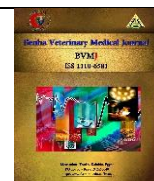




Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: <https://bvmj.journals.ekb.eg/>



Since 1990

Original Paper

The effects of coatings made from cereal and leguminous flour on chicken fillet and their antibacterial impact on some foodborne bacteria

Asmaa Y. Ragab; Abobakr M. Edris; Hemmat M. Ibrahim; Islam Ibrahim Sabike; Reham A. Amin

Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, Egypt.

ARTICLE INFO

Keywords

Batter
Chitosan
Flours
TBA(MDA)
Salmonella
Staph aureus.

Received 23/06/2025

Accepted 27/07/2025

Available On-Line
01/10/2025

ABSTRACT

Employing edible coatings composed of natural components with antioxidants and antibacterial properties may improve the keeping quality and shelf life of chicken fillets. Therefore, the present study focuses on assessing chitosan (0.2%), black cumin seed oil (0.1%), and five flour formulations developed from cereal grains and legumes: chickpea (*Cicer arietinum*), oat (*Avena sativa*), rice (*Oryza sativa* L.), soybean, and a control (wheat only), performed over 15 chilling days at 4 °C. Measuring the chicken fillet's keeping quality as pH, antioxidant capacity expressed by MDA (malondialdehyde) values, and total bacterial count. Additionally, evaluating their effects on microorganisms' viability as Coliform, *Staphylococcus*, *Staphylococcus aureus*, and *Salmonella enterica*. The pH remained at the borderline until the third day of chilled storage, and MDA (malondialdehyde) values increased ($p < 0.05$) in all treatments except for the control, while all battering treatments reduced total bacterial count to fewer than 5 logs; coliform count reduced to less than 4 logs at 0 and 3 days, then increased over the course of 15 days. However, *Staphylococcus* counts remained below 2 log CFU/g, particularly for cereal grain flour batters (oat, rice) until the conclusion of the treatment period. Foodborne pathogens such as *Staphylococcus aureus* and *Salmonella enterica* were suppressed and eliminated when fortified with oat and rice flours, respectively. In conclusion, the flour formula developed with natural antimicrobial agents contains cereal grain flour (oat and rice) and can suppress most food poisoning bacteria in chicken fillets until the 15th day of chilling.

1. INTRODUCTION

Chicken meat is the most sensitive food, along with others, during the storage and cooking processes. Therefore, producers are focusing on new preservation and cooking techniques (Maskat et al., 2004).

Processed chicken meat is more susceptible to oxidative changes than raw chicken (Xiao et al., 2011). In addition to its nutritional value, with a high level of polyunsaturated fatty acids, which are highly susceptible to oxidative deterioration during storage, its shelf life is affected (Sohaib et al., 2017).

Currently, the replacement of synthetic antioxidants with natural antioxidants is receiving more attention in the poultry meat industry, influenced by consumer purchasing decisions (Gulcin et al., 2010) and the retardation of protein and lipid oxidation. This leads to shelf-life extension in processed meat products that utilize natural preservatives (El Wahab et al., 2019).

These natural coatings are classified as "generally recognized as safe" (GRAS) materials, which have an antimicrobial effect on foods (Erginkaya et al., 2014).

The antioxidant compounds that are present in high content in grains are fat-soluble and water-soluble. The phenolic compounds such as ferulic acid, tocopherols (tocopherols and tocotrienols), caffeic acid esters, and carotenoids are commonly identified antioxidant compounds in grains and water-soluble antioxidants (Miller et al., 2000).

Cereals have recently been accepted as functional foods, primarily due to their fibers, such as β -glucan and

arabinosyran, along with carbohydrates, proteins, energy, minerals, vitamins, and antioxidants needed for human health (Ötles et al., 2006).

Among the vegetables, legumes and their derivatives were rich in a varied spectrum of phytochemicals, with an approved antimicrobial effect (Elsheikh et al., 2025).

This study investigates the effects of coatings made from cereal and leguminous flours (wheat, chickpea, oat, rice, and soybean) on chicken fillet pH, antioxidant capacity expressed by MDA (malondialdehyde) values, Total Bacterial Count, and their antibacterial impact on selected foodborne bacteria.

2. MATERIAL AND METHODS

2.1. Ethic approval:

Experiment management and approved by Scientific research Ethical Committee, Faculty of Veterinary Medicine, Benha University (Ethical Approval Number: BUFVM 50-11-23).

2.2. Chemicals

Chitosan (de-acetylation 93%, viscosity >75 %), from nano tech. lab.

Glacial acetic acid.

Black Cumin seed oil (*Nigella sativa* L. seeds) essential oil was supplied from National research center.

Various flours of available grains and legumes: control (Wheat), Chickpea, Oat, Rice, Soybean.

Malondialdehyde (MDA)

* Correspondence to: asmaaayahia@gmail.com

Baird-Parker and XLD agar were procured from Sigma-Aldrich (St. Louis, USA)

2.3. The food poisoning pathogens preparation

Staphylococcus aureus and *Salmonella Enterica* isolates were used and obtained from the Animal Health Research Institute (AHRI) in Dokki, Egypt.

For *Staphylococcus aureus* preparation, isolated colonies were cultured in sterile peptone water at 37°C for 24 h Saeed et al. (2005), diluted to 10^{10} , and plated on Baird Parker agar, adjusting to 10^6 cfu/ml Kantachote et al. (2008). This level corresponds to a significant enterotoxin concentration ($>10^5$ cfu/g) (Stewart et al., 2003). *Salmonella Enterica* was cultured overnight at 37 °C, then diluted to 10^5 CFU/mL, injecting 2 mL/100 g of chicken fillet for a target level of 10^3 CFU/g.

2.4. Chitosan coating preparation

Chitosan edible coatings were created based on Caner et al. (2008) with slight modifications. A 1.5% chitosan solution was made by dissolving it in distilled water at 100 °C, cooled to 45 °C, and mixed with 1% acetic acid and glycerol as a plasticizer, then stirred for 15 minutes.

2.5. Different Flour preparation.

Grains bought from the market were inspected for foreign object and washed with running and clean water many times. Then were dried in a hot air oven at 60°C for 6 hours, ground to a fine powder with a grinder, and sifted through a household sieve.

2.6. Sample preparation and distribution

Fresh chicken fillets were brought from a local market then delivered to the laboratory for investigation. Samples weighing 60 ± 5 g (about 3×6 cm² and 1.5-cm thickness) were aseptically prepared. Then were randomly allocated to five treatments: no CH addition (wheat control) group (1st), other four groups were fortified with 0.2 ml/g of CH. The 2nd group coated Chickpea flour, the 3rd group coated oat flour, the 4th group coated rice flour & the 5th group coated soybean flour. The experiment included five treatments over the six checking chilling days. Each individual treatment had 90 slices divided into three replicates, each consisting of thirty pieces. Each replicate had five slices per day, was allocated over six checking days. The samples of four groups were directly supplied with 0.2 ml/g chitosan, then 500 mL of sterile distilled water were added into the zippered package, followed by a simple rotation for covering all samples. After 30 min, all samples replaced to sterilized steel sieve to dry then battering with different flours. The same procedures were employed for the control group, which just used sterile distilled water and battering with wheat flour. All slices of five groups were bagged and refrigerated in the cooling incubator at 4 ± 0.1 °C. The five groups: control (W, 1st), (CP 2nd), (O 3rd), (R 4th) and (SB 5th) separately evaluated for pH, antioxidant capacity expressed by MDA (malondialdehyde) values, and TBC in chicken fillets, additionally assessment the antibacterial activity and stability of various flours in chicken fillets against artificially inoculated food-borne pathogens over 0, 3, 6, 9, 12, and 15 days.

2.7. Assessment the Keeping Quality of chicken fillets a-pH analysis.

The samples of chicken fillet appointed for pH and bacteriological evaluation, over the time of storage, were individually tenfold diluted with sterile distilled water and analyzed for pH using pH-meter electrodes (Jenway 3510 pH-meter, Cole-Parmer, Staffordshire, United Kingdom).

The pH meter was calibrated at room temperature using two distinct pH levels (4, and 7) in conjunction with a temperature metal probe. (Elsheikh et al., 2024).

b-Malondialdehyde estimation

Malondialdehyde (MDA) levels in homogenized chilled chicken fillets were determined using HPLC (Agilent HP 1200 series system, USA) following (Abd-Elrazek et al., 2018). A 10% sample homogenate with ice-cold 0.1M Tris-HCl (pH 7.4) was prepared using an ice-cold homogenizer. The homogenate was centrifuged for 15 minutes at $2000 \times g$ at 4 °C to remove debris (Youssuf et al., 2025). One mM MDA stock was prepared by dissolving 25 µL of tetra-ethoxy-propane (TEP) in 100 mL of water. After that, the 20 nmol/working standard was made by dissolving 1mL of TEP stock solution in 50 mL of 1% sulfuric acid for 2 hrs at room temperature, which was then diluted with 1% sulfuric acid to yield a final concentration of 1.25 nmol/mL for analysis, results expressed as n M/g.

c-Determination of Total Bacterial Count.

A Total Bacterial Count (TBC) of chilled chicken fillets was evaluated according to ISO 4833-1(2013).

2.8. Assessment of the bacterial viability of chilled chicken fillets.

Bacteriological examination (Coliform, *Staphylococcus counts*) and experimental inoculation of *Staphylococcus aureus* and *Salmonella* in chicken fillets were assessed during storage at 4 °C in a cooling incubator (Binder KB, BINDER GmbH, Tuttlingen, Germany) for 15 days.

a-Coliform Count Deduction.

Two sterile violet red bile agar dishes received 1 mL of serial dilutions, incubated at 37 °C for 24 h for Coliform counting in chicken fillets ISO 4832(2006).

b-Determination of Staphylococci, Staphylococcus aureus and Salmonella Enterica viability.

Chicken fillets were tested for *Staphylococcus aureus* and *Salmonella Enterica* using surface-plating methods. *Staphylococcus aureus* was confirmed on Baird Parker agar plates ISO 6888-1(1999), which appeared as narrow white margins surrounded by a clear halo zone, but *Salmonella enterica* counts were determined on XLD plates ISO 6579-1(2018), with a black colony. Incubation was conducted for 30 minutes, inversion, and incubation at 37°C for 48&24 hours, respectively.

2.9. Assessment of the antibacterial activities of different flour formulations, Chitosan 0.2% with Black Cumin seed oil 0.1% of chilled chicken fillets.

Different flours from cereal and legumin were examined in-vitro in chicken breast fillets for antibacterial activity against artificial inoculated *Staphylococcus aureus* and *Salmonella enterica*. They were categorized into five treatment groups: control(wheat), CP, O, R, and SB. Chicken fillets samples of CP, O, R, and Sb groups were blended directly with chitosan 0.2 ml/g, whereas the first group was blended with wheat only as control. For artificial inoculation, chicken samples were immersed in a 100 ml sterile peptone water 0.1% solution containing a 24-hour-old culture of *Staphylococcus aureus* (about 10^6 CFU/ml) (Kantachote et al., 2008). During half an hour at an ambient temperature (25°C), it improves bacterial adhesion and uptake of the inoculated bacteria (Dubal et al., 2004). The infected specimens were kept in sterile beakers at 30 ± 2 °C, where the initial bacterial load of *Staphylococcus aureus* was determined before treatment. *Salmonella Enterica* was similarly prepared using the same protocol of (Elsheikh et al., 2025). On Oxford agar, *Salmonella* was plated at 37 °C for 24 hours, and colonies in Trypticase soy broth were cultured., cells were centrifuged

and suspended in saline for a bacterial solution generation, achieving approximately 10^9 CFU/mL. Each group received 2 mL of the *Salmonella* culture per 100 g of chicken cultured overnight at 37°C and sequentially diluted to 10^5 CFU/mL. After that, 10 g of each group was moved to a sterile glass flask (125 mL, rubber closure). Three glasses (three replicates) from every single treatment were distributed at random to the six checkpoints (0, 3, 6, 9, 12, and 15) days further treatment) and then chilled at $4 \pm 1^\circ\text{C}$ in the incubator (Binder, BINDER GmbH, Tuttlingen, Germany). For Biocompatible different flour formulations, Chitosan 0.2% with Black Cumin seed oil 0.1%, antibacterial properties investigations, 10 g of the infected chicken fillet samples were blended for sixty seconds in a sterile stomacher bag with 90 mL of sterile distilled water (Stomacher 400 R, Seward, UK). After homogenization and serial dilutions, 100 μL was applied to Baird parker and XLD agar plates to count *Staphylococcus aureus* and *Salmonella* Enterica, counting colonies throughout $37 \pm 2^\circ\text{C}$ after 48 and 24 hours.

2.10. Statistical analyses

Analyzed data was performed with SPSS Version 22. (SPSS Inc., Chicago, Illinois, USA). The fortified groups: control (wheat only), CP (Chickpea), O (Oat), R (Rice) and SB (Soybean) over chilling check point (0, 3, 6, 9, 12, and 15) days. Comparable statistical methods were applied to evaluate the bacteriological, antioxidant, and pH properties of chicken breast fillets. The outcomes are presented along with their averages and the overall standard errors of those averages. The statistical model utilized one-way ANOVA and one Tukey's b multiple comparison test to measure the effectiveness of various flour formulation and their different scales in relation to control, as well as to evaluate distinct monitoring point averages within the same group. Notable differences were identified with a p-value below 0.05.

4. RESULTS

The different flour coat treatments, their activity on the keeping quality parameters, and selected food pathogens of chilled chicken fillets over 15 chilling days, rather than the control, are illustrated in Tables 1 and 2. The effects of different flour coats were assessed, with a p-value of less than 0.05. Significant differences were found in the interaction between treatments and storage periods. The control (wheat) chicken fillet samples in upward grades spoiled after 6 days during the chilling period in pH compared to the chicken fillet's pH after treatment with

different flours, remaining at borderline until the third day of storage, then increasing in pH values until the 9th day of storage, but in the O, R, and SB treatment groups, lower pH values than the control group appeared on the 12th and 15th days.

Regarding Table (1), the initial TBARS values appear to be 64.06 ± 2.06 , 69 ± 3 , 63.77 ± 2.77 , 75.27 ± 3.27 , and 61.68 ± 2.68 in C, Cp, O, R, and SB, then increased significantly ($p < .05$) to 73 ± 2.08 , 64.38 ± 2.38 , 85.15 ± 3.15 , 94.5 ± 3.5 , and 88.2 ± 3.2 mg malonaldehyde/kg, respectively, at 15 days, leading to spoilage. All groups treated with flour significantly increased ($p < 0.05$) in MDA values compared to the control group.

Oxidation indicators MDA (nmol/mg) revealed that different flours coated chicken fillet had a fixed effect throughout the entire storage period, with a clearly rising curve. Additionally, the Cp flour group appeared lower in MDA (nmol/mg) values than other treated groups.

The bacteriological evidence and the longevity of chicken fillets shelf life during the 15-day chilling experiment may be affected by treatment scales, storage intervals, and interactions that significantly affected ($p < 0.05$) all indices. The flour modulation scales also retarded bacteriological indices as (TBC) table (1), (Coliform Count, *Staphylococcus* count) table (2), and experimental inoculation as (*Salmonella enterica*, *Staphylococcus aureus* count) table (2) from day 0 to day 15 of the storage period; this was contrasted with the control (wheat) group ($p < 0.05$). For quality investigation, all treated groups showed inhibition of TBC below 6 logs CFU/g for 15 days post-treatment, while the coliform count was at a level of less than 4 logs CFU/g at 0 and 3 days, then increased above that over the course of 15 days. Cereal grain flour batters (oat, rice) in chicken fillet showed significant differences ($p < 0.05$) in retardation of *Staphylococcus* growth until the 15th day of chilling storage, with counts ranging from 0.5 to 1 log CFU/g compared to the control (wheat) group.

The results also explain the antimicrobial impact of different flour battering scales over 15 days of chilling on experimentally inoculated chicken fillet groups with *Staphylococcus aureus* and *Salmonella enterica* compared to control (wheat) chicken fillets. Appeared statistically suppressed and completely eliminated to *Staphylococcus aureus* and *Salmonella* Enterica when fortified with oat and rice flours, respectively. ($P < 0.05$) on *Staphylococcus aureus* and *Salmonella enterica* compared to other scales and the control group Table (2).

Table 1: The effect of different flour treatments on the keeping quality of chilled chicken fillet samples ($4^\circ\text{C} \pm 1^\circ\text{C}$)

parameter	groups	Time (Days)					
		0	3	6	9	12	15
pH	Control	6.83±0.02 ^{Ac}	6.77±0.02 ^{Ac}	6.91±0.07 ^{Abc}	6.89±0.02 ^{Abc}	7.01±0.09 ^{Bb}	7.9167±0.05175 ^{Aa}
	Cp	6.45±0.08 ^{Bd}	6.77±0.098 ^{Ac}	6.9±0.06 ^{Ac}	6.67±0.06 ^{Bcd}	7.47±0.12 ^{Ab}	7.9867±0.03844 ^{Aa}
	O	6.55±0.01 ^{Bc}	6.43±0.12 ^{Bc}	6.94±0.04 ^{Aa}	6.81±0.04 ^{ABab}	6.74±0.01 ^{BCb}	6.9900±0.01000 ^{Ca}
	R	6.57±0.10 ^{Bb}	6.53±0.04 ^{ABb}	6.98±0.01 ^{Aa}	6.81±0.06 ^{ABa}	6.53±0.09 ^{Cb}	6.8567±0.02963 ^{Da}
	SB	6.51±0.02 ^{Bd}	6.6533±0.04177 ^{ABcd}	6.93±0.03 ^{Ab}	6.93±0.04 ^{Ab}	6.79±0.10 ^{BCbc}	7.1167±0.03480 ^{Ba}
MDA (nmol/mg)	Control	64.06±2.06 ^{Bb}	50.88±1.88 ^{Cc}	64.21±2.21 ^{Cb}	73.21±2.21 ^{Cb}	107.91±2.91 ^{Aa}	73.08±2.08 ^{Cb}
	Cp	69±3 ^{ABab}	63.54±2.54 ^{Bb}	73.18±3.18 ^{ABab}	76.04±3.04 ^{BCa}	71.65±2.65 ^{Cab}	64.38±2.38 ^{Bb}
	O	63.77±2.77 ^{Bc}	85.41±3.41 ^{Aa}	72.13±3.13 ^{ABbc}	83.33±3.33 ^{ABCab}	79.96±2.96 ^{BCab}	85.15±3.15 ^{Aa}
	R	75.27±3.27 ^{Abc}	72.9167±2.91667 ^{Bc}	81.54±3.54 ^{Abc}	94.79±3.79 ^{Aa}	87.23±3.23 ^{Bab}	94.50±3.50 ^{Aa}
	SB	61.68±2.68 ^{Bb}	68.7500±2.75000 ^{Bb}	83.63±3.63 ^{Aa}	87.5±3.500 ^{ABa}	87.23±3.23 ^{Ba}	88.26±3.26 ^{Aa}
TBC	Control	2.26±0.17 ^{Ab}	3.255±2.301 ^{Ab}	4.71±3.30 ^{Da}	4.60±4 ^{Bab}	4.54±3.69 ^{Aab}	4.51±4.43 ^{Aab}
	CP	0.17±0.00 ^{Bd}	3.01±1.47 ^{Bd}	5.21±3.39 ^{Bb}	5.66±4.30 ^{Aa}	4.17±3.69 ^{Ad}	4.84±4.00 ^{Ac}
	O	0.39±0.00 ^{Ba}	2.09±1.176 ^{Ca}	3.62±2.301 ^{Ea}	5.09±5.02 ^{Ba}	5.97±5.81 ^{Aa}	5.59±5.43 ^{Aa}
	R	0.00 ^{Bd}	1.54±1.176 ^{Cd}	5.37±3.17 ^{Aa}	5.26±3.69 ^{Bb}	4.84 ^{Ac}	4.87±4.39 ^{Ac}
	SB	0.17±0.00 ^{Bc}	2.41±1.00 ^{Cc}	5.01±3.30 ^{Cbc}	5.74±4.00 ^{Aa}	4.95±4.00 ^{Abc}	5.63±5.38 ^{Aab}

Control(C) Wheat only, Chickpea (CP), Oat (O)Oat, Rice(R) and Soybean (SB). Chroma color intensity, pH, TBA (MDA, Malondialdehyde) and TBC total bacterial count, SEM standard error of the mean, Different small letters within the row show significant changes across chilling times ($p < 0.05$), while different capital letters within the column indicate significant differences between treatments. TBC not more than 10^5 cfu/g sample and TBA not more than 0.9 mg MDA/Kg, (9×10^8 (nmol/mg) sample (chicken fillet) according to (EOS, 1651, 2019) , (nmol/mg) stands for nanomoles per milligram.

Table 2: The effect of different flour treatments on viability of Coliform Count, *Staphylococcus* Count and inoculated *Staphylococcus aureus*, *Salmonellae* (log CFU/g) in chilled chicken fillet, (4 °C ± 1 °C)

Bacterial spp.	groups	Time (Days)					
		0	3	6	9	12	15
Coliform Count	Control	0.39±0.00 ^{Ac}	2.47±1.72 ^{Ac}	4.99±3.30 ^{Cc}	5.81±4.65 ^{Ab}	6.11±5.04 ^{Ba}	6.21±5.33 ^{Ba}
	CP	0.00±0.00 ^{Ab}	2.30±1.44 ^{ABCB}	4.45±3.36 ^{Eb}	6.29±4.54 ^{Ba}	6.16±5.68 ^{Ba}	6.350±5.79 ^{ABa}
	O	0.00 ^{Ad}	2.19±1.42 ^{BCD}	4.90±3.00 ^{Dd}	6.07±4.39 ^{Cc}	6.31±4.69 ^{ABb}	6.42±4.90 ^{ABa}
	R	0.39±0.17 ^{Ad}	2.39±1.161 ^{ABd}	5.29±3.17 ^{Bd}	5.97±4.47 ^{Dc}	6.17±5.31 ^{Bb}	6.28±5.33 ^{ABa}
<i>Staphylococcus</i> Count	SB	0.00±0.00 ^{Ac}	2.12±1.06 ^{Cc}	5.51±3.17 ^{Ac}	6.40±4.30 ^{Eb}	6.42±4.39 ^{Aab}	6.47±5.38 ^{Aa}
	Control	0.87±0.39 ^{Ac}	2.43±0.95 ^{Aa}	2.17±1.69 ^{Bb}	2.54±1.69 ^{Ba}	1.73±0.90 ^{Abc}	1.85±1.04 ^{Abc}
	CP	1±0.00 ^{Ab}	1.46±0.39 ^{Cb}	2.91±2.09 ^{Aa}	1.69±1.30 ^{Cb}	1.27±1.02 ^{Bb}	1.32±1.09 ^{Bb}
	O	0.00 ^{Bb}	0.30±0.00 ^{Db}	1.17±0.69 ^{Ba}	ND	ND	ND
<i>Staphylococcus aureus</i>	R	ND	1.32±0.77 ^{CDa}	1.39±0.69 ^{Ba}	ND	0.30±0.00 ^{Bb}	0.87±0.00 ^{Bb}
	SB	0.74±0.39 ^{ABc}	2.12±1.04 ^{Bb}	ND	2.97±1.87 ^{Aa}	0.69±0.47 ^{Bc}	0.00 ^{Bc}
	C	ND	1.491±1.279 ^{Aa}	ND	ND	ND	0.5 ± 0.17 ^{ABc}
	CP	1 ± 0.00 ^{Ab}	0.653±0.398 ^{Cb}	1±0.00 ^{Aa}	0.69±0.69 ^{Cb}	ND	0.00±0.00 ^{Bb}
<i>Salmonellae</i> Count	O	ND	ND	ND	ND	ND	ND
	R	ND	ND	0.17 ± 0.00 ^{Ba}	ND	ND	0.17 ± 0.00 ^{Bb}
	SB	0.69 ± 0.47 ^{ABc}	0.17±0.00 ^{Bb}	ND	ND	ND	ND
	C	1.06±0.17 ^{Ac}	0.30±0.00 ^{Cc}	2.21±1.51 ^{Aa}	1.91±1.34 ^{Ab}	ND	ND
	CP	0.00±0.00 ^{Bb}	1.13±0.00 ^{Ca}	ND	0.30 ± 0.00 ^{Bb}	ND	ND
	O	ND	ND	0.69±0.69 ^{Ba}	0.47±0.00 ^{Ba}	0.30 ± 0.00 ^{Aa}	ND
	R	ND	ND	ND	ND	ND	ND
	SB	0.47±0.30 ^{Bb}	1.70±0.6 ^{Aa}	ND	1.31±0.81 ^{Bb}	1.33±1.13 ^{Ab}	ND

Control(C) Wheat only, Chickpea (CP), Oat (O)Oat, Rice(R) and Soybean (SB). TBC total bacterial count, Coliform count, *Staphylococcus*, *Staphylococcus aureus* and *Salmonellae*, ND mean not detected, SEM standard error of the mean; Different small letters within the row show significant changes across chilling times ($p < 0.05$), while different capital letters within the column indicate significant differences between treatments. *S.aureus* count less than 10^2 cfu / g sample (chicken fillet) according to (EOS, 1651,2019).

4. DISCUSSION

The spoilage in meat and meat products was commonly detected by microbiological analysis; additionally, the chemical changes may measure by the association of the growth of specific spoilage organisms (Dainty et al., 1996). The effectiveness of black seed extract has not been extensively researched in meat, but it has antioxidant potency to protect meat products from oxidation. Muzolf-Panek et al. (2020) reported that the bacteriological quality of chicken meat treated with black seed extract was maintained. Additionally, the color of the samples changed consequently, and meat pH was stabilized.

Ni et al. (2021) found that chitosan-related compounds exhibited limited antimicrobial activity with possible packaging applications. Thus, blending natural compounds and chitosan to form a coating structure via crosslinking seems to be a favorable alternative against the food poisoning microorganisms of greatest concern in meat, which are *Staphylococcus aureus*, *Salmonella*, and enteropathogenic *E. coli* (El-Kewaiey et al., 2015).

pH value is a valuable chemical factor because of its effect on shelf life and other quality parameters, such as water holding capacity, color, and texture of meat and meat products (Abd El-Qader et al., 2004). Verma et al. (2012) found the higher pH of the samples in O, R, and SB (pH 6.50) and chickpea flour (pH 6.4) on the zero-day due to their slight alkalinity. The samples coated with flours showed a reduction in pH due to the correlation between the edible protein and coating storage time. Adegoke et al. (2022) mentioned with Krol et al. (2017) that the protein of the edible coating solution affects the pH of treated samples and agreed with our results that the pH of the coated sample decreased at a slower trend compared to the wheat control samples, which is related to higher values of WHC in the coated samples with flour than in the wheat control samples and microbial growth during chilling storage.

Many microorganisms were found in meat, reflecting the safety and longevity of the food and the extent of risk posed to the consumers (Shakila et al., 2020). In this study the results in table (1) illustrate an increase in the total bacterial count in the control group but a decrease in the others till day

3, but in the (O) group till day 6, then all groups became higher than the control wheat one till the end of the experiment. These results are in agreement with Pina-Pérez et al. (2018), who reported that some herbs have shown antimicrobial properties against food spoilage and pathogens. On the other hand, natural herb oil that was added to all treatment groups exhibits inhibition in microbial growth. Also, Surkiewicz et al. (1967) reported that the bacterial counts can decrease upon a dry batter composed of flour.

The reason for increasing total bacterial counts and coliform counts in coated flour groups may be attributed to chicken fillets, processing environment, and food handlers as other sources of contamination. Additionally, increasing the carbohydrate in cereal flour and the incorporation of legume flour, which is high in protein, in meat products showed high microbial counts exceeding the acceptable limit (10^5 cfu/g and 10^4 cfu) in chilled chicken fillet (EOS, 1651, 2019).

Phenols and phenolic compounds are known to have antibacterial and antifungal properties, as reported by Uwumarongie et al. (2007). Extensive studies reported that some polyphenols had antimicrobial activity against a wide spectrum of microorganisms (Pina Perez et al., 2017). The two dominant phenolics in legumes specifically are flavonoids and epicatechins (Khang et al., 2016; Pina-Pérez et al., 2018). Chickpea (*Cicer arietinum*) can exhibit antioxidant and antimicrobial activity (Kanatt et al., 2011). Antimicrobial potential has also been associated with peptides from legumes (AMPs), including a glucanase, a chitinase, and a cyclophilin-like protein (Wong et al., 2010; Malaguti et al., 2014), generally active against Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Salmonellae* Count (Barari et al., 2015; Pina-Pérez et al., 2018). The composition, nature, and origin of chickpeas affect their antimicrobial activity (Kan et al., 2010) and their potency against *S. aureus* and *E. coli* (Kanatt et al., 2011). Pina-Pérez et al. (2018) reported that foodborne pathogen growth can be suppressed on solid surfaces using soybean as a natural antimicrobial substance, and it is used in industry nowadays for food packaging (Srey et al., 2013).

Whole grains such as rice, wheat, and oats have bioactive lectin compounds, which are healthy for humans (Van Buul et al., 2014), which act as bacteriostatic rather than bactericidal (Nair et al., 2013). However, it was determined that *A. sativa* oat flour had an impact on the growth of selected microbial strains, resulting in higher phenolic and flavonoid content, depending on its composition, and found that all samples coated by oat flour showed a greater bactericidal effect against *Staphylococcus aureus* than *Salmonella enterica* bacterial strains (Hamad et al., 2020). Regarding chicken fillet coated with rice flour, it had a bactericidal effect and eliminated more *Salmonella* than other coating materials. Hossain et al. 2016 reported that rice bran is a collection of numerous bioactive components, had public health and veterinary nutritionists, and found that the dietary supplementation of rice bran made greater mucosal protection against enteric infections in people and animals.

5.CONCLUSIONS

The current research examined the inhibition of *S. aureus* and *Salmonella* in various flour coatings mixed with 0.2% chitosan and black cumin seed oil compositions in chicken fillets stored at 4°C. The results indicate that cereal flours (O, R) had an effective antibacterial method to reduce and eliminate *S. aureus* and *Salmonella* compared to other flours. Additionally, this model defines validated processing criteria that ensure the safety and longevity of chicken fillet samples.

6.REFERENCES

1. Abd El-Qader, M.F., 2004. Quality improvement of chicken frozen burger formulated with some spices or their volatile oils, Doctoral dissertation, M. Sc. Thesis, Fac. Agric., Cairo Univ.
2. Abd-Elrazek, A. M., and Ahmed-Farid, O. A. H. 2018, Protective effect of L-carnitine and L-arginine against busulfan-induced oligospermia in adult rat. *Andrologia*, 50, 1, e12806. <https://doi.org/10.1111/and.12806>
3. Adegoke, S.C., Adrah, K., Nowlin, K. and Tahergorabi, R., 2022. Microstructural and physicochemical changes of coated and frozen fried chicken. *Journal of Food Processing and Preservation*, 46, 9, p.e16822. <https://doi.org/10.1111/jfpp.16822>
4. Barari, L., Mosavi, N., Asgharpour, F., Asadi, A., Moulana, Z. and Elmi, M.M., 2015. 20153350429, English, Journal article, Bulgaria, 2278-4357, 4, 10, Sofia, World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS) , (336-346) World Journal of Pharmacy and Pharmaceutical Sciences (WJPPS), Antibacterial and antifungal effect of chickpea (*Cicer arietinum*) aqueous seed extract.
5. Caner, C. and Cansiz, Ö. 2008. Chitosan coating minimizes eggshell breakage and improves egg quality. *Journal of the Science of Food and Agriculture*, 88, 1:56-61.
6. Dainty, R.H. 1996, Chemical/biochemical detection of spoilage. *Int. J. Food Microbiol.* 33: 19-34.
7. Dubal, Z.B., Paturkar, A.M., Waskar, V.S., Zende, R.J. and Latha, C. 2004. Effect of food grade organic acids on inoculated *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* in sheep/goat meat stored at refrigeration temperature. *J. Meat Sci.*, 66: 817-821
8. El Wahab, M.G.A., Sohaimy, S.A.E., Ibrahim, H.A. and El-Makarem, H.S.A., 2019. Effect of Natural Antioxidant Extracts on Oxidative and Microbiological Stability of Beef Burger. *Alexandria Journal of Veterinary Sciences*, 60, 1: 141-154.
9. El-Kewaiey, I.A. and AAL-SAID, A.M.A.L., 2015. Microbial and Chemical quality of retail minced meats. *Assiut Veterinary Medical Journal*, 61, 147, 95-105.
10. Elsheikh, Mai, Ali Osman, Shimaa Edris, Wesam Dawam, Mahmoud Sitohy, and Islam Sabeq. 2025, "Soybean Glycinin's Antibacterial Properties Provide a Feasible Natural Alternative for Improving the Overall Quality and Shelf-Life of Beef Steaks and Combating Foodborne Pathogens." *Food and Bioprocess Technology* 18, 2: 1777-1792.
11. EOS, Egyptian Organization for Standards and Quality. No. 1651:2019. Chilled poultry and rabbit meats.
12. Erginkaya, Z., Kalkan, S. and Ünal, E., 2014. Use of antimicrobial edible films and coatings as packaging materials for food safety. In *Food processing: Strategies for quality assessment* 261-295. New York, NY: Springer New York. https://doi.org/10.1007/978-1-4939-1378-7_10
13. Gülçin, İ., Huyut, Z., Elmastaş, M. and Aboul-Enein, H.Y., 2010. Radical scavenging and antioxidant activity of tannic acid. *Arabian journal of chemistry*, 3, 1, 43-53.
14. Hamad, A., Edris, S. and Matter, A.F., 2025. Amelioration of hypoxia and cold stress in Nile tilapia: comparative effect of *Chlorella vulgaris* and its nanoparticle dietary supplementation on performance, antioxidant, hepatic functions, and meat quality. *Aquaculture International* 33, 66. <https://doi.org/10.1007/s10499-024-01739-2>
15. Hamad, M.N.F., El-Bushuty, D.H. and El-Zakzouk, H.S., 2020. Chemical composition, antioxidant and antimicrobial activities of oat, barley, sweet lupin and lima bean. *Journal of Food and Dairy Sciences*, 11, 4, 97-103.
16. Hossain, M.M., Park, J.W., Nyachoti, C.M. and Kim, I.H., 2016. Effects of extracted rice bran supplementation on growth performance, nutrient digestibility, diarrhea score, blood profiles, and fecal microbial shedding in comparison with apramycin, (antibiotic growth promoter) in weanling pigs. *Canadian Journal of Animal Science*, 96, 4, 495-503.
17. ISO 6579-1, 2018. Microbiology of the food chain—horizontal method for the detection, enumeration and serotyping of *Salmonella*—part 1: detection of *Salmonella* spp.
18. ISO, 4832:2006: Microbiology of Food and Animal Feeding Stuffs — Horizontal Method for the Enumeration of Coliforms — Colony-Count Technique, International Organization for Standardization, Brussels, Belgium.
19. ISO, B., 1999. 6888-1, 1999: Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of coagulase-positive staphylococci, *Staphylococcus aureus* and other species. Part 1: Technique using Baird-Parker agar medium. International Organization for Standardization, Geneva :1-22.
20. ISO, 4833-1, 2013: Microbiology of the food chain - horizontal method for the enumeration of microorganisms.
21. Kan, A., Ozceli, B., Kartal, M., Ozdemir, Z.A., Ozgen, S. 2010. *In vitro* antimicrobial activities of (*Cicer arietinum* L) Chickpea. *Tropical Journal of Pharmaceutical Research*, 9, 5, 475-481.
22. Kanatt, S.R., Arjun, K. and Sharma, A., 2011. Antioxidant and antimicrobial activity of legume hulls. *Food Research International*, 44, 10, 3182-3187.
23. Kantachote, D. and Charemrjitrakul, W., 2008. Selection of lactic acid bacteria from fermented plant beverages to use as inoculants for improving the quality of the finished product. *Pakistan Journal of Biological Sciences: PJBS*, 11, 22:2545-2552.
24. Khang, D.T., Dung, T.N., Elzaawely, A.A. and Xuan, T.D., 2016. Phenolic profiles and antioxidant activity of germinated legumes. *Foods*, 5, 2, 27; <https://doi.org/10.3390/foods5020027>
25. Król, Ż., Kulig, D., Marycz, K., Zimoch-Korzycka, A. and Jarmoluk, A., 2017. The effects of using sodium alginate hydrosols treated with direct electric current as coatings for sausages. *Polymers*, 9, 11, 602; <https://doi.org/10.3390/polym9110602>.
26. Malaguti, M., Dinelli, G., Leoncini, E., Bregola, V., Bosi, S., Cicero, A.F. and Hrelia, S., 2014. Bioactive peptides in cereals and legumes: agronomical, biochemical and clinical aspects. *International journal of molecular sciences*, 15, 11:21120-21135.
27. Maskat, M.Y. and Kerr, W.L. 2004, "Effect of breeding particle size on coating adhesion in breaded, fried chicken breasts", *Journal of Food Quality*, 27, 2: 103-113.
28. Miller, F. Rigelhof, L. Marquart, A. Prakash, M. Kanter., 2000. Antioxidant content of whole grains breakfast cereals,

- fruits and vegetables. Journal of the American College of Nutrition, 19: 312S-319S
29. Muzolf-Panek, M., Kaczmarek, A., Tomaszewska-Gras, J., Cegielska-Radziejewska, R., Szablewski, T., Majcher, M. and Stuper-Szablewska, K., 2020. A chemometric approach to oxidative stability and physicochemical quality of raw ground chicken meat affected by black seed and other spice extracts. *Antioxidants*, 9, 903; <https://doi.org/10.3390/antiox9090903>
 30. Nair, M., & Sandhu, S.S. 2013. A Kunitz trypsin inhibitor from chickpea (*Cicer arietinum* L.) that exerts an antimicrobial effect on *Fusarium oxysporum* f.sp. *ciceris*. *Agricultural Sciences*, 4, 11, 585–594.
 31. Ni, Y., Shi, S., Li, M., Zhang, L., Yang, C., Du, T., Wang, S., Nie, H., Sun, J., Zhang, W. and Wang, J., 2021. Visible light responsive, self-activated bio nanocomposite films with sustained antimicrobial activity for food packaging. *Food Chemistry*, 362, 130201. <https://doi.org/10.1016/j.foodchem.2021.130201>
 32. Ötles, S. and Cagindi, Ö., 2006. Cereal based functional foods and nutraceuticals. *ACTA Scientiarum Polonorum Technologia Alimentaria*, 5, 1:107-112.
 33. Pina-Pérez, M.C. and Pérez, M.F., 2018. Antimicrobial potential of legume extracts against foodborne pathogens: A review. *Trends in Food Science & Technology*, 72, 114-124.
 34. Pina-Pérez, M.C., Rivas, A., Martínez, A., Rodrigo, A., 2017. Antimicrobial potential of macro and microalgae against pathogenic and spoilage microorganisms in food. *Food Chem.* 235, 34–44.
 35. Saeed, S. and Tariq, P. 2005. Antibacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*. *Pak. J. Bot.*, 37, 4: 997-1001.
 36. Shakila, R.J., and Jeyasekaran, G., 2020. Aquatic Food Quality and Safety Assessment Methods, 1st ed. CRC Press. <https://doi.org/10.1201/9781003107194>
 37. Sohaib, M., Anjum, F.M., Arshad, M.S., Imran, M., Imran, A. and Hussain, S., 2017. Oxidative stability and lipid oxidation flavoring volatiles in antioxidants treated chicken meat patties during storage. *Lipids in Health and Disease*, 16, 1, 27. <https://doi.org/10.1186/s12944-017-0426-5>.
 38. Srey, S., Jahid, I.K., Ha, S.D. 2013. Biofilm formation in food industries: a food safety concern. *Food Control*, 31, 572–585.
 39. Surkiewicz, P. F., Hundman, J. B. & Yancey, M. V. 1967. Bacteriological survey of the frozen prepared foods industry 11. Frozen breaded raw shrimp. *Applied Microbiology*, 15, 1–9.
 40. Uwumarongie, O. H., Obasuyi, O. and Uwumarongie, E. G. 2007. Phytochemical analysis and antimicrobial screening of the root of *Jatropha tanjorensis*. *Chem. Tech. J.* 3: 445-448.
 41. Van Buul, V.J. and Brouns, F.J., 2014. Health effects of wheat lectins: A review. *Journal of Cereal Science*, 59, 2:112-117.
 42. Verma, A. K., Banarjee, R. and Sharma, B. D. 2012. Quality of low-fat chicken nuggets: effect of sodium chloride replacement and added chickpea, *Cicer arietinum* L. hull flour. *Asian-Australasian Journal of Animal Science* 25: 291-298.
 43. Wong, J.H., Ng, T.B., Cheung, R.C.F., Ye, X.J., Wang, H.X., Lam, S.K., Lin, P., Chan, Y.S., Fang, E.F., Ngai, P.H.K., Xia, L.X., Ye, Y., Jiang, Y., Liu, F. 2010. Proteins with antifungal properties and other medicinal applications from plants and mushrooms. *Applied Microbiology and Biotechnology*, 7, 4, 1221–1235
 44. Xiao, S., Zhang, W.G., Lee, E.J., Ma, C.W. and Ahn, D.U., 2011. Lipid and protein oxidation of chicken breast rolls as affected by dietary oxidation levels and packaging. *Journal of Food Science*, 76, 4: C612-C617.
 45. Youssuf, H., Soror, E.I., Shehab, A., El-daim, A.M., Abo-Gamil, Z.H., Ahmed-Farid, O., Hamad, A., Edris, S. and Matter, A.F., 2025. Amelioration of hypoxia and cold stress in Nile tilapia: comparative effect of *Chlorella vulgaris* and its nanoparticle dietary supplementation on performance, antioxidant, hepatic functions, and meat quality. *Aquaculture International*, 33, 1, 66. <https://doi.org/10.1007/s10499-024-01739-2>