

Available online at www.sciencedirect.com



Food Chemistry

Food Chemistry 104 (2007) 1622-1628

www.elsevier.com/locate/foodchem

# Possible role of volatile amines as quality-indicating metabolites in modified atmosphere-packaged chicken fillets: Correlation with microbiological and sensory attributes

Christiana C. Balamatsia, Apostolos Patsias, Michael G. Kontominas, Ioannis N. Savvaidis\*

Laboratory of Food Chemistry and Food Microbiology, Department of Chemistry, University of Ioannina, GR-45110 Ioannina, Greece

Received 16 November 2006; received in revised form 10 January 2007; accepted 4 March 2007

## Abstract

The present work evaluated the possible role of volatile amines as indicator(s) of poultry meat spoilage. Fresh chicken meat (breast fillet) was packaged in four different atmospheres: air (A), vacuum (VP) and two modified atmospheres (MAs), namely M1, 30%/65%/5%  $(CO_2/N_2/O_2)$  and M2, 65%/30%/5%  $(CO_2/N_2/O_2)$ . All chicken samples were kept under refrigeration  $(4 \pm 0.5 \text{ °C})$  for a period of 15 days. Of the four treatments, the VP and M1 and M2 gas mixtures were the most effective for delaying the development of aerobic spoilage microbial flora. Pseudomonas spp. in chicken samples stored under M2 gas mixture and VP were significantly lower than all the other samples after 15 days of storage. Of the remaining bacterial species examined, lactic acid bacteria (LAB), Brochothrix thermosphacta, were dominant in the microbial association of both aerobically- and MA-packaged chicken, while yeasts contributed to a much lesser extent in the final microbial flora of chicken meat. On the basis of microbiological data (TVC), shelf-life extensions of 2, 4 and 9-10 days were achieved by VP and M1 and M2 gas mixtures. Results of the present work showed that the limit of sensory acceptability (a score of 6) was reached for the aerobically, vacuum-packaged and M1 gas mixture chicken samples approximately on days 6-7 and 9-10, respectively. Based on sensory (taste) analysis and with regard to chicken spoilage and freshness, TMA-N and TVB-N limit values of acceptability, namely 10.0 mg N/100 g and 40 mg N/100 g for chicken samples stored in air, may be proposed as the upper limit values for spoilage initiation of fresh chicken meat stored aerobically. Interestingly, the M2 gas mixture sample did not reach these limit values throughout the 15 day storage period. The formation of volatile amines during chill storage of chicken meat, under the packaging conditions examined in the present study, seemed to be in good agreement with the increase in microbiological count (TVC) and sensory taste score except for the M2 gas mixture.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Amines; Chicken meat; Packaging; Shelf-life; Spoilage; Quality

## 1. Introduction

Chicken is a highly perishable food and it usually deteriorates within 1 week of slaughtering, regardless of storage chill systems, and such spoilage is largely due to different types of microorganisms, including bacteria, such as *Pseudomonas* spp., *Shewanella putrefaciens* and yeasts, depending on the initial microbiological quality of the poultry carcasses (Russell, Fletcher, & Cox, 1996).

Consumers' concerns about the freshness of meat are continually increasing. Reliable methods for assessing the microbiological quality and/or freshness of meat would benefit both consumers and the meat industry. Traditionally, shelf-life studies of perishable meat and meat products are carried out by evaluating the microbiological and sensory quality of the product as a function of storage time. Because traditional methods (based on direct microbial analysis) are costly and time-consuming, alternative

<sup>\*</sup> Corresponding author. Tel.: +30 26510 98343; fax: +30 26510 98795. *E-mail address:* isavvaid@uoi.gr (I.N. Savvaidis).

 $<sup>0308\</sup>text{-}8146/\$$  - see front matter  $\circledast$  2007 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2007.03.013

methods, involving chemical changes due to microbial growth, have also been suggested as quality indicators of meat (Dainty, 1996).

Modified atmosphere packaging (MAP) of foods has gained considerable popularity as a modern method for packaging fresh meat (Jeremiah, 2001). The combination of CO<sub>2</sub>, N<sub>2</sub> and O<sub>2</sub> in modified atmosphere (MA) packs is able to suppress the aerobic spoilage flora of perishable foods, such as meat, fish and related products, and to sustain their visual appearance (Davies, 1995; Gill, 1996).

While the microbiological changes in MA-packaged meat are well established, the chemical changes accompanying the growth of bacteria on meat during storage, either aerobically or anaerobically, are not well investigated (Garcia de Fernando, Nychas, Peck, & Ordónez, 1995). It has been suggested that, in both cases, the metabolism of glucose, lactic acid, amino acids and nucleotides can occur during storage (Gill & Molin, 1991; Jay, 1986; Nychas, Dillon, & Board, 1988). It is now well established that the formation of amines, including non-volatiles, such as the biogenic amines (BAs), and volatile amines (VAs), such as trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N), is primarily a consequence of the enzymic decarboxylation of specific amino acids due to microbial enzyme activity (Bardócz, 1995; Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994; Ruiz-Capillas & Jimenez-Colmenero, 2004). Although the determination of BAs in poultry meat has been proposed as a useful indicator of spoilage (Balamatsia, Paleologos, Kontominas, & Savvaidis, 2006; Rokka, Eerola, Smolander, Alakomi, & Ahvenainen, 2004; Silva & Gloria, 2002), there is little information on the chemical changes of VAs occurring in meat (Al-Bachir & Mehio, 2001; Balamatsia, Rogga, Badeka, Kontominas, & Savvaidis, 2006; Byun et al., 2003), including poultry, or products during refrigerated storage.

The formation of BAs in breast chicken meat during storage was studied under aerobic and MAP conditions at 4 °C (Balamatsia, Paleologos, et al., 2006). Levels of putrescine and cadaverine increased linearly with storage time and were higher in aerobically packaged chicken samples. Spermine and spermidine levels were also detected in both aerobically- and MA-packaged chicken meat. Levels of tyramine in both chicken samples stored aerobically and or under MAP were low ( $\leq 10 \text{ mg/kg}$ ), whereas the formation of histamine was only observed after day 11 of storage. In another study, Silva and Gloria (2002) reported levels of BAs in chicken meat and chicken-based meat products. Immediately after slaughter, spermine and spermidine were detected in breast and thigh meat. The presence of putrescine, cadaverine, histamine, and tyramine was observed in chicken samples after 15 days of refrigerated storage, and levels of these amines were higher in breast than in thigh meat. Chicken-based meat products also contained spermine and spermidine (Silva & Gloria, 2002). Recently, it was concluded that tyramine, putrescine and cadaverine could serve as quality indicators of MA- packaged broiler chicken meat (Rokka et al., 2004). With regard to meat quality, only recently TVB-N limit values of ca. 20 and 30 mg N/100 g for beef and pork (corresponding to 8 and 10 days of refrigerated storage, respectively) have been proposed as indicators of meat quality (Byun et al., 2003). The objectives therefore, of the present study were to: (1) determine the changes (formation) of volatile amines (TVB-N, TMA-N) in breast chicken meat during storage under aerobic and MAP conditions at 4 °C, (2) correlate microbial and sensory changes in chicken meat under the same storage conditions with formation of volatile amines, and (3) investigate the possible role of VAs as chemical indicators of chicken meat spoilage.

# 2. Materials and methods

## 2.1. Preparation of samples and storage conditions

Chicken breast fillets, 24 h after slaughter, were provided by a local poultry meat processing company (PIN-DOS, S.A., Ioannina, Greece). The fillets were placed on ice in insulated polystyrene boxes, and transported to the laboratory within 1 h of the chopping process. The chicken breast fillet samples (ca.  $150 \pm 10$  g) were then placed in polyethylene/polyamide/low density polyethylene (LDPE/ PA/LDPE) barrier pouches (one fillet per pouch), 75 µm in thickness, having an oxygen permeability of  $52.2 \text{ cm}^3$  $m^{-2} day^{-1} atm^{-1}$  at 75% relative humidity (RH), 23 °C, a carbon dioxide permeability of 191 cm<sup>3</sup> m<sup>-2</sup> day<sup>-1</sup> atm<sup>-1</sup> at 0% RH, 23 °C and a water vapour permeability of 2.4  $g^{-2}$  day<sup>-1</sup> at 100% RH, 23 °C. The following gas mixtures were used: M1, 30%/65%/5% (CO<sub>2</sub>/N<sub>2</sub>/O<sub>2</sub>) and M2, 65%/30%/5% (CO<sub>2</sub>/N<sub>2</sub>/O<sub>2</sub>). Gas mixtures were prepared using a PBI-Dansensor model mix 9000 gas mixer (Ringsted, Denmark). Pouches were heat-sealed using a BOSS model N48 vacuum sealer (Bad Homburg, Germany) and kept under refrigeration  $(4 \pm 0.5 \text{ °C})$ . Identical chicken samples were vacuum- and aerobically packaged, the latter being used as control samples. Samples were analysed at predetermined time intervals, namely, 1, 3, 5, 7, 11 and 15 days of storage.

#### 2.2. Microbiological analysis

Chicken meat samples (25 g) were weighed aseptically, transferred to a stomacher bag (Seward Medical, London, UK), containing 225 ml of sterile quarter-strength Ringer's solution, and homogenized using a stomacher (Lab Blender 400, Seward Medical, London, UK) for 60 s at room temperature (25 °C). For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluent, and quarter-strength Ringer's solution) of chicken homogenates were spread on the surfaces of dry media.

Total viable counts (TVC) were determined using plate count agar (PCA, Merck code 1.05463, Darmstadt, Germany), after incubation for 3 days at 30 °C. Pseudomonads were determined on cetrimide fusidin cephaloridine agar (Oxoid code CM 0559, supplemented with selective supplement SR 0103, Basingstoke, UK) after incubation at 25 °C for 2 days (Mead & Adams, 1977). Brochothrix thermosphacta was determined on streptomycin sulphatethallous acetate-cycloheximide (actidione) agar, prepared from basic ingredients in the laboratory after incubation at 25 °C for 3 days (Gardner, 1966). For members of the family Enterobacteriaceae, a 1.0 ml sample was inoculated into 10 ml of molten (45 °C) violet red bile glucose agar (Oxoid code CM 0485, Basingstoke, UK). After setting, a 10 ml overlay of molten medium was added and incubation was at 30 °C for 24 h. The large colonies with purple haloes were counted (Mossel, Eelderink, Koopmans, & Rossem, 1979). Lactic acid bacteria (LAB) were determined on de Man Rogosa Sharpe medium (Oxoid code CM 0361, Basingstoke, UK) after incubation at 25 °C for 5 days. Yeasts were enumerated using Rose Bengal chloroamphenicol agar (RBC, Merck code 1.00467, Darmstadt, Germany) after incubation at 25 °C for 5 days in the dark.

## 2.3. Sensory evaluation

Frozen chicken breast fillet (ca. 150 g) was cooked in a microwave oven at high power (700 W) for 4 min, including time to defrosting. A panel of seven judges, experienced (laboratory-trained) in poultry evaluation, was used for sensory evaluation. All panellists who evaluated the sensory attributes of cooked chicken had previously participated in training sessions to become familiar with the sensory characteristics of cooked chicken. Panellists were asked to evaluate taste, odour and appearance intensities of the cooked samples. Along with the test samples, the panellists were presented with a freshly thawed chicken sample, stored at -30 °C throughout the experiment, and cooked as described before, this serving as the reference sample.

Acceptability as a composite of odour, taste and appearance was estimated using a scale ranging from 0 to 9. The scale points were: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (first off-odour, off-taste development) <6; a score of 6 was taken as the lower limit of acceptability. The product was defined as unacceptable after development of first off-odour or off-taste.

#### 2.4. Chemical analysis

The pH value was recorded using a Metrohm (Herisau, Switzerland), model 691, pH meter. Chicken samples were thoroughly homogenized with distilled water (1:10) and the homogenate used for pH determination. Trimethylamine nitrogen (TMA-N) analysis was carried out according to the method proposed by AOAC (1990). Total volatile basic nitrogen (TVB-N) was determined according to the method of Malle and Poumeyrol (1989). The TMA-N and TVB-N contents were expressed as mg of TMA-N and TVB-N per 100 g of chicken muscle.

#### 2.5. Statistical analysis

Experiments were replicated twice on different occasions with different chicken samples. Triplicate samples were analyzed per replicate. Data from each replication were averaged and log transformed (microbiological). These data were subjected to analysis of variance (ANOVA) using the software Statgraphics (Statistical Graphics Corp., Rockville, MD, USA). Means and standard deviations were calculated and, when *F*-values were significant at the p < 0.05 level, mean differences were separated by the least significant difference (LSD) procedure (SAS, 1999).

## 3. Results

## 3.1. Microbiological analysis

The initial TVC of chicken meat (Fig. 1) was ca. 4.9 log cfu/g (day 1). A TVC count of 7–8 log cfu/g has been used to mark the end of microbiological shelf-life of fresh poultry (Senter, Arnold, & Chew, 2000) and, in the present study, an arbitrary value for TVC of 7 log cfu/g was taken for the upper acceptability limit of fresh chicken meat. This value was exceeded on day 5 (A), on day 7 (VP) and on days 11 and 15 (M1 and M2 gas mixture-packaged samples, respectively). After 15 days of storage, the VP, M1 and M2 chicken samples had significantly lower (p < 0.05) TVC counts than the control (A) samples.

Initial population of Pseudomonads, Gram-negative contributor of natural flora of chicken meat was ca. 4.2log cfu/g while a count of 7log cfu/g was exceeded between days 7–11 of storage (control samples), whereas VP, M1 and M2 chicken samples did not reach this population throughout the entire experiment (15 days) (Fig. 2). After 15 days of storage the M2 gas mixture and VP samples had a significantly lower (p < 0.05) *Pseudomonas* spp. count (5.1–6.3log cfu/g) than all the rest samples.

Lactic acid bacteria were also part of the natural flora of chicken (results not shown). Initial LAB count was ca. 3.9log cfu/g while a count of 7log cfu/g was exceeded on day 5 (A) and on day 12 (VP) samples, respectively. The M1 and M2 gas mixture-packaged samples did not reach



Fig. 1. Changes in total viable counts of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\blacklozenge$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.



Fig. 2. Changes in *Pseudomonas* spp. counts of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\blacklozenge$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.

this value throughout the 15-day storage period. Of the remaining facultative anaerobic species, examined in this study, both Enterobacteriaceae and *B. thermosphacta* were also dominant bacterial groups in chicken spoilage (Figs. 3 and 4, respectively), producing lower (p < 0.05) counts for VP and MAP samples in comparison to air-packaged chicken samples throughout the entire storage period. Finally, yeasts were less numerous (3.1–4.1 log cfu/g) than bacteria in chicken samples stored in air, under VP and MAS (results not shown).

## 3.2. Sensory evaluation

Sensory scores for odour and taste of microwavecooked chicken decreased with time of refrigerated storage (Fig. 5a and b). A score of 6 was taken as the lower limit of



Fig. 3. Changes in Enterobacteriaceae counts of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\blacklozenge$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.



Fig. 4. Changes in *Brochothrix thermosphacta* counts of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\blacklozenge$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.



Fig. 5. Changes in off-odour (a) and off-taste scores (b) of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\bigcirc$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.

acceptability, corresponding to initial off-odour or off-taste development. Odour and taste showed similar patterns of decreasing acceptability (Fig. 5a and b). All vacuum- and MA-packaged chicken samples received higher sensory scores (p < 0.05) than air-packaged samples, as judged by the above attributes. This trend was more apparent after day 3 and continued throughout the entire period of refrigerated storage. The limit of acceptability of odour was reached approximately on day 7 for the air- and vacuum-packaged samples and on day 9 for the M1 gas mixture-packaged chicken, whereas the limit of acceptability for odour was not reached for chicken samples packaged under M2 gas mixture, even after 15 days of storage (Fig. 5a).

Sensory scores for taste were only recorded for chicken samples that had not exceeded the microbiological limit value of 7log cfu/g since these samples were judged as unfit to taste (Fig. 5b). The limit of acceptability for taste was reached approximately on days 6–7 and 10 for chicken samples packaged in air and under vacuum, respectively. Similarly to odour, the limit of acceptability for taste was not reached for samples packaged under M2 gas mixture even after 15 days of storage. Appearance scores for all chicken samples decreased at a slower rate than odour and taste scores, never reaching the lower acceptability limit of 6 (results not shown).

## 3.3. Chemical analysis

The pH of fresh chicken muscle was about 6.32. The values of pH showed no statistically significant (p > 0.05) changes for chicken packaged in air and under VP and MAs during the entire period of storage (results not shown). The TMA-N values for all chicken packaged

breast portions are presented (Fig. 6). The initial TMA-N content was low (1.14 mg N/100 g). After ca. day 3 of storage. TMA-N values of the control and vacuum-packaged samples were significantly higher (p < 0.05) than those of MAP chicken and increased at a higher rate than the corresponding values of all other groups of samples throughout storage period. The TMA-N reached the values of 21.5 and 23.2 mg N/100 g, respectively, for the air- and vacuumpackaged samples after 15 days of storage. Final TMA-N values for all chicken samples packaged under MAs were significantly lower (p < 0.05) than those of chicken samples packaged in air and under vacuum. In a similar trend, TVB-N values of chicken increased from an initial value of ca. 20.5 mg N/100 g to final values of 54.5, 45.8, 43.1 and 29.6 mg N/100 g for air-, vacuum-, M1 and M2 gas mixture-packaged chicken samples, respectively (Fig. 7). As previously noted for TMA-N, significantly lower  $(p \le 0.05)$  TVB-N values were recorded for all chicken samples packaged under MAs than for chicken samples packaged in air and under vacuum.

#### 4. Discussion

The development of aerobic and anaerobic (VP and MAs) bacterial association of chicken meat (breast portions), consisting mainly of *Pseudomonas* spp., LAB, Enterobacteriaceae, *B. thermosphacta* and yeasts was reported earlier for poultry meat and poultry products (Gill, Harris-



Fig. 6. Changes in trimethylamine nitrogen (TMA-N) values of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\bigcirc$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.



Fig. 7. Changes in total volatile basic nitrogen (TVB-N) values of chilled fresh chicken meat during storage in air ( $\blacksquare$ ), under VP ( $\blacklozenge$ ), and under M1 ( $\blacktriangle$ ) and M2 ( $\blacklozenge$ ) gas mixtures. Each point is the mean of three samples taken from two replicate experiments ( $n = 3 \times 2 = 6$ )  $\pm$  SD.

son, & Penney, 1990; Jimenez, Salsi, Tiburzi, Rafaghelli, & Pirovani, 1999; Kakouri & Nychas, 1994; Saucier, Gendron, & Gariepy, 2000). Both VP and MAs, as expected, delayed the development of aerobic spoilage microbial flora, due to the anaerobic micro-niche (VP storage) and the  $CO_2$  action. The  $CO_2$ , because of its bacteriostatic effect inhibits the growth of aerobic spoilage microorganisms such as *Pseudomonas* spp. and yeasts, as a result of an extension of the lag phase of growth and a decrease in the growth rate during the logarithmic phase (Farber, 1991).

Of the remaining bacterial species examined, LAB growth (facultative anaerobic species), was dominant in the microbial association of both aerobically- and MApackaged chicken, indicating that this bacterial group is of importance in chicken meat spoilage, in accordance with the findings of Jimenez et al. (1999) and Saucier et al. (2000). The development of the facultative anaerobic bacterium B. thermosphacta in chicken breast portions packaged under VP and MAs is in agreement with earlier reports (Drosinos & Nychas, 1996; Kakouri & Nychas, 1994), confirming its remarkable spoilage potential with regard to meats, including poultry and poultry products. Of the microbial associations developed in chicken meat under the packaging treatments examined in the present study (aerobic and MAP), yeasts contribute to a much lesser extent in the final microbial flora of chicken meat. Present results obtained for yeasts in chicken meat are in agreement with results obtained by Ismail, Deak, Abd El-Rahman, Yassien, and Beuchat (2000) for various chicken products. It is noteworthy that both *Pseudomonas* spp. and yeasts, species known to grow strictly under aerobic storage, gradually increased in VP chicken samples; this can be attributed to the oxygen transmission rate of the packaging material, as oxygen levels as low as 1% are enough to support the growth of these organisms under VP conditions.

On the basis of microbiological data (TVC) in our study, a shelf-life extension of 2, 4 and 9–10 days was achieved by VP and M1 and M2 gas mixtures, in agreement with results reported by Kakouri and Nychas (1994) and by Jimenez et al. (1999) for poultry meat stored under MAP.

Results of the present work show that the limit of sensory acceptability (a score of 6) was reached for the aerobically, vacuum-packaged and M1 gas mixture-packaged chicken samples approximately on days 6-7 and 9-10, respectively. For these treatments (air, VP, M1), the limit of overall sensory acceptability coincided with the time when the Pseudomonas spp., LAB, and Enterobacteriaceae counts were around 6 and 7log cfu/g. The use of VP and MAs, as shown in the present study, resulted in an extension of shelf-life of chicken breast portions by approximately 2-3 days (M1 gas mixture), and by more than 9 days (M2 gas mixture), respectively. Overall acceptability data of air-, vacuum- and MA-packaged chicken samples correlated rather well with TVC data. Chicken meat was better preserved under M2 gas mixture, maintaining acceptable odour/taste attributes, even on the final day of storage.

In other studies on raw poultry products, the use of MAP resulted in a shelf life extension of 14 days of chicken carcasses (Sawaya, Elnawawy, Abu-Ruwaida, Khalafawi, & Dashti, 1995). The same shelf-life was recorded for chicken breast portions stored under 70% CO<sub>2</sub>/30% N<sub>2</sub> (Jimenez et al., 1999) and for chicken breasts after dipping in phosphate-spice marinade mix prior to VP at 4 °C (Buses & Thomson, 2003). Similarly, a shelf-life extension of 4–7 and 8 days was achieved for raw poultry treated with EDTA-nisin and stored under VP and for chicken legs after dipping in 5-10% trisodium phosphate without affecting sensory quality, respectively (Cosby, Harrison, & Toledo, 1999; Kim & Marshall, 1999).

Few studies (Balamatsia, Paleologos, et al., 2006; Balamatsia, Rogga, et al., 2006; Byun et al., 2003; Nychas et al., 1988; Rokka et al., 2004) have been published on the use of chemical indicators for predicting the microbial quality of meat (including fresh poultry and poultry products) in contrast to the wealth of information that is available for various fish species (Connell, 1990). Limited information is available on production of volatile amines in meat, including chicken (Silva & Gloria, 2002). The VAs, such as TMA-N and TVB-N, have been used to assess the extent of spoilage and freshness of marine fish, and upper TMA-N and TVB-N limits of 1-15 and 30-35 mg N/100 g have been suggested for seafood freshness, respectively (Connell, 1990). With regard to meat quality, only recently TVB-N limit values of ca. 20 and 30 mg N/ 100 g for beef and pork (corresponding to 8 and 10 days of refrigerated storage, respectively) have been proposed as indicators of meat freshness and shelf-life (Byun et al., 2003).

Based on sensory (taste) analysis and with regard to chicken spoilage and freshness, a TMA-N value of ca. 10.0 mg N/100 g may be proposed as the upper limit forspoilage initiation of fresh chicken meat stored aerobically. Respective TVC (day 6) for aerobically stored chicken meat was ca.  $10^7$  cfu/g. This limit value was reached on days 7-8 and 11-12 for chicken samples packaged under vacuum and under M1 gas mixture. Interestingly, the M2 gas mixture sample did not reach this value throughout the 15 day storage period. When using 10 mg of TMA-N/100 g as a limit of acceptability for fresh chicken meat freshness, the chicken breast fillets stored in air, under VP and M1 gas mixture would have been rejected after ca. 6, 7–8 and 11–12 days, respectively, and, interestingly, during this storage period, TVC had reached ca. 7log cfu/g, accompanied by sensory deterioration of chicken samples. The results obtained in this study indicate that both VP and MAP (M1 and M2 gas mixtures) have a considerable effect on the production of TMA-N in chicken meat. It is likely that the restriction of bacterial growth (H<sub>2</sub>S-producing bacteria) and other species (responsible for production of volatile amines) may have a positive effect on the deterioration of the sensory attributes of chicken meat, though this bacterial group was not monitored in the present study.

Total volatile basic nitrogen is a product of bacterial spoilage and often used as a chemical index to assess the quality and shelf-life of seafood products (Connell, 1990). Because ammonia production increases due to the deamination of amino acids during spoilage, TVB-N has been proposed as an index of fresh meat quality and maximum acceptability limit values between of 20 and 30 mg N/100 g have been suggested for beef and pork, respectively (Singhal, Kulkarni, & Rege, 1997).

In the present study, fresh chicken samples packaged under aerobic condition were judged unacceptable between days 5-7 of storage, based on microbiological and sensory parameters. It must be noted that a good correlation was noted between sensory (taste) and microbiological (TVC) parameters of chicken meat packaged in air, under vacuum and MAs (M1 and M2 gas mixtures). Considering a TVB-N limit value of acceptability of 40 mg N/100 g (day 6 of storage) for chicken samples stored in air, by inspection of Fig. 7, it is apparent that, in the present study, for samples stored under vacuum and under M1 gas mixture, TVB-N levels surpassed 40 mg N/100 g, the recommended limit for palatability for fresh chicken between days 7-8 and days 11-12 of refrigerated storage. Interestingly, as previously for TMA-N, chicken samples packaged under M2 gas mixture never reached the maximum acceptability value of 40 mg N/100 g during the entire period of storage. Based on the TVB-N value of 40 mg N/100 g, it may be postulated that a shelf-life extension of 1–2 and 5–6 days was obtained for chicken samples stored under vacuum and under M1 gas mixture, respectively. Singhal et al. (1997) suggested that stored meat (beef, pork) is not necessarily unpalatable until the TVB-N value reaches 30 mg N/ 100 g.

To our knowledge, this is the first report of volatile amines (TMA-N and TVB-N) that could be used as potential chemical indicators in predicting the microbial quality of fresh chicken meat during chill storage under aerobic and MAP conditions ( $CO_2/N_2/O_2$ : gas mixtures, M1:30%/65%/5% and M2:65%/30%/5%). The formation of VAs during chill storage of chicken meat under the packaging conditions examined in the present study seemed to be in a good agreement with the increase in the microbiological counts (TVC) and sensory scores (taste) except for the M2 gas mixture.

## 5. Conclusions

It is important to stress, as a final point, that present recommendations on levels of amines as potential indices of fresh chicken meat quality correspond to samples from one poultry plant and thus their general application is yet to be verified. In conclusion, further studies are needed since limited information is available to date, with regard to establishing acceptability limit values of volatile amines, both TMA-N and TVB-N that could be used as potential indicators in predicting the freshness and quality of poultry meat.

#### Acknowledgements

We express our thanks to PINDOS S.A., Rodotopi, Ioannina, Greece for providing chicken samples. This work was funded by the General Secretariat of Research and Technology (GSRT), through a bilateral research programme between Greece and Romania (2003–2005, 2000SE01330005).

## References

- Al-Bachir, M., & Mehio, A. (2001). Irradiated luncheon meat: Microbiological, chemical and sensory characteristics during storage. *Food Chemistry*, 75, 169–175.
- AOAC (1990). *Official methods of analysis* (14th ed.). Washington, DC: Washington Association of Analytical Chemists.
- Balamatsia, C. C., Paleologos, E. K., Kontominas, M. G., & Savvaidis, I. N. (2006). Correlation between microbial flora, sensory changes and biogenic amines formation in fresh chicken meat stored aerobically or under modified atmosphere packaging at 4 °C: Possible role of biogenic amines as spoilage indicators. *Antonie van Leeuwenhoek Journal*, 89, 9–17.
- Balamatsia, C. C., Rogga, K., Badeka, A., Kontominas, M. G., & Savvaidis, I. N. (2006). Microbiological, chemical and sensory characteristics of chicken meat stored aerobically at 4 °C: Effect of lowdose irradiation. *Journal of Food Protection*, 69, 1126–1133.
- Bardócz, S. (1995). Polyamines in food and their consequences for food quality and human health. *Trends in Food Science and Technology*, 6, 341–346.
- Buses, H., & Thomson, L. (2003). Dip application of phosphates and marinade mix on shelf life of vacuum-packed chicken breast fillets. *Journal of Food Protection*, 66, 1701–1703.
- Byun, J. S., Min, J. S., Kim, I. S., Kim, J. W., Chung, M. S., & Lee, M. (2003). Comparison of indicators of microbial quality of meat during aerobic cold storage. *Journal of Food Protection*, 66, 1733–1737.
- Connell, J. J. (1990). Methods of assessing and selecting for quality. In J. J. Connell (Ed.), *Control of fish quality* (3rd ed., pp. 122–150). Oxford: Fishing News Books.
- Cosby, D. E., Harrison, M. A., & Toledo, R. T. (1999). Vacuum or modified atmosphere packaging and EDTA-nisin treatment to increase poultry product shelf-life. *Journal of Applied Poultry Research*, 8, 185–190.
- Dainty, R. (1996). Chemical/biochemical detection of spoilage. International Journal of Food Microbiology, 33, 19–33.
- Davies, A. R. (1995). Advances in modified-atmospheres-packaging. In G.
  W. Gould (Ed.), *New methods of food preservation* (pp. 304–320).
  London: Blackie Academic and Professional.
- Drosinos, E. H., & Nychas, G.-J. E. (1996). Brochothrix thermosphacta, a dominant organism in Mediterranean fresh fish (Sparus aurata) stored under modified atmosphere. Italian Journal of Food Science, 4, 323–329.
- Farber, J. M. (1991). Microbiological aspects of modified-atmosphere packaging technology: A review. *Journal of Food Protection*, 54, 58–70.
- Garcia de Fernando, G. D., Nychas, G.-J. E., Peck, M. W., & Ordónez, J. A. (1995). Growth/survival of psychrotrophic pathogens on meat packed under modified atmospheres. *International Journal of Food Microbiology*, 28, 221–231.
- Gardner, G. A. (1966). A selective medium for the enumeration of Microbacterium thermosphactum in meat and meat products. Journal of Applied Bacteriology, 29, 455–460.
- Gill, C. O. (1996). Extending the storage life of raw chilled meats. *Meat Science*, 43, S99–S109.
- Gill, C. O., Harrisson, J. C., & Penney, N. (1990). The storage life of chicken carcasses packed under carbon dioxide. *International Journal* of Food Microbiology, 11, 151–157.

- Gill, C. O., & Molin, G. (1991). Modified atmospheres and vacuum packaging. In N. J. Russell & G. W. Gould (Eds.), *Food preservatives* (pp. 172–199). London: Blackie Academic and Professional.
- Halász, A., Baráth, A., Simon-Sarkadi, L., & Holzapfel, W. (1994). BA's and their production by microorganisms in food. *Trends in Food Science and Technology*, 5, 42–49.
- Ismail, S. A., Deak, T., Abd El-Rahman, H. A., Yassien, A. M., & Beuchat, L. R. (2000). Presence and changes in populations of yeasts on raw and processed poultry products stored at refrigeration temperature. *International Journal of Food Microbiology*, 62, 113–121.
- Jay, J. M. (1986). Microbial spoilage indicators and metabolites. In M. D. Pierson & N. J. Sterm (Eds.), *Foodborne microorganisms and their* toxins: Developing methods (pp. 219–240). Basel: Marcel Dekker.
- Jeremiah, L. E. (2001). Packaging alternatives to deliver fresh meats using short- or long-term distribution. *Food Research International*, 34, 749–772.
- Jimenez, S. M., Salsi, M. S., Tiburzi, M. C., Rafaghelli, R. C., & Pirovani, M. E. (1999). Combined use of acetic acid treatment and modified atmosphere for extending the shelf-life of chilled chicken breast portions. *Journal of Applied Microbiology*, 87, 339–344.
- Kakouri, A., & Nychas, G.-J. E. (1994). Storage of poultry meat under modified atmospheres or vacuum packs: Possible role of microbial metabolites as indicator of spoilage. *Journal of Applied Bacteriology*, 76, 163–172.
- Kim, C. R., & Marshall, D. L. (1999). Microbiological, colour and sensory changes of refrigerated chicken legs treated with selected phosphates. *Food Research International*, 32, 209–215.
- Malle, P., & Poumeyrol, M. (1989). A new chemical criterion for the quality of fish: Trimethylamine/total volatile basic nitrogen (%). *Journal of Food Protection*, 50, 419–423.
- Mead, G. C., & Adams, B. W. (1977). A selective medium for the rapid isolation of *Pseudomonas* associated with poultry meat spoilage. *British Poultry Science*, 18, 661–670.
- Mossel, D. A., Eelderink, L., Koopmans, M., & Rossem, F. V. (1979). Influence of carbon source, bile salts, and incubation temperature on recovery of Enterobacteriaceae from foods using MacConkey-type agars. *Journal of Food Protection*, 42, 470–475.
- Nychas, G.-J. E., Dillon, V., & Board, R. G. (1988). Glucose, the key substrate in the microbiological changes occurring in meat and certain meat products. *Biotechnology and Applied Biochemistry*, 10, 203–231.
- Rokka, M., Eerola, S., Smolander, M., Alakomi, H. L., & Ahvenainen, R. (2004). Monitoring of the quality of modified atmosphere packedbroiler chicken cuts stored at different temperature conditions. B. Biogenic amines as quality-indicating metabolites. *Food Control*, 15, 601–607.
- Ruiz-Capillas, C., & Jimenez-Colmenero, F. (2004). Biogenic amines in meat and in meat products. *Critical Reviews in Food Science and Nutrition*, 44, 489–499.
- Russell, S. M., Fletcher, D. L., & Cox, N. A. (1996). Spoilage bacteria of fresh broiler chicken carcasses. *Poultry Science*, 75, 2041–2047.
- SAS (1999). Statistical analysis system, version 8.0.. Cary, NC: SAS Institute.
- Saucier, L., Gendron, C., & Gariepy, C. (2000). Shelf life of poultry meat under modified atmosphere. *Poultry Science*, 79, 1851–1856.
- Sawaya, W. N., Elnawawy, A. S., Abu-Ruwaida, A. S., Khalafawi, S., & Dashti, B. (1995). Influence of modified atmosphere packaging on shelf-life of chicken carcasses under refrigerated storage conditions. *Journal of Food Safety*, 15, 35–51.
- Senter, S. D., Arnold, J. W., & Chew, V. (2000). APC values and volatile compounds formed in commercially processed, raw chicken parts during storage at 4 and 13 °C under simulated temperature abuse conditions. *Journal of the Science of Food and Agriculture, 80*, 1559–1564.
- Silva, C. M. G., & Gloria, M. B. A. (2002). Bioactive amines in chicken breast and thigh after slaughter and during storage at  $4 \pm 1$  °C and in chicken-based meat products. *Food Chemistry*, 78, 241–248.
- Singhal, R. S., Kulkarni, P. R., & Rege, D. V. (1997). Handbook of indices of food quality and authenticity. Cambridge: Woodhead Publishing Ltd.