Biopreservative Efficacy of Bacteriocin BacFL31 in Raw Ground Turkey Meat in terms of Microbiological, Physicochemical, and Sensory Qualities

AHLEM CHAKCHOUK-MTIBAA, SLIM SMAOUI, NAOUREZ KTARI, IMEN SELLEM, SOUMAYA NAJAH, INES KARRAY-REBAI, AND LOTFI MELLOULI*

Laboratory of Microorganisms and Biomolecules, Center of Biotechnology of Sfax, Road of Sidi Mansour Km 6, P. O. Box 1177, 3018 Sfax, Tunisia

Received 12 March, 2015/Accepted 24 August, 2016

The effect of the semi purified bacteriocin BacFL31 at 200 and 400 AU/g on the shelf life of refrigerated raw ground turkey meat was investigated. The microbiological, physicochemical, and sensory properties of the meat samples were examined during refrigerated storage. The findings indicated that BacFL31 treatments were effective \( p<0.05 \) against the proliferation of various spoilage microorganisms and suppressed the growth of Listeria monocytogenes and Salmonella Typhimurium. The pH, % Met-MB, and TBA-RS values of the treated samples were lower \( p<0.05 \) than those of their control samples. The addition of BacFL31 extended the shelf life and enhanced the sensory attributes of the turkey meat samples during refrigerated storage. These results suggest that BacFL31 could be considered a promising candidate for future application as an additive to preserve the raw turkey meat during storage at 4°C.

Key words : BacFL31 / Bio-preservation / Raw ground turkey escalope / Shelf life.

INTRODUCTION

The consumption of poultry products has been steadily increasing worldwide, not only because of their relatively low cost but also for the high nutritional value they contain (Barbut, 2002). Poultry meat can, however, be easily contaminated by microorganisms and support the growth of pathogens causing foodborne diseases (Anang et al., 2007). L. monocytogenes, Salmonella, and Staphylococcus aureus are the most commonly reported pathogens implicated in foodborne outbreaks (Jofré et al., 2008). L. monocytogenes has been detected in a wide variety of poultry and meat products (Esteban et al., 2008) and is known as the causative agent of listeriosis, a disease particularly dangerous to certain risk groups, such as pregnant women, newborns, the elderly, and immuno-compromised patients (Kiran and Osmanagaoglu, 2014). Salmonellosis caused by Salmonella is a serious foodborne illness, mainly associated with eggs and poultry products (Fujikawa and Sakha, 2013). Staphylococcal food poisoning is a gastrointestinal illness caused by foods contaminated with toxins produced by S. aureus (Landgraf and Destro, 2013).

The growth of spoilage microorganisms on the surface of food products can cause undesirable reactions that deteriorate their flavor, aroma, color, sensory, and textural properties (Lucera et al., 2012). Contaminated food products have lower quality, which shortens their commercial life and entail subsequent industrial and economic losses. Accordingly, recent research has increasingly become interested in the search for viable ways to control these harmful microorganisms and improve the safety of poultry products.

Refrigeration is one of the most common methods of food preservation. Although it has been instrumental in maintaining the microbial quality of poultry products and reducing food related diseases, the refrigeration technique has been limited by its limited time of storage. Several chemical additives are currently used in meat and poultry products to extend the shelf life of refrigerated items. Growing concerns have, however, emerged around the possible adverse health effects from the
presence of chemical additives in foodstuff (Castro et al., 2011). Researchers have, therefore, become increasingly interested in the search for novel natural alternatives to chemical compounds (Cleveland et al., 2001; Devlieghere et al., 2004). Recent research indicates that lactic acid bacteria (LABs) and their natural products can offer promising opportunities for the development of efficient food preservation (bio-preservation) strategies (Settanni and Corsetti, 2008; Leelavatcharamas et al., 2011).

LABs are generally recognized as safe (GRAS microorganisms) and play an important role in food and feed fermentation and preservation, either as natural microflora or as starter cultures added under controlled conditions (Leelavatcharamas et al., 2011). Among the natural products of LABs, bacteriocins have often been reported to be potent natural bio-preservatives for use in meat. Bacteriocins are a heterogeneous class of non-pathogenic, antimicrobial peptides or proteins that show potential in the biological control of foodborne pathogens and spoilage microorganisms (Gálvez et al., 2008). The antimicrobial effects of bacteriocins from LABs, such as enterocins, have been successfully demonstrated against various foodborne pathogens (Khan et al., 2010). Enterococci are a wide group of bacteriocins produced by species of Enterococci, such as Enterococcus faecalis and Enterococcus faecium. Two enterocins, namely enterocins A and B, have been reported to display potent activity for the preservation of deboned chicken breasts (Aymerich et al., 2000). A previous work by the authors has recently reported on the purification and characterization of a bacteriocin (BacFL31) produced by a newly isolated bacterium Enterococcus faecium sp. FL31 (Chakchouk-Mtibaa et al., 2014a). The BacFL31 bacteriocin was noted to possess a broad antimicrobial spectrum against pathogenic Gram-positive and Gram-negative bacteria. This bacteriocin was completely stable to heat (90 min at 100°C) and exhibited optimal antibacterial activity at pH values ranging between 5 and 7. The present study aimed to investigate the effect of partially purified BacFL31 on the microbiological counts, physicochemical changes, and sensory qualities of raw ground turkey during storage at 4°C.

**MATERIALS AND METHODS**

**Bacterial strain, media and culture conditions**

The BacFL31 producer strain, Enterococcus faecium FL31 (Chakchouk-Mtibaa et al., 2014a), was grown in a De Man, Rogosa and Sharp (MRS) broth medium at 37°C for 18 h (De Man et al., 1960). L. monocytogenes ATCC 19117, Salmonella Typhimurium ATCC 14028 and Staphylococcus aureus ATCC 6538 were used as target strains in a microbiological challenge test. Those bacteria were grown and stored frozen at -80°C in Luria-Bertani (LB) media containing 20% (v/v) glycerol. L. monocytogenes ATCC 19117 was cultured on a Palynmyxin Acriflavin Lithium-Chloride Ceftazidime Aesculin Mannitol (PALCAM) agar (LAB M Ltd, U.K.) at 37°C for 24 h; S. Typhimurium ATCC 14028 was grown on Xylose lysine deoxycholate (XLD) agar (Oxoid); and S. aureus ATCC 6538 was cultivated on a Chapman medium (Oxoid) at 37°C for 24 h. The bacteria were enumerated by the plate count method. Concerning meat experiments, growth values were measured as CFU/g on agar plates. The data represent results from three replicates.

**Bacteriocin BacFL31 preparation**

A partially purified BacFL31 was obtained from a 900 mL of an 18-h-old culture of E. faecium FL31 using a first purification step wherein the supernatant was heat-treated for 15 min at 90°C and then cooled at room temperature prior to pelleting the denatured proteins by centrifugation at 4500 × g for 30 min in accordance with Chakchouk-Mtibaa et al. (2014a). The obtained active solution was neutralized (pH 6.5) with NaOH to eliminate the effect of the organic acids produced by this strain, concentrated to one-tenth of the original volume in a Rotavapor at 70°C, sterilized by passage through 0.45-µm pore size filters (Millipore), and submitted to antimicrobial activity evaluation against L. monocytogenes ATCC 19117 using the agar well diffusion assay (AWDA) (Tagg and McGiven, 1971). In this assay, two-fold serial dilutions of the active solution were prepared and 50 µl aliquots of the various dilutions were loaded into separate wells. The plates were incubated overnight at 37°C, and bacteriocin activity was determined as the reciprocal of the highest dilution showing a definite zone of inhibition around the well. The bacteriocin activity in the concentrated extract was expressed in arbitrary units per milliliter (AU/mL) according to Kouakou et al., (2009) calculated by multiplying the reciprocal of the critical dilution by 1000/v (v = 50 corresponding to the volume in µl seeded in the well). In our case, the bacteriocin activity in the concentrated extract was 300 AU/mL. However, it should be noted that arbitrary units per milliliter (AU/mL) depends on several factors such as the determination method and the indicator strain. Consequently, in the results and discussion part, we have compared BacFL31 results with those of BactTN635 and BacJ1 previously described by Smaoui et al., (2014) and Chakchouk-Mtibaa et al., (2014b) respectively. In fact, arbitrary units per milliliter (AU/mL) of concentrated BactTN635 and BacJ1 were determined under the same conditions as for BacFL31.
Meat preparation and sampling

Fresh turkey meat was purchased from a local supermarket, immediately transported to the laboratory at 4°C, and ground in a sterile grinder. The ground meat was divided into three equal lots (600 g each). Lot E1 was the control lot without the BacFL31 addition and Lot E2 and Lot E3 were supplemented with partially-purified BacFL31 at 200 and 400 AU/g, respectively. BacFL31 was used at 300 AU/mL and to get a concentration of 200 AU/g in 600 g of meat and 400 mL of the active solution at 300 AU/mL were used and concentrated in a Rotavapor at 70°C to 20 mL. Ingredients were homogenized in a mixer grinder (Moulinex Mixer Grinder LM2421) for 10 min. They were packaged in sterile plastic bags and stored under refrigeration at 4°C. Samples were withdrawn at 0, 3, 7, 10 and 14 days for microbiological, sensory, physicochemical, and textural analysis.

Microbiological analysis

At each sampling time, a 10 g of raw ground turkey was taken and deposited in a stomacher bag to which 90 mL of 0.1% peptone sterile water was aseptically added. The mixture was macerated in the stomacher for 2 min at room temperature. After serial decimal dilution, appropriate dilution samples (1 mL) were poured onto total count and selective agar plates. Aerobic plate counts (APC) were determined on plate count agar (PCA; Difco), incubated at 30°C for 48 h. Psychrotrophic counts (PTC) were determined as described above for APC, except for the incubation at 7°C for 10 days (Cousin et al., 1992). Enterobacteriaceae counts were enumerated by the pour plating method on Violet Red Bile Glucose Agar (VRBGA; Oxoid). The plates were overlaid with a virgin layer of the same growth medium before incubation at 37°C for 24 h. All experiments were performed in triplicate.

Plates containing 25-250 colonies were selected and counted, and the average number of CFU/g was calculated. Staphylococcus aureus, L. monocytogenes, and Salmonella Typhimurium were counted in accordance with the methods of the International Organization for Standardization, namely ISO 6888-3: 2003c; ISO 11290-1: 1996c; and ISO 6579: 2002, respectively.

Microbiological challenge tests

In order to determine the efficacy of BacFL31 to repress the growth of spoilage and pathogenic microorganisms, three samples of raw ground turkey (the control and two samples in which BacFL31 was added at 200 or 400 AU/g) were inoculated with one of the pathogenic bacteria, L. monocytogenes ATCC 19117, S. Typhimurium ATCC 14028, or S. aureus ATCC 6538, at 10⁵ CFU/g. Portions were taken from each sample every three hours along a period of thirty hours. L. monocytogenes ATCC 19117 was detected and enumerated through plating the diluted homogenate on PALCAM agar (LAB M Ltd, U.K.). S. Typhimurium ATCC 14028 was spotted and enumerated by pour-plating on XLD agar (Oxoid). S. aureus ATCC 6538 was identified and counted on Chapman agar (Oxoid).

Physicochemical analysis

pH measurements

The pH value was determined by homogenizing 5 g of raw ground turkey in 50 mL distilled water (pH 7.00) and using a pH-meter (pH 210 Microprocessor pH Meter, HANNA instruments, Germany) at each sampling point.

Lipid oxidation

2-thiobarbituric acid reactive substances (TBA-RS) were determined according to a slightly modified version of the method of Eymard et al. (2005) first described by Salih et al. (1987). In brief, two grams of the sample were mixed with 100 μL of butylated hydroxytoluene (BHT) in ethanol (1 g/L) and 16 mL of trichloroacetic acid (TCA 50 g/L). The mixture was homogenized by a kitchen blender for 10 min and then filtered. Two milliliters of filtrate (or 2 mL of TCA for blank) were added to 2 mL of thiobarbituric acid (TBA) solution (20 mol/L) and placed in tightly closed tubes that were heated at 70°C for 30 min and rapidly cooled in ice.

Absorbance was read against the blank at 508, 532, and 600 nm with a spectrophotometer (T 60UV-visible Spectrophotometer, PG instruments). The absorbance measured at the maximum (532 nm) was corrected for the baseline drift as follows: A532 nm corrected = A532 nm − [(A508 nm − A600 nm) × (600 − 532)]/(600/508) − A600 nm. The results were expressed as mg of malonaldehyde (MDA) equivalents per kg of sample (mg/kg) using the molar extinction coefficient of the MDA-TBA adduct at 532 nm (1.56 × 10⁵ M⁻¹ cm⁻¹) according to Buege and Aust (1978). The MDA was determined using the following formula: mg MDA eq/kg = (A corrected × VTCA × 2 × MMDA,10⁻²)/(1.56 × m).

Metmyoglobin (Met-Mb) analysis

Metmyoglobin (Met-Mb) content was determined according to the method described by Krzywicki (1982). Raw ground turkey meat (5 g) was placed into a 50 mL polypropylene centrifuge tube and homogenized with 25 mL of ice-cold phosphate buffer (pH 6.80, 40 mM) by a homogenizer for 1 min. The homogenized sample was left to rest at 4°C for 1 h and centrifuged at 4500 × g for 30 min at 4°C. The supernatant was filtered through 0.45-µm pore size filters (Millipore), and absorbance was read at 572, 565, 545, and 525 nm.
nm by scanning the visible spectrum with a spectrophotometer (T 60UV- visible Spectrophotometer, PG instruments). The Met-Mb percentages were then calculated based on those absorbance values according to the method described by Krzywicki (1982) using the following formula:

$$\text{Met-Mb} \% = [\frac{-2.51 \times (A_{572} \text{nm}/A_{525} \text{nm}) + 0.777 \times (A_{565} \text{nm}/A_{525} \text{nm}) + 0.8 \times (A_{545} \text{nm}/A_{525} \text{nm}) + 1.098}{A}] \times 100$$

where A refers to the corresponding absorbance.

**Color measurement**

Color was measured by a Konica Minolta CR-300 Chroma Meter to determine $L^*$, $a^*$, and $b^*$ values. The $L^*$ value referred to color lightness, ranging from 0 (black) to 100 (white); the $a^*$ value related to the span of red-green color, ranging from -100 (greenness) to +100 (redness); and the $b^*$ value indicated the extent of yellow-blue color, ranging from -100 (blueness) to +100 (yellowness). All measurements were performed in triplicate.

**Sensory evaluation**

Sensory evaluation was carried out according to the method of Ogunbanwo and Okanlawon (2006). The sensory attributes were evaluated by a panel of 25 individuals. Each person was requested to mention levels of color (golden brown or pale), texture (soft or hard), odor (rancidity or agreeable) and overall acceptability of raw ground turkey (with or without the BacFL31 addition) that was stored at 4°C. The judges were not informed about the experimental approach, and the samples were blind-coded with 3-digit random numbers. A nine-point hedonic scale (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely) was used for the evaluation of overall acceptability. A score of 4 was taken as the lower limit of acceptability.

**Statistical analysis**

All experiments performed on the different fresh ground turkey meat samples were carried out in triplicate. The effects of treatment and storage time were analyzed for each sample using the Analysis of Variance (ANOVA) procedure of SPSS, version 19.0. Microbial data were transformed into logarithms of the number of colony forming units (CFU/g) and submitted to ANOVA. Means and standard errors were calculated. Differences among the mean values of the various treatments and storage periods were determined by the least significant difference test, and significance was defined at $p<0.05$. Relationships amongst microbiological analysis, physicochemical change and sensory evaluation were estimated using Pearson’s correlation coefficients.

**RESULTS AND DISCUSSION**

**Microbiological evaluation**

**Aerobic plate counts (APC).** The counts of aerobic microorganisms examined in the present study are shown in Fig.1A. The initial (APC) value recorded for the control sample was approximately 2.22 log CFU/g, which was indicative of the good quality of the raw ground turkey. The APC value increased with the increase of the storage time at 4°C and reached 7.04 log CFU/g after 7 days of storage, which is considered as the upper limit for fresh meat as defined by the French Association for Standardization (2004). The control sample started to deteriorate on day 7, indicating that the control raw ground turkey had a shelf life of 7 days. The treatment of the samples with the partially-purified BacFL31 was noted to significantly ($p<0.05$) inhibit the growth of APC. The treatment using 400 AU/g of BacFL31 was more effective than that of 200 AU/g, and the shelf life of the sample treated with 400 and 200 AU/g was up to 14 days and approximately 14 days, respectively (Fig.1A). The reduction in microbial counts by bacteriocin has previously been demonstrated by Smaoui et al. (2014). These authors proved that chicken breast meat treated with 1000 AU/g semi purified bacteriocin BacTN635 produced by Lactobacillus plantarum TN635 could extend the shelf-life of chicken breast to 21 days during storage at 4°C. In our case, the concentration of 200 AU/g of BacFL31 was able to extend the shelf life of raw ground turkey to 14 days.

**Psychrotrophic Counts (PTC).** As indicated in Fig.1B, a reduction in the PTC count was observed for samples treated with BacFL31 as compared to the control ($p<0.05$). While the PTC of the two treated samples never exceeded the maximal limit of 7 log CFU/g recommended for raw ground turkey throughout the 14 days of refrigerated storage, they started to deteriorate after 8 days of storage for the control samples. Treatment with BacFL31 (200 and 400 AU/g) reduced the PTC counts approximately by 3 and 4 logs, respectively, during the 14 days of storage as compared to the control samples ($p<0.05$) (Fig.1B). The reduction in psychrotrophic counts on poultry meat has been reported by Smaoui et al. (2014), who proved that a treatment with 1000 AU/g of bacteriocin BacTN635 could extend the shelf life of chicken breast to 28 days whereas the control samples started to deteriorate after 14 days of storage.

**Enterobacteriaceae counts.** The results presented in Fig.1C indicated that the growth of Enterobacteriaceae was slower than APC and PTC. The initial Enterobacteriaceae count recorded for the control...
than the typical limit value of 2 log CFU/g (ISO 4833, 2003b; ICMSF, 1986). This indicated that the addition of 200 AU/g of bacteriocin was able to reduce the growth of Enterobacteriaceae and extend the shelf life of raw ground turkey to 10 days, which reached 14 days with concentrations of 400 AU/g.

On the other hand, the results indicated that the Staphylococcus aureus, L. monocytogenes, and Salmonella spp detection tests were negative throughout the whole storage period of both the control and treated raw ground turkey samples (data not shown), thus confirming the conformity of the studied product to the criteria recommended by the APHA norms (APHA, 1992) and its suitability for human consumption.

**Microbiological challenge tests**

**Listeria monocytogenes ATCC 19117.** As shown in Fig. 2A, the psychrotrophic L. monocytogenes ATCC 19117 count increased during storage at 4°C, reaching 7.1 log CFU/g after 30 hours for the control sample. The addition of bacteriocin BacFL31 at concentrations of 200 and 400 AU/g significantly reduced the counts of this bacterium population to 1 log CFU/g after 18 and 12 hours of storage at 4°C, respectively ($p<0.05$). Similar results were previously observed by Smaoui et al. (2014), who reported that BacTN635, a bacteriocin secreted by Lactobacillus plantarum sp.TN635, repressed the growth of L. monocytogenes when added to raw chicken breast meat at a concentration of 500 AU/g after 10 hours of storage. Other bacteriocins have been isolated and characterized from Enterococcus species and reported to have antilisterial effects on meat products during refrigeration, including pork (Aymerich et al., 2000) and chicken breast (Nazef et al., 2008) meat.

**Salmonella Typhimurium ATCC 14028.** As indicated in Fig. 2B, the counts of S. Typhimurium ATCC 14028 underwent a slight decrease from 4 to 3.55 log CFU/g, which was inhibited by the storage temperature in the control sample. The presence of BacFL31 at concentrations of 200 and 400 AU/g in the treated samples significantly suppressed the growth of S. Typhimurium ATCC 14028 after 24 and 12 hours of storage, respectively ($p<0.05$). Several bacteriocins that were isolated and characterized from Enterococcus species were effective in controlling S. Typhimurium, including the enterocin P-like bacteriocin produced by E. faecium GM-1 (Kang and Lee, 2005) and enterocin 012 bacteriocin produced by Enterococcus gallinarum 012 (Jennes et al., 2000). This demonstrates the wide range of antimicrobial activity of bacteriocins produced by Enterococci, such as the BacFL31 bacteriocin produced by Enterococcus faecium FL31 presented in this work.

In a previous study, we proved that the addition of the sample increased from 1.54 log CFU/g on day 0 to a count of 4.3 log CFU/g by day 14, whereas it reached significantly lower count values of 2.2 and 2.02 log CFU/g in the samples treated with BacFL31 at 200 and 400 AU/g, respectively (Fig. 1C). The Enterobacteriaceae count of the sample treated with 200 AU/g of BacFL31 on day 7 was approximately 1.80, which was lower than the typical limit value of 2 log CFU/g (ISO 4833, 2003b; ICMSF, 1986). This indicated that the addition of 200 AU/g of bacteriocin was able to reduce the growth of Enterobacteriaceae and extend the shelf life of raw ground turkey to 10 days, which reached 14 days with concentrations of 400 AU/g.
The results revealed that BacFL31 not only displayed a wide spectrum of inhibitory activity against several Gram-positive and Gram-negative bacteria, but also showed attractive bactericidal effects, rapidly suppressing the growth of a wide range of pathogens. *Staphylococcus aureus* ATCC 6538. As shown in Fig. 2C, the growth of *S. aureus* ATCC 6538 in the control sample remained nearly constant throughout the storage period. The levels of *S. aureus* ATCC 6538 in the treated samples were significantly lower than in the control ($p<0.05$). The concentration of 400 AU/g of BacFL31 was more effective than 200 AU/g, reducing the number of *S. aureus* ATCC 6538 to 2.2 log CFU/g after 30 hours of storage. Compared to its activity against *S. Typhimurium* ATCC 14028 and *L. monocytogenes* ATCC 19117, the BacFL31 treatment of raw turkey meat was much less effective against *S. aureus* ATCC 6538.

**Physicochemical parameters**

**pH measurements**

Table 1 shows the effect of bacteriocin BacFL31 on the pH of the raw ground turkey meat samples during refrigerated storage at 4°C for 14 days. The turkey meat samples had an initial pH of 5.83, which is in accordance with the values previously determined for fresh poultry meat (Pränderl et al., 1988). The pH value of the control increased from 5.83 to 7 at the end of storage. This increase in pH reflected meat spoilage due to protein breakdown and free amino acid production, leading to the formation of NH$_3$ and amines, compounds of the alkaline reaction. Compared to the control, the sample treated with BacFL31 at 200 AU/g, pH underwent a slighter increase to 6.48. The pH remained almost stable for the sample treated with 400 AU/g of BacFL31.

**Lipid oxidation**

Lipid oxidation represents one of the major factors causing the progressive deterioration of meat product quality and limiting the shelf life during storage. It can be reduced by the addition of antimicrobial agents to the meat products. The TBA-RS method has been widely used to determine the degree of lipid oxidation. Table 1 represents the effect of BacFL31 on the TBA-RS values of refrigerated raw ground turkey samples. Throughout the storage period, the TBA-RS values in the treated samples were lower than those in control samples. A TBA-RS value of 0.96 mg MDA/kg meat was recorded for the control sample after 7 days of storage, and the emergence of an unpleasant odor was observed. The TBA-RS value remained relatively low in the two treated samples, reaching 0.7 mg MDA/kg meat by day 7 (Table 1). While the control meat samples were unacceptable beyond 7 days of storage, the
samples treated with 200 or 400 AU/g of BacFL31 remained acceptable up to 10 days of storage. These results showed that BacFL31 significantly retarded the development of oxidative rancidity. In fact, the TBA value has often been considered as an indicator of rancidity in fat products. Overall, the acceptability limit of the TBA-RS number in the present study was near 1 mg MDA/kg of raw ground turkey. Verme and Sahoo (2000) used MDA concentrations between 1.0 and 2.0 mg/kg as threshold values for rancidity in meat. As indicated in Table 1, no significant differences (p>0.05) were observed between the lipid oxidation of samples containing 200 AU/g and 400 AU/g of BacFL31.

Metmyoglobin (Met-Mb) analysis

Meat color depends on the chemical state of myoglobin. The undesirable discoloration of meat during preservation is often attributed to the oxidation of myoglobin to metmyoglobin (Chaijan, 2008). The changes in the metmyoglobin content in the raw ground turkey during storage at 4°C are presented in Table 1. The initial (% Met-Mb) was 28% in the three studied samples. After 7 days of storage, %Met-Mb increased (p<0.05) with all samples, reaching 43% in the control (without BacFL31 addition) and 36% and 32.8% in the samples treated with 200 and 400 AU/g of BacFL31, respectively. It is worth noting that consumer rejection of Met-Mb in meat products occurred at 40% (Sancoban and Yilmaz, 2014), with the onset of deterioration being recorded after 7 days of storage for the control sample and after about 9 and 10 days of storage for the samples treated with 200 and 400 AU/g of BacFL31, respectively (Table 1).

Color measurement

The color values recorded for the raw ground turkey meat samples in the presence or absence of BacFL31 are shown in Table 2. The L* values, referring to lightness, were noted to range from 49.6 to 52.92 (Table 2), which is in accordance with the values previously determined for poultry meat (Qiao et al., 2002). In a previous study, Qiao et al. (2001) separated broiler breasts into three groups of lightness characteristics as follows: lighter than normal, L*> 53; normal, 48 < L*< 51; darker than normal L* < 46. In the present study, the initial L* value showed normal lightness (49.80). The control sample displayed a slight decrease in lightness, with an L* value reaching 48 at the end of the storage period. Under this condition, a light dark tint was observed for the raw ground turkey meat, which could be associated with the onset of muscle protein decomposition (McDougall, 1982). Conversely, the L* values underwent a significant (p<0.05) increase during storage in the presence of BacFL31 at 200 and 400 AU/g and remained lower than 53 till the end of the experiment.

The analysis of a* (redness) values showed that the three turkey samples showed a decrease in redness during storage at 4°C (Table 2). Nevertheless, this decrease was more remarkable for the control than the BacFL31 treated samples. The reduction in red color intensity during storage could presumably be attributed to the interdependence between lipid oxidation and color oxidation in meats (Lynch and Faustman, 2000). Kennedy et al. (2005) reported on a decrease in a* values corresponding to decreases in the redness of meat due to myoglobin oxidation and metmyoglobin formation.

In terms of the b* values, referring to yellowness,
TABLE 2. Effect of semi-purified BacFL31 on instrumental color parameters (CIE L*, a*, b*) of raw ground turkey meat with and without semi-purified BacFL31 during 14 days of storage at 4°C.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>0 days</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>L* value</td>
<td>Control</td>
<td>49.80±2.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.73±1.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.02±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.02±1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48±1.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>49.70±2.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.77±1.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.10±1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52±1.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52±1.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>49.66±2.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.00±1.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.85±1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.92±1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.92±1.60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>a* value</td>
<td>Control</td>
<td>3.87±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.83±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.48±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>3.89±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.25±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.56±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>3.88±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.33±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.88±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>b* value</td>
<td>Control</td>
<td>7.24±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.20±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.64±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.3±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>7.25±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.08±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.21±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>7.28±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.90±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.89±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.78±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean±SD: Means and standard deviation of three replicates.

<sup>a-c</sup>: Means with different letters in the same column are significantly different (<i>p</i>&lt;0.05).

TABLE 3. Sensory analysis of raw ground turkey meat samples with and without semi-purified BacFL31 during 14 days of storage at 4°C.

<table>
<thead>
<tr>
<th>Sensory evaluation parameters</th>
<th>Treatments</th>
<th>0 days</th>
<th>3 days</th>
<th>7 days</th>
<th>10 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>Control</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.5±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.3±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.4±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.6±0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.6±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colour</td>
<td>Control</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.0±0.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.1±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.0±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Texture</td>
<td>Control</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.3±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5±0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.1±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.6±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.6±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>Control</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (200AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.5±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.5±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>BacFL31 (400AU/g)</td>
<td>9±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.25±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean±SD: Means and standard deviation of three replicates.

<sup>a-c</sup>: Means with different letters in the same column are significantly different (<i>p</i>&lt;0.05).

there were no significant differences between the control and treated samples (<i>p</i>&gt;0.05) (Table 2). The b* values of the control and two BacFL31 treated (200 and 400 AU/g) samples remained constant during storage at 4°C.

**Sensory evaluation**

Due to the growing consumer awareness and safety concerns worldwide, the poultry meat industry has increasingly been interested in the enhancement of the safety, value and quality of its products. Accordingly, knowing consumers’ preferences and perceptions of the sensory attributes of food products is very important to food manufacturers. The odor, color, texture, and overall acceptability values of the raw ground turkey meat samples treated with 200 and 400 AU/g of BacFL31 during storage at 4°C are shown in Table 3. At the beginning of the experiment, the best score obtained for sensory attributes was 9.0 (Table 3). This score declined with storage time at 4°C. Compared to the control, however, the sensory parameters of the two treated samples were noted to improve (<i>p</i>&lt;0.05).
TABLE 4. Correlation of microbiological, physicochemical, and sensory parameters of raw ground turkey meat.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>APC</th>
<th>PTC</th>
<th>Enterobacteriaceae</th>
<th>pH</th>
<th>TBA-RS value</th>
<th>a* value</th>
<th>b* value</th>
<th>% Met-Mb</th>
<th>Odor</th>
<th>texture</th>
<th>color</th>
<th>overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTC</td>
<td>0.969*</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>0.911**</td>
<td>0.950**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.912**</td>
<td>0.939**</td>
<td>0.909**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBA-RS value</td>
<td>0.866**</td>
<td>0.823**</td>
<td>0.771**</td>
<td>0.822**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L* value</td>
<td>-0.206</td>
<td>-0.215</td>
<td>-0.360</td>
<td>-0.127</td>
<td>0.214</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a* value</td>
<td>-0.806**</td>
<td>-0.789**</td>
<td>-0.725**</td>
<td>-0.837**</td>
<td>-0.978**</td>
<td>-0.317</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b* value</td>
<td>-0.545*</td>
<td>-0.579*</td>
<td>-0.490</td>
<td>-0.515*</td>
<td>-0.687**</td>
<td>-0.272</td>
<td>0.667**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% MetMb</td>
<td>0.896**</td>
<td>0.862**</td>
<td>0.852**</td>
<td>0.874**</td>
<td>0.961**</td>
<td>-0.007</td>
<td>-0.992**</td>
<td>-0.587**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Odor</td>
<td>-0.954**</td>
<td>-0.922**</td>
<td>-0.861**</td>
<td>-0.887**</td>
<td>-0.963**</td>
<td>-0.021</td>
<td>0.917**</td>
<td>0.691**</td>
<td>-0.956**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>texture</td>
<td>-0.866**</td>
<td>-0.840**</td>
<td>-0.787**</td>
<td>-0.861**</td>
<td>-0.991**</td>
<td>-0.212</td>
<td>0.985**</td>
<td>0.696**</td>
<td>-0.965**</td>
<td>0.962**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>color</td>
<td>-0.841**</td>
<td>-0.817**</td>
<td>-0.753**</td>
<td>-0.828**</td>
<td>-0.968**</td>
<td>-0.257</td>
<td>0.984**</td>
<td>0.706**</td>
<td>-0.954**</td>
<td>0.947**</td>
<td>0.991**</td>
<td>1</td>
</tr>
<tr>
<td>overall acceptability</td>
<td>-0.904**</td>
<td>-0.813**</td>
<td>-0.763**</td>
<td>-0.809**</td>
<td>-0.965**</td>
<td>0.009</td>
<td>0.914**</td>
<td>0.555**</td>
<td>-0.956**</td>
<td>0.965**</td>
<td>0.955**</td>
<td>0.937**</td>
</tr>
</tbody>
</table>

*** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)

Correlations among microbiological, physicochemical, and sensory parameters of turkey meat

The correlations among microbiological, physicochemical, and sensory parameters of raw ground turkey are illustrated in Table 4. The results indicated that the microbiological parameters (APC, PTC, and Enterobacteriaceae) showed significant positive correlations between their values (p<0.01). APC, PTC, and Enterobacteriaceae were positively correlated with pH, TBA-RS, and %Met-Mb (p<0.01) and negatively correlated with a*, b* (p<0.01), and L* values (p>0.05). These results clearly indicated that microbiological data correlated well with physicochemical data.

The microbiological parameters also showed significant negative correlations (p<0.01) with sensory data. The pH was positively correlated to TBA and %Met-Mb (p<0.01), indicating a strong correlation between physicochemical values. These values (pH, TBA, and %Met-Mb) were negatively correlated (p<0.01) with sensory parameters (texture, odor color, and overall acceptability).

ACKNOWLEDGMENTS

This research was funded by the Tunisian Ministry of Higher Education and Scientific Research.

REFERENCES


ture, water holding capacity, and emulsification capacity. 