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Microbial evaluation of smoked turkey as influenced by storage temperature and vacuum packaging

Abd El-Rady A. Mousa

Bot. Dept., Fac. Arts and Sci., Kufra, Benghazi Univ., Libyan

Bdalradymsy51@gmail.com

ABSTRACT

The development of microbial association of vacuum-packed smoked turkey during storage at 0, 5, 10 and 15 ºC for up to 30, 20,10 and 5 days, respectively was examined. Total viable count (TVC), lactic acid bacteria (LAB), yeasts and moulds (Y&M), Pseudomonads, total staphylococci, members of the *Enterobactericeae* group, *Escherichia coli*, and the pathogenic bacteria *Listeria* spp. and *Salmonella* spp. were enumerated.

The population of lactic acid bacteria (LAB), Staphylococci, Pseudomonades, *Enterobacteriaceae* and yeast/moulds was low (below 10^2 CFU/g), while no pathogenic bacteria e.g., *Listeria* and *salmonella* was detected by enrichment technique.

Temperature and sampling time were significantly influenced on TVC and LAB counts. At the end of storage, the microbial association in smoked turkey was dominated by lactic acid bacteria regardless of the storage temperature.

The Gram-negative flora (e.g., *Enterobacteriaceae* and pseudomonads) was not found to increase in numbers while staphylococci and yeasts/moulds remained at low level (< 2 log CFU/g) in smoked turkey regardless of the packing conditions. The pH value of smoked turkey was 6.72 to 6.75 in the beginning of storage, and the values of pH reached to 5.72 to 6.45 at the end of storage at 15 and 0 ºC respectively. It could be concluded, on the basis of the results that, the vacuum packaging of smoked turkey and chilling temperature conditions (0 and 5 ºC) were effective methods for the preservation and safety of this product for extending the shelf life.

Keywords: Smoked turkey, microbiological analysis, storage, bacteria, lactic acid bacteria

الملخص العربي

التقييم الميكروبي لشرائح الرومي المدخن تحت تأثير درجات حرارة التخزين والتعبئة تحت تفريغ

عبد الراضي موسى

قسم علم النبات –كلية الآداب والعلوم – الكفرة – جامعة بنغازي – ليبيا

تم اختبار الحمل الميكروبي المرتبط لشرائح اللحم الرومي المدخن والمعبأ بالتفريغ أثناء التخزين في درجات حرارة 0 و5 و10 و15 درجة مئوية لمدة تصل إلى 30 و20 و10 و5 أيام على التوالي. تم تقديرالعدد الكلي للبكتيريا الحية (TVC)، وبكتيريا حمض اللاكتيك (LAB)، الخمائر والفطريات (Y&M)، Pseudomonads، مجموع المكورات العنقودية، ومجموعة البكتيريا المعوية *Enterobacteriaceae*، *Escherichia coli*، والبكتيريا المسببة للأمراض *Listeria* spp. و*Salmonella* spp. في شرائح اللحم الرومي المدخن والمحفوظة على دراجات الحرارة السابقة.

كان عدد بكتيريا حمض اللاكتيك (LAB) والمكورات العنقودية والسيدوموندس والبكتريا المعوية والخميرة / الفطريات منخفضًا (أقل من 10^2 CFU / جم)، بينما لم يتم الكشف عن البكتيريا المسببة للأمراض مثل الليستريا والسالمونيلا بتقنية الاكثار في البيئات المتخصصة والانتقائية.

أثرت درجات الحرارة المستخدمة في الحفظ ووقت أخذ العينات بشكل كبير على تعداد TVC و LAB. في نهاية التخزين، سيطرت بكتيريا حمض اللاكتيك على الحمل الميكروبي الموجود في شرائح اللحم الرومي المدخن بغض النظر عن درجة حرارة التخزين.

لم يتم اكتشاف وتقدير البكتيريا السالبة لجرام (على سبيل المثال، *Enterobacteriaceae* و pseudomonads) أو حدوث زيادة في الأعداد بينما ظلت المكورات العنقودية والخمائر / الفطريات عند مستوى منخفض (<2 Log CFU / جم) في شرائح الرومي المدخن والمعبأة تحت تفريغ والمحفوظة بغض النظر عن درجات الحرارة المستخدمة في الحفظ. كانت قيمة الرقم الهيدروجيني لشرائح الرومي المدخن تتراوح ما بين 6.72 الي 6.75ووصلت هذه القيم الي 5.72 إلى 6.45عند درجات حفظ 15 وصفر مئوي على التوالي.

يمكن الاستنتاج، على أساس النتائج، أن التعبئة تحت تفريغ لشرائح الرومي المدخن والحفظ عند دراجة درجة حرارة التبريد (0 و 5 درجة مئوية) كانت طرق فعالة للحفاظ على هذا المنتج وسلامته وزيادة فترة صلاحيته.

الكلمات المفاتحة: لحم الرومي المدخن– التحليل الميكروبيولوجي ، تخزين ، بكتيريا ، بكتيريا حمض اللاكتيك

1. Introduction

There is no doubt that each food product harbours its own specific and characteristics microflora at specific points in time during production and storage. These microorganisms are functions of the processing, preservation and storage conditions. Theoretically, at the end of manufacturing, indigenous flora of pasteurised, cooked and smoked meats is present at low levels. According to **(Rubio, et al., 2006; Zwirzitz, et al., 2021),** cross- contamination during chopping or slicing and packaging leads to an increase of total viable microflora.

Contamination of ready-to-eat (RTE) meat with foodborne pathogens due to malpractices in handling, slicing and storage conditions remains an important public health issue, because it can lead to illness if it is consumed without further heating **(Halagarda, et al., 2022).** The most important pathogen is *Salmonella*. Data from the European Union (EU) show that in 2001, there were 157 822 reported cases of human salmonellosis originating from poultry meat consumption (**Cavitte, 2003).** During storage, environmental factors, such as temperature, pH, gaseous atmosphere and NaCl

influence certain bacteria, and affect their growth rate and activity **(Farber and Peterkin, 1991; Membre et al., 2005; Stellato, et al., 2016; Zwirzitz, et al., 2021).** For vacuum-packaged, chill-stored meat products, the absence of oxygen restricts the growth of organisms such as *Pseudomonas* and *Enterobacteriaceae* so that spoilage which typically occurs is associated with the growth of lactic acid bacteria (LAB**) (Borch, et al., 1996; Taylor, 1996; Korkeala and Bjorkroth, 1997; Holley, 1997; Hansen and Bautista, 2000; Nychas and Drosinos, 2000; Stellato, et al., 2016).** But other Gram positive (e.g., *Brochothrix)* and Gram-negative bacteria (e.g., *Enterobacteriaceae)* can occur in relatively large number in vacuum packaged poultry meat **(Mead, 2004).** The development of LAB under anaerobic conditions suppresses the Gram-negative bacteria *Enterobacteriaceae* and *Pseudomonads* **(Simard, et al., 1983).**

However, vacuum packaging has been shown to be very effective in extending the shelf life of perishable food **(Silla and Simonsen, 1985; Church, and Parsons, 1995).** The shelf life of sliced vacuum-packed cooked meat is 18-20 days at a storage temperature of 4 ºC **(Ambrosiadis, and Georgakis, 1993)**. The average shelf life for cured, cooked, sliced turkey fillets and cooked sausage is 2 and 1 week at 4 and 10°C, respectively **(Pexara, et al., 2002).** Knowledge and control of the two groups of spoilage and pathogenic microorganisms are essential to produce and store vacuum packaged sliced smoked turkey that satisfies the characteristics of hygienic quality, microbial criteria and food safety. Although, there are several studies on the microbial profiles of food products at low temperatures **(Holley, 1997; Babji, et al., 2000; Gill and Badoni, 2002; Cayré, et al., 2005).** However, the studies on microbial evolution on sliced smoked turkey stored under vacuum conditions at low and high temperatures are limited. Therefore, the purpose of this study was to evaluate the microbial loading of vacuum packaging of smoked turkey sliced during storage under different temperatures (0, 5, 10 and 15◦C). The determined shelf life was based on the total viable counts, lactic acid bacteria and the presence of pathogenic bacteria (*Staphylococcus aureus, Listeria* and *Salmonella*).

2. Materials and methods

2.1. Sampling

The vacuum packaging of smoked turkey samples was processed at a local meat plant according to standard practices in Egypt. The smoked turkey meat prepared commercially by pasteurisation &smoking and cooked, were purchased from supermarket and examined. The product was divided into four groups based on the following temperature regimes=0, 5, 10 and 15 ºC. All the samples were examined to characterize the rates of microbial growth and pH changes at different storage temperatures.

2.2. Microbiological analysis

For microbiological analysis, the samples (25g) were transferred aseptically to a stomacher bag (Sewared, London, UK), 225ml of sterile Ringer's solution (Lab 100 Z) was added and homogenized for 60 seconded with a stomacher (Lab. Blender 400, Seward Medical, London, UK) at room temperature. Decimal dilution was prepared in

Ringer's solution and duplicate 1ml or 0.1 ml samples of appropriate dilutions were poured or spread on non-selective or selective agar plates.

Total viable count (TVC) was determined on Plate Count Agar (PCA; Merck, 1.05463), incubated at 25 ºC for 72h; lactic acid bacteria (LAB) on de Man, Rogosa, Sharpe (MRS Biolife) overlayed with 5 ml of the same medium and incubated at 25 ºC for 72 h. *Enterobacteriaceae* on Violet Red Bile Dextrose Agar (Biolife), with double layer of the same media, incubated at 37 ºC for 24 h. Yeasts and moulds on Rose Bengal Chloramphenicol agar (Lab M, 36 supplemented with chloramphenicol, X009), incubated at 25 ºC for 5 days. Pseudomonads on Cetrimide-Fucidin-Cephaloridene (CFC Agar Lab M supplemented with selective supplement X109), incubated at 25 ºC for 48 h. Staphylococci on Baired Parker agar (Biolife) supplemented with egg yolk, incubated at 37 ºC for 48 h. and to detect *S. aureus*, the plates were examined for the typical black colonies, convex colonies, with a light halo, and these were tested for positive coagulase reaction (Bactident Coagulase Biolife). TBX (LAB M) for *Escherichia coli* incubated at 37 ºC for 4 h, and directly incubated at 44 ºC for 37 h. *Listeria* Palcam Agar (Biolife) for *Listeria* spp, incubated at 30ºC for 48 h. XLD Agar (Merck, 1.05287) for *Salmonella* spp., incubated at 37ºC for 24 h.

Presence of *Listeria* was determined by suspending 25 g of smoked turkey into 225 ml *Listeria* Enrichment broth (Merck) followed by incubation at 30 ºC for 48 h. Then the culture was streaked on Palcam agar (Biolife) and incubated at 30 ºC for 48 h. The detection of *Salmonella* was achieved by suspending 25g of the products into 225 ml buffered peptone water (BPW) (Merck), and then incubated at 37 ºC for 24 h. Following incubation, 0.1ml of each BPW incubated was transferred into culture tubes containing 10 ml of Rappaport Vassiliadis (RV) enrichment broth and incubated again at 42 ºC for 24 h. The culture was then streaked on XLD Agar (Merck, 1.05287).

2.3. pH assay

For all the samples the PH value was measured using a PH meter model RL150 by blending 25 g of sample with 225 ml Ringer's solution.

2.4. Statistical analysis

Analysis of variance technique was used to determine the significant difference (P<0.05) in microbial counts and pH value changes during storage at different temperatures of both products. Mean logarithmic were separated by Tukey's multiple range test using statistical software SPSS 10.0 for windows.

3. Results and Discussion

Listeria spp., *E. coli* and *Salmonella* were absent in 25 g of smoked turkey at all temperature storage and under of vacuum conditions. During storage, the pathogenic bacteria (*Listeria* spp., *Salmonella*, *E. coli* and *S. aureus*) remained at blow $\log_{10}$ 2 cfu/g in all the four temperature regimes, as well as the colonies of staphylococci were negative to coagulase test. This mean that this product has been processed under safety condition and all management practices have been applied. **Pexara et al., (2002)** and **Zwirzitz, et al., (2021)** observed that during storage of vacuum sliced turkey breast fillets and other meat product (at 4 and 10 °C), *Listeria* spp. was not detected in 25g as well as pathogenic bacteria (staphylococci and *Listeria)* were remained at low or zero initial levels in all samples. The behaviours of spoilage microorganisms during storage of vacuum-packed smoked turkey at 15, 10, 5 and 0 °C and the log number of the spoilage micro-flora were calculated and examined.

Fig. 1, 2, 3 and 4 shows the wide range of the microbial contamination between the temperatures. Thus, during storage, the high storage temperatures (10 and 15 ºC) show highest growth on the total viable counts (TVC) and the lactic acid bacteria (LAB) **(Figures 1 and 2).** On the contrary, the low storage temperatures (0 and 5 ºC) show highest growth inhibition on the TVC and the LAB during the storage periods **(Figures 3 and 4). Membre et al.,** (**2005)**and **Lee et al.,** (**1983**)observed that temperature is the most important parameter for the growth of LAB and other organisms, as would be expected in view of the known optimum temperature for the growth of these micro-organisms in meat. Independently storage temperatures and storage periods significantly ($P < 0.05$) affected the total viable counts (TVC) and Lactic acid bacteria (LAB). In a final experiment, the LAB would remain as the dominant flora in the smoked turkey stored under vacuum at the four temperature conditions. Also, the development of LAB under the four temperature conditions and its growth is in agreement with those reported in the literature for meat processing **(Borch, et al., 1996; Holley, 1997; Stellato, et al., 2016).** They found that lactic acid bacteria are the major bacterial group associated with the spoilage of cooked, cured meat products packed in vacuum or modified atmosphere and stored at chilled temperatures.

Initially, the cooked smoked-turkey had a significantly lower microbial load in comparison to the control in both packaging materials. The lowest total viable counts (TVC) and Lactic acid bacteria (LAB) were observed at 0 days for all the four-temperature condition (≤2 log cfu/g). Also, the other microorganisms were below < 2-log cfu/g. The greatest development of the TVC of the smoked turkey was shown at 15, 10, 5 and 0 ºC after 4, 10, 18, 30 days respectively, while the LAB of the smoked turkey was determined at 15, 10, 5, and 0 ºC after 4.5, 10, 20 and 30 days respectively. So, during storage at 0 ºC the total viable counts on the smoked turkey were not significantly different between 0 up to 6 days of storage. On the other hand, the increase of the total viable counts on the smoked turkey after 9 days was significant in the comparison with the period 0 up to 9 days. Between 14 and 20 days of storage at 5 ºC for the product, there was no significant difference in TVC and LAB. The same pattern was also observed at 10 ºC between 5 and 6 days.

During storage of the smoked turkey at 0 ºC, there was no significant increase in TVC and LAB between 21 and 30 days. This proofed that the shelf life of this product stored at low temperatures was higher than when stored high temperatures. The smoked turkey stored at 0 ºC had the highest shelf life and were acceptable for consumption up to 30 days. At the end of storage of these products at 0ºC after 30 days, the total viable counts and lactic acid bacteria was not reached (7.0 log cfu/g). This is in agreement with **(Mano, et al., 1995)** who reported that the appearance of spoilage, the level of total viable count e g. 10^7 $_{cfu}$/cm^2. **Gill, (1983)** reported that meat spoilage does not occur until the total bacterial count reaches a level 6-8 log cfu/g The time necessary to reach 7.0 log cfu/g depends on the storage temperatures. During storage at 5, 10 and 15ºC the lactic acid reached > 7.0 log cfu/g after 14, 5 and 4 days respectively. **Ambrosiadis and Georgakis (1993)** reported that the shelf life of sliced vacuum-packed cooked meat is 18-20 days at storage temperature of 4 ºC.

In comparison to the yeasts & moulds, *Enterobacteriaceae*, Pseudomonads, and staphylococci, they show a different development pattern during storage periods and temperature regimes. *Enterobacteriaceae* numbers were generally <1 log cfu/g in all the storage periods and all the storage temperatures, except after 3 days at 0 ºC their numbers were increased significantly. The same pattern was observed at 10 ºC after 7 and 9 days of storage. Yeast& moulds, Pseudomonads and staphylococci on the smoked

turkey remained <2-log cfu/g in all the period of storage at 0ºC except, Yeast& moulds and staphylococci that increased significantly after 3 days. The same trend was shown during storage at 10 ºC after one day. The numbers of yeast& moulds and staphylococci increased significantly. The development of Pseudomonades on the smoked turkey at 10 and15ºC after one day was clear and the increase was significant. While, after one day of storage at 10 and15 ºC of the smoked turkey showed a dramatic decline in their numbers and remained below <2-log cfu/g at all the storage period. On the contrary, the number of Pseudomonads on the smoked turkey remained below <2-log cfu/ g during storage at 10 and 15ºC. At 5 ºC storage of the smoked turkey, yeast& molds, Pseudomonades and staphylococci remained below < 2 log cfu/g and the increase of yeast& moulds in the smoked turkey after 16 and 20 days was insignificant. This may be due to the condition of packaging and growth of some micro-organisms which develop during cold storage of the vacuum –packed smoked turkey. The development of lactic acid bacteria under anaerobic conditions suppresses the Gram-negative bacteria (*Enterobacteriaceae* and *Pseudomonads*). Some lactobacilli produce antimicrobial agents, including hydrogen peroxide, which are inhibiting to the Gram-negative microorganisms **(Simard, et al., 1983).** Many studies show that lactobacilli restricted the growth of other microorganisms when fresh meat or meat products were vacuum –packaged. Many factors such as pH change, temperature, the presence of inhibitory substances, product composition and packaging materials as well as environment inside the package has been suggested to contribute to the decline of *Pseudomonades, Enterobacteriaceae*, staphylococci and yeasts & moulds during storage at low temperatures (0 and 5ºC) **(Taylor, 1996; Korkeala and Bjorkoroth, 1997; Hansen and Boutista, 2000; Nychas and Drosinos, 2000; Stellato, et al., 2016).**

During storage the smoked turkey at 10 and 15ºC respectively, it was observed a different development picture for staphylococci. Staphylococci were present at different time of storage i.e., 4,7,8,9 and 10 days on the smoked turkey at 10 ºC. This may be due to contamination of some slices of smoked turkey during slicing and packaging. **Rubio, et al., 2006** and **Zwirzitz, et al., (2021**) showed that cross- contamination during chopping or slicing and packaging leads to an increase of total viable microflora.

The changes in the pH values of the smoked turkey during storage at 0, 5,10 and 15 ºC are presented in **Table 1.** The initial pH values of the smoked turkey ranged between 6.72 to 6.75. At the end of storage time, it was realized that pH values of the product decreased with increased the storage temperatures (10 ºC and 15 ºC). This data is correlated with the growth observed for LAB at 10 and 15 ºC where population reached high final cell counts. No significant differences (P >0.05) in pH values of the smoked turkey were found during storage at 0.0 ºC. pH value of the smoked turkey significantly decreased after 18 days of storage at 5 ºC. Significant correlations were found between pH, TVC and LAB counts at all the storage temperatures for each product. The low correlations coefficient (r= -0.324) found between pH and lactic acid bacteria of the smoked turkey, are in agreement with results of **Silla and Simonsen, (1985)** who reported no correlation between pH change and the number of lactobacilli. The same pattern was observed for the total viable counts on the product.

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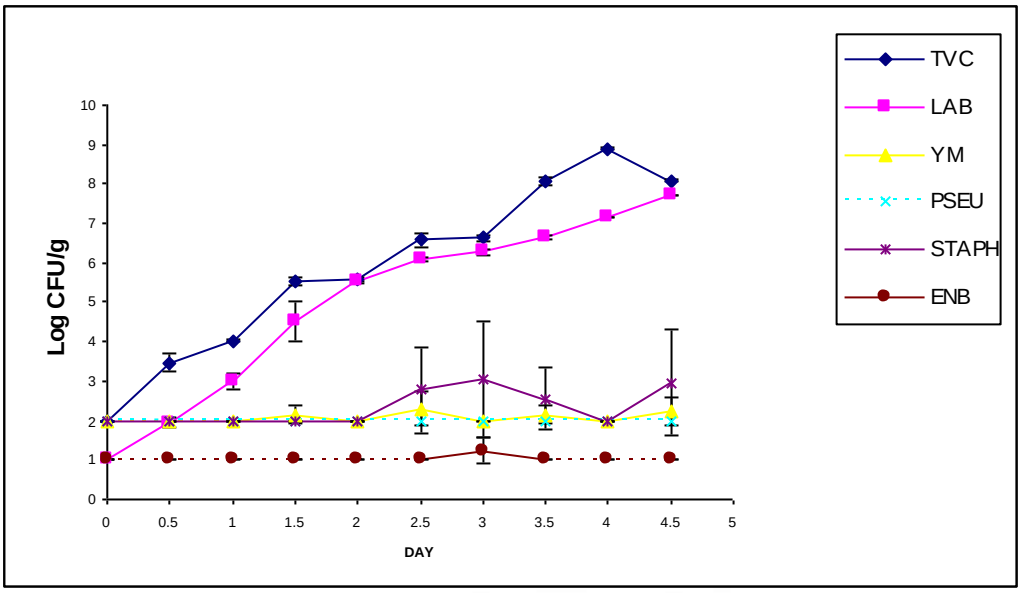

Fig (1): Effect of storage temperature at 15 °C on total viable count (TVC), lactic acid bacteria (LAB), yeasts& moulds (YM), pseudomonads (PSEU), staphylococci (STAPH), and *Enterobacteriaceae* (ENB) in vacuum packaged smoked turkey

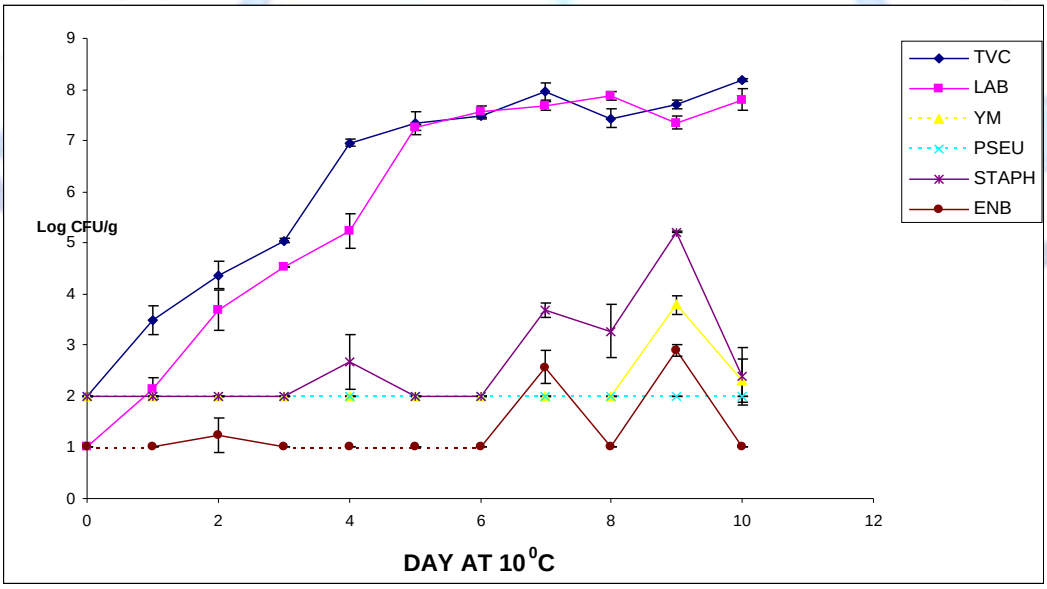

Fig (2): Effect of storage temperature at 10 °C on total viable count (TVC), lactic acid bacteria (LAB), yeasts& moulds (YM), pseudomonads (PSEU), staphylococci (STAPH), and *Enterobacteriaceae* (ENB) in vacuum packaged smoked turkey.

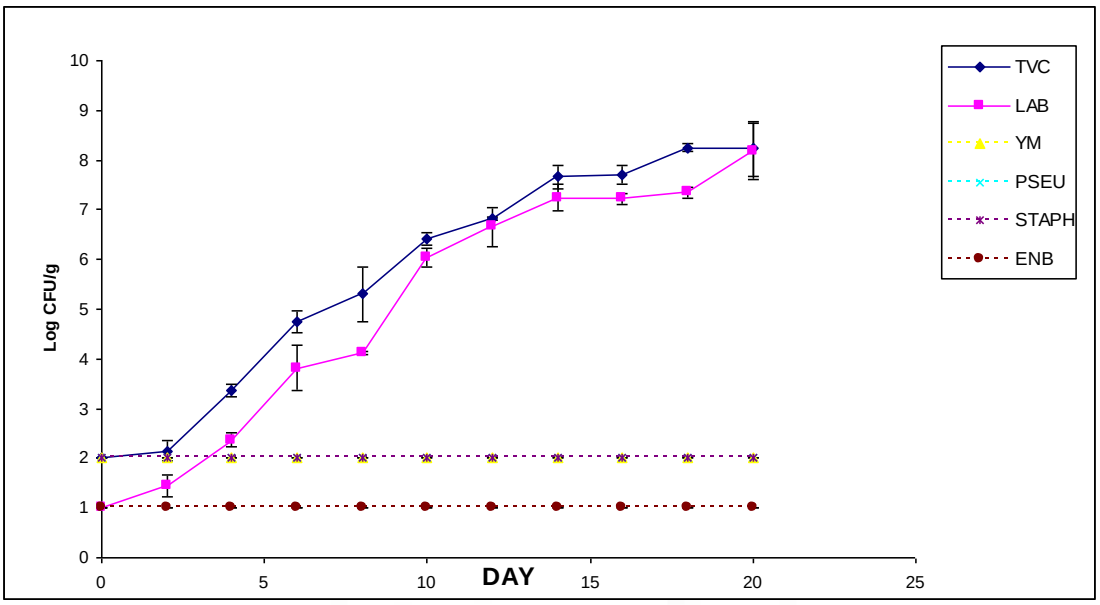

Fig (3): Effect of storage temperature at 5 °C on total viable count (TVC), lactic acid bacteria (LAB), yeasts& molds (YM), pseudomonads (PSEU), staphylococci (STAPH)and *Enterobacteriaceae* (ENB) in vacuum packaged smoked turkey.

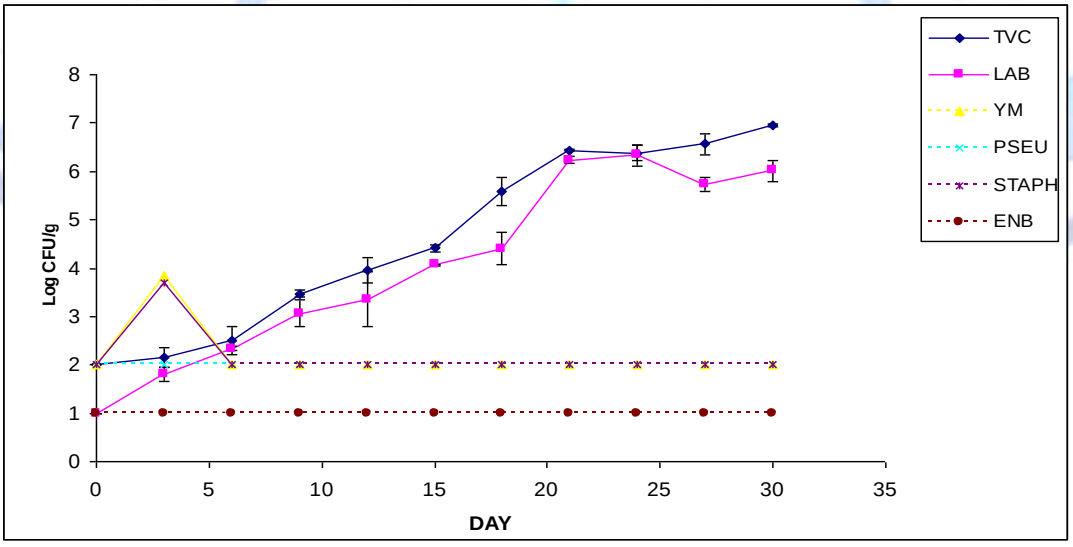

Fig (4): Effect of storage temperature at 0.0 °C on total viable count (TVC), lactic acid bacteria (LAB), yeasts& moulds (YM), pseudomonads (PSEU), staphylococci (STAPH) and *Enterobacteriaceae* (ENB) in vacuum packaged smoked turkey.

Table 1: Changes in the vacuum packaged smoked turkey pH during storage at 0.0, 5.0, 10.0 and 15°C.

Time (day)	pH at 0.0 °C	pH at 5.0 °C	pH at 10.0 °C	pH at 15.0 °C
0.0	6.73±0.19	6.72±0.12	6.73±0.13	6.75±0.14
2	6.73±0.13	6.71±0.34	6.23±0.54	6.24±0.44
4	6.72±0.62	6.70±0.45	6.11±0.42	6.23±0.32
6	6.71±0.11	6.56±0.31	5.98±0.41	6.21±0.36
8	6.68±0.12	6.51±0.38	5.96±0.54	6.19±0.45
10	6.58±0.14	6.43±0.37	5.93±0.65	6.17±0.46
12	6.55±0.13	6.42±0.36	5.89±0.53	5.99±0.38
14	6.49±0.15	6.33±0.36	5.89±0.56	5.99±0.44
16	6.48±0.18	6.32±0.38	5.86±0.51	5.98±0.35
18	6.47±0.11	6.31±0.32	5.83±0.42	5.78±0.43
20	6.45±0.16	6.29±0.45	5.88±0.34	5.72±0.41