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Evaluation of Some Natural Antioxidant Sources in Broiler Diets: 2-Effect on Chemical and Microbiological Quality of Chilled and Frozen Broiler Meat

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Abstract: This study was conducted to evaluate the efficiency of aqueous extract of ginger root (GAE), aqueous extract of beetroot (BAE) and tomato puree (TP) as natural antioxidant sources in broiler diets during summer season. Three hundred twenty 1-d-old Arbor Acres broiler chicks (mixed sex) were randomly allocated into 32 wire cages that were then randomly divided into 8 groups (treatments, 4 cages per treatment). Chicks fed on corn-soybean meal basal diet (Con, contained 50 IU of Vit. E/Kg) supplemented with either 50 IU /Kg vitamin E (E-100) or one of the additives (GAE, BAE and TP) at levels of 0.5 or 1.0% from 1 to 40 d of age. The total phenols contents (as Gallic acid equivalent) of GAE and BAE were 44 and 31 µg/L, respectively and lycopene content in TP was 155 mg/Kg. At 40 d of age, 32 birds (4 birds/treatment) were slaughtered and carcass characteristics were recorded. Refrigerated (up to 4 days at 4°C) and frozen (60 days at -20°C) meat samples were examined for total phenols content, antioxidant activity (through two scavenging assays: DPPH and TBARS) and microbiological status. The obtained results showed that neither antioxidant source nor level affected on dressing %, thickness of breast meat, triglycerides and cholesterol compounds of chilled breast meat. On the other side, source of antioxidant have affected strongly on oxidative stability especially after freezing for 60 d. Among examined sources, using BAE was less efficient in reducing oxidation rate than both GAE and TP. The microbiological examination showed strong effect of both tested sources and levels of natural antioxidant additives on decreasing count of total bacteria and *Staph. aureas* in refrigerated and frozen broiler meat. Using BAE failed to decrease count of *Staph. aureas* in frozen meat as GAE and TP. According to results of DPPH and TBARS assays and microbiological examination, adding GAE or TP to broiler diets could protect meat safe and healthy even with prolonged storage by freezing to 60d.

Key words: Broiler, antioxidant, ginger, beetroot, tomato, summer season, meat

INTRODUCTION

However synthetic antioxidants have been widely used as food and feed preservatives, because of their effectiveness to delay lipid oxidation with relatively low cost, there are increases of consumers' preferences for natural ingredients over synthetic compounds (Ahn *et al.*, 2002). This situation increased the demand of discovering the efficacy of different natural sources that can reduce lipid oxidation in poultry products. Some natural sources, rosemary, sage, ginger, grape seed and green tea, contain large amounts of antioxidant compounds, showed high antioxidant activities, decreased lipid peroxidation and extend the shelf life of broiler meat under different storage conditions (Botsoglou *et al.*, 2002; Rababah *et al.*, 2004; Jang *et al.*, 2007; Sahin *et al.*, 2010). Many studies showed that exposing broiler to high ambient temperature increase free radical formation and accelerate lipid peroxidation and that considered to be the major cause of quality deterioration in meat, meat products and egg yolk (Sahin and Kucuk, 2003; Sahin *et al.*, 2006;

Mujahed *et al.*, 2007). Khan *et al.* (2012) showed in a review that feed additives are an effective strategy to alleviate heat stress, improve poultry performance and decrease lipid peroxidation of poultry meat and egg yolk. Ginger (*Zingiber officinale*) is widely used in many countries as a food condiment and as a medical herb and showed strong antimicrobial, antioxidant and various pharmacological effects due to its content of phenylpropanoid-derived compounds including gingerols and shogaols (Kundu *et al.*, 2009; Rababah *et al.*, 2004; Sasidharan and Menon, 2010). Chrubasik *et al.* (2005) showed that the preparation procedures of ginger product affected its clinical efficacy. When ginger is dried gingerol undergoes a dehydration reaction forming shogaols, which are about twice as pungent as gingerol (Ling *et al.*, 2010). These facts increase of using ginger as natural effective feed additive in poultry diets (Janz *et al.*, 2007; Sudrashan *et al.*, 2010; Awadein *et al.*, 2012) to improve growth performance, enhance activities of antioxidant enzymes (enhance the scavenging capacity of free radicals) and reducing

malondialdehyde (MDA) as indicator of lipid peroxidation (Zhang *et al.*, 2009; Akbarian *et al.*, 2011; Sadeghi *et al.*, 2012).

Beet root was evaluated as one of the 10 most potent vegetables, whereas it showed high total phenol content and its extract showed strong antioxidant effects *in vitro* (Kahkonen *et al.*, 1999).

Beet root extract contains betanines or betalains compounds, vulgaxanthin I, vulgaxanthin II, indicaxanthin, betanin, prebetanin, isobetanin flavonoids and neobetanin, which are natural dye used in different food industries as natural antioxidant and antimicrobial food additive (Pedreno and Escribano, 2001; Kujala *et al.*, 2001; Zhong *et al.*, 2005). Although, some researcher reported that the bioavailability of betalains is at least as high as flavonoids, which are well-accepted as natural antioxidants (Romer *et al.*, 2010), using betalains as feed additive has been limited because some factors, such as temperature and pH, influence on the pigment stability (Socaciu, 2008). So betalains is exposed to degradation immediately after extraction and that degradation is accelerated by raising the pH, temperature and water (%) (Sturzoiu *et al.*, 2011). On the other side, Socaciu (2008) modified the extraction and storage condition of beet root aqueous extract to get high concentration and more stable betalains.

Tomato, tomato products and tomato by products are natural sources of antioxidant components like lycopene, ascorbic acid, phenolics, flavonoids potassium as well as some vitamin A and E (Abushita *et al.*, 1997; Agarwal and Rao, 1998, Campbell *et al.*, 2004; Assi and king, 2007). Lycopene is a potent antioxidant that provides protection against cellular damage caused by reactive oxygen species (Atasoy, 2012). So many researchers could retard lipid peroxidation in postmortem tissue during long-term frozen storage or heating of poultry meat as a result of increased alpha-tocopherol in broiler diets (King and Zeidler, 2003; Assi and king, 2007, 2008). Another group of researchers reported that Lycopene is the most important antioxidant component in tomato due to its high efficacy of free radical scavenging, retarding lipid peroxidation and lowering oxidative damage of DNA (Stacewicz-Sapuntzakis and Bowen, 2005; Sahin *et al.*, 2008). Agarwal *et al.* (2001) and Capanoglu *et al.* (2008) reported increased availability of lycopene in tomato products which exposed to relatively high temperature during preparation process of tomato products such as paste and puree than fresh tomato. Shi and Maguer (2000) explained that increased availability of lycopene in processed tomato products by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix.

So this study aimed to study the effect of inclusion of three natural antioxidant sources, ginger root aqueous extract (GAE), beet root aqueous extract (BAE) and

tomato puree (TP) in broiler diets during summer season on the quality of chilled and frozen broiler meat.

MATERIALS AND METHODS

Three hundred and twenty 1-d old unsexed Arbor Acres broiler chicks were individually weighed and divided into 8 treatments of 4 replicates each (10 chicks each). Chicks fed on basal diets based on corn-soybean meal during starting (1-10 d), growing (11-24 d) and finishing (25-40 d) periods. Basal diets were formulated to be 3100 kcal of ME/kg and 23% CP, 3110 kcal of ME/kg and 21% CP and 3200 kcal of ME/kg and 19% CP and contained whole strain requirement from the rest of macro and micro nutrients during starting, growing and finishing periods. There were two control groups the first fed on basal diet (Con) which contained 50 IU of vitamin E/kg of diet (the strain requirement of vit. E) and supplemented with either 50 IU /kg vit. E as alpha-tocopherol acetate (E-100, to be the second control group) or one of the natural antioxidant additives (GAE, BAE and TP) at levels of 0.5 or 1.0% from 1 to 40 d of age (Table 1). All birds were kept under similar management conditions. Criteria of growth performance were recorded during the experimental period and the environmental temperature and humidity surrounding birds were recorded daily during the experimental period and were ranged between 36-41°C and 20-55%, respectively.

Antioxidant sources: Aqueous extract of ginger and beetroot were prepared in Poultry Nutrition Department Labs, APRI, before starting the growth trail while pasteurized TP was obtained from local commercial company of tomato products.

GAE: It prepared, to reach the maximum free radical scavenging activity (94.4%) and 0.94 protecting factor, according to Kishk and El Sheshetawy (2010), then filtered and was frozen to -20°C until it was used. Total phenols content of the prepared GAE was determined using Folin-Ciocalteu (FC) assay according to Wright *et al.* (2000) and Atoui *et al.* (2005) and was 44 µg/mL as Gallic acid equivalent.

BAE: It was prepared from fresh beetroot and kept in acidic media [ascorbic acid (1g/L)+citric acid (2g/L)] according to Sturzoiu *et al.* (2011) then filtered and was frozen to -20°C until it was used. Total phenols contents

Table 1: Experimental design

	Supplementation to basal* diet (1-40 d of age)							
	1	2	3	4	5	6	7	8
Con	--							
Vit. E(IU/kg)		50						
GAE (%)			0.5	1.0				
BAE (%)					0.5	1.0		
TP (%)							0.5	1.0

Basal diet contained 50 IU of Vit. E

of the prepared BAE determined using Folin-Ciocalteu (FC) assay by applying method of Wright *et al.* (2000) and Atoui *et al.* (2005) and was 31 µg/L in Gallic acid equivalent.

TP: The determined value of lycopene content in TP was 155 mg/Kg using procedures described by Bunghez *et al.* (2011).

Slaughtering and sampling: At 40 d of age, 32 birds (4 birds per treatment which were around the average body weight) were slaughtered and carcass characteristics including dressing % and weights of liver, gizzard, heart, abdominal fat and intestine as percentage from life body weight and thickness of breast muscles were recorded. After slaughtering thighs of each carcass were taken to antioxidant examinations. The first thigh was refrigerated (up to 4 days at 4°C) while the second thigh was frozen (60 days at -20°C) to carry out assays of total phenols content, antioxidant activity [through two scavenging assays: 1,1-Diphenyl-2-Picrylhydrazyl radical-scavenging assay (DPPH) and 2-thiobarbituric acid-reactive substances assay (TBARS)] and microbiological status. Samples of breast muscles were collected and stored for 24 h at 4°C to estimate pH then stored up to 4 days at 4°C to estimate lipid profile of meat.

Laboratory analysis of broiler meat: Ultimate pH (pHu) was measured at 24 h post mortem of chilled breast muscles on 4°C using pH meter, provided by a temperature control system, by probe method. The minimum depth to adopt was 1 cm after incision of the muscles. Lipid profile including total cholesterol (TC), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglycerides (TG) of meat were determined in refrigerated samples (4 days at 4°C) by colorimetric methods using analytical kits produced by Biodiagnostic Company.

Measurements of antioxidant status: Measurements of antioxidant status included total phenol content (TPh), DPPH and TBARS, as indicator of malondialdehyde content, were determined in refrigerated (4 days at 4°C) and frozen (60 days at -20°C) broiler thigh samples (4 samples/treatment/storing condition).

Tph: Samples of broiler thigh were prepared and analyzed for TPh according to procedures of Jang *et al.* (2007). Gallic acid was used as the standard and the obtained results were expressed as mg gallic acid/100 g meat.

DPPH: DPPH assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. The method is based on the

spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an oxidant (Pyrzynska and Pekal, 2013). The DPPH radical scavenging activity was estimated with the aqueous supernatant obtained from thigh meat according to the method of Blois (1958) and with modifications and calculation equation of the percentage of DPPH radical scavenging reported by Jang *et al.* (2007) as : Radical-scavenging activity = $[1 - (\text{absorbance value of testing solution} / \text{absorbance value of control solution})] \times 100$.

TBARS: Each meat sample (5 g) of broiler thigh meat was homogenized in 15 mL of distilled water. Sample homogenate (5 mL) was transferred to a test tube and lipid oxidation was determined as the 2-thiobarbituric acid-reactive substance (TBARS) value by the described method of Ahn *et al.* (1999). Lipid oxidation was reported as milligrams of malondialdehyde per kilogram of meat (Jang *et al.*, 2007).

Microbiological traits: To investigate the effect of examined natural antioxidant on the safety of broiler meat stored by chilling or freezing the count of total bacteria and *Staphylococcus aureus* in refrigerated (4 days at 4°C) and frozen (60 days at -20°C) breast meat samples were carried out according to Gouda (2002).

Statistical analyses: Data of GAE, BAE and TP treatments were subjected to two way analysis of variance to detect the effects of antioxidant source and supplemental level. Data of all experimental treatments, including Con and E-100, were subjected to one way analysis of variance to detect the differences between them. Variables showing a significant F-test ($p = 0.05$) were compared to each other's using Duncan's Multiple Range Test (Duncan, 1955). The statistical procedures were computed using SAS (1997).

RESULTS AND DISCUSSION

The recorded values of the environmental temperature and relative humidity surrounding birds during the experimental period were ranged between 36-41°C and 20-55%, respectively and showed that birds were exposed to continuous high temperature from 1 to 40 d of age.

Carcass characteristics: Results of carcass characteristics (Table 2) showed that neither antioxidant source nor level of supplementation had any significant effect on dressing, liver, gizzard, heart, abdominal fat and intestine weight as percentage from life body weight and thickness of breast muscles. Also, the same trend was observed in results of all experimental treatments including Con and E-100 treatments. Conversely, some previous reports showed beneficial effects of adding

ginger to broiler diets on carcass characteristics including dressing (Javed *et al.*, 2009; Zhang *et al.*, 2009), while El-Deek *et al.* (2002), Moorthy *et al.* (2009) and Onu *et al.* (2010) could not detect any significant effect of diet supplementation with ginger at levels from 0.1 to 1.0% on carcass characteristics of broilers. On the

other side, Lee *et al.* (2009) recorded significant reduction of fat pad of mice fed on diet supplemented with red beet leaf at level of 8% as antioxidant source.

Lipid profile and pH of chilled meat: As shown in Table 3, the recorded values of pHu of breast meat of

Table 2: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) as natural antioxidants on carcass characteristics of fresh broiler meat at 40 d of age

	Dressing (%)	Liver (%)	Gizzard (%)	Heart (%)	Abd. Fat (%)	Intestine (%)	Breast depth (mm)
Main factors							
Antioxidant source							
GAE	65.2	2.38	1.52	0.49	1.73	5.84	46.8
BAE	65.2	2.32	1.54	0.46	1.83	5.53	48.1
TP	65.1	2.45	1.65	0.46	1.54	5.46	50.0
Supplemental level							
0.5%	65.1	2.29	1.62	0.47	1.62	5.55	47.5
1.0%	65.3	2.47	1.51	0.47	1.78	5.67	49.1
P-value							
Antioxidant	0.9690	0.7287	0.3286	0.4291	0.3302	0.1728	0.5119
Level	0.7109	0.1965	0.1700	1.000	0.3258	0.4805	0.4541
Interactions	0.8875	0.6108	0.6795	0.1086	0.0413	0.8405	0.7768
Mean of SE±	0.158	0.062	0.038	0.0142	0.088	0.083	0.127
Treatments							
Con	64.5	2.22	1.71	0.56	1.96	5.95	47.5
E-100	64.8	2.32	1.55	0.54	1.67	5.70	45.0
GAE 0.5	65.2	2.25	1.61	0.46	1.93	5.83	45.0
GAE 1.0	65.2	2.50	1.42	0.53	1.52	5.84	48.7
BAE 0.5	65.3	2.20	1.55	0.46	1.72	5.40	47.5
BAE 1.0	65.2	2.46	1.52	0.46	1.95	5.65	48.7
TP 0.5	64.9	2.45	1.70	0.50	1.21	5.41	50.0
TP 1.0	65.3	2.44	1.60	0.41	1.86	5.51	50.0
Mean of SE±	1.840	0.054	0.033	0.014	0.073	0.074	0.950
P-value	0.4896	0.7486	0.4620	0.0818	0.1113	0.7223	0.8422

Table 3: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) as natural antioxidants on pH and lipid profile of chilled (4 days at 4°C) broiler meat

	pHu	Lipid profile of chilled meat (mg/100g meat)			
		TC	HDL	LDL	TG
Main factors					
Antioxidant source					
GAE	6.38	178	110.5	6.93	303.5 ^b
BAE	6.38	229	101.0	5.76	611.0 ^a
TP	6.45	162	108.6	4.86	399.0 ^b
Supplemental level					
0.5%	6.41	224	122.0	6.66	485
1.0%	6.40	174	91.9	5.04	387
P-value					
Antioxidant	0.7191	0.4423	0.9303	0.2842	0.0381
Level	0.8556	0.2243	0.1573	0.1399	0.2646
Interactions	0.8083	0.3726	0.3136	0.8330	0.3945
Mean of SE±	0.03	8.18	9.63	0.51	16.42
Treatments					
Con	6.02 ^b	235	75.0	19.1 ^a	704 ^a
E-100	6.05 ^b	204	100.3	7.96 ^b	482 ^b
GAE 0.5	6.42 ^a	176	105.6	6.47 ^{bc}	321 ^b
GAE 1.0	6.34 ^a	179	115.0	7.40 ^{bc}	286 ^c
BAE 0.5	6.38 ^a	269	110.3	6.50 ^{bc}	759 ^a
BAE 1.0	6.39 ^a	190	93.0	5.03 ^{bc}	462 ^b
TP 0.5	6.44 ^a	166	140.3	6.07 ^{bc}	419 ^b
TP 1.0	6.46 ^a	157	77.0	3.63 ^c	379 ^{bc}
Mean of SE±	0.07	61.23	7.52	0.99	14.13
P-value	0.0001	0.4203	0.4665	0.0001	0.0433

Con and E-100 were significantly lower than those recorded for samples related to the other groups, which proved that natural antioxidant sources, GAE, BAE and TP, were more efficient for increasing pHu of breast meat than vitamin E. Among natural antioxidant treatments, there was no significant effect on pHu due to antioxidant source or level of supplementation. This result confirmed those reported by Jang *et al.* (2007) who recorded increase of pH of breast meat due to adding medicinal herb extract to broiler diets at levels 0.3 and 1.0%. While Tavarez *et al.* (2011) found no effect of supplemental synthetic antioxidant (ethoxyquin) to broiler diets on pH of breast meat (5.71 vs. 5.69 for control group). Herawati and Marjuki (2011) reported increased pH of broiler meat (6.11 vs. 5.80 of control samples) as a result of inclusion ginger in diet at levels 0.5, 1.0, 1.5 and 2.0%. An early study by (Offer and Knight, 1988) showed that an increase in muscle pH has been shown to improve water holding capacity of meat from muscle by increasing the electrostatic repulsions between myofibrillar protein. Also there was strong negative correlation between drip loss and ultimate pH reported for broiler meat (Le Bihan-Duval *et al.*, 2001). So the reported increase of pHu in this experiment might reflect decrease of drip loss and increase of water holding capacity of breast meat in groups fed diet supplemented with GAE, BAE or TP. Classification of some lipid components showed that TC, HDL and LDL of chilled broiler breast meat did not affected by adding any of GAE, BAE or TP at levels of 0.5 or 1.0% to diets (Table 3). While TG values decreased

significantly in chilled broiler breast meat of birds fed on either GAE or TP. Results of one way analysis of variance showed significant decrease in LDL and TG of chilled broiler breast meat of birds fed on any experimental diet compared to that of Con group. The lowest LDL in breast meat recorded for birds fed on diet supplemented with 1.0% TP, while the lowest TG value recorded for birds fed on GAE 1.0%. However some previous studies concerned effect of using ginger or tomato products on blood cholesterol compounds (Nasiroslami and Torki, 2010; Saeid *et al.*, 2010; Mohamed *et al.*, 2012), there was limited work focused on effect of those natural antioxidants on cholesterol compounds of broiler meat. Lopez *et al.* (2010) compared adding mixture of rosemary and sage (500 mg/kg) as antioxidant with diet enriched in alpha-tocopheryl acetate (200 mg/kg) on quality of broiler meat. They found that meat of broilers fed diet containing spice extracts had smaller concentrations of total cholesterol oxidation products (COPS) than meat from the control group ($p < 0.05$). Supplemental alpha-tocopheryl acetate reduced the COPS concentrations to a greater extent than did spice extracts ($p < 0.05$). Likewise, Stanacev *et al.* (2011) could reduce cholesterol of chicken tissues by adding garlic powder to broiler diet.

Antioxidant status of thigh meat: Results of antioxidant status of refrigerated (4 days at 4°C) presented in Table 4 showed general increase of TPh and the activity of DPPH radical scavenging in broiler meat as a result

Table 4: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) on antioxidant status of chilled (4 days at 4°C) and frozen (60 days at -20°C) broiler meat

	Chilled meat (4 days at 4°C)			Frozen meat (60 days at -20°C)		
	TPh. (mg/100g)	DPPH (%)	TBARS (mg/kg)	TPh. (mg/100g)	DPPH (%)	TBARS (mg/kg)
Main factors						
Antioxidant source						
GAE	1.10	12.30	22.57 ^b	0.37	6.95 ^a	29.97 ^b
BAE	1.04	11.63	31.06 ^a	0.40	4.93 ^b	51.09 ^a
TP	1.16	11.34	24.12 ^b	0.29	0.87 ^c	28.82 ^b
Supplemental level						
0.5%	1.08	11.09 ^b	25.61	0.35	4.19	37.78
1.0%	1.14	12.43 ^a	26.23	0.35	4.31	35.47
P-value						
Antioxidant	0.0668	0.1447	0.0355	0.0904	0.0001	0.0001
Level	0.4723	0.0028	0.8124	0.9628	0.7494	0.3455
Interactions	0.1572	0.4503	0.7541	0.4073	0.1069	0.3588
Mean of SE±	0.02	0.24	1.39	0.02	0.56	2.42
Treatments						
Con	0.87 ^c	10.67 ^c	26.30 ^{ab}	0.24 ^b	2.81 ^{cd}	34.50 ^b
E-100	1.12 ^{ab}	12.79 ^{ab}	19.95 ^b	0.36 ^{ab}	6.24 ^a	26.28 ^b
GAE 0.5	1.08 ^{ab}	11.30 ^{bc}	21.75 ^{ab}	0.37 ^{ab}	6.86 ^a	28.65 ^b
GAE 1.0	1.12 ^{ab}	13.31 ^a	23.40 ^{ab}	0.38 ^{ab}	7.04 ^a	31.30 ^b
BAE 0.5	0.98 ^{bc}	11.23 ^{bc}	32.10 ^a	0.37 ^{ab}	4.35 ^{bc}	53.60 ^a
BAE 1.0	1.10 ^{ab}	12.03 ^{abc}	30.00 ^{ab}	0.44 ^a	5.51 ^{ab}	48.60 ^a
TP 0.5	1.12 ^{ab}	10.73 ^c	22.95 ^{ab}	0.27 ^b	1.35 ^{de}	31.10 ^b
TP 1.0	1.19 ^a	11.95 ^{abc}	25.30 ^{ab}	0.32 ^{ab}	0.39 ^e	26.55 ^b
Mean of SE±	0.02	0.24	1.22	0.017	0.461	1.92
P-value	0.0016	0.0425	0.1713	0.0789	0.0001	0.0001

of adding any of examined antioxidant substance to basal diet with exception of DPPH value of meat in TP 1% group and this increase did not accompanied by reduction of lipid peroxidation as TBARS did not affected. The obtained results became clearer after long period of freezing storage (60 days at -20°C). Both GAE and TP could depress the lipid peroxidation, so TBARS values decreased, more than BAE in both refrigerated (4 days at 4°C) and frozen (60 days at -20°C). Also GAE was the most effective antioxidant for increasing DPPH scavenging of free radicals in frozen broiler meat (60 days at -20°C), while inclusion of TP in broiler diets recorded the lowest DPPH scavenging activity of free radicals. Raising level of supplemental antioxidant from 0.5 to 1.0% increased activity of DPPH radicals scavenging of refrigerated (4 days at 4°C) significantly ($p = 0.0028$). Also increasing storage period up to 60 days resulted in decreasing of TPh and the activity of DPPH radical scavenging in broiler meat and increased lipid peroxidation even the storage was on -20°C (Table 5). Samples of TP group recorded the greatest depression percents of TPh and DPPH (75 and 92.3%) and the lowest increase percent of TBARS value (19.5%) compared with GAE and BAE. The obtained results in this study confirmed those reported previously by Shariatmadari *et al.* (2011) who recorded decrease of MDA of broiler thigh meat when fed broilers on diet containing 100 IU Vit. E/kg diet and recorded increase of MDA of chilled (4°C) meat when birds exposed to heat stress (33°C). Also they recorded gradual increase of MDA by increasing days of cold storage from 0 to 6d. Furthermore, these results were in match with those reported in the first part of our study whereas adding TP to broiler diet at levels of 0.5 or 1.0% decreased plasma MDA and increase plasma TAOC compared to GAE, BAE or Con groups. The same trend reported in many previous studies concerned by reducing lipid peroxidation of broiler meat using antioxidant sources (Lopez *et al.*, 1998; Sahin *et al.*, 2010; Tavarez *et al.*, 2011; Yesilbag *et al.*, 2011; Saemi *et al.*, 2012). Sahin *et al.* (2008) studied the effect of heat stress and inclusion of tomato powder at levels 0, 2.5 and 5% in quail diet on growth performance and lipid peroxidation. They found that heat stress increased MDA values of both breast muscles and liver and detected linear reduction of MDA value by increasing dietary tomato powder level. The obtained increase of both TPh content and DPPH scavenging activity and decrease of TBARS of broiler meat as a result of adding GAE, BAE or TP in diet was in match with those results reported by Jang *et al.* (2007) when supplemented broiler diet with mixture of medical plants (Mulberry leaf, Japanese honeysuckle and goldthread) at levels of 0.3 and 1.0%. Also, inclusion of red beet leaf at level of 8% in high fat and high cholesterol mice diet reduced TBARS of liver,

Table 5: Effect of supplemental antioxidant source and level on change of antioxidant status (%) of broiler meat after freezing for 60 days at -20°C

	TPh.	DPPH	TBARS
Main factors			
Antioxidant source			
GAE	↓ 66.4	↓ 42.7	↑ 32.8
BAE	↓ 61.6	↓ 57.6	↑ 64.5
TP	↓ 75.0	↓ 92.3	↑ 19.5
Supplemental level			
0.5%	↓ 67.6	↓ 62.2	↑ 47.5
1.0%	↓ 68.5	↓ 65.3	↑ 35.2

kidney and heart after one week of feeding and increased antioxidant enzymes in plasma and liver (Lee *et al.*, 2009).

There were different explanation of the relationship between T Ph content of natural antioxidant and reduced lipid peroxidation. The first is, phenolic compounds present in natural plants react with lipid and hydroxyl radicals and convert them into stable products (Kahkonen *et al.*, 1999). Another suggested by Sahin *et al.* (2010) who reported that polyphenols act as modifiers for signal transduction pathways through enhancing Keap-1 dissociation from Nrf2 (transcription factor that control the cellular antioxidant response against oxidants) that occur in response to stressors, which are accompanied by suppression of lipid peroxidation and elevation of antioxidant enzyme activities Sahin *et al.* (2013). That explanation confirmed the report of Lian and Wang (2008) suggesting that Lycopene may increase nuclear Nrf2 which lead to enhanced expression of antioxidant enzymes protein.

The obtained results showed that phenolic compounds in BAE were less effective than those in GAE and TP in reducing lipid peroxidation whereas there was no significant difference in TPh content of antioxidant sources, there were significant differences in TBARS values of chilled and frozen broiler meat, and BAE group recorded the highest TBARS values. This result may be indicting that although Socaciu (2008) modified the extraction and storage condition of BAE to protect betalaines from degradation, these compounds failed to delay lipid peroxidation especially after freezing meat for 60 d on -20°C. On the contrary, many aforementioned researches confirmed the strong antioxidant activity of beet root pigments in reducing lipid peroxidation of human food (Tytti *et al.*, 2000; Georgiev *et al.*, 2010; Rababah *et al.*, 2011).

Microbiological traits: Inclusion of any of examined antioxidant substances in broiler diets decreased counts of both total bacteria and *Staphylococcus aureus* in refrigerated (4 days at 4°C) and frozen (60 days at -20°C) breast meat samples compared to those counted in samples of unsupplemented Con group (Table 6). In refrigerated (4 days at 4°C) samples of TP group contained the largest total bacteria count, while

Table 6: Effect of ginger aqueous extract (GAE), beetroot aqueous extract (BAE) and tomato puree (TP) on count of Total bacteria count (TBC) *Staph. aureus* count of chilled (4 days at 4°C) and frozen (60 days at -20°C) broiler meat

	Chilled meat		Frozen meat	
	TBC (X 10 ⁸)	<i>Staph. aureus</i> (X 10 ⁴)	TBC (X 10 ⁸)	<i>Staph. aureus</i> (X 10 ⁴)
Main factors				
Antioxidant source				
GAE	12 ^b	10.0 ^a	11	8.6 ^b
BAE	10 ^b	7.0 ^b	7.5	100 ^a
TP	23 ^a	2.5 ^b	12	3.1 ^b
Supplemental level				
0.5%	18	7.4 ^a	12.4 ^a	76 ^a
1.0%	12	4.2 ^b	8.2 ^b	2.2 ^b
P-value				
Antioxidant	0.0512	0.001	0.1478	0.0007
Level	0.1506	0.0001	0.0456	0.0013
Interactions	0.5382	0.0001	0.7927	0.0011
Mean of SE±	15	3.9	10.3	30
Treatments				
Con	260.0 ^a	3100 ^a	39 ^a	1500 ^a
E-100	20.0 ^b	400 ^b	13 ^b	40 ^b
GAE 0.5	14.0 ^b	200 ^b	13 ^b	17 ^b
GAE 1.0	10.0 ^b	9.7 ^b	10 ^b	0.23 ^b
BAE 0.5	11.3 ^b	13 ^b	9.3 ^b	20 ^b
BAE 1.0	9.3 ^b	1 ^b	5.7 ^b	6 ^b
TP 0.5	29.0 ^b	3 ^b	15 ^b	5.7 ^b
TP 1.0	17.0 ^b	1.9 ^b	9 ^b	0.6 ^b
Mean of SE±	19	290	2.4	130
P-value	0.0009	0.0619	0.0030	0.0056

the largest number of *Staphylococcus aureus* counted in samples of GAE group. After 60 days of freezing on -20°C significant differences in total bacteria count disappeared and BAE samples contained the largest number of *Staphylococcus aureus*. Increasing supplemental level of any of examined antioxidant substances from 0.5 to 1.0% reduced count of *Staphylococcus aureus* in both refrigerated and frozen breast samples and reduced total bacteria count in frozen breast meat samples. These results confirmed the obtained results in the first part of this study whereas adding GAE, BAE or TP to broiler diet at levels of 0.5 or 1.0% decreased count of both total bacteria and *Staphylococcus aureus* in broiler intestine. Previous studies of Awadein *et al.* (2012) showed antibacterial effect of ginger when added to layer diets at level of 1% and the same trend reported by Sadeghi *et al.* (2012) in broiler diet when used at level of 0.75%. In the same way Sudrashan *et al.* (2010) recorded antibacterial effect of aqueous extract of ginger oil on count of *Staphylococcus aureus* when diluted with water in ratios of 1:150, 1:250 and 1:500 to chicken meat. The most effective dilution ratio was 1:150. Lee *et al.* (2004) reported that the antimicrobial activity of essential oils made them as alternative to antibiotics in many applications such as poultry industry and suggested that phenolic compounds in essential oils could penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilicity (Helander *et al.* 1998). These finding may explain the reduction of

Staphylococcus aureus count of breast meat by increasing the inclusion level of GAE, BAE or TP from 0.5 to 1.0%.

Conclusion: The obtained overall results of the current study confirmed the efficacy of using some natural antioxidant sources in improving the quality of broiler meat during summer season. Both GAE and TP could decrease TG of chilled thigh meat and depress lipid peroxidation of both chilled and frozen breast meat than BAE. Increasing level of inclusion rate from 0.5 to 1.0% resulted in significant reduction of *Staphylococcus aureus* count of both chilled and frozen breast meat.

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