The Effects of Butylated Hydroxyanisole, Ascorbic Acid, and α-Tocopherol on Some Quality Characteristics of Mechanically Deboned Chicken Patty during Freeze Storage

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Abstract

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In this study, the effects were evaluated of butylated hydroxyanisole (BHA), ascorbic acid (AA) and α-tocopherol (TO) on the stability of raw mechanically deboned chicken patties stored at –20°C for 6 months. pH, thiobarbituric acid reactive substance (TBARS), haem iron (mg/kg), metmyoglobin formation (%) and colour (*L**, *a**, *b**, *C** and *h* values) of patties were measured for 0, 2, 4, and 6 months of storage time. pH values were found to be the highest in the initial storage period. TBARS values were observed to range between 0.33 and 2.40 mg malondialdehyde/kg of sample and the *L**, *a**, and *b** values of the patty samples during the storage period were found to range between 38.14 and 49.52, 9.01 and 20.87, and 7.28 and 14.62, respectively. The haem iron and metmyoglobin contents were found to range between 8.39 and 10.87 mg/kg and 19.26% and 45.91%, respectively. As a result, it is suggested that *L*-ascorbic acid and α-tocopherol can be added into chicken patty samples in view of the storage quality parameters mentioned above.

Keywords: mechanically deboned chicken meat; patty; antioxidant; metmyoglobin; haem iron; tocopherol; ascorbic acid

Meat and meat products are usually marketed in small butcher shops or in the minced form in Turkey, and most people prefer to consume meat and meat products in the minced form. Therefore, many meat products, such as patties and kebabs prepared from minced meat, are consumed in Turkey (Ulu 2006). Freezing is an excellent process for preserving the quality of meat and fish and of the products of them for long periods. However, deterioration of quality caused by chemical or physical factors can occur. Many studies have shown that lipid oxidation is one of the primary causes of quality losses of frozen stored meat and meat products (Gökalp *et al.* 1983). The colour alters as the pigments oxidise and the flavour and aroma also change as a result of the accumulation of secondary volatiles. Biologically active compounds can be destroyed and, in some cases, toxic and carcinogenic substances represented by hydroperoxides, radicals, epoxides, aldehydes, acids, etc. accumulate (Balev *et al.* 2005). The consumption of poultry meat and poultry meat products is currently growing and the increased production of cut up and processed meat provides considerable quantities of parts suitable for mechanical deboning. Mechanically deboned

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chicken meat (MDCM) has higher haem and fat contents than does hand deboned chicken meat (HDCM) (Froning 1976). During freeze storage of mechanically deboned turkey meat, extensive autooxidation occurs resulting in a decreased functionality of the meat (Mielnik *et al*. 2003). However, the storage life of mechanically deboned meat can be extended with antioxidants (Barbut *et al*. 1985). The most important process in the oxidation of lipids in meat is the peroxidation of polyunsaturated fatty acids from cell membranes (Keller & Kinsella 1973). The major catalysts are transitional metal ions, such as Fe^{2+} and Cu^{+}. Haem compounds of meat can also contribute to this process due to the participation of haem iron in accelerating lipid peroxidation (Decker & Welch 1990; Pikul 1992).

In industrial processing, mainly synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate are used to prolong the storage stability of food. However, the demand for natural antioxidants has recently increased because of the toxicity and carcinogenicity of synthetic antioxidants (Juntachote *et al*. 2006). In addition, synthetic antioxidants have limited applications because of their low water solubility (Bekhit *et al*. 2003). BHA, however, has a high solubility in animal fats (Allen & Hamilton 1994; Coppen 1994).

In meat, the functions of ascorbic acid include maintaining the cured colour and preventing the off-flavour (Bauernfiend 1985). The antioxidation mechanism of ascorbic acid is to activate the primary antioxidants, to be an oxygen scavenger, and to inactivate prooxidants (Bauernfiend & Pinkert 1970). At low concentrations, ascorbic acid promotes lipid oxidation; however, at high concentrations it inhibits lipid oxidation (Decker & Xu 1998). α-Tocopherol is also a significant antioxidant and it can decrease lipid oxidation and colour changes in meat products and animal fats (Morrisey *et al*. 1994). The positive effect of α-tocopherol in the protection of precooked meats against off-flavour is generally acknowledged (Bertelsen *et al*. 2000).

The colour of meat changes from red to brown due to the oxidation of oxymyoglobin to metmyoglobin. The relationship between lipid oxidation and pigment oxidation is significant (Kanner & Harel 1985). Free radicals produced by lipid oxidation can initiate the reaction of oxidising oxymyoglobin to metmyoglobin. Similarly, hydrogen peroxide activates metmyoglobin to form ferrylmyoglobin radicals which catalyse lipid oxidation in muscle foods (Kanner *et al*. 1987).

BHA, ascorbic acid, and α-tocopherol have been investigated for their antioxidant activities in many studies, but no data are available on their effects on the colour, metmyoglobin formation, and haem iron content of raw mechanically deboned chicken patties during freeze storage. The objective of this research was to determine the effects of various antioxidants on some quality characteristics of raw mechanically deboned chicken patties during freeze storage conditions.

MATERIALS AND METHODS

Materials. Mechanically deboned chicken meat (moisture 70.30%, protein 19.43%, and fat 9.02%) used in the production of patties was obtained from BEYPI Co. (Bolu, Turkey). The fresh meat was transported to the laboratory as 9 kg blocks under refrigeration. All the chemicals and reagents used for the study were of analytical grade and were procured from Sigma Chemical Co. (St. Louis, USA).

Preparation of patties. Each patty group sample was prepared so as to include 88.50% mechanically deboned chicken meat, 10% rusk flour, and 1.5% salt. Antioxidant material and salt were added into the patty dough as a mixture to achieve homogeneity. The mechanically deboned chicken meat samples used in the production of patties were separated into four groups and the antioxidants were added into these groups to prepare the patty dough batches as: (1) Control (no added antioxidant), (2) 200 mg/kg BHA, (3) 300 mg/kg L-ascorbic acid, and (4) 300 mg/kg α-tocopherol. Synthetic antioxidant (BHA) was incorporated at the permitted level (200 mg/kg). Each natural antioxidant was used at a medium level (300 mg/kg). Each antioxidant was mixed with salt (1.5%) and 20 ml water and then added to each batch of 1.6 kg of mechanically deboned chicken meat (MDCM). The patty batches prepared were kneaded by hand until achieving homogeneity and then a total of 240 patties of uniform weight (about 40 g) were formed with a diameter of 4 cm and thickness of 1.1 cm. The prepared experimental raw patty samples were placed on styrofoam trays. The patty samples on trays were aerobically packaged in sealed polyethylene pouches (individually). These packed patty samples were stored raw in a deep

freeze (Ugur 300 BK Deep-freezer, Nazilli-Aydin, Turkey) for 6 months at –20°C except for the samples allocated to the day zero analysis. One of the packs was opened for subsequent analysis for each formulation every two months of the freeze storage. Moisture, protein, and fat content analyses were performed in the first step. pH, TBARS, colour, haem iron, and metmyoglobin analyses were also performed with the frozen stored patty samples at 0, 2, 4, and 6 months. The periodical sampling for the analyses of the frozen samples was carried out at 2 month intervals. The frozen samples were thawed overnight at 4 ± 1°C prior to analysis. All analyses were performed twice with three replicates each.

Proximate analyses and pH. Moisture (hot air oven), protein (Kjeldahl, N × 6.25), and fat (ether-extractable) were determined according to AOAC Official Method (2000). For pH determination, the sample (10 g) was homogenised in 100 ml of distilled water for 1 min using a blender (Waring Commercial Blendor®, Springfield, USA). Then, pH was measured using a pH meter (pH 315i/SET WTW, Germany) (Ockerman 1985).

Lipid oxidation determination. TBARS values were expressed as mg malondialdehyde/kg sample and estimated colourimetrically using 2-thiobarbituric acid (Tarladgis *et al.* 1960) with a UV-visible spectrophotometer (Hitachi U-1800 Model, Japan). The mixture of 10 g of blended sample, 2.5 ml of concentrated HCl, and 97.5 ml of distilled water was distilled and 5 ml of the distillate was treated with 5 ml of TBA reagent. After boiling for 35 min, optical density was measured at 532 nm. The standard curve was prepared using 1.1.3.3-tetraethoxypropane (TEP). The TBARS values were calculated by multiplying the absorbance readings at 532 nm by a factor of 7.03 determined from the standard curve.

Metmyoglobin (%). The analysis of metmyoglobin content was performed as described by Krzywicki (1982). A lump of minced chicken raw meat (5 g) was placed into a 50 ml polypropylene centrifuge tube, and 25 ml of ice-cold phosphate buffer (pH 6.80, 40mM) was added into the tube. The mixture in the tube was homogenised for 10 s at 13.500 rpm with an Ultra-Turrax T25 (Janke & Kinkel, Staufen, Germany). The homogenised sample was allowed to stand at 4°C for 1 h and centrifuged at 5000 rpm for at 4°C using a centrifuge (Nuve, NF-800-R Model, Ankara, Turkey). The supernatant was filtered through Whatman #1 filter paper and the absorbance was read at 572, 565, 545, and 525 nm by scanning the visible spectrum with a spectrophotometer (Hitachi U-1800 Model, Tokyo, Japan). The percentage of metmyoglobin was calculated using the following equation (Krzywicki 1982):

$$\%\text{Metmyoglobin} = [-2.51(A_{572}/A_{525}) + 0.777(A_{565}/A_{525}) + 0.8(A_{545}/A_{525}) + 1.098] \times 100$$

Determination of haem iron content. Haem iron was determined by means of acidified acetone extraction followed by spectrophotometry (Clark *et al.* 1997). The minced chicken meatball sample (2 g) was transferred into a 50 ml centrifuge tube and 9 ml of acid acetone mixture (90% acetone, 8% deionised water, and 2% HCl) was added. The mixture was macerated using a glass rod and allowed to stand for 1 h at room temperature. The extract was centrifuged at 2200 g for 10 minutes. The supernatant was filtered through Whatman #42 filter paper, and the absorbance was read at 640 nm against the acid acetone blank. The total pigments were calculated as haematin using the following formula (Lee *et al.* 1999):

$$\text{Total pigment (mg/kg)} = A_{640} \times 680$$

and haem iron was calculated as follows (Clark *et al.* 1997):

$$\text{Haem iron (mg/kg)} = \text{total pigment (mg/kg)} \times 8.82/100$$

Colour measurements. Colour measurements were performed on chicken patty samples at room temperature (20 ± 2°C) using a chromameter CR-400 (Konica Minolta, Inc., Osaka, Japan) with illuminate D65, 2° observer, Diffuse/O mode, 8 mm aperture of the instrument for illumination and 8 mm for the measurement. The chromameter was standardised with a white ceramic tile [L^* = 98.11, a^* = –0.53 and b^* = 2.21] before the measurements. The L^*, a^* (redness) and b^* (yellowness) colour coordinates were determined according to the CIELab colour space system. The visual impression of colour is formed from hue-angle [$h = \tan^{-1}(b^*/a^*)$] and chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$]. For the colour measurements, American Meat Science Association guidelines were followed (Hunt *et al.* 1991). The average of three replicate measurements was used to calculate the hue-angle (h) which represents the relative position of colour between redness and yellowness and chroma (C^*)

which assesses the colour intensity. The colour stability was expressed as the rate of change (the slope of the fitted linear model) in L^*, h and C^*. Colour properties (L^*, a^* and b^* values) of the chicken patty samples were measured at 0, 2, 4, and 6 days of storage. The measurements were made directly upon the samples and carried out three times.

Statistical analysis. Each parameter was tested in the samples with three replications. The conventional statistical methods were used to calculate the means and standard deviations. The collected data was subjected to statistical analysis using Minitab Statistical Software, Release 14® for Windows (Pensilvania, USA). Multifactor analysis of variance (ANOVA) was used to evaluate the effects of the treatments (Control, 200 mg/kg BHA, 300 mg/kg L-ascorbic acid, and 300 mg/kg α-tocopherol) and storage time (0, 2, 4, and 6 months) as the main effects, and of all their interactions. The results of the statistical analyses are shown in the tables as the mean values and standard deviations. Also, the results of the interaction data are shown in the tables as the mean values and standard deviations. When a significant ($P < 0.01$; $P < 0.05$) main effect was found, the mean values were further analysed using Duncan's Multiple Range Test (MSTATC Vers. 4.00) (Snedecor & Cochran 1989).

RESULTS AND DISCUSSION

Proximate analyses and changes in pH

Chicken patties were found to contain approximately 61.58–63.29% of moisture, 19.37–20.71% of protein, and 7.79–8.28% of fat. The effects of the treatment and storage time on pH value of raw frozen mechanically deboned chicken patties are shown in Table 1. Both the antioxidant treatment and storage time had a statistically significant ($P < 0.01$) effect on pH values. Control samples had generally higher pH values than the other samples throughout the storage time. The sample containing AA had a lower pH value than the control sample and those containing BHA and α-tocopherol ($P < 0.01$). During storage, pH value of frozen raw chicken patties decreased until 2 months after storage, and then it began to increase (Table 1). Such an increase in pH is due to the accumulation of metabolites caused by bacterial action in meat and deamination of proteins (Jay 1996). McCarthy *et al.* (2001) and Biswas *et al.* (2004) reported similar findings in pork patties containing BHA/BHT antioxidants during refrigeration and freeze storage. An interaction ($P < 0.01$) between the treatment and the storage time was observed in relation with pH values as shown in Table 2. According to this, in the analysed frozen raw chicken

Table 1. The main effect of pH values, TBARS values, haem iron and metmyoglobin formations of raw chicken patties with different treatments during frozen storage

Main effect	pH	TBARS	Metmyoglobin (%)	Haem iron (mg/kg)
Treatment (A)	**	**	**	**
Control	6.25 ± 0.02^a	2.26 ± 2.16^a	33.57 ± 4.94^c	9.95 ± 0.54^a
BHA	6.19 ± 0.05^b	1.71 ± 1.22^b	33.78 ± 3.31^b	9.71 ± 0.72^a
AA	6.16 ± 0.05^d	0.45 ± 0.11^d	23.77 ± 3.68^d	9.81 ± 0.30^a
TO	6.17 ± 0.05^c	1.41 ± 0.94^c	34.49 ± 4.18^a	9.35 ± 0.57^b
Storage time (months) (B)	**	**	**	**
0	6.25 ± 0.01^a	0.33 ± 0.07^d	28.23 ± 6.19^d	10.01 ± 0.57^a
2	6.15 ± 0.05^d	0.84 ± 0.26^c	33.66 ± 5.27^a	10.09 ± 0.27^a
4	6.18 ± 0.06^c	2.25 ± 1.24^b	32.60 ± 6.88^b	9.13 ± 0.23^c
6	6.21 ± 0.04^b	2.40 ± 1.99^a	31.11 ± 4.13^c	9.59 ± 0.54^b
A × B interaction	**	**	**	**

$^{a–d}$means within a column with different letters are significantly different ($P < 0.01$); means based on six values ($n = 24$); control – no antioxidant added; BHA – 200 mg/kg butylated hydroxyanisole; AA – 300 mg/kg L-ascorbic acid; TO – 300 mg/kg α-tocopherol; TBARS – mg malondialdehyde/kg sample

Table 2. Effect of treatments and storage time on the pH values, TBARS values, metmyoglobin formations and haem iron contents of raw chicken patties with different treatments during frozen storage

Parameter	Month	Control	BHA	AA	TO
		M ± SD			
pH	0	6.25 ± 0.01^{a,A}	6.25 ± 0.01^{a,A}	6.23 ± 0.01^{a,B}	6.24 ± 0.01^{a,B}
	2	6.23 ± 0.01^{b,A}	6.13 ± 0.01^{c,B}	6.11 ± 0.01^{c,B}	6.11 ± 0.02^{d,B}
	4	6.26 ± 0.01^{a,A}	6.19 ± 0.01^{b,B}	6.11 ± 0.01^{c,D}	6.14 ± 0.01^{c,C}
	6	6.27 ± 0.01^{a,A}	6.21 ± 0.01^{b,B}	6.18 ± 0.01^{b,C}	6.18 ± 0.01^{b,C}
TBARS	0	0.386 ± 0.08^{c,A}	0.331 ± 0.04^{d,A}	0.368 ± 0.03^{c,A}	0.248 ± 0.02^{d,B}
	2	0.621 ± 0.02^{c,C}	1.245 ± 0.01^{c,A}	0.595 ± 0.01^{a,D}	0.913 ± 0.03^{c,B}
	4	2.374 ± 0.04^{b,B}	3.606 ± 0.12^{a,A}	0.326 ± 0.01^{d,C}	2.681 ± 0.04^{a,B}
	6	5.669 ± 0.02^{a,A}	1.638 ± 0.04^{b,C}	0.503 ± 0.02^{b,D}	1.800 ± 0.05^{b,B}
Metmyoglobin	0	26.42 ± 0.05^{d,C}	32.12 ± 0.04^{c,B}	19.26 ± 0.03^{d,D}	35.15 ± 0.08^{b,A}
	2	31.85 ± 0.06^{c,C}	36.04 ± 0.05^{b,B}	26.41 ± 0.04^{c,D}	40.36 ± 0.05^{a,A}
	4	37.93 ± 0.03^{b,A}	37.59 ± 0.05^{a,B}	21.32 ± 0.03^{b,D}	33.54 ± 0.10^{c,C}
	6	38.07 ± 0.04^{a,A}	29.37 ± 0.03^{d,B}	28.09 ± 0.03^{a,D}	28.92 ± 0.03^{d,C}
Haem iron	0	10.42 ± 0.38^{a,A}	9.96 ± 0.33^{a,AB}	9.82 ± 0.22^{b,B}	9.86 ± 0.42^{a,B}
	2	10.34 ± 0.20^{a,A}	10.33 ± 0.33^{a,A}	9.84 ± 0.24^{b,A}	9.56 ± 0.61^{a,AB}
	4	9.26 ± 0.35^{c,A}	9.03 ± 0.46^{b,AB}	9.40 ± 0.38^{c,A}	8.85 ± 0.26^{b,AB}
	6	9.80 ± 0.38^{b,B}	9.52 ± 0.39^{ab,B}	10.18 ± 0.23^{a,A}	8.85 ± 0.27^{b,C}

$^{a-d}$means within a column with different letters are significantly different ($P < 0.05$); means based on six values ($n = 6$); $^{A-D}$means within a row with different letters are significantly different ($P < 0.05$); control – no antioxidant added; BHA – 200 mg/kg butylated hydroxyanisole; AA – 300 mg/kg L-ascorbic acid; TO – 300 mg/kg α-tocopherol

patties, pH values decreased until 2 months after storage and then they began to increase (Table 2). Similar to our results, pH values of buffalo meat with the use of lactic acid, clove oil, and vitamin C during retail display were reported to decrease and then to increase (Naveena *et al*. 2006). During storage, the pH values of raw chicken patties initially decreased and then significantly ($P < 0.01$) increased with a few exceptions at the end of storage. The initial decrease might be attributed to the acid treatment, whereas the final increase in pH may be attributed to the microbial metabolites (Goddard *et al.* 1996). As stated by Gill (1983), bacteria on exhaustion of stored glucose utilise amino acids released during protein breakdown and, as a product of amino acid degradation, ammonia accumulates and pH rises.

Lipid oxidation

As shown in Table 1, the treatment and storage time had a statistically significant effect ($P < 0.01$) on TBARS values of raw frozen chicken patties. The lowest TBARS values occurred in the chicken patties with the addition of ascorbic acid (0.45 mg MDA/kg sample) ($P < 0.01$), while the highest TBARS values (2.26 mg MDA/kg sample) occurred with the control sample ($P < 0.01$). TBARS values were lower ($P < 0.01$) in all treated samples as compared to the control. The antioxidant effectiveness of AA was higher than that of the other antioxidants used in this research throughout the whole time of storage. According to these results, ascorbic acid exhibited the highest antioxidant effect compared to other treatments. The efficiency of various antioxidants inhibiting lipid oxidation throughout freeze storage was in the following order: ascorbic acid > tocopherol > BHA > control ($P < 0.01$). This data did not confirm the results of King *et al.* (1995) and Serdaroglu and Yildiz-Turp (2004), who found ascorbic acid inactive in preventing lipid oxidation on poultry at the end of the storage at –20°C for 6 weeks. When used in meat products at 0.025–0.05%, ascorbic acid and sodium ascorbate inhibited lipid oxidation

and preserved the desirable meat flavour (Rhee *et al.* 1997). Ascorbic acid and ascorbates influence lipid oxidation by reducing or interacting with metal ions (Kanner *et al.* 1977).

During freeze storage (–20°C), TBARS values of chicken patties increased steadily during 6 months of storage, but the increase rate was faster after 2 months of storage ($P < 0.01$) (Table 1). Similar to our results, Lai *et al.* (1991) reported that freeze storage increased malondialdehyde concentration in chicken patties.

The interaction ($P < 0.01$) between the treatment and storage time observed for TBARS values is shown in Table 2. According to the table, with all treatments occurred an increase in TBARS value in both the control samples and in those treated with antioxidants (except added ascorbic acid), this increase being the most rapid between 2 and 4 months of storage. The TBARS values of the samples increased at the beginning of storage and then began to decrease. According to Melton (1983), although malonaldehyde is a secondary product of lipid oxidation, this does not necessarily mean that the TBARS value continues to increase throughout the storage. These low TBARS values are thought to be the result of malondialdehyde reactions with proteins. During freeze storage, the chicken patties treated with ascorbic acid had significantly slower TBARS increases than the other groups. The highest concentration of malondialdehyde was observed to occur in control samples as expected. According to these results, out of the antioxidants studied, ascorbic acid was significantly more effective in inhibiting the formation of TBARS.

Formation of metmyoglobin

The formation of metmyoglobin in raw frozen chicken patties is shown in Table 1. There were significant ($P < 0.01$) differences between both the treatment groups and storage time. Metmyoglobin formation was time-dependent when chicken patties were stored for 6 months at –20°C. The samples treated with BHA, AA, and TO inhibited ($P < 0.01$) metmyoglobin formation for as long as the first 4 months of storage (Table 1). The sample treated with AA was more effective in the inhibition of metmyoglobin formation than those with the other antioxidants ($P < 0.01$). The reducing activity of ascorbic acid plays a role in maximising the muscle colour stability through metmyoglobin reduction (Lee *et al.* 1999).

Renerre *et al.* (1992) reported that the susceptibility of myoglobin to autoxidation is the main factor in explaining colour stability in meat and meat products. In the meat systems, the identities of the substances capable of oxidising OMb to MMb are not clear. Several authors concluded that O^{2-} can initiate lipid peroxidation, leading to the formation of prooxidant substances capable of reacting with OMb which results in MMb formation (Acton *et al.* 1993). They postulated that OMb can be oxidised not only by lipid-oxy radicals but also by other prooxidant radicals generated by O^{2-}. Several authors reported visually noticeable percentages of metmyoglobin or consumer rejection levels of metmyoglobin. Van Der Oord and Wesdorp (1971) reported the detection at 50% metmyoglobin. Harrison (1977) found that metmyoglobin was visible when it represented 30% to 40% of total pigment. Greene *et al.* (1971) indicated that consumer rejection occurred at 40% metmyoglobin.

Haem iron content

Muscle tissue contains a considerable amount of iron bound to proteins. Myoglobin is the most abundant hemoprotein in the muscle tissue. In our study, total iron content of mechanically deboned chicken meat was 11.15 mg/kg and haem iron content of chicken patties subjected to the treatments ranged from 9.35 mg/kg to 9.95 mg/kg wet weight, respectively, corresponding to 83.84–89.23% haem iron of total iron (data not shown). The haem iron content in raw frozen chicken patties is shown in Table 1. Significant ($P < 0.01$) differences occurred between both the treatment groups and storage time. During the first 4 months of storage, the haem iron content considerably decreased. Thereafter, haem iron content of raw chicken patties treated with antioxidants (except the control sample) increased ($P < 0.01$) as the storage time increased (Table 1). This might be due to the higher soluble haem pigment in fresh meat as caused by autolysis. This might contribute to the greater extractability of haem pigments. The decreased haem iron content observed with an extended storage time is presumably due to the release of free iron from haem. As a result, less haem iron was retained. Additionally, the lowered

haem pigment extractability with the increasing storage time also resulted in a lower iron content in the haem extracted. Our results are in agreement with those of Benjakul and Bauer (2001), and Gomez-Basauri and Regenstein (1992a, b) who reported that the decrease in haem iron was inversely related to haem iron content. Purchas *et al.* (2003) reported that the drip from meat released during storage contained significant quantities of iron and particularly soluble haem iron.

Colour

Colour is an important factor for consumer acceptance of meat and its products. The shelf life and quality of meat products are strongly influenced by the initial meat quality, additives, packaging parameters, and storage conditions. The treatment (except L^* and b^* values) and storage time had a statistically significant effect ($P < 0.01$) on the colour parameters of raw frozen chicken patties (Table 3). As seen in Table 3, all values except L^* and b^* ($P > 0.05$) were affected by the addition of antioxidants. The treatments showed significant effects on redness, hue-angle, and chroma ($P < 0.01$). The lowest values for parameter a^* were found in TO added patties ($P < 0.01$). No significant differences were found between the control samples and those with added BHA and AA ($P > 0.05$). The effects on chroma values of raw chicken patties were similar to the effects on a^* value. b^* values of the patties changed within narrow limits and the lowest value was noted for the control samples. b^* value of chicken patties was statistically insignificant ($P > 0.05$). The effects on hue-angle values of raw chicken patties showed a trend opposite to a^* and chroma values. Chroma values calculated in this study followed similar trends as a^* values in the respective treatments. This can be explained by the increased water retention associated with hygroscopic materials, and because these antioxidants were prepared as dry powder, they absorbed free water in the product, subsequently decreasing the lightness values. This relation between free water in and lightness of meat and meat products has been reported by several authors (Fernandez-Lopez *et al.* 2000; Pipek *et al*. 2008).

Freeze storage (–20°C) times, lightness (L^*), redness (a^*), yellowness (b^*), hue-angle, and chroma values were significantly different ($P < 0.01$) (Table 3). The lowest L^* value was found after 4 months of storage, while the highest L^* value was found after 6 months. Values L^* with chicken patties decreased steadily until 4 months of storage ($P <$

Table 3. The main effect of L^*, a^*, b^*, hue-angle (h) and chroma (C^*) of raw chicken patties with different treatments during frozen storage

Main effect	L^*	a^*	b^*	Hue-angle (h)	Chroma (C)
Treatment (A)	NS	**	NS	**	**
Control	44.27 ± 4.53	14.27 ± 4.60^a	10.76 ± 2.75	37.53 ± 8.27^b	18.03 ± 4.63^a
BHA	44.81 ± 3.33	13.69 ± 4.07^a	10.89 ± 2.90	38.63 ± 4.76^b	17.55 ± 4.79^a
AA	44.85 ± 4.19	14.30 ± 2.87^a	10.98 ± 2.66	37.33 ± 5.58^b	18.10 ± 3.51^a
TO	45.07 ± 3.84	12.25 ± 3.61^b	10.99 ± 2.62	42.19 ± 9.00^a	16.66 ± 3.64^b
Storage time (months) (B)	**	**	**	**	**
0	47.97 ± 0.93^a	19.45 ± 1.75^a	14.00 ± 0.75^a	35.83 ± 1.76^b	23.98 ± 1.76^a
2	42.25 ± 1.26^b	12.90 ± 1.90^b	9.22 ± 0.52^c	35.85 ± 3.93^b	15.89 ± 1.63^c
4	39.99 ± 1.62^c	11.18 ± 1.04^c	7.63 ± 0.55^d	34.42 ± 3.39^b	13.56 ± 0.87^d
6	48.79 ± 1.12^a	10.96 ± 1.79^c	12.78 ± 1.02^b	49.57 ± 5.44^a	16.91 ± 1.30^b
A × B interaction	**	**	**	**	**

$^{a–d}$means within a column with different letters are significantly different ($P < 0.01$); means based on six values ($n = 24$); control – no antioxidant added); BHA – 200 mg/kg butylated hydroxyanisole; AA – 300 mg/kg L-ascorbic acid; TO – 300 mg/kg α-tocopherol; NS – not significant ($P > 0.05$)

0.01) and then they began to increase ($P < 0.01$) (Table 2). The initial a^* value (19.45) decreased during storage, and reached 10.96 after 6 months of storage. The decrease of a^* value during storage is probably due to oxymyoglobin oxidation to metmyoglobin. Up to 4 months of storage, the hue-angle was stable, and then it increased.

The interaction ($P < 0.01$) between the treatment and storage time noted for the colour parameters (L^*, a^*, b^*, hue-angle and chroma) is shown in Table 4. In all samples, redness (a^*) decreased as the storage time progressed ($P < 0.01$). This is not surprising as the meat which has been stored for a longer time is expected to contain predominatly either OMB or MMb, as opposed to deoxymyoglobin (DMb), which in turn will predispose the meat to a faster browning rate (Hunt *et al.* 1999). At the end of storage (6 months), a^* values of all the samples were lower ($P < 0.01$) than those in month 0. Several authors studied the effects of different antioxidants on the colour of meat and meat products (Fernández-López *et al.* 2005) and reported that the meat oxidation decreases a^* values. Probably, this decrease in redness was associated with the effect of pH on the myoglobin proportion (Table 1). Trout (1989) reported that the effect of pH on the denatured myoglobin percentage was sufficient to produce obvious colour differences in cooked beef muscles. The lowest L^* value was found after 4 months, while the highest L^* value was found after 6 months of storage. Values L^* for chicken patties decreased steadily

Table 4. Effect of treatments and storage time on the lightness (L^*), redness (a^*), yellowness (b^*), hue-angle (h) and chroma (C^*) of raw chicken patties with different treatments during frozen storage

Parameter	Month	Control	BHA	AA	TO
		M ± SD			
L^*	0	$48.00 \pm 1.48^{a,A}$	$47.56 \pm 0.28^{a,A}$	$48.53 \pm 0.63^{a,A}$	$47.78 \pm 0.87^{b,A}$
	2	$42.28 \pm 1.22^{b,A}$	$43.09 \pm 1.49^{b,A}$	$41.90 \pm 1.30^{b,A}$	$41.77 \pm 0.80^{c,A}$
	4	$38.14 \pm 1.04^{c,B}$	$40.59 \pm 1.32^{c,A}$	$40.02 \pm 1.13^{b,AB}$	$41.20 \pm 1.26^{c,A}$
	6	$48.64 \pm 0.38^{a,A}$	$48.02 \pm 0.71^{a,A}$	$48.98 \pm 1.55^{a,A}$	$49.52 \pm 1.13^{a,A}$
a^*	0	$20.87 \pm 1.56^{a,A}$	$20.10 \pm 1.31^{a,AB}$	$18.89 \pm 0.48^{a,AB}$	$17.96 \pm 0.35^{a,B}$
	2	$14.39 \pm 1.54^{b,A}$	$11.60 \pm 1.29^{b,B}$	$13.76 \pm 0.73^{b,AB}$	$11.87 \pm 1.04^{b,AB}$
	4	$11.95 \pm 0.68^{bc,A}$	$10.70 \pm 0.99^{b,B}$	$11.92 \pm 0.54^{c,A}$	$10.16 \pm 0.61^{c,A}$
	6	$9.87 \pm 0.42^{c,B}$	$12.34 \pm 0.78^{b,A}$	$12.62 \pm 1.00^{bc,A}$	$9.02 \pm 1.16^{c,B}$
b^*	0	$14.28 \pm 0.55^{a,A}$	$14.62 \pm 0.73^{a,A}$	$13.89 \pm 0.51^{a,AB}$	$13.22 \pm 0.43^{a,B}$
	2	$9.17 \pm 0.76^{c,A}$	$9.38 \pm 0.41^{c,A}$	$9.22 \pm 0.50^{b,A}$	$9.12 \pm 0.45^{b,A}$
	4	$7.45 \pm 0.51^{d,A}$	$7.28 \pm 0.60^{d,A}$	$7.85 \pm 0.19^{c,A}$	$7.94 \pm 0.61^{c,A}$
	6	$12.15 \pm 0.61^{b,B}$	$12.30 \pm 0.39^{b,B}$	$12.97 \pm 1.26^{a,AB}$	$13.69 \pm 0.94^{a,A}$
h	0	$34.44 \pm 1.51^{b,A}$	$36.19 \pm 2.54^{bc,A}$	$36.33 \pm 0.98^{b,A}$	$36.36 \pm 1.24^{b,A}$
	2	$32.83 \pm 3.81^{b,B}$	$39.09 \pm 2.65^{b,A}$	$33.85 \pm 2.16^{b,AB}$	$37.64 \pm 3.57^{b,AB}$
	4	$31.94 \pm 2.14^{b,B}$	$34.28 \pm 2.92^{c,AB}$	$33.40 \pm 1.32^{b,B}$	$38.04 \pm 3.70^{b,A}$
	6	$50.89 \pm 1.78^{a,B}$	$44.95 \pm 2.11^{a,C}$	$45.74 \pm 4.07^{a,C}$	$56.71 \pm 2.34^{a,A}$
C^*	0	$25.29 \pm 1.52^{a,A}$	$24.87 \pm 2.19^{a,A}$	$23.45 \pm 0.58^{a,AB}$	$22.31 \pm 0.27^{a,A}$
	2	$17.09 \pm 2.40^{b,A}$	$14.93 \pm 1.17^{c,B}$	$16.57 \pm 0.64^{c,AB}$	$14.99 \pm 0.64^{c,B}$
	4	$14.09 \pm 0.66^{c,A}$	$12.95 \pm 0.95^{c,B}$	$14.27 \pm 0.47^{d,A}$	$12.92 \pm 0.23^{d,B}$
	6	$15.65 \pm 0.58^{bc,C}$	$17.44 \pm 0.58^{b,AB}$	$18.13 \pm 1.01^{b,A}$	$16.41 \pm 1.34^{b,BC}$

$^{a–d}$means within a column with different letters are significantly different ($P < 0.05$); means based on six values ($n = 6$); $^{A–C}$means within a row with different letters are significantly different ($P < 0.05$); control – no antioxidant sdded; BHA – 200 mg/kg butylated hydroxyanisole; AA – 300 mg/kg L-ascorbic acid; TO – 300 mg/kg α-tocopherol

until 4 months of storage ($P < 0.01$) and then they began to increase ($P < 0.01$) (Table 4). During storage, while the initial a^* values were higher after 6 months of storage they were lower. The decrease of a^* value during storage is probably due to oxymyoglobin oxidation to metmyoglobin. Up to 4 months of storage, the hue-angle was stable, and then it increased. In general, as the storage days number increased, a^* and b^* values decreased, the amount of discoloration increased, and the discoloration was darker. As the storage days number increased, the oxymyoglobin pigment form was shifted to metmyoglobin as lipid oxidation also increased. The iron in oxymyoglobin is in the reduced state (Fe^{2+}), but the iron in metmyoglobin is in the oxidised state (Fe^{3+}). The effectiveness of an antioxidant, in terms of colour stability, is determined by its ability to keep iron in the reduced state which results in a desirable colour. Once iron has been oxidised, it cannot be converted back to its reduced state, thus the shift from oxymyoglobin to metmyoglobin is permanent (Jenschke 2004).

CONCLUSIONS

Our results indicated that storage at –20°C for 6 months decelerated oxidation effectively. At the end of the storage time, the samples with added ascorbic acid had lowest TBARS values. Colour parameters changed significantly during the storage time. Ascorbic acid and α-tocopherol as antioxidants inhibited metmyoglobin formation and stabilised red meat colour when added to chicken patties. In addition, they inhibited lipid peroxidation and the degradation of haem pigments caused by storage. These actions of ascorbic acid and α-tocopherol showed that they may be useful as additives to chicken patties to maintain their stability and increase their shelf life.

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