XVIII EUROPEAN SYMPOSIUM ON THE QUALITY OF POULTRY MEAT

XII EUROPEAN SYMPOSIUM ON THE QUALITY OF EGGS AND EGG PRODUCTS

SYMPOSIUM PROCEEDINGS

PRAGUE SEPTEMBER 2-5 2007

SYMPOSIUM PROCEEDINGS

XVIII European Symposium on the Quality
of Poultry Meat
and
XII European Symposium on the Quality
of Eggs and Egg products

Prague, September 2 - 5, 2007
Czech Republic

Editor:

Czech Branch of WPSA

P-032

Biogenic amines formation on Light and Dark colour turkey meat stored at 0°C

M. J. Fraqueza, C. M. Alfaia, M. C. Ferreira, A. S. Barreto

Faculty of Veterinary Medicine. CIISA. UT Lisbon. Av. da Universidade Técnica. Polo Universitário. Alto da Ajuda. 1300-477 Lisbon. Portugal. e-mail:mjoaofraqueza@fmv.utl.pt

INTRODUCTION

The biogenic amines, tyramine, phenylethylamine, histamine, tryptamine, cadaverine, putrescine, spermine and spermidine have been detected in meat and meat products, occurring naturally from metabolic processes in muscle and connective tissues and mainly due to bacteria decarboxylation of amino acids (Bauer, 1995). Turkey meat spoilage is predominantly done by *Pseudomonas spp.* and other Gram negative *Enterobacteriaceae* (Fraqueza *et al.*, 2005) and also by *Achromobacter, Flavobacterium, Acinetobacter/Moraxella, Alcaligenes, Aeromonas, Brochotrix, Lactobacillus* and some yeast which induced changes on its organoleptic characteristics with slime and putrid odours (Cox *et al.*, 1998). According to Silla Santos (1996) bacteria as *Pseudomonas spp., Enterobacteriaceae* and *Lactobacillus* are descarboxylase-positive being one of the principal factors to the formation of biogenic amines. Tyramine, putrescine and cadaverine are frequently referred as suitable indicators of meat spoilage, however there are little information about the relationship between turkey meat quality, its spoilage and the production of biogenic amines.

This study intended to evaluate the formation of biogenic amines in light and dark colour turkey meat stored aerobically at 0°C establishing a relationship with the microbial evaluation in order to obtain data about the sensitivity of the spoilage indicator - biogenic amines content.

MATERIAL AND METHODS

Samples were collected from turkey carcasses slaughtered according to commercial practices of an industrial slaughterhouse. The meat quality evaluation was performed by pH_{24} (24 h *postmortem*) and colour (24 h *postmortem*) measurements.

Determination of pH was made directly in the *pectoralis major* muscle with a portable pH meter (HI9023) equipped with a pH electrode (FC 230B, Hanna Instruments, Italy). The colour was measured on the internal side of the *pectoralis major* muscle with a Minolta Colourimeter CR-300 (Minolta, Osaka, Japan) using the L*, a*, b* coordinates (CIELAB colour system). Breasts were selected according to Luminance (L*) and pH_{24}: L*≥51 and pH<5.8 for light colour, 43<L*<51 for intermediate colour, L*≤43 and pH>5.8 for dark colour (Fraqueza *et al.*, 2006).

Breast muscles from different colour categories were cut into slices in a deboning room. The meat was placed in a polyethylene bag and transported in an isothermal box to the laboratory in less than one hour. Sliced meat samples were individually packaged in aerobiosis, using polypropylene trays (Tecknopack plastics, S/L, Barcelona) and polyvinyl chloride (PVC) film. All samples were immediately stored in refrigeration (0±1 °C) in the dark for 12 days. Meat samples packaged in aerobiosis were evaluated for their microbiological characteristics and biogenic amines formation (putrescine (Put), cadaverine (Cad), histamine (His), tyramine (Tyr), spermidine (Spd) and spermine (Spm)) on days 0, 5 and 12 of storage. At least five replications were made on different working days.

Microbiological determinations: total mesophilic aerobic counts (Plate Count Agar, Sharlau, Spain) at 30 °C for 2 days; total psychrotrophic aerobic counts (Plate Count Agar, Sharlau, Spain) at 7 °C for 10 days; *Enterobacteriaceae* counts in Violet Red Bile agar (VRB agar, Merck, Germany) at 37 °C for 2 days; *Pseudomonas* spp. counts (cephaloridene, fucidin and cetrimide (CFC) agar base; Oxoid, UK) after incubation at 30 °C for 2 days, lactic acid bacteria (LAB) counts in Man Rogosa Sharpe Agar (Oxoid, UK) incubated at 30 °C for 3 days (ISO 15214:1998) and *Brochothrix thermosphacta* count in streptomycin, actidione, thallous acetate agar (STAA, Oxoid, UK) incubated for 2 days at 30 °C. Counts were expressed as log cfu.g^{-1}.

Biogenic amines determination: Biogenic amines, putrescine (Put), cadaverine (Cad), histamine (His), tyramine (Tyr), spermidine (Spd) and spermine (Spm), were separated and quantified by a high-performance liquid chromatographic method described by Eerola *et al.* (1993). The amines were extracted with perchloric acid and derivatized with dansyl chloride. The chromatographic separations were performed on a reversed-phase column (Supelcosil RP-18, 5µm, 250x4.6 mm, Supelco, PA), detection at 254 nm (UV) using 1,7-diaminoheptane as internal standard (IS). Biogenic Amine Index (BAI) was calculated from addition of Put, Cad and Tyr.

Statistical analysis: Data was analysed using SPSS 11.5 for Windows. The comparison between different colour quality meat samples, for microbial parameters, was performed by model adjustment of a "one-way" ANOVA for each day. The comparison between days, considering each package and colour meat condition, was made by t-test for dependent samples. A Pearson correlation test was performed.

RESULTS AND DISCUSSION

The initial *Pseudomonas* spp. counts in turkey meat were 4.6 log cfu. g^{-1} while *Enterobacteriaceae, Brochothrix thermosphacta* and lactic acid bacteria (LAB) counts were inferior to 3 log cfu.g^{-1}. Samples classified with different colour had no significant differences for all microflora groups counts on the 5th day, although on the 12th day the darker samples presented higher counts of total mesophylic and psychrotrophic aerobic flora than the lighter samples (Figure 1). This tendency was also observed on the *Enterobacteriaceae* and *Pseudomonas spp.* Counts (Figure 2). Independently of the colour turkey meat category the presence of Put, Cad, Tyr, Spd and Spm were detected since the first day of storage. After 5th day of storage a significant increase of Put, Cad and Tyr was observed with a content of 15.30, 32.50 and 7.64 mg.kg^{-1} respectively in the 12th day. However, the turkey meat of different colour categories packaged aerobically was not significantly different regarding its content on different biogenic amines quantified in different days of storage (Figure 3). Turkey meat under our study conditions did not present any toxicological risk associated to the increase of Tir, with a maximum mean concentration of 7.64 mg.kg^{-1}, and regarding histamine, which were above limit quantification (1.3 mg.kg^{-1}) in only two samples with a maximum value of 1.99 mg.kg^{-1}. Relationships between microbial parameters and biogenic amines in turkey meat was observed (Table 1) with significant correlations ($p<0.01$) among *Pseudomonas* spp. (0.607), *Enterobacteriaceae* (0.577) and *Brochothrix thermosphacta* (0.565) and an increased concentration of Cad, while higher concentrations of Put were correlated with *Pseudomonas* spp. (0.516) and *Brochothrix thermosphacta* (0.529). The increase of Tyr was associated to the growth of *Enterobacteriaceae* (0.472). BAI was significantly higher correlated ($p<0.01$) with all microbial groups analysed than each biogenic amine with them, exception made to LAB. This was indicative of a lower contribution from LAB group to biogenic amines formation in turkey meat under aerobic package, and good suitability of BAI as spoilage indicator. A BAI value of 50.67 mg.kg^{-1} corresponded to turkey meat with signs of putrefaction (Figure 3). Since at 5th day of storage dark and intermediate colour samples surpass the limit of acceptability (5x10^6 cfu.g^{-1}) was proposed as indicative of spoilage, a BAI value superior to 25-30 mg.kg^{-1}.

Figure 1: Mesophylic (A) and psychrotrophic aerobic total (B) counts in sliced turkey meat different colour (Light, Intermediate, Dark) categories during storage time at 0°C.

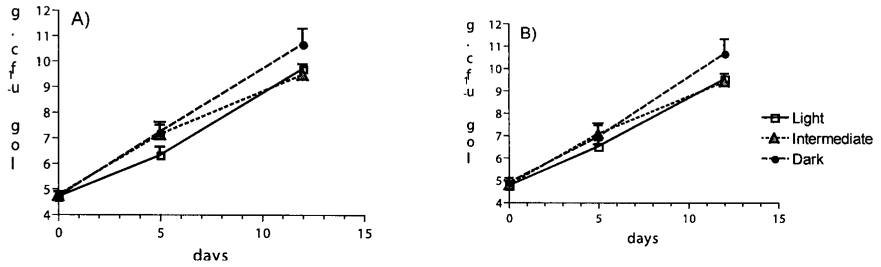

Figure 2: *Pseudomonas* spp. (A) and *Enterobacteriaceae* (B) counts in sliced turkey meat different colour (Light, Intermediate, Dark) categories during storage time at 0°C.

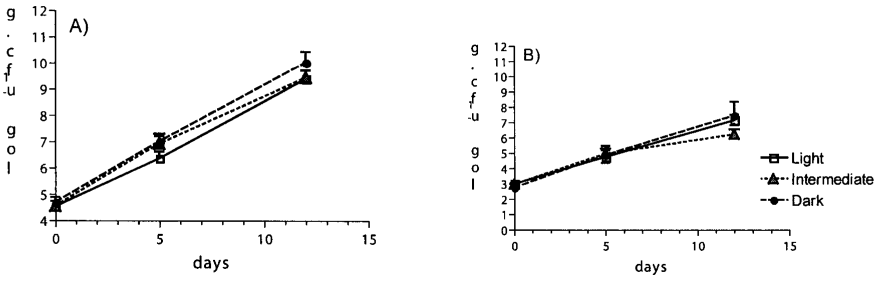

Figure 3: Biogenic Amine Index in sliced turkey meat of different colour (Light, Intermediate, Dark) categories during storage time at 0°C.

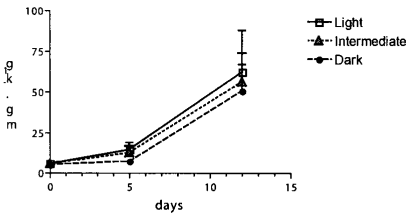

Table 1: Pearson Correlation between biogenic amine values, microflora counts and pH on sliced turkey meat during storage time at 0°C (n ≥85).

	Put	Cad	His	Tyr	Spd	Spm	BAI
Total mesophylic count	0.511(**)	0.602(**)	0.052	0.249(*)	0.543(**)	0.210(*)	0.626(**)
Total psychrotrophic	0.513(**)	0.607(**)	0.049	0.458(**)	0.567(**)	0.224(*)	0.658(**)
Enterobacteriaceae	0.473(**)	0.577(**)	0.153	0.472(**)	0.598(**)	0.397(**)	0.626(**)
Pseudomonas spp.	0.516(**)	0.607(**)	0.037	0.450(**)	0.548(**)	0.206	0.657(**)
LAB	0.349(**)	0.105	-0.208	-0.188	0.154	-0.062	0.134
Brochothrix thermosphacta	0.529(**)	0.565(**)	-0.033	0.246(*)	0.457(**)	0.115	0.606(**)
pH	0.386(**)	0.402(**)	-0.271(*)	-0.024	0.163	-0.172	0.383(**)

** Pearson correlation significant at 0.01; * Pearson correlation significant at 0.05.

REFERENCES

Bauer, F. 1995. Chemical changes during storage and spoilage of meat and meat products. In: Shelf life of meat and meat products. pp57-74. Ed. F. Bauer and S. Burt ECCEAMST Foundation. Utrecht. Netherlands.

Cox, N.A.; Russell, S.M.& Bailey, J.S. 1998. The microbiology of stored poultry. In: The microbiology of meat and poultry. pp.266-286. Ed. A. Davies and R. Board. Blackie Academic & Professional. London.

Eerola S., Hinkkanen, R., Lindfors, E., Hirvi T. 1993. Liquid Chro-

matographic Determination of Biogenic Amines in Dry Sausages. Journal of AOAC International, Vol.76, No. 3, pp. 575-577.
Fraqueza, M.J., Ferreira, M.C., Barreto, A.S. 2005. "Shelf life of different colour breast turkey meat under modified atmosphere package". Book of Abstracts 51th International Congress of Meat Science and Technology, ICoMST. Baltimore, Maryland. USA. pp. 41.
Fraqueza, M.J., Cardoso, A.S., Ferreira, M.C., Barreto, A.S. 2006. "Incidence of pectoralis major turkey muscles with light and dark color in a Portuguese slaughterhouse". Poultry Science, 85:1992-2000.
Silla Santos, M.H. 1996. Biogenic amines: their importance in foods. International Journal of Food Microbiology 29, 213-231.

Keywords: Biogenic amines, turkey meat, spoilage, colour, quality.

P-033

Comparing the yolk pigmentation efficacy of various red carotenoids and their in-yolk cooking stability

E. Fru[1], P. Philipps[1], R. Aureli[1], J. Schierle[2]

[1]*DSM Nutritional Products, Boulevard d'Alsace, F-68128 Village-Neuf, France;* [2]*DSM Nutritional Products Ltd., Kaiseraugst, Switzerland*

fidelis.fru@dsm.com

INTRODUCTION

Since carotenoids are mainly synthesized by plants, animals therefore, fully rely on dietary supplies to cover their needs (Grashorn and Steinberg, 2002). Egg yolk pigmentation does not affect the nutritional quality of eggs, it however plays a major role in consumer acceptability. Apart from recent reports showing that carotenoids possess some anti-oxidative effects (Surai *et al.*, 2003) the major reasons for supplementing laying hen's feed with carotenoids have been to achieve the level of pigmentation preferred by consumers and ensuring uniformity in yolk colour (Blount *et al.*, 2000). These substances, mainly carotenoids, have over the years been used to acquire desired yolk colour hue (*Bornstein, 1966; Hencken, 1992, Fru et al., 2006).* Yolk colour stability also plays a key role in acceptability to consumers and therefore of interest to the egg industry. It is a common observation that after cooking, most yolks lose some of their colour as such certain consumers associate this loss of colour to a poor egg quality. In this study we investigate the egg yolk pigmentation efficacy and the cooking stability of the pigments, of 3 canthaxanthin products produced in China (coded C1, C2 and C3) comparing them to CAROPHYLL® Red 10% (CR).

MATERIALS AND METHODS

The trial was carried out at the Research Centre for Animal Nutrition (DSM Nutritional Products France, F-68128 Village-Neuf) and was in accordance with the norms as stipulated by the official French instructions for experimentation with live animals. Laying hens of the line "Lohmann Brown" were used. The hens were 46 weeks old at the beginning of the trial and were kept in individual battery cages in an environmentally controlled room.

Prior to trial, hens were fed a low carotenoid basal diet with no supplementation of pigmenting substances for 28 days. Details on the composition of the basal diet are presented in Table 1.
During the trials, the control treatment (C0) replicated by four small groups of three hens each continued receiving the low carotenoid basal diet, while the rest of the animals were divided into treatment groups, each replicated as in the control treatment, and fed test diets supplemented with the appropriate pigmenting substance for three weeks. Animals had *ad libitum* access to water and feed, fed in mash form. In the test diets, adequate amounts of the following canthaxanthin products were added to obtain in-feed canthaxanthin inclusion levels of 2.0, 4.0 and 8.0 mg per kg feed:

- CR - CAROPHYLL® Red 10%, (commercial product form with min. 10% canthaxanthin,)
- C1 - product 1 (commercial product form with min. 2.5% canthaxanthin)
- C2 - product 2 (commercial product form with min. 10% canthaxanthin,)
- C3 - product 3, (commercial product form with min. 10% canthaxanthin)

In addition, all test diets were supplemented with 3.0 mg apo-ester (CAROPHYLL® Yellow 10%: min. 10% apo-ester) per kg feed to provide a good yellow basis for the pleasant golden orange colour in egg yolk required. Appropriate amounts of the products were mixed with 1 kg of the basal feed as a premix which was then added onto the feed to get the final concentration, according to the treatment.

The last four eggs of each hen were collected after three weeks of feeding. Half of the 12 eggs per replicate were hard-boiled (using a Krups egg cooker, according to the producer's recommendation). All (six raw and six hard-cooked) egg yolks per replicate group were used for measurements. The canthaxanthin content of feed samples and egg yolks were determined by HPLC. Pigment deposition rates were calculated as percentages of pigment consumed per day that was deposited in the egg.

The egg yolk colour was determined by reflectance colourimetry (CIE-Lab system, Xenocolor Chromameter) using standard conditions and also by visual scoring using the DSM yolk-colour-fan (DSM-YCF). Data were analysed using the GLM (factors: product and dose) of SPSS version 12.0.1 for Windows and GraphPad Prism version 3.0 for Windows, and treatment means were compared in which $P \leq 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Although laying rate was measured and products mainly did not affect the laying rate (87 ±14 %), the duration of the experiment was too short to draw any binding conclusion.

The analysed concentrations of canthaxanthin in experimental diets were similar to the targets. For all products, analysed concentrations of canthaxanthin in the egg yolk increased linearly with increasing dietary inclusion levels. Averagely, the canthaxanthin deposition rate in egg yolk, as shown on Fig. 1, of CAROPHYLL® Red (35.1±2.7 %) was significantly better than those of C0 (0.0%), C1 (30.1 ±3.4 %), C2 (26.8 ±2.8 %) and C3 (23.7 ±2.2 %), while C1 was also significantly better than C0, C2 and C3, suggesting a better bioavailability of canthaxanthin in CAROPHYLL® Red compared to the other canthaxanthin products. Bioavailability of formulated products could be influenced by many factors, including but not limited to, matrix type and structure, formulation particle size, possibility of formation of non-absorbable complexes with matrix components and degradability of carrier.