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Antioxidant Activity of Marjoram Extract and its Effect on the Antioxidative Properties of Broilers' Chicken Meat

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Abstract

The antioxidant properties of marjoram ethanolic extract (MEE) were evaluated in the present study .The total antioxidant capacity of the extract was found to be 323.86 mg ascorbic acid equivalent/g, total phenolic content was 177.08 mg gallic acid equivalent/g and total flavonoids content was 54.43 mg querectin equivalent/g .A total number of ninety Ross broiler chicks (one day old) were distributed into three groups of three replicates each .The chicks were reared for 36 days and the dietary treatments consisted of a corn-soybean meal basal diet (control) ;the test diet was supplemented with 100 ppm marjoram extract (T1); and the second test diet was supplemented with 500 ppm marjoram extract (T2). At the end of the experimental period, breast and thigh meat samples were separately stored and refrigerated at 4°C. The meat samples were analyzed at three storage periods (day 1, 3, and 7) for their proximate composition and antioxidative activity .The results showed that the supplementation of MEE into broilers' diet did not have any

significant influence on the proximate analysis of breast and thigh meat. On the other hand, the total phenolic content of groups T1 and T2 was significantly ($p<0.05$) higher than that of the control group . The addition of MEE resulted in a significant) ($p<0.05$) improvement in the -1,1 Diphenyl - 2 - picrylhydrazy (DPPH) radical - scavenging activity of chicken meat in T1 and T2 as compared to control. The meat samples of T2 retained their high scavenging activity up to storage day 7. Also, the supplementation of diets with MEE caused a significant ($p<0.05$) delay in lipid oxidation of chicken meat in T1 and T2 as compared to control. The results of the present work suggest that the dietary inclusion of marjoram extract in broilers diet can improve their antioxidative activity during refrigerated storage .

Introduction

Poultry meat is one of the most important protein sources in human nutrition. According to the Food and Agriculture organization (FAO), chicken meat has many benefits as it is healthy and more affordable than other meats beside of its positive contributions to the diet of those on low incomes ***(Farrel, 2008)***. Chicken meat can be stored refrigerated for only two days as it is usually susceptible to oxidative deterioration due to its high content of polyunsaturated fatty acids ***(Zhang et al., 2015) (FDA, 2015).*** Broilers tend to deposit polyunsaturated fatty acids in their tissues similar to those present in the diet therefore, oxidation products result in decreased shelf life of poultry meat ***(Tavárez et al., 2011).***

In this respect, the use of either synthetic or natural antioxidants is one of the major strategies for preventing lipid oxidation ***(Sampaio et al., 2012).***The addition of antioxidants into

poultry diets has been implemented to achieve optimal growth performance, reproduction, and chicken meat quality ***(Delles et al., 2014).*** Conventionally, broiler feed is manufactured by using Vitamin E and synthetic antioxidants like butylated hydroxyl-anisole (BHA), butylated hydroxyl- toluene (BHT) and ethoxyquin ***(Shahid et al., 2016).*** Dietary antioxidant supplementation causes a protective barrier against oxidation of broiler breast meat which is associated with enhanced cellular antioxidant enzymatic activity and reduced reactive oxygen species (ROS) propagation ***(Delles et al., 2014).***

In the recent years, the use of synthetic antioxidants has come into debate due to their supposed carcinogenic potential ***(Fratianni et al., 2010).*** Also there is an increasing consumers' awareness about the safety and toxicity of these synthetic antioxidants ***(Jang et al., 2008).*** For these reasons many researchers focused their work on finding natural antioxidants that could be effective as well as safe to be used in broiler's diet instead of synthetic antioxidants.

Antioxidants from plant essential oils or extracts have received much attention as natural antioxidants for broilers. ***Wang et al. (2008)*** reported that *Forsythia suspensa* plant extract have good antioxidative and free-radical scavenging properties and can be used to reduce the risks of peroxidation and improve nutrient digestibility and growth performance of broiler chickens. ***Fratianni et al.(2010)*** found that balm and thyme essential oils can be used as effective natural preservatives and have the ability to increase the shelf life of chicken breast meat. The beneficial effect of using dietary curcumin on meat quality and antioxidant profile of breast muscle in broilers was also reported by ***Zhang et al. (2015).***

In our previous researches, we have reported the growth-promoting and antioxidative effects for some natural extracts. Our results indicated that addition of pomegranate peel extract into broilers' diet resulted in an improved productive performance and promotion of chickens' health ***(Hamady et al., 2015).*** Also we have reported that the use of chamomile leave extract as a natural preservative for chicken breast meat showed promising results. chamomile extract ***(Hassanin et al., 2015a).***

The objective of the present study was to evaluate the effect of dietary inclusion of marjoram extract as natural antioxidant in broilers' diet on meat quality and antioxidative properties of chicken breast and thigh meat stored in refrigerator for seven days.

Materials and methods

Preparation of marjoram extract

Dried marjoram was purchased from local market in Egypt. The extraction was done by adding 250 ml of ethanol (80%) to 100 g of dry marjoram and keeping the mixture on magnetic stirrer overnight. The resulting extract solution is then collected and the solvent is evaporated. The dry marjoram extract (MEE) was then kept in the freezer until used.

Determination of the total antioxidant capacity of marjoram extract

The total antioxidant capacity of MEE was determined by the "phosphomolybdenum" method ***(Prieto et al.,1999).*** The results were calculated from a standard curve using ascorbic acid as a reference

antioxidant material and results were expressed as mg ascorbic acid equivalent/g of extract (mg AAE/g).

Determination of the total phenolic content of marjoram extract

The total phenols content of MEE was determined using the Folin-Ciocaleau method ***(Turkmen et al., 2006).*** A calibration curve of gallic acid was prepared and the results, determined from regression equation of the calibration curve were expressed as mg gallic acid equivalents per gm of the extract (mgGAE/g).

Determination of the total flavonoids of marjoram extract

The total Flavonoids content of the extract was determined by the aluminium chloride test ***(Mohdaly et al., 2010)*** using quercetin as standard and the results were calculated as mg quercetin equivalent/g of extract (mgQE/g).

Experiment design

A total of 90 Ross broiler chicks (one-day-old) were randomly divided into three groups of three replicates each, and reared for 36 d. The chicks were fed on a corn-soybean meal basal diet (control), a basal diet with 0.01% MEE (T1), and a basal diet with 0.05% MEE (T2). The birds were fed experimental starter diets (3000 kcal of MEE/kg and 22% CP) until 28 d of age and finisher diets (3100 kcal of MEE/kg and 19% CP) until 36 d of age ***(Table 1) (NRC;1994).*** At the end of the feeding period, the chickens were slaughtered and cut and thigh and breast meat samples were excised and stored in a refrigerator (4°C). The antioxidative properties and the proximate composition of meat samples were analyzed on storage days 1, 3, and 7, respectively.

Proximate analysis of chicken meat

Proximate composition of chicken meat samples were determined according to by the standard procedures of ***AOAC (2006).*** The moisture content of samples was determined after drying at 105°C for 24 h to constant weight. Crude protein was determined by Kjeldahl method, ash was determined after burning at 550°C and crude fat was analyzed by soxhlet extraction using diethyl ether.

Antioxidative properties of chicken meat

The antioxidative properties of chicken meat samples were estimated following the procedures described by ***Jang et al. (2008)*** as follows:

Chicken meat samples were homogenized in distilled water and then chloroform was added to the homogenates and the mixture was shaken vigorously 2 to 3 times to separate the lipids. The supernatant was collected and used for the determination of the 1, 1-Diphenyl-2picrylhydrazyl (DPPH) radical-scavenging activity and total phenols content of meat samples.

The DPPH scavenging activities of meat samples were determined by mixing 1 ml of the previously prepared supernatant with 1 ml of DPPH solution (0.2 m*M*) and allowing the mixture to stand for 30 minutes before its absorbance was measured at 517nm using a SpectroD 250 plus spectrophotometer (Analytik jena).

The total phenols content of chicken meat samples was estimated by the Folin-Ciocalteu method. Quantification was done based on the standard curve generated with gallic acid.

Thiobarbituric Acid-Reactive Substances (TBARS)

The lipid oxidation of the meat was estimated using the thiobarbituric acid reactive substances (TBARS) test following the procedure described by ***Racanicci et al., (2008).***

Results and discussion

The results of total antioxidant capacity, total phenols and total flavonoids of marjoram extract are presented in **table 2**. It can be noticed that marjoram extract contains appreciable amounts of phenolic compounds and flavonoids which contribute to its antioxidant activity. It has been reported that phytochemical constituents such as phenols and flavonoids commonly found in plants have several biological activities responsible for antioxidant activity in preventing a number of diseases through free-radical scavenging activity ***(Kannan et al., 2010).*** There is high correlation between antioxidant activity and phenolic content and also phenolic compounds play an important role in stabilizing lipid peroxidation ***(Hassanin et al., 2015b).***

The proximate composition results for breast and thigh meat of chickens fed dietary marjoram extract as natural antioxidant are given in **table 3**. Dietary addition of marjoram extract did not significantly affect the proximate composition of breast and thigh meat. This is in agreement with the findings of ***Jang et al., (2008)*** who reported that the supplementation of medicinal herbs in chickens diets did not affect their meat composition. Similarly, ***Shirzadegan*** and ***Falahpour, (2014)*** found that the dietary supplementation with different levels of medicinal herb extract mixture not show significant differences on proximate composition of chicken samples.

Table 4 shows the results for total phenols content of breast and thigh meat of chickens fed dietary marjoram extract. The total phenols content of breast and thigh meat in the treated groups was significantly higher ($p<0.05$) than that of the control group. This trend was observed starting from day 1 of storage up till day 7. It was also generally noted that by increasing the addition level of MEE, the total phenols content of chicken meat was significantly increased ($p<0.05$).These results indicate that the antioxidative activity in breast and thigh meat of broiler chickens can be increased by dietary MEE, which is attributed to the phenols content of the extract ***(Shirzadegan*** and ***Falahpour, 2014).***

The antioxidative properties of chicken meat were further tested using the DPPH scavenging assay to estimate the efficiency of MEE as an antioxidant and the results are shown in table 5. The results of DPPH free scavenging assay of breast and thigh meat showed that there is significant effect for the dietary addition of MEE in broilers' diet. It can be seen from table 5 that during the three studied storage days the DPPH-scavenging activity of T2 diet was significantly greater ($P<0.05$) than T1 diet. Also, the free radical inhibition percentage of breast and thigh meat of control group was significantly lower ($p<0.05$) than the treated groups T1 and T2. According to ***Zhang et al., (2015)*** the high efficiency of antioxidants in the free radical scavenging assays could be attributed to either its direct capacity to neutralize stable free radicals or an indirect role as a hydrogen donor. Our results suggest that the dietary addition of marjoram extract as a potential antioxidant, improved the antioxidant properties of breast and thigh meat in broilers, possibly by the enhanced hydrogen- donating ability and free radical scavenging

capacity. This is in agreement with the results of ***Fratianni et al., (2010); Sohaib et al., (2012)*** and ***Zhang et al., (2015).***

Lipid peroxidation results from oxidative deterioration of fatty acids in meat and leads to the production of off-flavors and off- odors and the reduction of food shelf life ***(Fratianni et al., 2010).*** In the present study, table 6 presents the TBARS values of chicken breast and thigh meat as influenced by the dietary addition of MEE. The results show that dietary addition of MEE succeeded in delaying the lipid oxidation of broiler chicken breast and thigh meat. The TBARS values of T1 and T2 were significantly lower ($p<0.05$) than those of the control group. The presence of phenolic compounds in marjoram extract can react with lipid and hydroxyl radicals and convert them into stable products so they can extend the shelf life and improve the quality of meat products ***(Jang et al., 2008).***

Conclusion

The present study proved that marjoram extract could be a potential natural antioxidant that can be used as feed additive. The dietary addition of marjoram extract increased the oxidative stability of breast and thigh meat in broiler chickens. The natural antioxidant investigated succeeded in extending the refrigerated storage time of chickens' meat up to seven days without any deterioration in meat quality. Further work could be done to study the effect of other natural additives on the antioxidative properties of chicken meat.

Table 1: Composition and determined analysis of the basal starter and finisher diets

Ingredient (%)	Starter diet	Finisher diet
Corn 7.3%	58.475	64.300
Soybean meal (44%)	28.500	24.00
Gluten (60%)	7.00	4.5
Corn oil	2.00	3.00
Di-Calcium phosphate %	2.030	1.825
Limestone%	0.570	0.990
Vitamin and mineral*	0.400	0.400
Sodium chloride	0.300	0.300
Choline chloride (70%)	0.075	0.075
DL-Methionine	0.260	0.270
L-Lysine	0.390	0.340
Total	100.00	100.00
Calculated analysis (%)		
Crude protein%	22.28	19.18
Metabolizable energy Kcal	3034	3128
L-Lysine%	1.4	1.21
DL-Methionine %	0.55	0.50
Methionine%+cystine%	1.04	0.90
Ca%	0.95	0.85
Non-Phytate P	0.47	0.43

(*) vitamin and mineral supplied per Kg of diet: Vit. A, 12000 I.U., Vit. D3, 2000I.U. ; Vit.E, 10mg ;Vit.K3 , 2mg; Vit.B1, 1 mg; Vit.B2, 5 mg; Vit. B6, 1.5 mg; Vit. B12, 10 ug; Biotin, 50ug; Choline chloride,500mg; Pantothenic acid , 10 mg; Niacin,30mg; Folic acid,1mg; Manganese, 60mg; Zinc,50mg; Iron,30mg; Copper,10mg; Iodine,1mg; Selenium,0.1mg and Cobalt,0.1mg.

Table 2: Total antioxidant capacity, total phenolic content and total flavonoids of marjoram extract

Total antioxidant capacity (mg AAE/g)	323.86±2.31
Total phenolics content (mg GAE/g)	177.08±1.54
Total flavonoids content (mg QE/g)	54.43±0.32

Mean± standard deviation

Table 3: Proximate composition (%) of breast and thigh meat from chickens fed the dietary MEE

Breast meat				
	Moisture	Protein	Fat	Ash
Control	74.89 ± 0.015	22.81 ± 0.015	1.81 ± 0.005	0.49 ± 0.02
T1	74.83 ± 0.011	22.89 ± 0.01	1.79 ± 0.01	0.50 ± 0.02
T2	74.8 ± 0.015	22.93 ± 0.015	1.78 ± 0.015	0.5 ± 0.03
Thigh meat				
	Moisture	Protein	Fat	Ash
Control	70.89 ± 0.05	18.88 ± 0.02	9.41 ± 0.01	0.84 ± 0.03
T1	70.81 ± 0.02	18.99 ± 0.02	9.36 ± 0.02	0.84 ± 0.02
T2	70.78 ± 0.01	19.13 ± 0.04	9.29 ± 0.01	0.80 ± 0.05

Mean± standard deviation

Table 4: Total phenols content (ppm) of breast and thigh meat from chickens fed the dietary MEE

Storage period	Breast			Thigh		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control	49.89^{c} ±0.015	47.50^{c} ±0.3	45.33^{c} ±1.52	51.38^{c} ±0.015	49.47^{c} ±0.21	47.03^{c} ±0.25
T1	64.05^{b} ±0.02	63.50^{b} ±0.2	62.17^{b} ±0.115	69.20^{b} ±0.2	66.43^{b} ±0.25	62.17^{b} ±2.51
T2	75.13^{a} ±0.153	74.03^{a} ±0.05	73.03^{a} ±0.03	78.15^{a} ±0.03	76.60^{b} ±0.2	74.03^{a} ±0.06

^{a,b,c} Means within a column with different letters are significantly different (P<0.05)

Table 5: DPPH scavenging activity (%) of breast and thigh meat from chickens fed the dietary MEE

Storageperiod	Breast			Thigh		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control	20.86^{c} ±0.02	19.95^{c} ±0.02	16.97^{c} ±0.025	20.77^{c} ±0.02	18.82^{c} ±0.2	16.97^{c} ±0.152
T1	23.39^{b} ±0.015	22.47^{b} ±0.02	21.25^{b} ±0.025	22.1^{b} ±0.1	21.20^{b} ±0.115	20.03^{b} ±0.251
T2	25.40^{a} ±0.1	24.83^{a} ±0.115	22.91^{a} ±0.021	24.9^{a} ±0.11	23.53^{a} ±0.1	22.91^{a} ±0.25

^{a,b,c} Means within a column with different letters are significantly different (P<0.05)

Table 6: TBARS (%) of breast and thigh meat from chickens fed the dietary MEE

Storage period	Breast			Thigh		
	Day 1	Day 3	Day 7	Day 1	Day 3	Day 7
Control	0.650[a] ±0.02	0.780[a] ±0.02	0.92[a] ±0.02	0.810[a] ±0.005	0.960[a] ±0.03	1.020[a] ±0.01
T1	0.566[b] ±0.001	0.590[b] ±0.01	0.63[b] ±0.03	0.600[b] ±0.01	0.710[b] ±0.02	0.780[b] ±0.005
T2	0.401[c] ±0.025	0.450[c] ±0.005	0.51[c] ±0.02	0.550[c] ±0.02	0.600[c] ±0.015	0.650[c] ±0.02

[a,b,c] Means within a column with different letters are significantly different (P<0.05)

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دراسة النشاط المضاد للاكسده لمستخلص البردقوش و تأثيره على الخواص المضاده للاكسده في لحم دجاج التسمين

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الملخص العربي

تم اجراء الدراسه الحاليه لتقييم الخواص المضاده للاكسده لمستخلص البردقوش. وقد وجدت نتائج النشاط الكلي المضاد للاكسده 323.86 ملجم مكافئ لحمض الاسكوربيك/ جم و نسبة الفينولات الكليه 177.08 ملجم مكافئ لحمض الجاليك/ جم و الفلافونيدات الكليه 54.43 ملجم مكافئ للكويرستين/ جم. تم بعد ذلك استخدام 90 كتكوت من نوع روس عمر يوم واحد و تم تقسيمها الى ثلاث مجموعات من ثلاث مكررات المجموعه الاولي (كونترول) والثانيه بأضافة 100جزء في المليون من مستخلص البردقوش ((T1 والثالثه بأضافة 500 جزء في المليون من مستخلص البردقوش ((T2 في العليقه وبعد فترة 36 يوم تم ذبح الطيور وتقسيمها الي صدور وأوراك وحفظها في الثلاجه على 4 درجه مئوية وحفظها لمدة 1 و 3 و 7 ايام. ثم تم قياس كفاءة النشاط المضاده للاكسده في لحوم الدجاج عند فترات التخزين المختلفه. و قد اوضحت النتائج ان التحاليل العامة للحم الدجاج لم تتأثر بإضافة المستخلص. اما بالنسبه للنشاط المضاد للاكسده فقد ادت إضافة المستخلص إلى زيادة معنويه في القدره على تثبيط الشوارد الحره بالنسبه للحوم المجموعات T1 و T2 مقارنه بالمجموعه الكونترول. كما أدت إضافة المستخلص في العلائق إلى تأخر تأكسد الدهون للمجموعات T1 و T2 مقارنه بالمجموعه الكونترول حتى 7 ايام من التخزين بالتبريد. هذا و تشير النتائج المتحصل عليها من الدراسة الحاليه أن إضافة مستخلص البردقوش في علائق دجاج التسمين يمكن أن تؤدي إلى تحسن النشاط المضاد للاكسده للحم الدجاج عند تخزينه بالتبريد حتى 7 ايام.