

## QUALITY IMPROVEMENT OF CHICKEN FILLETS SUPPORTED WITH PROBIOTICS

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### ABSTRACT

This study examined the microbiological and physicochemical characteristics of raw chicken fillets that had been dipped in both conventional and probiotic yoghurt that included *Lactobacillus Acidophilus* La-5 and *Bifidobacterium longum* ATCC15707 and had been stored for eight days at 4°C. In this regard, samples of chicken breast fillets were subjected to sensory, chemical (pH, cooking loss percentage, and thiobarbituric acid reactive chemicals), and microbiological analysis (APC, *E.coli*, *S.aureus*, *Campylobacter*, and *Salmonella* count) during storage at 4±1°C for 8 days. In comparison with control samples, the mean values of (APC, *E. coli* and *S. aureus*, counts, pH index, malondialdehyde value, and cooking loss percentage) in the chicken breast fillets treated with regular (RY) and probiotic yogurt (PY) at day 8 of storage, were (5.17±0.35, 4.53±0.37; 1.57±0.32, 1.33±0.27 and 1.54±0.55, 1.29±0.25 log CFU/g; 4.78±0.02, 4.72±0.03; 1.25±0.05, 0.89±0.02 and 49 ± 0.05, 45 ± 0.05), respectively, that showing significant reduction ( $P < 0.05$ ), particularly that treated with probiotic yogurt (PY) which considered the best group showed remarkable decrease in all values compared with other groups. In the current study, there were no counts of *Salmonella* or *Campylobacter* in the chicken fillet samples. The study's findings indicated that probiotics inhibited the development of microorganisms, enhanced physicochemical quality, and extended chicken meat fillets' shelf life during storage and cooking. Therefore, it is recommended to use probiotics as one of the biological preservation systems for foods.

**Keywords:** Probiotics, regular, yogurt, chicken breast Fillets, quality and shelf life.

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### INTRODUCTION

Chicken meat is considered the most widely used and unrelated to any

cultural or religious taboos, it is consumed at a leading rate worldwide, in addition to having high biological value proteins and amino acids, vitamins, and other necessary elements. The acceptance of the product can be greatly influenced by the quality and nutritional content of the chicken meat and the produced goods. Chickens are a major potential source of food-borne disease (Heredia and García, 2018). Fresh meats

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encourage the development of pathogenic and spoilage bacteria and are extremely perishable. Food-borne infections continue to pose a significant threat to public health in both developing and developed nations, despite the implementation of several controls and preventative measures (Zhou *et al.*, 2010). The use of probiotics as microbial preservatives has gained much interest recently since consumers are becoming more conscious about artificial additives. Probiotics are essential for maintaining human health. Additionally, they can stop the spread of pathogens and increase the chicken meat's shelf life (Kazemi, 2014). In this sense, probiotic foods mostly include bifidobacteria and lactic acid bacteria (LAB) (Gaggia *et al.*, 2011). Probiotics are utilized in two different ways as a type of biological preservation technique to increase the shelf-life of chicken meat. Utilizing antimicrobial compounds made by LAB causes the environment to be altered to defeat bacteria. The compounds that LAB produces carry out antimicrobial activity. These compounds can be classified as organic acids, diacetyl, hydrogen peroxide, reuterin, and bacteriocins (Sharma *et al.*, 2022). One example of how lactic acid bacteria work is that they lower the pH of the medium and enhance the permeability of the cell membrane. It enhances the effects of other antimicrobial agents in this way. In the presence of oxygen, LAB and the enzyme flavoprotein oxidase combine to create hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The target cell's lipid membrane and cellular proteins are oxidized by the H<sub>2</sub>O<sub>2</sub> molecule that builds up in the environment since the catalase enzyme is absent from LAB. As a result, it has an antagonistic impact on viruses, mould, yeast, and bacteria. When the lactoperoxidase enzyme, which is present in milk, is present, the H<sub>2</sub>O<sub>2</sub> molecule reacts with the thiocyanate chemical, producing an antibacterial effect at non-lethal concentrations. Some lactic acid bacteria (LAB) synthesize amino acids that are used to produce bacteriocins, which are antibacterial peptides or proteins that are produced extracellularly and impede the

development of pathogenic microorganisms resistant to traditional antimicrobials. Furthermore, bacteriocins are adaptable antibacterial agents that have positive effects on the digestive system and general health in addition to being employed as a bio-preservative. Nisin is a polypeptide bacteriocin that exhibits acidic qualities and functions as an antibacterial. It is generated during the fermentation of modified milk. Between pH 3 and 7, it exhibits a better tolerance to temperature. Moulds, yeasts, and Gram-negative bacteria are ineffective against it, despite its effectiveness against some spore-producing and Gram-positive bacteria (Raman *et al.*, 2022). Several modes of action are used by bacteriocins. Certain substances have the capacity to induce porosity in the target microorganism's cell membrane, hence augmenting its permeability. Additionally, these substances may prevent the production of the cell wall. Some can enter the bacterium's cytoplasm and release RNA or DNA. Only strains closely related to the generating organism can be inhibited by bacteriocins, which have a limited spectrum of inhibitory action. However, they can also inhibit a variety of Gram-positive microbes (Betancur-Hurtado *et al.*, 2022). The microbiological characteristics of raw chicken fillets submerged in yogurt containing *L. casei* and kept at 4°C for nine days were investigated by Masoumi *et al.* (2022). They showed that the probiotic yogurt-preserved chicken fillets had lower levels of filamentous fungus, yeast, fecal coliforms, and *S. aureus*. Because they produce bacteriocins, which help to preserve meat and meat products. According to Silva *et al.* (2018), bacteriocins are physiologically active compounds with comparable peptide structures that are produced by ribosomal proteins. In Egypt, marinating chicken meat with yoghurt is fairly common, since it improves the flavour and texture of the meat. Yogurt-marinated chicken fillets are used as a barbecue or culinary element in the Middle East. The purpose of this study was to look at how utilizing ordinary and probiotic yoghurt affected the microbiological characteristics

and physicochemical features of chicken fillets kept in the refrigerator for eight days at 4°C.

## MATERIALS AND METHODS

### 1. Collection and preparation of samples:

This experiment was performed in the Animal Health Research Institute's Damamhur lab. Three kilograms of fresh, raw, boneless chicken breast fillet samples were gathered from poultry abattoirs in the province of El Behera, which is close to Damamhur city. The samples were then securely transported to the laboratory in sterile polyethylene bags. In an hour, they will be placed in different boxes with cooling packs and kept at 4±1°C until further examination. The samples, each weighing one kilogram, were divided into three groups. The first group's cut, untreated chicken meats were kept in the refrigerator as control samples. While, the effects of ordinary or probiotic yoghurt on the sensory, chemical, and microbiological quality and shelf-life of the other two sets of chicken meat samples were evaluated.

### 2. Bacterial Strains:

*Lactobacillus Acidophilus La-5* and *Bifidobacterium longum ATCC15707* were obtained from the Faculty of Agriculture at Ain Shams University in Egypt.

#### 2.1. Preparation of Starter Cultures

*Lactobacillus casei* (Lactobacillus CaseI 431®) 0.1% (w/v) was added to the necessary volume of milk to create a probiotic yoghurt starter (fermented milk). Following that, the pH was raised to 4.6 by incubating the conventional and probiotic yoghurt starters at 40°C and 37°C, respectively. The yoghurt samples served as starting cultures and were kept in a refrigerator at 4°C.

### 3. Preparation of Yogurts (Masoumi *et al.*, 2022)

**3.1. Regular yogurt (RY):** To make regular yoghurt (RY), combine 1000 milliliters of

milk with 3 milliliters of regular yoghurt starter. Then incubate for six hours at 40°C. The mixture's pH was checked every 60 minutes until the yoghurt's pH reached 4.6 by pH meter (Metrohm 827, Switzerland).

### 3.2 Preparation of Probiotic yogurt (PY) (Masoumi *et al.*, 2022) and (Rahmani *et al.*, 2021):

Lyophilized probiotic bacteria including *Lactobacillus acidophilus La-5* and *Bifidobacterium longum ATCC15707* used in this study were added to a sterile MRS broth medium and incubated in an aerobic and anaerobic jar at 37°C for 48 h for *Lactobacillus acidophilus* and *Bifidobacterium longum*, respectively. Bacterial cultures were harvested by centrifugation at 4,000 × g for 10 min at 4°C, washed twice with sterile saline, and collected by centrifugation. Optical density bacterial suspensions were prepared, the culture biomass was used as inoculum and cell numbers were determined using surface plate counting techniques by serial dilution and plating on MRS agar. Plates were then incubated as described above for *L. acidophilus* and *B. longum ATCC15707* under aerobic and anaerobic conditions for 2 days at 37°C, respectively. Bacteria counts were calculated by counting bacterial colonies.

Probiotic yogurt (PY) was prepared after blending 2 milliliters of probiotic yoghurt starting (which imparts probiotic qualities and fragrance to yoghurt) and 1 milliliter of yoghurt starter (which coagulates and ferments milk to produce a hard gel), 1000 milliliters of milk were added. After that, this mixture was incubated for 8 hours at 37°C, achieving a pH of 4.6. Cell numbers were determined using surface plate counting techniques by serial dilution of probiotic yoghurt and plating it on acidified MRS agar to ascertain the probiotic enumerations and viability. The amount of *B. longum ATCC15707* and *L. Acidophilus La-5* in probiotic yoghurt samples reached 4×10<sup>8</sup> CFU/ml for all groups after 6 hours of incubation, according to the results.

#### 4. Sample Preparation

As control samples, the sliced, untreated chicken meats were kept in the refrigerator. The fillets were marinated at room temperature for one minute, twice a day, for two minutes each in two lit of ordinary or probiotic yoghurt. After the extra yoghurt was drained off,  $10\% \pm 0.2$  (w/w) of the marinated fillets were smeared with yoghurt. Each sample was stored at  $4^{\circ}\text{C}$  and was sealed in sterile plastic bags made of polystyrene. Days 0, 2, 6, and 8 of storage were used for sampling to conduct microbiological, chemical, and sensory analyses.

#### 5. Sensory analysis

The chicken breast samples were given to fifteen adult-trained specialist panelists, who were asked to rate their sensory attributes. The panelists were not aware of the experimental methodology; the samples were blind-coded using unique codes. When the items were still fresh (uncooked), they were asked to rate each overall acceptability. A descriptive nine-point scale was employed (Lawless and Heymann, 2010).

#### 6. Chemical analysis

##### 6.1. Measurement of pH

An electronic pH meter (Digital, Jenco 609) was used to confirm the measurement of pH according to (ES 63-11/2006). By mixing a 10 g sample with 90 ml deionized water for two minutes, the pH was determined. A digital pH meter was used to determine the suspension's pH (ES 63-11/2006)

##### 6.2. Measuring Cooking Loss

Chicken fillet samples were weighed and cooked at  $75^{\circ}\text{C}$  to quantify the cooking loss, then reweighed after cooling down (Pelicano *et al.*, 2003).

##### 6.3. Measurement of Thiobarbituric acid reactive substance (TBARS)

A ten-gram sample and forty-eight milliliters of distilled water were combined. Add two milliliters of 4% ammonium chloride (to raise the pH to 1.5), blend the components for two minutes, and let the mixture sit at

room temperature for ten minutes. Following a wash with an additional 50 mL of distilled water, an antifoaming preparation, and a few glass beads, the liquid was quantitatively placed into Kjeldal flasks. After the flask was heated to  $50^{\circ}\text{C}$ , the Kjeldal distillation apparatus was assembled together. Distillates were collected ten minutes after the boiling started. After mixing the distillates (50 mL), a glass Stoppard tube was pipetted with the mixture. After adding 5 milliliters of TBA reagent (0.2883/100 milliliters of glacial acetic acid), left in a water bath for 35 minutes. Similar to the sample, a blank was made by mixing 5 mL of TBA reagent with 5 mL of distilled water, and it was handled the same way. The tube was heated and then allowed to cool for ten minutes under tap water. A portion of the sample was moved to a curette, and then a spectrophotometer (Perkin Elmer, 2380, USA) was set to measure the sample's optical density (D) against the blank at a wavelength of 538 nm. The TBA value (mg malondialdehyde/Kg of the sample) =  $D \times 7.8$   
D: the read of the sample against blank (ES 63/9-2006).

#### 7. Microbiological analysis

##### 7. 1. Preparation of serial dilutions

Using a heated spatula, samples of chicken breast flesh were first surface sterilized. Subsequently, the cauterized regions were extracted using a sterile scalpel and forceps. Finally, 225 milliliters of aseptic peptone water (0.1%) were added to a sterile homogenizer flask containing 25 grams of weighed chicken meat sample. To produce a  $10^{-1}$  dilution, the contents of each flask were homogenized for 2.5 minutes at 14000 rpm. Subsequently, 1 ml was transferred using a sterile pipette to a sterile test tube that held 9 ml of peptone water (0.1%). To account for the whole range of expected sample contamination, a decimal serial dilution was then prepared in increments of  $10^{-10}$ . For microbiological counting, the number of colonies in colony-forming units per gram (cfu/g) of meat samples was counted and recorded (APHA, 1992).

### 7.2. Total aerobic plate count (APC)

For the enumeration of (APC), 1 ml of the appropriate diluent was plated in triplicate using the pour-plate method on the plate count agar (Merck, Germany). After that, the plates were incubated for 48 hours at 32 °C and 10 days at 7 °C, respectively (Jay, 2005).

### 7.3. *E. coli* count

Duplicate plates of Eosin methylene blue (EMB) agar (OXOID, CM0 069) were equally spread with 100 µl of each previously made serial dilution using a sterile bent glass spreader. At 37 °C, the control and inoculation plates were incubated for a full day. There was a dark purple center to the greenish metallic colonies that were thought to be *E. coli*. Recorded were the quantity of colonies and their expression in log CFU/g of material (FDA, 2001).

### 7.4. *Staphylococcus aureus* count

Per the FDA (2001), the serial dilution was applied to egg yolk tellurite emulsion plates and left at 35°C for 48 hours. For morphological examination and biochemical identification, colonies that seemed dubious—black, glossy, and surrounded by a halo zone were chosen.

### 7.5. Detection of *Campylobacter* spp.:

Following ISO/TS 10272-2:2006 protocol, the sample was supplemented with double-strength Bolton Broth and incubated at 42°C for 48 hours under microaerophilic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>). Microaerophilic gas packs were used to provide these conditions. An initial count of *Campylobacter* spp. was obtained by streaking a loopful of Bolton Broth over modified Charcoal Cefoperazone

Deoxycholate (mCCD) agar and incubating it under microaerophilic conditions for 48 hours at 42°C. By staining, campylobacter was defined as Gram-negative cells with an S- or curved-shaped morphology.

### 7.6. Detection of *Salmonellae* spp.

After preparing the meal homogenate, incubate it at 37°C ± 1°C on 0.1% buffered peptone water for 18 hours ± 2 hours. Then, 10 milliliters of Muller-Kauffmann Tetrathionate/novobiocin broth (10 ml MKTTn) and 10 milliliters of Rappaport-Vassiliadis broth with Soya (RVs broth) each received 1 milliliter of pre-enrichment broth and 0.1 milliliter of pre-enrichment broth culture supplement. Thereafter, the two broths were incubated for 24 hours ± 3 hours at 41.5°C ± 1 °C. After serial dilution, a loopful of each MKTTn and RVS broth was applied to the surfaces of Xylose lysine Deoxycholate agar (XLD agar) and Brilliant Green (BG) agar by streaking. The samples were then incubated for 24 hours ± 3 hours at 37°C. TSI agar slants and urease streaking was used to validate suspected colonies. For further identification, suspected colonies were inoculated into a nutrient agar slant (ISO, 6579-1/2017).

### 8. Statistical Analysis:

Three duplicate samples (n = 3) were investigated for each attribute. The results were described using the mean and the standard deviation (SD) of the mean. One Way ANOVA was used to compare the means using SPSS software version 17.0, followed by Duncan's Multiple Range Test (Duncan, 1955). P<0.05 was regarded as significant when comparing mean differences using the least significant difference test.

## RESULTS

**Table 1:** The mean score for the sensory qualities of chicken breasts treated with probiotics and regular yoghurt during eight days of refrigeration at 4°C.

Descriptor	Sensory scores				
	Day 0	Day 2	Day 4	Day 6	Day 8
<b>1) Appearance</b>					
<b>Control</b>	5.98± 0.01 <sup>a</sup>	5.82 ± 0.03 <sup>a</sup>	5.62 ± 0.05 <sup>a</sup>	Spoiled	Spoiled
<b>Regular-Yogurt-marinated samples (RY)</b>	6.12 ± 0.03 <sup>a</sup>	5.92 ± 0.01 <sup>a</sup>	5.75 ± 0.32 <sup>a</sup>	5.44 ± 0.11 <sup>b</sup>	Spoiled
<b>Probiotic-Yogurt-marinated samples (PY)</b>	6.24 ± 0.03 <sup>a</sup>	5.97 ± 0.03 <sup>b</sup>	5.87 ± 0.25 <sup>a</sup>	5.64 ± 0.35 <sup>a</sup>	4.85 ± 0.42 <sup>c</sup>
<b>2) Tenderness</b>					
<b>Control</b>	5.36 ± 0.01 <sup>a</sup>	5.35 ± 0.05 <sup>a</sup>	Spoiled	Spoiled	Spoiled
<b>Regular-Yogurt-marinated samples (RY)</b>	5.67 ± 0.05 <sup>a</sup>	5.58 ± 0.07 <sup>a</sup>	5.42 ± 0.23 <sup>a</sup>	5.19 ± 0.32 <sup>b</sup>	Spoiled
<b>Probiotic-Yogurt-marinated samples (PY)</b>	5.75 ± 0.02 <sup>a</sup>	5.64 ± 0.02 <sup>a</sup>	5.55 ± 0.32 <sup>a</sup>	5.33 ± 0.25 <sup>a</sup>	4.84 ± 0.35 <sup>a</sup>
<b>3-Flavor</b>					
<b>Control</b>	5.85 ± 0.02 <sup>a</sup>	5.19 ± 0.03 <sup>a</sup>	Spoiled	Spoiled	Spoiled
<b>Regular-Yogurt-marinated samples (RY)</b>	6.52±0.02 <sup>a</sup>	6.45 ± 0.08 <sup>a</sup>	5.67 ± 0.32 <sup>a</sup>	5.27 ± 0.45 <sup>a</sup>	Spoiled
<b>Probiotic-Yogurt-marinated samples (PY)</b>	6.65±0.02 <sup>a</sup>	6.57 ± 0.03 <sup>a</sup>	5.95 ± 0.54 <sup>b</sup>	5.58 ± 0.85 <sup>b</sup>	5.44 ± 0.25 <sup>a</sup>

Data revealed as mean ± SD of 3 replicates. Values with different letters within the same row differed significantly at ( $P < 0.05$ ).

**Table 2:** Chicken breast pH after being chilled and stored at 4°C for 8 days with both regular and probiotic yoghurt.

Chicken breast	pH values				
	Day 0	Day 2	Day 4	Day 6	Day 8
<b>Control</b>	6.12±0.06 <sup>a</sup>	6.36±0.03 <sup>a</sup>	6.75±0.01 <sup>a</sup>	7.29±0.03 <sup>a</sup>	7.95±0.01 <sup>a</sup>
<b>Regular-Yogurt-marinated samples (RY)</b>	6.11±0.04 <sup>a</sup>	5.27±0.01 <sup>b</sup>	5.07±0.02 <sup>a</sup>	4.96±0.03 <sup>b</sup>	4.78±0.02 <sup>b</sup>
<b>Probiotic-Yogurt-marinated samples (PY)</b>	6.10±0.01 <sup>a</sup>	5.21±0.02 <sup>b</sup>	5.04±0.06 <sup>c</sup>	4.90±0.19 <sup>b</sup>	4.72±0.03 <sup>d</sup>

Data revealed as mean ± SD of 3 replicates.

Values with different letters within the same row differed significantly at ( $P < 0.05$ ).

**Table 3:** The TBARS values (MDA mg/kg) of chicken breasts treated with either regular or probiotic yoghurt were analyzed over an 8-day chilling period at 4°C.

Chicken breast	TBARS values (malonaldehyde mg/kg)				
	Day 0	Day 2	Day 4	Day 6	Day 8
Control	0.46±0.01 <sup>a</sup>	0.75±0.02 <sup>b</sup>	0.97±0.04 <sup>c</sup>	1.22±0.05 <sup>d</sup>	1.82±0.03 <sup>e</sup>
Regular-Yogurt-marinated samples (RY)	0.43±0.05 <sup>a</sup>	0.58±0.01 <sup>a</sup>	0.73±0.08 <sup>b</sup>	0.95±0.03 <sup>a</sup>	1.25±0.05 <sup>a</sup>
Probiotic-Yogurt-marinated samples (PY)	0.40±0.02 <sup>a</sup>	0.42±0.05 <sup>b</sup>	0.51±0.07 <sup>a</sup>	0.59±0.01 <sup>b</sup>	0.89±0.02 <sup>c</sup>

Data revealed as mean ± SD of 3 replicates.

Values with different letters within the same row differed significantly at ( $P < 0.05$ ).

**Table 4:** Cooking loss (%) of chicken breasts stored at 4°C for 8 days after being treated with regular and probiotic yoghurt.

Chicken breast	Cooking loss (%)				
	Day 0	Day 2	Day 4	Day 6	Day 8
Control	58 ± 0.03 <sup>d</sup>	59 ± 0.05 <sup>a</sup>	60 ± 0.07 <sup>c</sup>	61 ± 0.09 <sup>b</sup>	62 ± 0.05 <sup>a</sup>
Regular-Yogurt-marinated samples (RY)	58 ± 0.02 <sup>a</sup>	55 ± 0.03 <sup>d</sup>	51 ± 0.04 <sup>a</sup>	47 ± 0.06 <sup>b</sup>	49 ± 0.05 <sup>c</sup>
Probiotic-Yogurt-marinated samples (PY)	58 ± 0.01 <sup>a</sup>	54 ± 0.02 <sup>a</sup>	50 ± 0.03 <sup>a</sup>	44.53±0.32 <sup>a</sup>	45 ± 0.05 <sup>a</sup>

**Table 5:** Aerobic bacterial count (log<sub>10</sub>cfu/g) in chicken breasts treated with probiotics and regular yoghurts for eight days at 4°C during cooling

Chicken breast	Total aerobic bacterial count (log <sub>10</sub> cfu/g)				
	Day 0	Day 2	Day 4	Day 6	Day 8
Control	3.75±0.32 <sup>a</sup>	4.65±0.87 <sup>a</sup>	5.54±0.34 <sup>b</sup>	6.45±0.21 <sup>a</sup>	7.32±0.45 <sup>c</sup>
Regular-Yogurt-marinated samples (RY)	3.52±0.45 <sup>a</sup>	3.65±0.15 <sup>a</sup>	4.27±0.05 <sup>a</sup>	4.83±0.39 <sup>b</sup>	5.17±0.35 <sup>a</sup>
Probiotic-Yogurt-marinated samples (PY)	3.17±0.09 <sup>a</sup>	3.35±0.14 <sup>b</sup>	4.07±0.15 <sup>b</sup>	4.45±0.55 <sup>c</sup>	4.53±0.37 <sup>c</sup>

Data revealed as mean ± SD of 3 replicates.

Values with different letters within the same row differed significantly at ( $P < 0.05$ ).

**Table 6:** *E. coli* count (log<sub>10</sub>cfu/g) in chicken breast treated with Regular Yogurt and Probiotic-Yogurt during chilling storage at 4°C for 8 days.

Chicken breast	<i>E.coli</i> count (log <sub>10</sub> cfu/g)				
	Day 0	Day 2	Day 4	Day 6	Day 8
Control	1.35±0.02 <sup>a</sup>	1.47±0.05 <sup>a</sup>	1.79±0.25 <sup>a</sup>	2.28±0.25 <sup>a</sup>	2.63±0.45 <sup>a</sup>
Regular-Yogurt-marinated samples (RY)	1.17±0.03 <sup>a</sup>	1.25±0.35 <sup>a</sup>	1.32±0.22 <sup>a</sup>	1.45±0.09 <sup>b</sup>	1.57±0.32 <sup>c</sup>
Probiotic-Yogurt-marinated samples (PY)	1.09±0.02 <sup>a</sup>	1.12±0.35 <sup>a</sup>	1.17±0.05 <sup>a</sup>	1.29±0.24 <sup>b</sup>	1.33±0.27 <sup>b</sup>

**Table 7:** *S.aureus* count (log<sub>10</sub>cfu/g) in chicken breast treated with Regular Yogurt and Probiotic Yoghurt during chilling storage at 4°C for 8 days.

Chicken breast	<i>S. aureus</i> count (log <sub>10</sub> cfu/g)				
	Day 0	Day 2	Day 4	Day 6	Day 8
Control	1.29±0.15 <sup>a</sup>	1.42±0.19 <sup>d</sup>	1.67±0.25 <sup>c</sup>	2.07±0.12 <sup>a</sup>	2.52±0.01 <sup>b</sup>
Regular-Yogurt-marinated samples (RY)	1.18±0.02 <sup>d</sup>	1.23±0.34 <sup>b</sup>	1.34±0.09 <sup>a</sup>	1.47±0.33 <sup>c</sup>	1.54±0.55 <sup>d</sup>
Probiotic-Yogurt-marinated samples (PY)	1.02±0.07 <sup>b</sup>	1.09±0.46 <sup>a</sup>	1.14±0.03 <sup>b</sup>	1.21±0.92 <sup>a</sup>	1.29±0.25 <sup>c</sup>

Data revealed as mean ± SD of 3 replicates.

Values with different letters within the same row differed significantly at ( $P < 0.05$ ).

## DISCUSSION

Using sensory profiles, we can evaluate the quality of chicken flesh and sometimes identify unwanted contaminants (Rasooli, 2007). The results presented in Table (1) make it clear that the panelists saw that both treated and untreated samples of freshly cooked chicken breasts (day 0) performed well in every sensory category when they were given regular (RY) and probiotic (PY) yoghurt. The samples containing both ordinary and probiotic yoghurt scored higher than the control samples in every sensory characteristic, as per the results of the sensory evaluation. Several of the investigated parameters showed substantial change ( $P < 0.05$ ). The look, softness, and flavor of chicken breast fillets were much enhanced by the addition of both conventional and probiotic yoghurt until the end of the sixth storage day, notably for samples treated with probiotic yoghurt (PY). The chicken breast samples' sensory quality significantly declined on the fourth day of storage, particularly for the control sample, and the change in odor made the samples unfit for cooking. When compared to control samples and other chicken breast samples marinated with regular yoghurt (RY), the changes in sensory characteristics were less noticeable in the samples marinated with probiotic yoghurt (PY). The results of Masoumi *et al.* (2022), who discovered that probiotics and regular yoghurt improved the physicochemical quality of chicken fillets during cooking and storage and reduced microbial growth are also compatible with these findings. However, probiotic yoghurt (PY) outperformed regular yoghurt (RY) in

terms of improving the sensory qualities and shelf-life of chicken meat. Additionally, probiotic yoghurt (PY) enhances the acceptable sensory qualities of chicken meat, such as taste, colour, odour, texture, and overall acceptability, according to Angelovicova *et al.* (2013). Additionally, they noted that adding probiotics to chicken flesh somewhat improved its hardness, springiness, and chewiness. Furthermore, probiotic yoghurt (PY) can prolong the shelf life of chicken meat in addition to providing meals with the right color and flavor.

### 1. Chemical analysis

#### 1.2. pH

Table (2) reports the changes in pH values of the samples kept at 4°C for 8 days. The pH of the chicken fillets was 6.12±0.06 at the beginning and increased to 7.95±0.01 for the control group at the end of storage; however, after 8 days of storage, it dramatically decreased to 4.78±0.02 and 4.72±0.03 for the RY and PY treated groups, respectively. Total volatile basic nitrogen (TVB-N) and ammonia, two alkaline chemicals created by microbial spoiling, may be the cause of the elevated pH Mood in the control group (Ghollasi, 2017). The results of the present study are consistent with those of Masoumi *et al.* (2022), who found that chicken breast samples treated with both regular and probiotic yoghurt showed significantly lower levels of pH reduction, acidification, and chemical degradation. Notably, the samples treated with probiotic yoghurt (PY) had the lowest pH value. Furthermore, the finding of this study concurs with those of Grajales *et al.* (2012), who study how lactic acid



bacteria affect the flavour and chemical composition of pork roast. After seven days of storage, they discovered that the pH index of the treated samples decreased. According to Fraqueza et al. (2008) investigation of the rotting of turkey flesh, the medium turns acidic due to the overabundance of lactic acid bacteria activity over proteolytic bacteria. Yogurt-coated chicken meats showed a lower pH, according to the findings of another study conducted by Gˆoˆgˆus *et al.* (2004). The presence of organic acids produced by probiotics may have contributed to the lowering of pH in treated chicken breast samples treated with probiotic yoghurt (PY) during storage. These acids have an antibacterial effect, preventing the growth of many food-borne pathogens (Bolivar *et al.*, 2018).

### 1.3. TBARS:

Fresh chicken meat's high protein and moisture content, as well as its almost neutral pH, make it particularly vulnerable to lipid oxidation. In general, incorrect sensory qualities of meat products are caused by secondary products of lipid oxidation like malondialdehyde (Kostaki *et al.*, 2009). The mean values of TBA in the control samples increased from  $0.46 \pm 0.01$  mg MDA/kg on day zero of storage to  $1.82 \pm 0.03$  mg MDA/kg on day eight of storage, according to the data shown in Table (3). On the eighth day of storage, the treated chicken breast with (RY) TBA levels rose from  $0.43 \pm 0.05$  mg MDA/kg on the zero-day to  $1.25 \pm 0.05$  mg MDA/kg. Ultimately, TBA measurements for chicken breast treated with (PY) rose from  $0.40 \pm 0.02$  mg MDA/kg on day zero of storage to  $0.89 \pm 0.02$  mg MDA/kg on day eight. When storage time was extended, TBARS levels increased regardless of treatment, however, TBA levels in treated samples significantly reduced malondialdehyde levels relative to the control sample. There was no oxidative rancidity during the storage period in the treated and control chicken samples, which showed moderate levels of lipid oxidation with lipid oxidation levels below 0.5 mg MDA/kg. TBA in poultry meat should not

be more than 0.9 mg/kg of poultry meat, according (ES 1651/2005).

The TBARS value of the control samples rose over the storage period as predicted, while in the RY- and PY-treated samples, it stayed mostly constant or even dropped. Zhang (2011) investigated the antioxidative activity of lactic acid bacteria in yoghurt and identified two important defense mechanisms, enzymatic and non-enzymatic, to slow down oxidation. Antioxidant enzymes neutralize the harmful effects of superoxide anions and scavenge hydroxyl and hydrogen peroxide in the context of enzymatic defense. On the other hand, the nonenzymatic route allows cells and organisms to acquire defense mechanisms, such as reduction activity and metal ion chelating ability, which can get rid of active oxygen (Wang *et al.*, 2017).

### 1.4. Cooking Loss

One of the main factors influencing how chicken meat looks and is accepted is cooking loss. During the storage period, the control group showed a continuous rise in cooking loss. However, this increase did not reach statistical significance (Table 4). Conversely, during storage, the percentage of cooking loss in treated chicken fillets dropped dramatically (to %45 for PY-treated fillets and %49 for RY-treated fillets, respectively). When the meat's center temperature exceeds  $75^{\circ}\text{C}$  during the cooking process, it is said to have had "cooking loss." The denaturation of proteins at pH values near the isoelectric point (IP) may be the primary factor causing the rise in the percentage of cooking loss and the decline in the treated samples' ability to hold water. According to the findings of Murphy and Marks (2000) and Barbanti and Pasquini (2005), denaturation of myofibrillar proteins during the cooking process results in the shrinkage of muscle fiber and an increase in cooking loss. The reduction in cooking loss and water holding capacity (WHC) in samples treated with regular and probiotic yoghurt was caused by several factors, including the presence of ionic calcium in

yoghurt, maintaining a pH close to IP  $\sim$  5.5, and breaking down protein structures. These findings were reported by Barbut (1993) and Northcutt *et al.* (1994).

## 2. Microbiological analysis

### 2.1 Total aerobic plate count (APC):

Elevated APC can be linked to many sources of contamination in chicken meat, inadequate processing, and improper storage conditions (Zahran, 2004). The aerobic plate count mean values of the control samples varied from  $3.75 \pm 0.32$  at zero-day to  $7.32 \pm 0.45$  log<sub>10</sub> cfu/g at day 8 of storage, according to data shown in Table (5). On day zero, the mean APC values of chicken breast fillets treated with RY and PY were  $3.52 \pm 0.45$ ,  $3.17 \pm 0.09$ , and on day eight of storage, they reached  $5.17 \pm 0.35$ ,  $4.53 \pm 0.37$ , respectively. Samples treated with both types of yoghurt (RY and PY) showed a significant reduction in the count of aerobic bacteria, compared to the control group. Of these, the samples treated with Probiotic-Yogurt (PY), which is regarded as the best group, showed a significant reduction in the count of APC, compared to other groups. A total of  $10^5$ /g is the maximum number of bacteria that should be present (ES 1651/2005). On day 4, the APC of control samples was  $5.54 \pm 0.34$ , exceeding the maximum recommended limit and indicating a shelf-life of less than 4 days for the untreated control chicken breast samples. On day 1, the APC of control samples was  $4.65 \pm 0.87$ , which was close to the maximum limit of APC recommended by (ES 1651/2005). Probiotic-Yogurt-marinated samples (PY) showed a greater reducing effect in the total bacterial count, extending the shelf-life to 8 days during chilling storage. The APC values for the samples treated with RY were still valid for consumption until day 6 of storage. In contrast, the samples treated with PY showed delayed growth for APC until day 8. Amani, (2012) and Reham, (2012), who found that probiotics significantly reduced the total viable count of minced beef during refrigerated storage, provided support for

this finding. Even after being stored for seven days, treated samples containing both probiotic bacteriocin and lactic acid bacteria (*L. acidophilus*) did not surpass the allowable limit of  $10^5$  cfu/g. This could be brought on by the probiotics' antibacterial properties, particularly those in their condensed form (bacteriocins and nisin). Similar results have also been reported by (Ibrahim and Desouky, 2009) on fish-based food items (Gelman *et al.*, 2001) using metabolites generated by probiotics (*Lactobacillus*) to enhance the microbiological aspects (TCC) and safety of frozen fish fillets and fresh meat products made from veal (Raman *et al.*, 2022).

### 2.3 *E. coli* count

Since *E. coli* is a normal resident of both warm-blooded animals and humans' digestive systems, its presence in chicken flesh is a reliable indicator of faecal contamination. Additionally, it suggests a potential intestinal pathogen contamination. Contamination of raw or undercooked chicken meat can occur during primary production, such as during slaughter, or subsequent processing and handling (Adeyanju and Ishola, 2014). Therefore, most safety regulations include the enumeration of *E. coli*. Table (6) displays the microbiological counts of chicken fillet samples as a function of storage period at 4°C. The findings indicate that both ordinary yoghurt and probiotic yoghurt significantly reduce the amount of *E. coli* during storage. The aforementioned findings demonstrated that on day eight of storage, the mean value of *E. coli* counts in the control samples increased from  $1.35 \pm 0.02$  log<sub>10</sub> cfu/g to  $2.63 \pm 0.45$  log<sub>10</sub> cfu/g. While treated chicken breast samples with RY and PY *E. coli* count were slightly increased from  $1.17 \pm 0.03$ ,  $1.09 \pm 0.02$  at day zero to  $1.57 \pm 0.32$ ,  $1.33 \pm 0.27$  log<sub>10</sub> cfu/g at day 8 of storage respectively. Treatment with yogurt with both type (RY and PY) produced a significant decrease in *E. coli* count, compared to the control sample, especially that treated with Probiotic-Yogurt (PY) which was considered the best group,

showed a significant reduction in the count of *E. coli* compared with other groups. Amal and Soher (2010), Amani (2012), Reham (2012), Arena *et al.* (2016), and Masoumi *et al.* (2022) have also published similar results, demonstrating that probiotics significantly decreased the amount of *E. coli* in treated beef samples. The organic salts could be used in combination with probiotics to inhibit the growth of *E. coli*. The antibacterial properties of lactic acid strains are demonstrated in conjunction with mineral elements. For example, it has been demonstrated that the combination of copper and lactic acid may eradicate food-borne pathogens like *Salmonella* and *E. coli* O157:H7 (Gyawali and Ibrahim, 2012).

#### 2.4 *S. aureus* count:

The identification of *S. aureus* in chicken flesh suggests that food handlers and improperly maintained equipment may have contaminated the meat (ICMSF, 1996). The data shown in Table (7) indicate that the control samples' mean *S. aureus* count grew from  $1.29 \pm 0.15$  log<sub>10</sub> cfu/g on day zero to  $2.52 \pm 0.01$  log<sub>10</sub> cfu/g on day eight of storage. On day eight of storage, the mean *S. aureus* count of the treated chicken breast samples with RY increased marginally from  $1.18 \pm 0.02$  at day zero to  $1.54 \pm 0.55$  log<sub>10</sub> cfu/g. Ultimately, the mean *S. aureus* count of the probiotic-yogurt (PY)-treated chicken breast samples increased marginally, from  $1.02 \pm 0.07$  at day zero to  $1.29 \pm 0.25$  log<sub>10</sub> cfu/g on day eight of storage. This indicates that the best group had a considerable decrease in *S. aureus* counts compared with other groups. These findings are consistent with the findings of Reham (2012); Bahni and Dhar (2013) and Masoumi *et al.* (2022), who reported a highly significant ( $p < 0.01$ ) decrease in the staphylococci count in the inoculated minced fish meat that had previously been treated with LAB. The staphylococci count decreased from 2.40 to 1.46 log<sub>10</sub>cfu/g over the course of the storage period, and the reduction was significant after the 14th day of storage. Nevertheless, several authors have documented the potential use of specific

Probiotics (LAB) as bioprotective cultures to inhibit the growth of foodborne pathogens, including *S. aureus* in sausage Lucke (2000), beef burger Mohsen *et al.* (2009), and numerous meat products Batdorj *et al.* (2007) Pilet and Leroi (2011). The antibacterial metabolites of LAB, such as organic acids (which cause pH to drop quickly below 5.3), H<sub>2</sub>O<sub>2</sub> (*S. aureus* is 2–10 times more sensitive to H<sub>2</sub>O<sub>2</sub> than most LAB), bacteriocins (which work better against Gramme positive bacteria than Gramme negative bacteria), and bacteriocin-like substances, may be the cause of the growth inhibition of *S. aureus* (2007). Different bacteriocins work in different ways. For example, some can generate gaps in the target microorganism's cell membrane to improve its permeability, while others can prevent the production of the cell wall. Some can enter the bacterium's cytoplasm and release DNA or RNA, which inhibits the growth of gram-positive and spore-forming microorganisms and a wide range of microorganisms (Betancur-Hurtado *et al.*, 2022).

#### 2.5 Detection and enumeration of *Campylobacter* Spp

The human pathogen *Campylobacter* has been connected to chicken and poultry products. In the US, it is regarded as one of the most frequent causes of foodborne disease (Centers for Disease Control and Prevention, "CDC," 2018). One of the main causes of human food-borne illnesses linked to *Campylobacter* is chicken flesh. According to Marder *et al.* (2018), *campylobacter* is thought to be one of the primary agents of bacterial food-borne GIT disease (enteritis) worldwide. *Campylobacter* microorganisms can contaminate chicken flesh breast at any point during the production process, from the farm where the food is first cultivated to the customer's consumption. According to Ananchaipattana *et al.* (2012), this includes contamination that could happen during primary production on the farm, during transit of live poultry, during slaughtering procedures, in the abattoir environment, and

even throughout storage until it is consumed. The samples of chicken breast fillets that were analyzed in this experiment did not contain any *Campylobacter* species. These findings concur with ES 1651/2005. *Campylobacter Spp* and other foodborne pathogens must not be present in chicken flesh. Probiotics are useful in this aspect for lowering the population of *Campylobacter spp*. According to Deng *et al.* (2020), probiotics have the required physiological properties and anti-*Campylobacter* actions. Probiotics that inhibit *Campylobacter* colonization in the gastrointestinal tract (GIT) use many mechanistic techniques, including immunomodulation, antagonism, and competitive exclusion. In vitro, probiotics demonstrated the predicted anti-*Campylobacter* action (Kobierecka *et al.*, 2017; Dec *et al.*, 2018). The generation of antimicrobial metabolites including organic acids, H<sub>2</sub>O<sub>2</sub>, and bacteriocins is one of the antagonistic effects of probiotics. Since many potential probiotics are Lactic Acid Producing Bacteria (LAB), probiotics frequently generate enough organic acid production to change the pH of the surrounding environment and lower infections (Chaveerach *et al.*, (2004). By generating organic acids and anti-*Campylobacter* proteins, probiotics prevented the development of *Campylobacter* with a co-culture in vitro (Neal-McKinney *et al.*, 2012).

## 2.6. Detection and enumeration of *Salmonella spp*

According to Ahmed (2014), the predominant bacterial pathogen responsible for causing foodborne diseases in chicken flesh is *Salmonellae*. In many underdeveloped nations, chicken products have historically been the main source of salmonellosis (Yang *et al.*, 2011). The amount and kind of *Salmonella* present in retail food, together with the storage and preparation circumstances, all affect the risk of contracting salmonellosis from chicken flesh. Because the residual bacteria from processing live birds is injected into the poultry production system, salmonella

infection is a possible risk at every level of the processing process. Hence, throughout the production processes, *salmonella* may transfer from carcass to carcass (Nidaullah *et al.*, 2017). According to Ananchaipattana *et al.* (2012), contamination can arise at any point in the production process, including during primary production on the farm, live poultry transportation, slaughtering procedures, the abattoir environment, and even storage until the product is consumed. This could explain the presence of *Salmonella* in chicken breast fillets. When live birds are processed, the bacteria may be introduced into the poultry production system. Hence, throughout the production processes, salmonella may transfer from carcass to carcass (Nidaullah *et al.*, 2017).

*Salmonella spp* was not found in the samples of chicken breast fillets that were investigated in this study. These findings concur with ES 1651/2005. *Salmonella Spp* and other foodborne pathogens must not be present in chicken flesh. Probiotics have been shown to be successful in this respect in lowering the population of *Salmonella spp*. This is consistent with earlier research. According to Kizerwetter-Rwida and Binek (2016), probiotic *Lactobacillus* isolates with the greatest capacity to prevent *Salmonella* Enteritidis from growing, Probiotic usage is on the rise and has been shown to be an effective strategy for preventing *Salmonella* infections (Herich *et al.*, 2010 and Soncini 2011). Additionally, I concur with Maragkoudakis *et al.* (2009), who investigated how applying live lactic acid bacteria affected the raw chicken meat's microbiological quality. It shown that the probiotics prevent spoiling by slowing the growth of *Salmonella enteritidis* and *Listeria monocytogenes* while maintaining nutritional value. The bio-preservative impact of LAB bacteria in chicken products has been demonstrated by the reduction of *L. monocytogenes* and *Salmonella* development by 85 and 92%, respectively, during the course of six days of refrigerated storage (Sakaridis *et al.*, 2012). According to Gyawali and Ibrahim (2012), foodborne

organisms including *Salmonella* and *E. Coli O157:H7* have been demonstrated to be eliminated when copper and lactic acid are combined.

## CONCLUSIONS

Probiotics can prolong the shelf life of chicken breast fillets and postpone microbiological and chemical changes. They can also improve the flavor, color, texture, and general acceptance of the product. According to the findings, when compared to control samples, regular and probiotic yoghurt may considerably lower the amount of APC, *E. coli*, *S. aureus*, pH index, malondialdehyde value, and cooking loss percentage in chicken breast fillets. The microbiological and physicochemical characteristics of marinated chicken fillets differed significantly between those treated with RY and PY. When compared to other groups, the PY-treated chicken fillets that were deemed to be in the best category showed a notable decrease in all metrics. The samples of chicken breast fillets that were evaluated in this experiment did not contain any *Salmonella* spp or *Campylobacter* spp. The study's findings indicated that probiotics inhibited the development of microorganisms, enhanced the physicochemical quality, and extended the shelf life of chicken meat fillets during both storage and cooking. Probiotics stop common food-borne bacteria from growing. Consequently, taking into account the inclination of consumers towards natural additives creates novel opportunities for the use of bio-preservation in meat products. Probiotics are found in chicken breast and other animal products as a natural antibacterial.

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### تحسين جودة فيلية الدجاج المدعم بالبروبيوتك

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في هذه الدراسة، تمت دراسة الخواص الميكروبية والفيزيائية والكيميائية لشرائح الدجاج النيئة المغموسة في الزبادي العادي واللبن البروبيوتيك المحتوي على اللاكتوباسيلس اسيدوفيليس Lactobacillus acidophilus La-5 و البيفيدوبكتيريوم لونجلم Bifidobacterium longum ATCC15707، المحفوظة عند 4 درجات مئوية لمدة 8 أيام. وفي هذا الصدد، تم إجراء التحليل الميكروبي (العد البكتيري الكلي للميكروبات الهوائية APC، و عد كلي الإشيريكية القولونية *E. coli*، عد كلي للمكورات العنقودية الذهبية *S. aureus*، عزل لميكوب الكمبيلوباكتر *Campylobacter*، عزل لميكروب السالمونيلا *Salmonella*) والتحليل الفيزيائي للخواص الحسية والفحص الكيميائي (الرقم الهيدروجيني، نسبة فقدان الطهي والمواد المتفاعلة مع حمض الثيوباربيتوريك) والتحليل الحسي لعينات شرائح صدور الدجاج. مدة التخزين عند درجة حرارة 4±1 درجة مئوية لمدة 8 أيام. بالمقارنة مع العينات الضابطة بالمجموعة المقارنة السلبية، تم قياس متوسط قيم (العد البكتيري الكلي للميكروبات الهوائية APC، و عد كلي الإشيريكية القولونية *E. coli*، عد كلي للمكورات العنقودية الذهبية *S. aureus*، مؤشر قيم الأس الهيدروجيني pH، قيمة المالونديالدهيد لحامض الثيوباربيتوريك TBARS، ونسبة فقدان الطبخ *Cooking Loss*) في شرائح صدور الدجاج المعالجة بالزبادي العادي (RY) والبروبيوتيك (PY). (في اليوم الثامن من التخزين، كانت (5.17 ± 0.35، 4.53 ± 0.37، 1.57 ± 0.32، 1.33 ± 0.27 و 1.54 ± 0.55، 1.29 ± 0.25، 4.78 ± 0.02، 4.72 ± 0.03، 1.25 ± 0.05، 0.00 ± 0.02 و 0.49 ± 0.05) على التوالي، والتي أظهرت انخفاضاً معنوياً ( $P > 0.05$ )، وخاصة تلك المعالجة بالزبادي بروبيوتيك (PY) والتي تعتبر المجموعة الأفضل التي أظهرت انخفاضاً ملحوظاً في جميع القيم مقارنة مع المجموعات الأخرى. في الدراسة الحالية، لم تكن هناك أي أعداد لميكروبي السالمونيلا أو الكمبيلوباكتر في عينات شرائح فيلية الدجاج التي تم تحليلها. أشارت نتائج الدراسة إلى أن البروبيوتيك يمنع تطور الكائنات الحية الدقيقة، ويعزز الجودة الفيزيائية والكيميائية، ويطيل العمر الافتراضي لشرائح فيلية الدجاج أثناء التخزين والطهي لذا ينصح باستخدام البروبيوتك كأحد أنظمة الحفظ الحيوي للاغذية.