of the genus of *E.coli* and the enteric genera of pathogenic *Salmonella* and *Shigella*, these results are similar with those obtained by Chan *et al*. (2014).

## Recommendations

The role of spices is not only added to food as flavor but also play an important role as natural preservatives which improve the shelf life of various food items. From these investigation, we found that addition of black pepper spices to meat

products (luncheons) had an effect in reducing the product spoilage. The black pepper extract in found to be better effective in enhancing the product preservation from the hole seeds. However, the use of the extracted active ingredient (piperine) in the black pepper showed considerable difference from the control and hole seed in the time taken for the luncheons to spoil. This calls for further investigation on effects of using the specific ingredient and such products formula to be more effective as a preservative due to the homogeneity control and its greater degree of spicy (higher pungency content) and better penetration into the luncheon process.

## Conflicts of Interest

The authors declare no conflict of interest.

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# التقييم الكيميائي والميكروبيولوجي للفلفل الأسود كمواد حفظ غذائية طبيعية

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## الملخص العربي

استخدام المواد الحافظة في إنتاج الأغذية الصناعية أصبح الآن ممارسة شائعة. ومع ذلك، هناك قلق متزايد بين المستهلكين بشأن الآثار الضارة للمواد الحافظة الكيميائية الشائعة. ونتيجة لذلك، هناك طلب متزايد على الأغذية التي خضعت لقليل من المعالجة أو لم تخضع لأي معالجة. في الآونة الأخيرة، كان هناك اهتمام كبير بإيجاد بدائل للمواد الحافظة الكيميائية لاستخدامها في صناعة اللحوم، في شكل مكونات طبيعية. تشمل الحلول الممكنة استخدام المستخلصات النباتية والزيوت الأساسية كعوامل مضادة للميكروبات. تقدم هذه الورقة حلاً عمليًا لاستبدال المواد الحافظة الاصطناعية في بعض منتجات اللحوم بمركبات كيميائية تقليدية من أصل طبيعي. بينما تم التحقيق من البيبيرين المستخرج من بذور نبات الفلفل الأسود كمادة حافظة طبيعية ضد البكتيريا المسببة لفساد الطعام المرتبطة بمنتجات اللحوم. حيث تمت دراسة تأثير الخصائص المضادة للميكروبات للفلفل الأسود على بعض الميكروبات المسببة للتلف في بعض منتجات اللحوم (اللانشون). في حين تم معالجة اللانشون الخام بالبيبيرين كمادة حافظة طبيعية. كان التركيب الكيميائي للانشون الخام والمعالج مختلفًا بشكل كبير في الرطوبة ودرجة الحموضة وكان هناك اختلافات كبيرة في كل من الدهون الكلية والبروتينات عند ($p < ٠,٠٥$). كما انخفض إجمالي الأعداد الكلية للكائنات الدقيقة في الأيام الأولى بعد إضافة الإضافات الطبيعية وأظهرت اختلافات كبيرة ($p < ٠,٠٠١$) للنوعين من اللانشون (الدجاج ولحم البقر)، وكان إضافة البيبرين المركز أكثر فاعلية من إضافة الحبوب الكاملة والمطحونة من حيث إجمالي الأعداد الكلية للميكروبات مما أدى إلى تقليل حدود المنتج المدروس من $٣,٤ \times ٢١٠^{2}$ إلى $٨,٥ \times ٢١٠^{2}$ بعد ٩٦ ساعة. وقد أظهرت النتائج ارتباطًا مهمًا بوجود علاقة عكسية بين تركيز البيبرين ونمو البكتيريا بمرور الوقت وزيادة الوقت المستغرق لفساد اللانشون. علاوة على ذلك، تساعد التوابل في الحفاظ على الطعام وتحسين الطعم ومدة صلاحيته. فقد تم إجراء قياسات التجانس ومدة الحفظ كدالة للوقت من أجل التحقيق في الخصائص الكيميائية الحيوية لعينات لانشون اللحوم والدجاج المعالجة بتركيبة البيبيرين لتكون أكثر فعالية كمادة حافظة بسبب درجتها العالية كمادة حريفة على مقياس سكوفال (محتوى أعلى من الحرقة) وكذلك تعطى تجانس أفضل خلال عملية تصنيع اللانشون.