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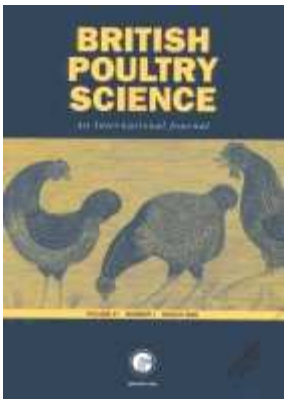


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# Spoilage of light (PSE-like) and dark turkey meat under aerobic or modified atmosphere package: microbial indicators and their relationship with total volatile basic nitrogen

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RUNNING TITLE: **PSE-LIKE AND DARK MEAT SPOILAGE**

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**Abstract** 1. The aim of this work was to evaluate the shelf life of turkey meat from different colour categories (light (PSE-like), intermediate and dark), packaged under aerobic or modified atmosphere (MAP) conditions; also to establish a relationship between microbial quality and total volatile basic nitrogen (TVB-N), evaluating its capacity for shelf life determination.

2. Breasts were selected according to luminance (L*) and $pH_{24}$: $L \geq 51$ and $pH < 5.8$ for light colour, $43 < L < 51$ for intermediate colour, $L \leq 43$ and $pH > 5.8$ for dark colour. Sliced meat was packaged under aerobic or MAP conditions with 50% $N_2$ and 50% $CO_2$, then stored in the dark at 0 ± 1ºC for periods during 12 or 25 d. Meat under aerobic conditions was evaluated for microbiological characteristics and TVB-N on d 0, 5 and 12. This evaluation was extended to include d 19 and 25 when samples were under MAP conditions.

3. Dark meat group after 12 d of storage in aerobiosis presented significantly higher plate counts of aerobic mesophilic, psychrotrophic microorganisms and higher TVB-N than other meat colour categories. The shelf life of turkey meat under MAP was one week longer for intermediate and light colour meat (20 d) than for dark meat. TVB-N values of 20-30 mg $NH_3$/100 g turkey meat correspond to advanced spoilage stages. We proposed 14 mg $NH_3$/100 g as the limit of freshness acceptability for turkey meat.

4. TVB-N was an indicator of turkey meat microbial spoilage but was not a suitable early predictor for microbial spoilage and in particular for turkey meat stored under MAP conditions because counts of microorganisms were moderately correlated (*Pseudomonas* spp. and *Enterobacteriaceae*) with this index, as they were inhibited by MAP gas mixture and storage temperature used in the present study.

## INTRODUCTION

The packaging of fresh meat in small portions is frequently used to facilitate its preparation and distribution with ease to retailers and consumers. The simple aerobic package, with polystyrene trays and polyvinyl chloride (PVC) film, protect meat against possible contaminants and allows labelling with information such as meat origin, to be provided for the consumers benefit. Furthermore, vacuum and modified atmosphere packaging are technological innovations in the second level transformation of pork, beef and recently in poultry, extending meat shelf life and improving presentation, storage, distribution, selling and ease of use by consumers (Farber, 1991; Church, 1994; Ohlsson, 1994; Smolander *et al*., 1997). The success of this technology depends on several factors such as gas mixture composition, nature of and initial microbial meat quality, temperature control, packaging properties and the efficiency of the equipment used (Taylor, 1996). Modified atmosphere (MAP) does not justify a lack of hygiene during production and handling of the meat, as it only delays the process of meat spoilage. Microorganisms are the main cause of meat spoilage and their development is affected by intrinsic characteristics such as pH and water activity (aw) and by extrinsic characteristics such as temperature and atmosphere composition. Intrinsic characteristics depend upon several *ante-* and *post-mortem* factors related to the handling of the birds, slaughter conditions and carcase cooling which give rise to raw meat variability characteristics related to pH, water holding capacity and colour (Santé *et al*., 1998; Rathgeber *et al*., 1999), independent of initial flora contamination but affecting its development. The extrinsic factors such as temperature (refrigeration) applied simultaneously to modified atmospheres packaging enriched by $CO_2$ increase poultry shelf life

because Gram-negative psychrotrophic dominant flora, the majority constituted by *Pseudomonas* spp., are inhibited (Blakistone, 1999).

According to meat preservation conditions, the commercial shelf life period corresponds to a variable storage time before the presentation of spoilage signs or other biotic or abiotic modifications are exhibited. The expiry point occurs when the acceptable maximum limit of the existing bacteria is exceeded or when meat appearance, odour and taste are unacceptable. When bacterial counts exceed 7-8 log cfu.cm$^{-2}$ the related cellular growth, metabolite production and exogenous enzymes action give rise to bad odours, viscosity and chemical modifications in the meat, such as oxidation responsible for colour modifications. It is mainly due to microbial activity that meat organoleptic modifications occur with shelf life limitation. There is a pressing need to decide the end of the shelf life period. Several methods are used to arrive at that decision and to predict with some accuracy the end of that period. The plate count of specific micro organisms (indicators of spoilage or related to it) is a precise method but needs 2 d or more for incubation and results. Despite this, it can help with the prediction of shelf life by giving information about the behaviour of microorganisms that have been quantified. The relationship between spoilage resulting from bacteria multiplication and chemical indexes able to reveal it, could be an auxiliary technique used to make a decision or predict with some accuracy the end of meat shelf life (Dainty, 1996).

Total Volatile Basic Nitrogen (TVB-N) determination by the method of Conway microdiffusion is a routine, rapid and low-cost method used to access freshness of fish muscle with established limits indicative of fish products freshness (95/149/EC). TVB-N includes compounds such as $(CH_3)_3N$, $(CH_3)_2N$

and $NH_3$, volatile compounds from spoilage microbial production. TVB-N levels are considered a potential indicator of fish spoilage with potential use as an on-package sensor evaluating real time freshness (Gram and Dalgaard, 2002, Pacquit *et al*., 2006).

The aim of this work was to evaluate shelf life of turkey meat of different colour categories packaged in aerobiosis and modified atmosphere (with a gas mixture of 50% $CO_2$ and 50% $N_2$) and to establish a relationship between the microbial quality and the biochemical parameter total volatile basic nitrogen, evaluating its capacity for shelf life determination.

## MATERIAL AND METHODS

### Selection and collection of samples

Samples were collected on different days from turkey carcases randomly evaluated after slaughtering bird flocks according to commercial practices of an industrial slaughterhouse. All birds (male turkeys, BUT9 and BIG6, 16 to 18 wk old) were electrically stunned with a current of 205 V to 225 V for 3 s, bled, scalded in a vertical bath with a temperature of 82.8ºC for5 min., defeathered, eviscerated and showered. The carcases were fast-cooled in a tunnel (-2ºC/2 $m.s^{-1}$/90% R.H.) for 2 h and kept in a refrigeration chamber (0ºC/85% R.H.) until deboning (approximately 24 h *postmortem*). The meat quality evaluation was performed by $pH_{24}$ (24 h *postmortem*) and colour (24 h *postmortem*) measurements.

Determination of pH was made directly on the *Pectoralis major* muscle with a portable pH meter (HI9023) equipped with a pH electrode (FC 230B, Hanna Instruments, Italy). Each value was an average of three determinations on the muscle. The colour was measured on the internal side of the *Pectoralis major*

muscle with a Minolta colorimeter CR-300 (Minolta, Osaka, Japan) using the $L^*$, $a^*$, $b^*$ coordinates (CIELAB colour system). Each value resulted from the arithmetic mean of 9 determinations.

Breasts were selected according to Luminance ($L^*$) and $pH_{24}$: $L^* \geq 51$ and $pH < 5.8$ for light colour, $43 < L^* < 51$ for intermediate colour, $L^* \leq 43$ and $pH > 5.8$ for dark colour (Fraqueza *et al*., 2006). Breast muscles (total n = 20) from different colour categories were cut into slices (10-11 slices for each breast, from cranial, medium and caudal side) approximately 1-1.5 cm deep and with a surface area of 90-100 $cm^2$ in a deboning room, according to commercial practices. The meat was placed in a polyethylene bag and transported in an isothermal box to the laboratory in less than 1 h.

Sliced meat samples were individually packaged in aerobiosis, using polypropylene trays (Tecknopack plastics, S/L, Barcelona) and polyvinyl chloride (PVC) film, and in modified atmosphere with 50% $N_2$ and 50% $CO_2$.

For modified atmosphere packaged meat, polypropylene trays (Tecknopack plastics, S/L, Barcelona) were used and polylaminated plastic bags "HBX-070" (R. Bayer, Germany) with high impermeability to $O_2$ and $CO_2$ (permeability: $O_2$ = 7.5 $cm^3/m^2$.d.bar 75% R.H. 23ºC, $CO_2$ = 32 $cm^3/m^2$.d.bar 75% R.H. 23ºC, $N_2$ = 3 $cm^3/m^2$.d.bar 75% R.H. 23ºC and water steam = 0.77 $g/m^2$.d) due to a high barrier layer of ethylene vinyl alcohol (EVOH). The packages were sealed in a EVT-7-CD machine (Tecnoprip, Barcelona) after a vacuum of 97% and an introduction of gas mixture at 60%.

All samples (n = 160) were immediately stored under refrigerated conditions (0 ± 1ºC) in the dark for 12 or 25 d.

Meat samples packaged under aerobic conditions were evaluated for their microbiological characteristics and total volatile basic nitrogen on d 0, 5 and 12 of storage. This evaluation was extended to 19 and 25 d when samples were stored under modified atmosphere packaging.

For each meat colour categories and packaging condition at least 5 replications were made on different days.

**Determination of microbiological characteristics**

The preparation of meat samples for microbial analysis was done in accordance with ISO 6887-1:1999. Microbial determinations were carried out to: total mesophilic aerobic counts (Plate Count Agar, Sharlau, Spain) at 30ºC for 2 d, in accordance with ISO 4833:2003; total psychrotrophic aerobic counts (Plate Count Agar, Sharlau, Spain) at 7ºC for 10 d (ISO/DIS 6730:2005); anaerobic count at 7ºC for 10 d (Brewer Anaerobic Agar, Merck, Germany); *Enterobacteriaceae* counts in Violet Red Bile agar (VRB agar, Merck, Germany) at 37ºC for 2 d (ISO 21528-2:2004); *Pseudomonas* spp. counts (cephaloridene, fucidin and cetrimide (CFC) agar base; Oxoid, UK) after incubation at 30ºC for 2 d (ISO 13720:1995), lactic acid bacteria (LAB) counts in Man Rogosa Sharpe Agar (Oxoid, UK) incubated at 30ºC for 3 d (ISO 15214:1998) and *Brochothrix thermosphacta* count in streptomycin, actidione, thallous acetate agar (STAA, Oxoid, UK) incubated for 2 d at 30ºC (ISO 13722:1996; Santé *et al*., 1994). Counts were expressed as log cfu.$g^{-1}$.

**Determination of total volatile basic nitrogen (TVB-N)**

The method of Conway microdiffusion was used for total volatile basic nitrogen determination according to NP-1848 (1987) and Pearson (1970) for meat and meat products. The extraction of volatile bases was performed from 50 g meat

sample with 100 ml of trichloroacetic acid solution, and 1 ml of the filtrate was pipetted into the outer annular space of the Conway unit. The liberation of ammonia was made by alkalinisation with saturated potassium hydroxide solution (1 ml). Diffusion and reception of the ammonia was into a boric acid solution (1 ml) and titration realised with hydrochloric acid solution 0.02 N.

**Statistical analysis**

Data were analysed using SPSS 11.5 for Windows. The comparison between different packaging conditions and different colour quality meat samples, for microbial parameters, was performed by model adjustment of a one-way ANOVA for each day. If the *F* test from ANOVA was significant, a Least Significant mean Difference of a *post hoc* multiple comparisons test was performed. The comparison between d, considering each package and colour meat condition, was done by t-test for dependent samples (Pestana and Gageiro, 2003). Pearson's correlation analysis was performed for relationship evaluation of analytical parameters.

## RESULTS

**Meat quality evaluation**

Table 1 shows $pH_{24}$ and $L^*$ characteristics of turkey breasts selected and classified as light, intermediate and dark colour. Dark meat was characterised by a significantly ($P < 0.001$) lower Luminance ($L^*$) value and higher pH than intermediate and light colour meat. Light colour meat pH and $L^*$ characterised it as PSE-like meat (Barbut, 1996, 1997; Fraqueza *et al*., 2006).

Table 1 near here

**Microbial development in light (PSE-like) and dark turkey meat under aerobic and modified atmosphere packaging**

Figures 1 and 2 represent the evolution of microbial flora during storage of sliced turkey meat from different colour categories, packaged in aerobiosis and MAP (50% $CO_2$ and 50% $N_2$). The initial contamination of turkey meat with aerobic mesophilic, psychrotrophic and anaerobic psychrotrophic microorganisms was not significantly different in the three colour categories (light, intermediate and dark). Aerobic mesophilic and psychrotrophic plate counts were 4.7 and 4.8 log cfu.$g^{-1}$ respectively, whereas anaerobic psychrotrophic plate counts were 3.3 log cfu.$g^{-1}$. After 5 d of storage all samples from different colour categories presented aerobic mesophilic plate counts over $5x10^6$ cfu.$g^{-1}$. Dark colour meat presented significant ($P$<0.05) higher counts of aerobic mesophilic and psychrotrophic micro organisms (about 1 log cfu.$g^{-1}$) than intermediate and light colour meat after 12 d of storage in aerobiosis at 0ºC (Figure 1A and B). The microbial plate counts in MAP meat till the d 5 of storage were not significantly different from those initially observed.

Figures 1 and 2 near here

After 12 d of storage at 0ºC, a significant increase was registered ($P$<0.05) in mesophilic and psychrotrophic aerobic plate counts in MAP dark colour meat (from 4.88 to 5.95 log cfu.$g^{-1}$ and from 4.97 to 5.56 log cfu.$g^{-1}$, respectively). The psychrotrophic anaerobic plate counts also significantly ($P$<0.05) increased in MAP meat after twelve d of storage (Figure 1). Their growth after this storage time was clearly shown in dark meat more than in the other meat colour categories although this difference was not significant between MAP samples of different colour categories. There is a part of anaerobic facultative flora that seems to be inhibited by the presence of $CO_2$ and pH meat condition (Figure 1).

Between different analytical periods (d 12, 19 and 25) the total mesophilic and psychrotrophic aerobic plate counts difference was 2 log cfu.$g^{-1}$ in dark meat and 1 log for light and intermediate colour meat.

After 25 d of storage significantly ($P$<0.05) higher aerobic mesophilic and psychrotrophic plate counts were noted in dark meat, with a difference of 2 log cfu.g$^{-1}$ compared to other colour meat categories.

The results of specific flora analysed are reported in Figure 2. The initial flora (d 0) was composed of high plate counts of *Pseudomonas* spp. (4.5 log cfu.g$^{-1}$) while *Enterobacteriaceae*, lactic acid bacteria and *Brochothrix thermosphacta* plate counts were lower, 2.9 log cfu.g$^{-1}$, 2.6 log cfu.g$^{-1}$ and 1.9 log cfu.g$^{-1}$, respectively.

Microflora in general showed an increase of 2 log cfu.g$^{-1}$ in meat packaged in aerobiosis after 5 d of storage, except for lactic acid bacteria (Figure 2C), which presented a slow growth. From the enumerated flora, *Pseudomonas* spp. continues to dominate in the microbial population with a plate count of 6.9 log cfu.g$^{-1}$ (Figure 2A). The turkey meat in aerobiose packaging after 12 d of refrigeration presented high counts of Gram negative psychrotrophic *Pseudomonas* spp. and *Enterobacteriaceae*. In addition, the Gram-positive flora *Brochothrix thermosphacta* (Figure 2D) growth was outstanding. *Pseudomonas* spp. and *Enterobacteriaceae* growth were inhibited in MAP meat during storage period (Figure 2A and B).

The growth of LAB in 50% $CO_2$ and 50% $N_2$ was faster in dark and intermediate categories than in light meat, presenting plate counting of 4.14, 4.03 and 3.06 log cfu.g$^{-1}$ respectively on d 12 of storage d (Figure 2C). Nevertheless, this difference of 1 log disappeared after this period of storage with the counts not being significantly different between meat colour categories on d 25 of storage (≈ 5 log cfu.g$^{-1}$).

*Brochothrix thermosphacta* growth was similar on different colour turkey meat categories under MAP. Even though significant differences were not registered between them, dark meat had plate counts with a difference superior to 1 log cfu.$g^{-1}$ compared to light and intermediate meat on 25$^{th}$ d of storage (Figure 2D).

**TVB-N evolution in light (PSE-like) and dark turkey meat under aerobic and modified atmosphere packaging**

Figure 3 shows the evolution of total volatile basic nitrogen (TVB-N) in meat samples packaged according to study conditions and stored at 0ºC. The initial mean value of TVB-N for turkey meat was 12.42 ± 2.08 mg $NH_3$/100 g. During storage time, light colour meat did not have an increase of TVB-N but intermediate and dark meat registered a significant ($P < 0.05$) increase from the 5$^{th}$ d to the 12$^{th}$ d of storage, particularly dark meat. Sliced turkey meat packaged with 50% $CO_2$ and 50% $N_2$ did not present significant differences of TVB-N either between colour categories or during storage time, with a mean value of 12.93 ± 2.10 mg $NH_3$/100 g.

Figure 3 and Table 2 near here

**Relationship between microbial spoilage indicators and TVB-N**

Table 2 shows the Pearson correlation coefficients between microbial parameters in turkey meat packaged under study conditions and TVB-N. The microbial group presenting the higher correlation value with TVB-N irrespective of packaging conditions and storage time was the psychrotrophic aerobes plate count. The correlation between TVB-N and microbial groups were moderate but associate part of the $NH_3$ production to psychrotrophic microorganisms' metabolism relating *Pseudomonas* spp. and *Enterobacteriaceae* to a high production of $NH_3$.

*Pseudomonas* spp. and *Enterobacteriaceae* plate counts in fresh turkey meat could explain 30 and 29% respectively of the variation observed in TVB-N.

## DISCUSSION

The results of turkey meat initial aerobic mesophilic and psychrotrophic plate counts were similar to those observed by Zeuthen and Mead (1996) and Smolander *et al.* (2004) in poultry meat. The anaerobic psychrotrophic plate counts have shown that a large part of the flora was psychrotrophic and facultative anaerobic. The initial values enumerated for *Pseudomonas* spp., *Enterobacteriaceae* plate count and lactic acid bacteria have been usually observed in fresh meat and similar to that stated by Erkmen (2000) and Smolander *et al*. (2004). The initial microflora was not different in the three colour turkey meat categories.

After 5 d of storage all samples from different colour categories, packaged in aerobiosis, presented aerobic mesophilic plate counts over the limit of acceptability ($5x10^6$ cfu.$g^{-1}$) recommended by French Government (Anonymous, 1998), for microbial criteria of animal products. The initial high contamination of mesophilic and psychrotrophic flora (4.7 and 4.8 log cfu.$g^{-1}$, respectively) on turkey meat under this study conditions, was one of the factors contributing to the high level of mesophilic counts after 5 d of storage. In fact, the temperature of storage was 0ºC, but is seems that part of the meat flora was psychrotrophic (e.g. *Pseudomonas* spp.) being able to develop in this temperature condition through 5 d of storage.

The dark colour meat under aerobic packaging presented earlier signs of spoilage than the others colour categories (data not shown) with putrefactive smell, slime and higher plate counts after 12 d of storage. This was according to

results reported by Allen *et al*. (1997, 1998) stating that a more rapid perception of abnormal odours in dark poultry meat (broiler) corresponded to a higher psychrotrophic plate counts and shorter shelf life period. These authors did not find significant differences in psychrotrophic flora between light and dark poultry meat after seven d of storage at 7ºC. Nevertheless dark meat developed a more intense odour than light meat.

The difference noticed in microbial population in turkey meat of different colour categories after 12 d of storage was not relevant since, for all different meat categories, total aerobic plate counts were higher than the acceptable hygienic quality limit.

The turkey meat in aerobiosis packaging after storage presented high counts of Gram negative psychrotrophic *Pseudomonas* spp. and *Enterobacteriaceae* and also Gram positive *Brochothrix thermosphacta*. Of the two aerobic species selected for analysis it appears that *Pseudomonas* spp. predominates in spoilage flora of refrigerated turkey meat under aerobiosis packaging, without influence of meat pH on their development in spite of the observed higher count in dark colour meat. This is in agreement with Gill (1983) and Arnaut-Rollier *et al.* (1997) who found that *Pseudomonas* spp. growth rate is higher than that of their usual competitors. Labadie (1999) stated that *Pseudomonas* spp. is always dominant after some d in storage at temperatures between 0ºC and 7ºC, whatever the type of meat. *Enterobacteriaceae* and *Brochothrix thermosphacta* are also mentioned as contributors to meat spoilage (Blakistone, 1999, Russo *et al*., 2006).

Roseiro (1999), in her study on the microbial quality of PSE and DFD pork packaged in aerobiosis, stated that there were no significant differences in aerobic mesophilic and LAB plate counts between these meat categories. There was a

higher growth rate of *Pseudomonas* spp. in the initial phase of DFD meat storage. However the plate counts of those micro organisms were significantly higher in PSE meat after 7 d of storage. Nevertheless, it is stated that many strains of *Moraxella/Acinetobacter*, as they are part of dominant aerobic flora meat spoilage, are influenced by pH. Meat of higher pH (6.2) when stored at temperatures equal to or less than 10ºC presented earlier signs of putrefaction caused by *Pseudomonas, Acinetobacter* and *Moraxella*, while the growth of these two micro organisms is inhibited when pH is equal to or less than 5.3 (Gill and Newton, 1982; Gill, 1983).

Light turkey meat showed pH values which were not so low as those presented by PSE pork, so dominant flora including *Pseudomonas, Enterobacteriaceae* and *Brochothrix thermosphacta* in turkey meat were not influenced by pH values associated to light and dark colour meat when stored at 0ºC and packaged in aerobiosis.

The gas mixture used on turkey meat in a modified atmosphere package had an inhibiting effect on microbial development till the d 5 of storage, attributed to $CO_2$ (Blakistone, 1999), and this extended the latency phase (lag) of the microbial growth curve. The dissolution of $CO_2$ in the meat water phase and consequent carbonic acid formation changed the bacteria cell's internal pH affecting the biologic system balance and inducing cellular inactivation without wall damage (Erkmen, 2000). The inhibiting effect of $CO_2$ was supported by significantly ($P < 0.001$) lower Gram-negatives aerobic total plate counts in turkey meat under MAP, than those observed in aerobically packaged meat.

Despite the anaerobic conditions and inhibitory effect of $CO_2$ there occurred a significant increase in mesophilic and psychrotrophic aerobic plate counts in

MAP dark colour meat after 12 d of storage at 0ºC but there is a part of anaerobic facultative flora that seems to be inhibited by the presence of $CO_2$ and pH meat condition.

The gas mixture, 50% $CO_2$ and 50% $N_2$ had an inhibitory effect on the growth of *Pseudomonas* spp., and *Enterobacteriaceae*. The sensitivity of *Brochothrix thermosphacta*, microaerophilic bacterium, to $O_2$ absence was well marked in light and intermediate colour meat. LAB was responsible for the spoilage of turkey meat under MAP since this slower growth group was not inhibited by anoxic conditions of package as stated by Santé *et al.* (1994). The turkey meat pH associated to its colour had no effect in LAB development. However others anaerobic facultative psychrotrophic bacteria had grown in dark meat colour under MAP with 50% $CO_2$ and 50% $N_2$ when *Pseudomonas* spp. and *Enterobacteriaceae* were inhibited, being responsible for meat spoilage. Their growth was promoted by intrinsic conditions related to dark meat with a pH equal or above 6 and by their micronutrients content. Differences in flora development in dark turkey meat compared to light (PSE-like) meat could be related to differences in meat pH (Allen *et al*., 1997 and 1998) when other external factors are introduced such as temperature and modified atmosphere packaging, changing the type of flora and inducing other interactions between competitive micro organisms. According to Boulianne and King (1998) and Soidla *et al*. (1998) dark meat is richer in iron. This nutrient is very important for non-siderophore strains, being used rapidly without energetic losses by siderophores species, which promotes their growth (Champomier-Vergès *et al*., 1996; Gram *et al*., 2002). In dark meat, the development of flora is higher than in intermediate and light meat because in these the lower pH constitutes an intrinsic hurdle, added to the

extrinsic hurdles created by anaerobiosis and refrigeration at 0ºC. Blickstad and Molin (1983), Cox *et al*. (1998) and Labadie (1999) referred to the inhibition of *Enterobacteriaceae* and *Brochothrix thermosphacta* by pH and temperature.

The increase of TVB-N may have been due to a combination of bacterial growth and an enzymatic proteolytic action with liberation of ammonia compounds. After glucose depletion bacteria proliferate using the amino acid and developing a proteolytic action. During storage time intermediate and dark meat under aerobiosis packaging registered a significant ($P < 0.05$) increase of TVB-N. These meat samples (intermediate and dark colour) exceed the values for beef indicated by Mathews *et al*. (1990) as an acceptable limit, 16.5 mg $NH_3$ /100 g of meat.

Dark meat in aerobiosis registered higher plate counts of micro organisms and higher values of TVB-N. The greater attack on nitrogen compounds by microbial flora in dark meat is related not only to an early exhaustion of glycogen reserves (Gill, 1983; Drosinos and Board, 1994; Kakouri and Nychas, 1994) but also to the adaptation of microbial flora with a minor lag phase and higher growth rate because they are not inhibited by pH and have available micronutrients. The siderophore and non-siderophore spoilage bacteria will have mineral nutrients such as iron available in more abundance in dark meat than in light and intermediate colour meat (Champomier-Vergès *et al*., 1996; Boulianne and King, 1998; Gram *et al*., 2002).

Only when there were evident signs of spoilage caused by *Pseudomonas* spp., *Enterobacteriaceae* and *Brochothrix thermosphacta* was an increase of TVB-N registered, which limited this parameter as an early indicator of spoilage

and particular in meat under MAP because these micro organisms were inhibited by the MAP gas mixture under study.

The mean TVB-N value of turkey meat when there were no spoilage signs or microbial plate counts higher than $10^6$ cfu.g$^{-1}$ was 13 mg de $NH_3$/100 g. This value is lower than that referred to for well preserved fresh meat, 20 mg de $NH_3$/100 g (Technical rule 1/81, IQA). However the rule that the double value corresponds to advanced spoilage stages applies because values of 20-30 mg de $NH_3$/100 g in turkey meat revealed advanced putrefaction. A TVB-N value of 14 mg $NH_3$/100 g is proposed as a limit of acceptability for turkey meat freshness.

## CONCLUSIONS

The initial microbial flora did not differ between the three colour turkey meat categories. Between meat samples of different colour categories after storage according to the study conditions, significant differences were observed only in aerobic mesophilic and psychrotrophic plate counts. The main spoilage flora was Gram negative psychrotrophic, with *Pseudomonas* spp dominating. Dark colour meat presented significantly higher plate counts of those aerobic mesophilic and psychrotrophic micro organisms and TVB-N than other meat colour categories after 12 d of storage at 0ºC. In turkey meat packaged in aerobiosis at 0ºC the others microbial groups analysed were not influenced by initial pH meat differences since there were not significant differences on microbial population growth rate and final plate counts.

The shelf life of dark turkey meat under MAP with 50% $CO_2$ and 50% $N_2$ is shorter than that for intermediate and light meat. According to hygienic microbial criteria (Unnamed, 1998), dark turkey meat under MAP after 12 d of storage at 0ºC was outside the limit of acceptability and not suitable for consumption. The

shelf life period of sliced turkey meat under MAP was one week longer for intermediate and light colour meat (20 d) than for dark meat according to these study conditions.

TVB-N values of 20-30 mg $NH_3$/100 g of turkey meat correspond to advanced spoilage stages. A limit of freshness acceptability for turkey meat is proposed as being 14 mg de $NH_3$/100 g.

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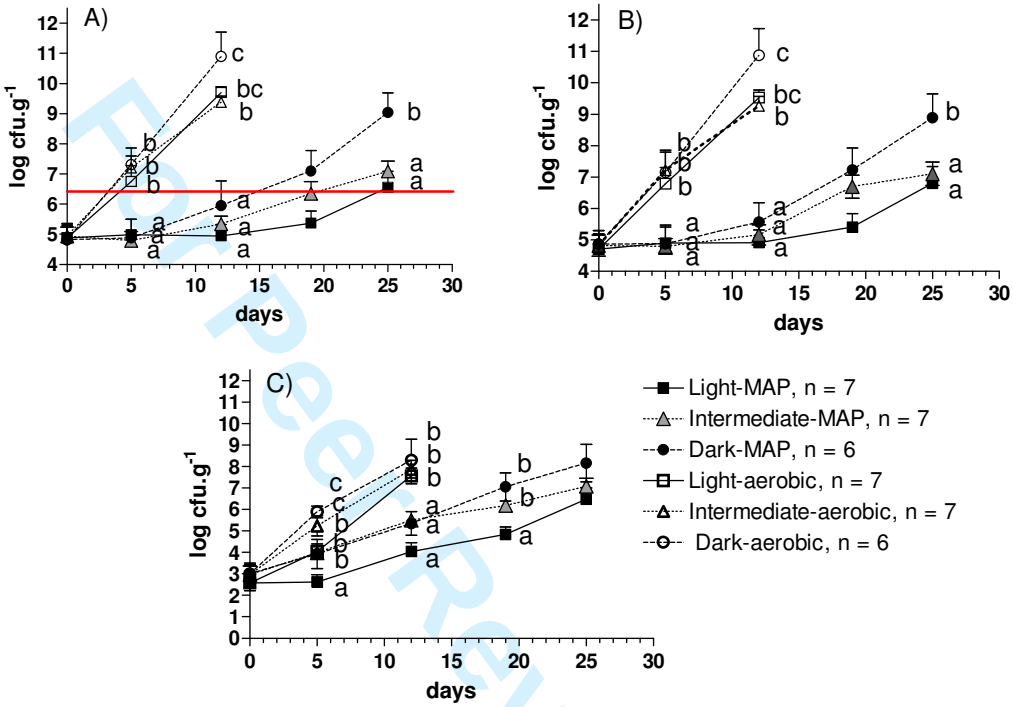


**Figure 1**. *Total mesophilic aerobic (A), total psychrotrophic aerobic (B) and anaerobic (C) counts evolution in light, intermediate and dark turkey breast meat during storage at 0ºC under aerobic or modified atmosphere (MAP) packaging ($^{abc}$ values on the same day of storage not sharing a common superscript were significantly different: $P < 0.05$).*

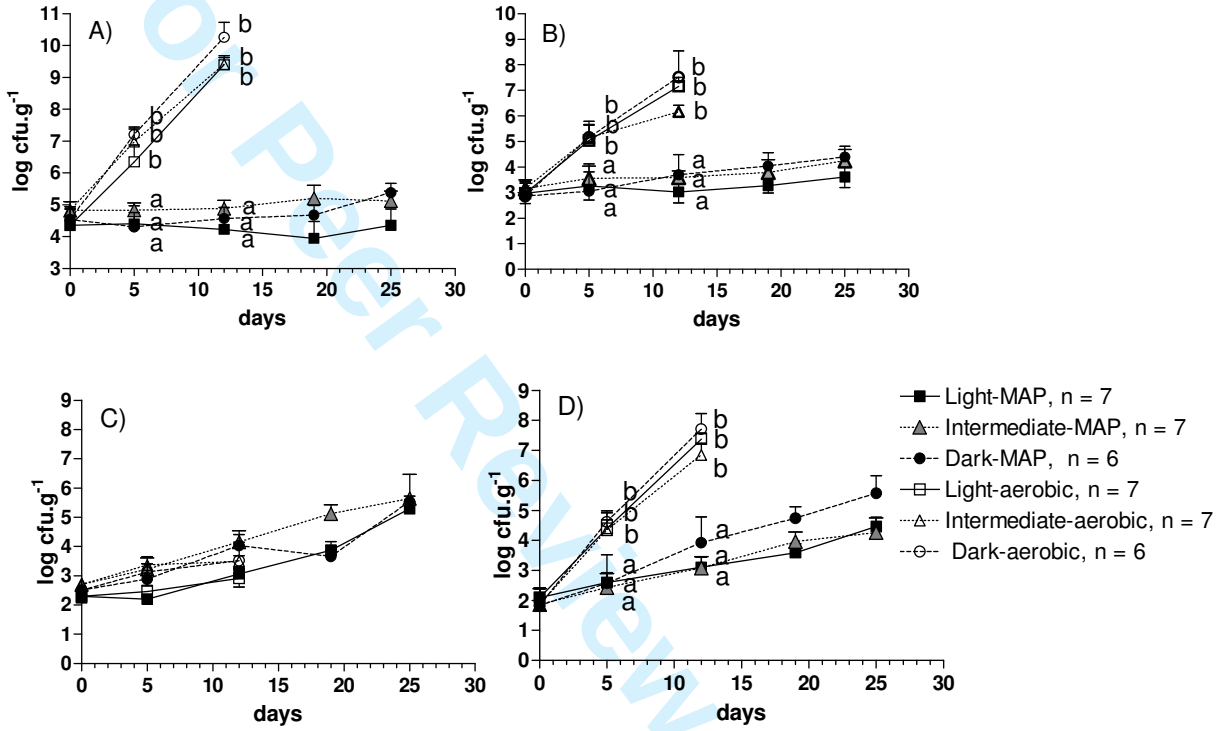


**Figure 2.** *Pseudomonas spp. (A), Enterobacteriaceae (B), lactic acid bacteria (C) and Brochothrix thermosphacta (D) counts evolution in light, intermediate and dark turkey breast meat during storage at 0ºC under aerobic or modified atmosphere (MAP) packaging ($^{ab}$ values on the same day of storage not sharing a common superscript were significantly different: $P < 0.05$).*

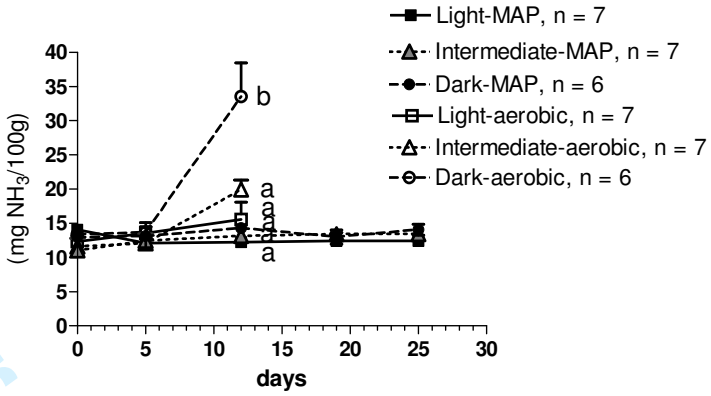


**Figure 3.** *TVB-N production in light, intermediate and dark turkey breast meat during storage at 0ºC under aerobic or modified atmosphere (MAP) packaging ($^{abc}$ values on the same day of storage not sharing a common superscript were significantly different: $P < 0.05$).*

**Table 1.** *Characteristics of selected breast turkey samples M. Pectoralis major*

| | Colour categories | | | |
|---|---|---|---|---|
| Breast samples | Light n = 7 | Intermediate n = 7 | Dark n = 6 | Sig. |
| pH | 5.69 ± 0.07[a] | 5.85 ± 0.03[b] | 6.05 ± 0.13[c] | *** |
| L* | 51.73 ± 0.60[c] | 46.78 ± 1.47[b] | 41.33 ± 1.31[a] | *** |

[abc] means within a row with different superscript letters are significantly different.

*** $P < 0.001$.

**Table 2.** *Pearson's correlation coefficients between microbial determinations and TVB-N in turkey meat (n = 160)*

| Microbial determinations | TVB-N | pH 24 h |
|---|---|---|
| Total mesophilic aerobic counts | 0.612 (**) | 0.084 |
| Anaerobic count at 7ºC | 0.499 (**) | 0.110 |
| Total psychrotrophic aerobic counts | 0.627 (**) | 0.056 |
| *Enterobacteriaceae* counts | 0.609 (**) | -0.059 |
| *Pseudomonas* spp. counts | 0.617 (**) | -0.015 |
| Lactic acid bacteria counts | 0.110 | 0.194 (*) |
| *Brochothrix thermosphacta counts* | 0.574 (**) | 0.095 |
| pH 24 h | 0.167 (*) | 1.000 |

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