EFFECT OF AMMONIA AND SODIUM ERYTHORBATE ON THE PINK COLOR DEFECT IN TURKEY MEAT

AMONYAK VE SODYUM ERİTORBAT UYGULAMALARININ HİNDİ ETİNDE GÖRÜLEN PEMBELEŞME PROBLEMİ ÜZERİNE ETKİLERİ

Birol KILIÇ[1], Orhan Onur AŞKIN
Süleyman Demirel Üniversitesi, Ziraat Fakültesi, Gıda Mühendisliği Bölümü, Isparta

ABSTRACT: In this study, the effect of ammonia exposure and contamination, sodium erythorbate treatment and exposure to room atmosphere for possible bacterial contamination on pink color development in uncured cooked turkey meat were investigated. Turkey deli breast was used as the model system. Color, pH, oxidation reduction potential (ORP), oxymyoglobin and cytochrome c levels were measured in raw, tumbled, cooked and stored turkey deli breast meat samples. It was shown that ammonia exposure and contamination, and addition of sodium erythorbate resulted in pink color development in final product ($p<0.05$). However, exposure to room temperature for possible bacterial contamination did not have any significant effect on development of pink color.
Key words: Pink color defect, ammonia, sodium erythorbate

ÖZET: Yapılan araştırmada, amonyak gazı uygulaması, sıvı amonyak kontaminasyonu, sodyum eritorbat kullanımı ve çiğ etin oda sıcaklığında bekletilme işleminin kür edilmemiş pişmiş hindi etinde pembeleşme problemi üzerine etkileri araştırılmıştır. Hindi göğüs eti model sistem olarak kullanılmıştır. Çiğ, marine edilmiş, pişmiş ve depolanmış hindi göğüs eti örneklerinde, renk, pH, oksidasyon redüksiyon potansiyeli (ORP), oksimyoglobin and sitokrom c seviyeleri ölçülmüştür. Amonyak gazı uygulaması, sıvı amonyak kontaminasyonu ve sodyum eritorbat uygulaması son üründe pembeleşmeye neden olmuştur ($p<0,05$). Fakat oda sıcaklığında bekletme işleminin pembeleşme problemi üzerine bir etkisi görülmemiştir.
Anahtar Kelimeler: Pembe renk kusuru, amonyak, sodyum eritorbat.

INTRODUCTION

The color of meat is affected by various interrelated factors such as the content of hemepigments (myoglobin, hemoglobin and cytochrome c), oxidative influences on pigments, reactions of the pigments with gaseous elements or compounds, and the structural properties of muscle proteins (1). However, the concentrations and the chemical state of the pigment are the most important factors for meat color development. The major pigment found in poultry meat is myoglobin and the amount of the myoglobin in meat is related with animal type, age, sex, and type of muscle within a carcass (2).

Consumers evaluate many characteristics when making decisions to purchase meat products. Color of the product is one of the most important factors in acceptance and sale of meat products. When color is different from expected, consumers would prefer not to purchase the meat product. Uncured poultry products do not contain added nitrite which is used for *Clostridium botulinum* control and development of traditional pink color in cured products. Therefore, pink color evolvement in traditionally white uncured poultry products is an undesirable characteristic (3). The poultry industry has had incidents where well-cooked, uncured poultry products possessing a pink color result in product rejection. It has especially been a problem in turkey breast meat products. Even though the products may have been fully cooked, many consumers, retailers and brokers

[1] E-posta: birol@ziraat.edu.tr

believe that the products are undercooked or contaminated. Even though it is a visual problem, it causes product rejection as well as loss of consumer confidence in the quality of this food supply. Eventually, this can create economic problems for producers and processors.

A number of probable causes for the pink color defect have been suggested, including nitrite and nitrate contamination in feeds, drinking or chilling water (4), exhaust fumes during transportation of birds prior to slaughter (5), carbon monoxide and nitric oxide generated by oven fumes, poorly ventilated gas ovens (6), low end-point cooking temperatures (7), concentrations and oxidation states of pigments (2), and irradiation (8).

It was reported that small amounts (e.g. 10 ppm) of nitrite present in poultry meat may form and produce pink color problems in processed poultry meat (4). In addition, chill water containing as little as 0.7 ppm nitrite can produce a pink color reaction in cooked meat (9). It was observed that the concentrations of nitrate and nitrite normally found in turkey breast meat was very low and not enough to produce a color problem (10).

Pool (6) suggested that substances in the oven gases can interact during the cooking period and produce a pink color. Cornforth (11) reported that while pinking was seen on turkey rolls exposed to as little as 0.4 ppm nitrogen dioxide, carbon monoxide and nitric oxide did not cause pink color development.

End-point cooking temperature has an important role in controlling the meat color. Redness values increase when end-point temperatures are below 71 °C (7). Time and temperature were found to be factors in determining the amount of undenatured pigment present in the cooked meat (4). It was found that the brown cooked pigment could be partially reversible and develop red color at refrigerated temperature storage (12). The pink color due to refrigerated storage can fade quickly if meat is exposed to either light or oxygen (13). Short-time refrigerated storage decreased redness whereas short-time frozen storage increased redness (14). It was found that the amount of pigment denaturation by temperature was increased by lowering the pH. The addition of phosphates reduced the amount of denaturation due to pH increase (15). The addition of sodium chloride increased the heat denaturation rate of myoglobin. The reverse effect of sodium chloride and phosphates were observed with cytochrome c (16). Spray-dried albumin was found to increase meat pinkness more than pan-dried albumin (17). It was suggested that diethylenetriamine pentaacetic acid, ethylenedinitrilo-tetraacetic acid disodium salt, trans 1,2-diaminocyclohexane-N,N,N',N' tetraacetic acid monohydrate, and calcium reduced nonfat dried milk provided reduction in pink color development with presence of sodium nitrite and especially nicotinamide (18). It was reported that added myoglobin (0 and 0.4 mg/g of meat) and cytochrome c (0 and 0.1 mg/g of meat) increased pinkness but cytochrome c was much more effective than myoglobin (19). Undenatured myoglobin and oxymyoglobin may be present in sufficient concentration to cause red color in meats cooked to 71°C, if pH is greater than 6.0 (20, 21). It was reported that cytochrome c had a much higher denaturation temperature than myoglobin (22). Therefore, cytochrome c is still quite active at normal heat processing conditions and may be responsible for pink color formation in cooked poultry meat.

This study was intended to understand and solve the industry-wide sporadic pink color defect in uncured cooked poultry products, and explore the chemistry existing behind it to bring consumers confidence back to these food supplies, which will translate into fewer product refusals and greater profitability. Ammonia is naturally present in live animal production housing, refrigeration systems, and sanitation supplies and can be produced by common bacteria. Addition of reductants such as sodium erythorbate in manufacturing meat products is also common application. Therefore, the purpose of this study was to investigate the effect of ammonia contamination and exposure, sodium erythorbate and exposure to room atmosphere for possible bacterial contamination on the formation of pink color. Turkey breast meat was used as the model to investigate pink color defect reactions possibly caused by a variety of ingredients, chemical reactions, and processing practices.

MATERIALS AND METHODS

Raw material measurements

Fresh turkey breast meat bought from commercial poultry processors was used to prepare oven-prepared turkey deli breasts. Oxidation reduction potential (ORP), pH, and color of raw meat were measured initially.

Brine pH measurement also was taken prior to injection. The pH values were determined using an platinum redox electrode (Cole Parmer, Chicago, IL 60648). For enstrumental color, CIE L* (Lightness), a* (Redness), and b*(Yellowness) values were measured on the surface of freshly-sliced breast meat with a Minolta Chroma Meter (Model CR-200, Minolta Corp., Ramsey, NJ 07446). ORP were determined using a spear tip electrode.

Preperation of turkey deli breast and analysis

The deli products were fabricated using three portions of meat totaling 1000 g very similar to turkey breast delis prepared by the poultry industry. To prepare brine solution, sodium chloride (16.5 g) and phosphate (5.5 g) were disolved in 10% of added water. Freshly prepared brine mixture was injected into the meat using injection syringe. The meat and brine mixture was placed into 17 x 17 x 21 cm Servin Saver containers (Rubbermaid, Inc., Wooster, OH 44691), which have two 1 x 5 x 20 cm plastic baffles increasing tumbling action. Each container lid had a 1 cm hole in the center to provide evacuation from the containers. After closing the lids, the containers were sealed with duct tape to prevent the lid from coming off during tumbling. The individual Servin Saver containers were placed horizontally into a large Model AR–500 vacuum tumbler (Miltona Brand Meat Machinery, Miltona, MN 56354) and held in cooler with 4 °C. The turkey breast was tumbled for protein extraction and brine uptake using a schedule of 30 min tumbling and 30 min stationary for 4 h. After tumbling, meat pH was measured. Then, turkey breast meat was placed into Cryovac oven-prepared cook-in barrier bags (W.R.Grace&Co, Duncan, SC 29334). Thermal processing was carried out in a ched Mini-Smoker (Model 450-UA, ched, Lodi, WI 53555). A step-up cooking schedule was followed as given in Table 1.

Table 1. Step-up cook schedule for turkey breast

Dry Bulb Temperature (°C)	Cooking Time (Min)
54	60
66	60
77	60
82	until 71°C internal

Turkey deli breast products were cooked at an internal temperature of 71 °C. The loaves were placed in a 4 °C cooler for overnight. The deli products were then analyzed for pH, ORP, pigment and color. The breasts were removed from the bags and the first half of each product was vacuum packed and stored in a cooler for 8 days. The second half of each products was placed into an inert atmosphere (helium) glove bag. While in the glove bag, a fresh cut was made into the interior of the deli breast. Five color measurements for L^*, a^*, and b^* values were taken on the newly exposed surface by using the Minolta Chroma Meter and the average of these data was recorded. ORP measurements were recorded in 10 min following color measurements after placing the spear tip into meat. Pigment analysis in cooked turkey deli breast was carried out to determine oxymyoglobin and cytochrome c contents of cooked meat. A modified method of Warriss (23) for measuring meat pigments was used. Meat samples were minced into 3 mm cubes. Five grams of each sample was placed in 50 ml polypropylene centrifuge tubes containing 25 ml ice cold phosphate buffer (pH 6.8, 0.04 M), and homogenized for 40–45 sec at low speed using the small diameter head of a polytron homogenizer. After holding samples for 1 h at 0 °C (on ice), the samples were centrifuged at 9000 RPM for 45 min. Supernatant was filtered through Whatman no. 1 filter paper. Absorbance values of the filtrate were taken between 400 and

700 nanometer using phosphate buffer as a blank. To determine presence of cytochrome c content, 30 mg of sodium dithionite was added to 3 ml extract in a cuvette, and mixed gently 10 sec prior to reading absorbance values between 400 and 700 nanometers (24).

Effects of ammonia, reductant, and exposure to room temperature for possible bacterial contamination on pink color development were investigated. This experiment was consisted of seven groups including reductant (sodium erythorbate) (SE), liquid ammonia contamination before (ABC) and after (AAC) cooking, ammonia exposure before (AEBC) and after (AEAC) cooking, exposure to room temperature (BC), and control. For liquid ammonia contamination before cooking, the turkey breast meat was soaked in 400 ml of ammonium hydroxide in a sealed container for 30 min to allow sufficient penetration of ammonium hydroxide into meat. Ingredients used were the same as described above. For liquid ammonia contamination after cooking, meat loaf was removed from package after cooking and soaked into 400 ml ammonium hydroxide for 30 min in a sealed container, and vacuum packaged a second time. To evaluate the reductant group effect, additional 550 ppm sodium erythorbate was added to brine. To permit bacterial contamination, raw turkey breast meat was removed from the package, and exposed to room temperature for 24 h. For ammonium exposure before cooking, turkey breast meat was placed in desicator which has a beaker containing 20 ml ammonium hydroxide. Meat was held for 30 min with ammonium hydroxide and were fabricated with the other groups in the same way as the described in the procedure for preparing turkey deli breast formulation. For ammonia exposure after cooking, meat loaf was removed from package after cooking and exposed with ammonia for 30 min in desicator which had a beaker containing 20 ml ammonium hydroxide. ORP, pH, color and meat pigment measurements were taken as described above following overnight storage at 4 °C. These measurements were repeated following 8 days storage in a 4 °C cooler.

Statistical analysis

Mixed model procedure of analysis of variance of SAS (25) was applied to analyse data from 3 replications. Mean values were separated using the least significant difference procedure.

RESULTS AND DISCUSSION

The redness (a*) values of turkey breast meats are given in Table 2. Redness values of treatments ranged from 4.4 to 10.8. As storage time advanced, there was an increase in a* values for each group ($p<0.05$). This resulted in increased pinkness in final product. At the end of the 8 days of storage, ABC had the highest a* values followed by AEBC, AAC, SE, AEAC, BC, and control, respectively. Increase in a* values can be a result of combined effect of changed pH and ORP due to various treatments.

The pH values (Table 3) were approximately 6.0 in all raw turkey breast meat. Ammonia contamination before cooking (ABC) gave rise to the greatest pH increase ($p<0.05$). AAC, AEBC and AEAC also had a significant impact on increased pH values of turkey deli product ($p<0.05$). Meat pH gradually increased during storage (SE, ABC, AEBC) ($p>0.05$) excluding ammonia exposure after cooking (AEAC) which increased relatively faster ($p<0.05$). We observed that significantly increased pH favored the formation of a pink color.

Very low ORP (Table 4) was provided by ammonia contamination ($p<0.05$) in cooked breast meat. Lower ORP values were collected for every treatment after 8 days of storage. Low ORP favors pink color formation because in low ORP, all the heme iron is in the ferrous state which can form pink hemochromes. It has been also reported earlier that the pink defect was promoted by reducing conditions and prevented by oxidizing conditions (26).

Pigment analysis indicated that oxymyoglobin was the dominant meat pigment in each treatment (Table 5). Because of reducing conditions higher oxymyoglobin level was observed for SE compared the other groups. Absorption spectra showed that ammonia created different peak than oxymyoglobin which is indicative of various hemochromes of myoglobin or hemoglobin. A higher cytochrome c level was observed in ammonia contaminated or exposed groups except AEAC group which had lower pH compare to ABC, AAC, and AEBC.

Table 2. Instrumental color values of oven-cooked turkey breast with different treatment

	CIE values (Cooked)			CIE values (8 days storage)		
Treatment	L^*	a^*	b^*	L^*	a^*	b^*
Control	84.73±0.86^a	4.37±0.47^a	11.30±0.51^a	87.53±0.71^a	4.40±0.36^a	10.49±0.35^a
SE	85.52±0.74^a	5.16±0.39^b	9.49±0.47^b	80.99±0.43^b	7.72±0.34^b	8.18±0.44^b
ABC	81.43±0.55^b	9.82±0.32^c	8.73±0.33^c	83.40±0.65^c	10.84±0.76^c	7.15±0.47^c
AAC	80.52±0.58^b	5.47±0.29^b	9.75±0.49^b	80.30±0.22^b	8.47±0.05^{bd}	6.99±0.51^c
AEBC	80.39±0.19^b	7.50±0.17^d	8.85±0.33^c	84.12±0.43^c	8.70±0.72^d	5.93±0.55^d
AEAC	85.24±0.17^a	4.52±0.28^{ab}	11.30±0.61^a	86.69±0.39^a	6.67±0.28^e	9.86±0.27^a
BC	80.92±0.42^b	4.63±0.5^{ab}	10.29±0.47^b	82.26±0.41^{bc}	5.18±0.13^a	8.93±0.43^{ab}

Means with the same letter within a column are not significantly different ($p<0.05$).

Table 3. pH values of raw, tumbled, cooked and stored turkey breast meat samples

Treatment	Raw	Tumbled	Cooked	8 days storage
Control	6.0±0.12^a	6.0±0.06^a	6.5 ±0.03^a	6.5±0.08^a
SE	6.0±0.12^a	6.0±0.12^a	6.4±0.19^a	6.5±0.28^a
ABC	5.9±0.14^a	9.2 ±2.07^b	10.4 ±0.26^b	10.7±0.02^b
AAC	6.0±0.1^a	6.1±0.05^a	10.1±0.15^c	10.1±0.21^c
AEBC	5.9±0.04^a	6.0±0.07^a	8.2±0.08^d	8.3±0.14^d
AEAC	5.9±0.02^a	6.0±0.07^a	6.3±0.16^a	7.3±0.13^e
BC	5.9±0.02^a	6.0±0.05^a	6.3±0.03^a	6.3±0.03^a

Means with the same letter within a column are not significantly different ($p<0.05$).

This can be explained by elevated pH in ammonia treated samples resulting lower denaturation of meat pigments. Higher oxymyoglobin and cytochrome c levels favored pink color defect in final product.

It was observed that using sodium erythorbate in the formulation increased pink color development in uncured cooked turkey delies (Table 2) and slightly increased the raw meat pH (Table 3) ($p<0.05$) . The possible reason is that sodium erythorbate produce reducing conditions enhancing the pink color development, and under coditions, all the ferric heme iron can be converted to the ferrous form, which is the most effective iron to form the pink color (26). Because of this effect, the reducing agents can convert brown metmyoglobin to red myoglobin gradually with refrigerated storage.

Liquid ammonia contamination prior to cooking has resulted in a very pink color in final product (highest a^* values) ($p<0.05$). This color development would be due to high pH and reducing conditions provided by ammonia. At high pH, the iron of heme is predominantly in the ferrous state which is active in production of

Table 4. Oxqidation - reduction potential (ORP) of cooked turkey breast meat samples

	ORP(mV)		
Treatment	Raw	Cooked	8 days storage
Control	90±5 [a]	–14.9±14.2 [a]	–26.7±15.3 [a]
SE	89±6 [a]	–56±8.1 [b]	–79.3±5.8 [b]
ABC	86.7±7.6 [a]	–319.7±24.8 [c]	–334.3±27.7 [c]
AAC	93.7±1.5 [b]	–196±11.5 [d]	–185.7±5.5 [d]
AEBC	81.3±7.7 [a]	–85±11.5 [c]	–97.3±7.5 [c]
AEAC	77.3±2.3 [c]	–61.3±6.7 [b]	–82.7±7.5 [b]
BC	80.7±3.1 [a]	–24±6 [a]	–31,.7±5.5 [a]

Means with the same letter within a column are not significantly different (p<0.05).

Table 5. Oxymoglobin and cytochrome C levels of oven-cooked turkey breast.

	Cooked		8 days storage	
Treatment	OxyMb (gr/ml)	Cyt (µr/ml)	OxyMb(gr/ml)	Cyt(µr/ml)
Control	7.9±0.63 [a]	2.7±0.5 [a]	7.5±1.96 [a]	2.6±0.95 [a]
SE	9.9±0.36 [b]	4.3±0.48 [b]	11.7±0.9 [b]	4.1±0.95 [b]
ABC	ND	7.7±0.37 [c]	ND	6.6±0.68 [c]
AAC	ND	5.3±1.37 [d]	ND	6.6±0.68 [c]
AEBC	ND	5.3±1.37 [d]	ND	6.5±1.86 [c]
AEAC	ND	3.1±0.42 [a]	ND	3.1±0.65 [a]
BC	7.2±0.18 [a]	3.1±0.2 [a]	6.9±0.55 [a]	3.2±0.15 [a]

Means with the same letter within a column are not significantly different (p<0.05).
OxyMb: Oxymyoglobin, Cyt: cytochrome c, ND: not determined.

pink color, and low pH accelerates ferrous iron conversion to the ferric state (16). In addition, high pH (higher than 6.0) stabilizes myoglobin to heat, prevent myoglobin denaturation, leading to red color after cooking (20). However; ammonia contamination after cooking resulted in slight pink color development on the surface of the product. Pink color was stronger after 8 days of storage and was uniformly present in the whole product (Table 2) (p<0.05). Color, ORP, pH, and pigment values of samples with ammonia contamination after cooking were lower than those of ammonia contamination before cooking (Table 2,3,4,5). This can be explained by decreased penetration of ammonia into meat and increased pigment denaturation due to cooking application. A comparison of absorpsion spectra has clearly indicated that the ammonia derived color is not a nitrosohemochrome (cooked cured) nor residual undenurated oxymyoglobin. Ammonia may produce the

spesific hemochrome of myoglobin or hemoglobin. It is important to identify this specific pigment formed by ammonia.

It was shown that ammonia exposure before cooking was another important factor which affect pink color formation. Samples exposed to ammonia before cooking increased pH and a* values, decreased ORP values significantly ($p<0.05$). Pink color development was also created by ammonia exposure after cooking ($p<0.05$), however, this effect was not as strong as that of ammonia exposure before cooking.

The results of this study showed no indication of pink color development as a result of exposing the raw meat to room temperature openly for 24 h time period for possible bacterial contamination and oxidation.

CONCLUSIONS

The results of this study showed that ammonium contamination and exposure before and after cooking and addition of sodium erythorbate contributed pink color development in uncured cooked turkey deli breast. On the contrary, exposure to room atmosphere for possible bacterial contamination did not show this effect.

A number of research was conducted to understand and solve the pink color development in uncured cooked poultry products, and some of the suggestions such as controlling nitrate and nitrite contamination has provided some improvements. However, because of the complicated nature of the problem, the industry-wide sporadic pink color defect in uncured cooked poultry meats still exists as an economical problem for the poultry industry. To solve this problem, as this research intends, all the factors reported (over and undercooking, nitrate and nitrite contamination, pH etc.) or suspected (packaging environments, cytochrome c, and ORP etc.) which might contribute pink color development should be identified. According to our results, new processing and handling practices should be employed into poultry meat industry.

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