



INHIBITION OF LIPID PEROXIDATION AND MICROBIOLOGICAL IMPROVEMENT OF BROILER THIGH QUALITY AND CHARACTERISTICS BY ADDING CYPERUS ROTUNDUS POWDER AND VITAMIN E TO CHICKEN FEED

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Article info	Abstract
Received: 2024-10-24 Accepted: 2024-12-26 Published: 2024-12-31	Different amounts of <i>Cyperus rotundus</i> tuber powder and vitamin E were added to broiler feed during the breeding period to investigate their effects on the thigh meat cuts of the chicken carcasses. The findings show that adding the powder in 2.5, 5, and 7.5 g/kg feed maintained the pH stability of the thigh meat as well as significantly enhanced its water-retention ability. The findings also showed that all the different concentrations of the additives prevented fat from oxidizing in the chicken thigh meat. This was evidenced by the significantly lower MDA, PV, and FFA levels ($P<0.05$) compared to the control treatment without the additives. The treatments that prevented lipid oxidation the most were the 5 and 7.5 g/kg feeds. Also, the thigh meat from the 7.5 g/kg treatment had better taste and microbial characteristics over the 5 g/kg treatment while those receiving the 2.5 g/kg vitamin E or the control treatment did not show any significant effects. The results also showed that chicken thigh meat in the 5 and 7 g/kg <i>C. rotundus</i> tuber powder treatment was better in terms of taste, juiciness, and overall acceptance. Finally, adding the powder
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stopped the production of free radicals and free fatty acids in the chicken thigh meat, which meant that the fat oxidation markers declined.

Keywords: Cyperus rotundus, Vitamin E, Broiler, Thigh meat, Microbiological.

تثبيط بيروكسيدة الدهن وتحسين الصفات الميكروبية وجودة لحم فخذ فروج اللحم بإضافة مسحوق السعد وفيتامين E

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الخلاصة

هدفت الدراسة إلى التعرف على تأثير تراكيز مختلفة من مسحوق درنات *Cyperus rotundus* وفيتامين E في عليقة فروج اللحم خلال فترة التربية في قطيعات الفخذ بعد جزر الطيور، إذ أظهرت النتائج أن إضافة مسحوق *Cyperus rotundus* بتراكيز 2.5 و 5 و 7.5 غرام / كغم علف الى العليقة لعب دوراً ايجابياً في المحافظة على قيم الرقم الهيدروجيني للحم الفخذ، كما حسنت الاضافة معنوياً من قابلية اللحم على حمل الماء، في حين أنها خفضت من كمية الفقد الحاصل خلال الطبخ. كما دلت النتائج إلى أن جميع الإضافات بتراكيزها المختلفة أدت إلى كبح في أكسدة الدهون في لحم فخذ فروج اللحم إذ سجلت معاملات التجربة انخفاضاً معنوياً ($P < 0.05$) في قيم المألوالديهايد وقيمة البيروكسيد والاحماض الدهنية الحرة عند مقارنتها بمعاملة السيطرة الخالية من أي إضافة. وأظهرت المعاملتين 5 و 7.5 غرام/ كغم علف اعلى فعالية تثبيط أكسدة الدهون في لحم الفخذ. كما دلت نتائج التجربة على تفوق الصفات الحسية والميكروبية للحم فخذ الدجاج المغذى على عليقة تحتوي 7.5 غرام/ كغم من مسحوق *Cyperus rotundus* تلى ذلك الدجاج المغذى على عليقة تحتوي 5 غرام/ كغم، في حين لم يلاحظ تأثير معنوي على قطيعات فخذ لدجاج المغذى على عليقة تحتوي 2.5 غرام/ كغم وفيتامين E ولا حتى معاملة السيطرة. كما بينت النتائج تفوق لحم فخذ الدجاج المغذى على عليقة تحوي 5 و 7 غرام/ كغم مسحوق درنات *Cyperus rotundus* في صفات النكهة والعصيرية والتقبل العام، كما أظهرت النتائج دور إضافة مسحوق *Cyperus rotundus* في كبح تكوين الجذور الحرة والاحماض الدهنية الحرة في لحم فخذ الدجاج مما أدى إلى خفض مؤشرات أكسدة الدهن.

كلمات مفتاحية: درنات السعد، فيتامين E، فروج اللحم، لحم الفخذ، مايكروبايولوجي.

Introduction

Cyperus rotundus Linn is a sedge plant of the family Cyperaceae that grows all over the Mediterranean basin. There are many of these plants in the northeast, center, and south of Tunisia (43). They grow naturally in tropical, subtropical, and cold spots. *C. rotundus* is a traditional Indian, Chinese, and Japanese medicine plant that is used to treat stomach problems, cramps, and inflammatory bowel diseases (19). Studies have demonstrated the presence of phenolic chemicals in the aerial parts of the plant, yet their potential to shield cells from damage and combat free radicals remains largely unknown (25 and 26). *C. rotundus* L. is called "Nut grass." Old Ayurveda referred to it as "musta moola churna" (34), while Chinese traditional medicine refers to it as "Xiangfu" or "Xiangfuzi" (47).

A wide variety of herbs have been used for their health benefits, which include pain relief, and antibacterial, antidiarrheal, anti-inflammatory, antioxidant, antipruritic, antisaturative, appetite-stimulating, digestive, lacto-depurative, thirst-relieving, and calming effects (32 and 38). Investigations into the phytochemicals in *C. rotundus* L. revealed the presence of sesquiterpenes, flavonoids, phenylpropanoids, phenolic acids, alkaloids, (41) and saponins (39). Also, the root of the plant was found to have rutin, ipolamiide, and 6- β -hydroxyipolamiides. Scientists also note that the ethyl acetate and methanolic fractions could help treat liver damage caused by CCl₄ in rats (8).

In 2014, Lydia J. and Sudarsanam found 15-Hydroxy-4-oxo-10-pentadecynoic acid lactone in *C. rotundus* L. (31). Rotunduside is a phenolic molecule found in the methanol extract of CRR, and was recently shown in mice models to have antidepressant properties (29). Due to the stress on farm animals, the use of antioxidants as feed additives is growing. This is because stress causes protein and fats to oxidize in the body, which lowers the quality and safety of the final product (33). Rotunduside is safe for animals, improves the quality of animal products, and makes them more appealing to customers. There is increasing interest in the nutritional value of adding plant-based antioxidants that are high in phenols and polyphenols. These compounds reduce the harmful effects of lipid peroxide by lowering monoaldehyde and raising the level of tocopherol in tissues, as well as enhancing animal products (10 and 30).

The European Union allows the use of several plants as food additives (preservatives) in meat and meat products due to their demonstrated antioxidant properties, which help to stop or slow down oxidative reactions. These plants are also on the GRAS list in the United States (27). The Sad plant is one of the best natural sources of antioxidants. It is an annual grass plant with only one pair of leaves. There are about 5600 species and 109 groups in this family around the world. There are many species in the genus *rotundus* (16 and 24). The tubers are small, white, squamous, oval, or oblong, covered with fibrous veins, dark reddish-brown on the outside, and reddish-white on the inside, and they have a strong smell. The roots are what are used (8 and 22). Pasta was made from the starch taken from *C. rotundus*, which were also used to season cooked meat. Experts have demonstrated its tubers can extend the freshness and safety of meat and prepared meat products (11 and 44).

In addition to being used as a spice, it is also added to food to make it taste better and keep it fresh because it has antioxidants like flavonoids and polyphenols (18, 21 and 42).

Research indicates that the extract of *S. rotundus* has significant effectiveness against many gram-positive and gram-negative bacteria, including *Bacillus subtilis*, *B. pumilus*, *Pseudomonas aeruginosa*, *Shigella flexneri*. It has also been noted to have antifungal activity against *C. albicans* and *A. niger* (40). The researchers also showed that adding *C. rotundus* powder to poultry diets before slaughter or to meat before freezing had a positive effect on preserving the distinctive characteristics of broiler chicken carcasses and increasing their shelf life. The minced chicken meat remained frozen at -18 °C for over 30 days (5 and 6). It was also shown to help remove H₂O₂ and attach to Fe⁺² ions, which greatly increases the number of microbes and improved its chemical and physical properties. However, the product's sensory quality suffered when the powder was added in large amounts (17).

Materials and Methods

Cyperus rotundus tubercles purchased from local markets were washed and dried and then ground with an electric grinder. The powder was stored in bags until it was incorporated into the chicken feed. The treatments tested were:

T1- First treatment (control): normal diet (no powder addition).

T2- Second treatment: diet fortified with vitamin E at 300 mg/kg/feed.

T3- Third treatment: diet fortified with *Cyperus* tubers powder at 2.5 g/kg feed.

T4- Fourth treatment: diet fortified with *Cyperus* tubers powder at 5 g/kg feed.

T5- Fifth treatment: diet fortified with *Cyperus* tubers at 7.5 g/kg feed.

The birds were raised for 42 days and then slaughtered. The carcass thighs were cut open and their physical, chemical, and microbial properties studied.

pH assessment: The pH of the chicken thigh samples was assessed by homogenizing 10 g of meat with 90 ml of distilled water with an electrolytic homogenizer and measured with a pH meter (12).

Water holding capacity: The water-holding capacity was estimated according to the method described by (45) by taking 2 g of thigh meat samples and confining them between two filter papers placed within two glass plates. A 1-kg weight was placed on the plates and the results calculated based on the difference between the water amount before and after pressure according to the equation:

Water holding capacity = moisture content of meat (%) - moisture content lost after pressing (%).

Loss during cooking: The loss rate from cooking the thigh meat was estimated by using 20 g of cut thigh cubes and placing them in trays covered with cellophane paper and then grilling them at 180 °C for 10 minutes. They were then removed from the oven, dried, and their cooking-weight loss calculated according to the equation (28):

$$\text{Loss when cooking} = \frac{\text{Weight of meat before cooking} - \text{Weight of meat after cooking}}{\text{Weight of meat before cooking}} \times 100$$

Estimation of malondialdehyde (MDA) in the thighs: Fat oxidation was assessed using the modified approach by (2) by quantifying malondialdehyde, an indicator of fat oxidation, via the determination of TBA values.

Estimation of free fatty acids and peroxide values: The amount of free fatty acid and peroxide in the thighs was estimated using the method mentioned by (15).

Microbiological analysis: Total microbial count was determined by mixing 11 g of thigh samples with 99 ml of 1% peptone water, which had been sanitized in a sterile stomacher bag. The combination was blended for 2 minutes in a stomacher, and this solution was labeled as the first dilution. Dilutions were prepared up to the fifth dilution, from which 1 ml was taken from each and transferred to Petri dishes, and nutrient agar added to enumerate the total bacterial count. In contrast, MacConkey agar was utilized to determine the amount of coliform bacteria. Plates were incubated at 37 °C for 48 hours. PDA media was used to count yeasts and molds, and the plates incubated at 25 °C for 5-7 days. At the end of the incubation period, the number of colonies was counted and subsequently multiplied by the inverse dilution value (1).

Sensory evaluation: The treatments were evaluated after cooking at 180 °C for 15 minutes and graded from 1-7 based on flavor, juiciness, tenderness, public acceptance, and appearance.

Results and Discussion

pH assessment: Table 1 presents the impact of a diet incorporating *C. rotundus* powder and vitamin E on the pH values of the thigh meat of the birds after slaughter. The fifth and fourth treatments demonstrate a statistically significant superiority in pH ($p < 0.05$) compared to the other treatments. The table makes it evident that higher amounts of *C. rotundus* powder as a nutritional supplement in diets enhances the inherent qualities of the meat, demonstrating its beneficial effects.

The table clearly indicates that increased concentrations of *C. rotundus* powder in animal feed correlates with enhanced pH stability, demonstrating that adding it as a nutritional supplement improves the quality of the meat's natural qualities. This could be due to the phenolic compounds and volatile oils in the plant which protect meat protein and reduce chemical degradation that could result in an unusual decline in pH. In addition, it stops proteins from denaturation, which protects the muscle fibers of the meat thereby enhancing its taste and consumer appeal (36).

Table 1: Effect of adding *C. rotundus* powder and vitamin E to broiler feed on some physical characteristics of their thigh meat.

Property	Treatment					Sig. level	Avg. characteristics	Avg. SE
	T1	T2	T3	T4	T5			
pH	5.96 d	6.02 b	6.03 b	6.04 ab	6.05b	<.0001	6.02	0.016
Water holding capacity %	22.2 d	23.2 c	22.7 c	23.8 b	24.3 a	<.0001	23.24	0.179
Cooking loss %	24.7 a	19.1 b	18.3 b	17.8 b	15.6 b	0.4716	19.1	1.53

**** n.e.: no significant differences between the treatments at significance level ($P \leq 0.05$).
a - d: different letters in the same row indicate significant differences between the treatments at significance level ($P \leq 0.05$).**

Water holding capacity: Table 1 also indicates the positive effects of a diet supplemented with *C. rotundus* powder on the water-holding capacity of avian meat. The results indicate that the fifth treatment had significantly superior water retention capacity ($p < 0.05$) over the others, specifically the fourth, followed by the third and second treatments (which contained vitamin E), which registered no significant differences. The control group demonstrated the lowest water-holding capacity. This might be because of the way *C. rotundus* tubers are made, containing chemicals that protect tissues from damage, making them better able to keep their shape.

The presence of carbohydrates and certain active compounds, such as flavonoids, enhances water binding to proteins, resulting in decreased moisture loss and reduced loss during cooking. Furthermore, these active compounds might interact with meat proteins like actin and myosin, enhancing the tissues' capacity to retain water (7). Adding *C. rotundus* powder to the feed makes meat better at holding water by raising the concentration of active compounds in the bird's body, boosting its immune system, and stopping the activity of free radicals. This protects muscle fibers, which improves the quality of meat, makes it more tender and better at holding water, all of which are important factors determining consumer preferences.

Loss during cooking: Table 1 illustrates the positive effects of feeding birds with a diet enriched with *C. rotundus* powder in terms of a reduction of chicken thigh weight during cooking. While the fifth treatment recorded the least loss, it provides evidence that the higher the amount of *C. rotundus* in the feed, the better the meat's ability to retain weight during cooking, while the control treatment recorded the greatest loss. This is due to the active compounds contained in *C. rotundus*, which led to improving the binding strength of meat tissues and increasing bound water, thus reducing loss during cooking. The active compounds in *C. rotundus* enhance meat quality by limiting cooking loss. These compounds help retain water content in the meat, protect muscle cells, and improve protein composition, thereby promoting muscle cohesion and reducing water loss at elevated temperatures (4). The natural oils and compounds in the plant improve the quality of feed and keep meat proteins and fats from going bad. This keeps nutrients and weight from being lost during cooking, which is important for the industry (14).

Malondialdehyde in the thigh: Table 2 presents the results of malondialdehyde as an indicator of oxidation. Adding different amounts of *C. rotundus* powder in the chicken feed improved the control of fat oxidation in its thigh meat. All experimental treatments demonstrated statistically significant reductions ($P < 0.05$) in MDA values compared to the control treatment, which contained no additives, with the fourth and fifth treatments exhibiting the greatest antioxidant activity. The inclusion of *C. rotundus* tubers in the diet led to a decrease in oxidative stress by inhibiting free radical damage to biofilms (3).

Free fatty acids and peroxide value: Table 2 also shows a significant superiority ($p < 0.05$) in the peroxide values (PV) of chicken thigh meat samples in the control compared to the other treatments, with the lowest significant values found in the fourth and fifth treatments (9). The high PVs in Table 2 indicate increased levels of oxidation, which affects the quality and shelf life of the meat. The table also shows

that adding *C. rotundus* powder to the bird feed reduced PVs. Also, there was a significant superiority ($p < 0.05$) in PV of chicken thigh meat in the control compared to the other treatments, while the lowest values appeared in the fourth and fifth treatments. The addition of *C. rotundus* powder to bird feed significantly reduced PVs, thereby decreasing the oxidation of thigh meat due to its phenolic and flavonoid constituents. When added to feed these compounds act as antioxidants and retard the oxidation of meat fat, as seen in the meat's PVs (13).

Table 2: Antioxidant effect on broiler thigh meat from adding *C. rotundus* powder and vitamin E to chicken feed.

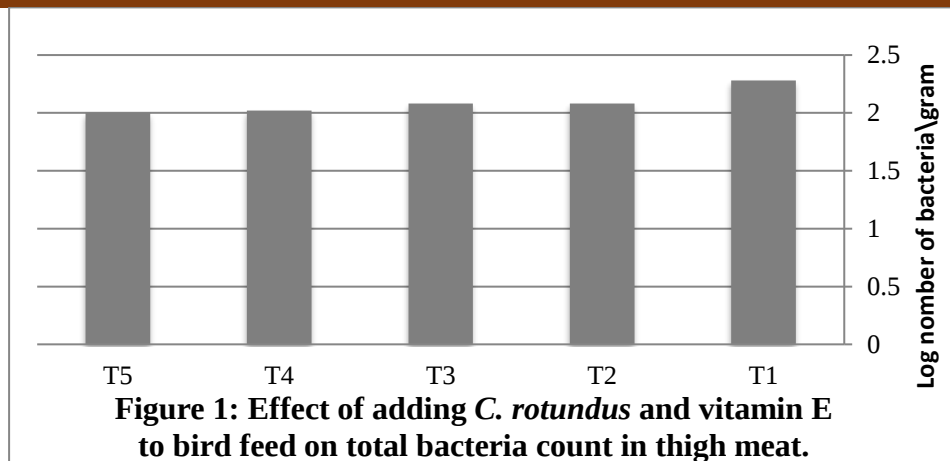
Property	Treatment					Sig. level	Avg. characteristic	Avg. SE
	T1	T2	T3	T4	T5			
MDA mg/kg meat	0.574 a	0.280 b	0.246 b	0.166 c	0.148 c	<.0001	0.283	0.041
PV milliequivalent/kg meat	1.41 a	0.780 b	0.616 c	0.470 d	0.433 d	<.0001	0.743	0.096
% FFA	0.400 a	0.333 ab	0.326 abc	0.263 bc	0.253 c	0.0060	0.315	0.016

**** n.e.: no significant differences between the treatments at significance level ($P \leq 0.05$).**

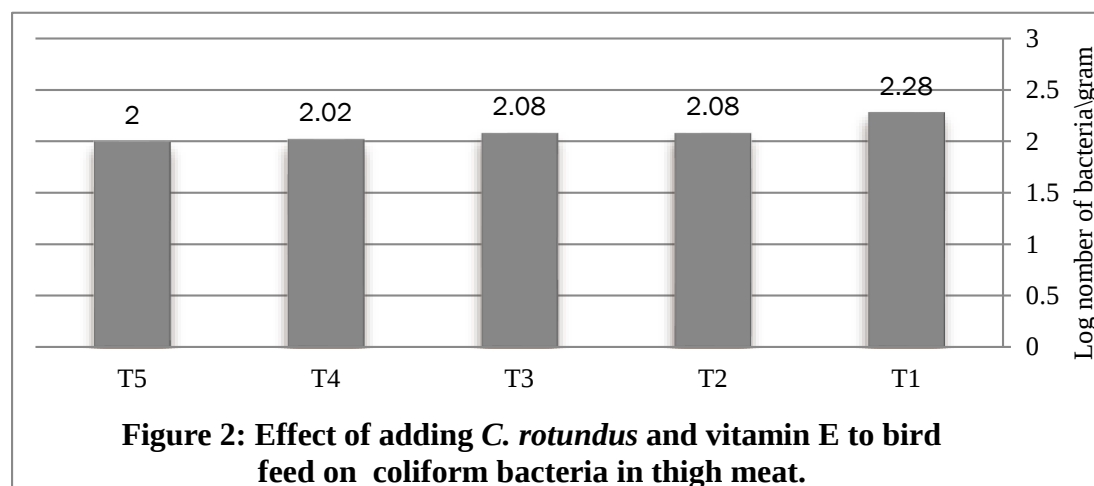
a – d: Different letters in the same row indicate significant differences between the treatments at significance level ($P \leq 0.05$).

Furthermore, the addition of *C. rotundus* powder into the feed enhanced the quality characteristics of the resultant meat by decreasing the concentration of free fatty acids. Table 2 demonstrates a significant superiority ($p < 0.05$) in the percentage of free fatty acids in the control, second, and third treatments relative to the fourth and fifth treatments, resulting in a diminished rancid odor of the meat. The active ingredients regulate the free fat content in chicken meat by enhancing muscle development and diminishing fat accumulation. This reduces the concentration of free fatty acids in the broiler organism while simultaneously strengthening immunity, thereby enhancing the health of broilers, and reducing fat oxidation. This is positively evidenced by the reduction of resultant fatty acids (35).

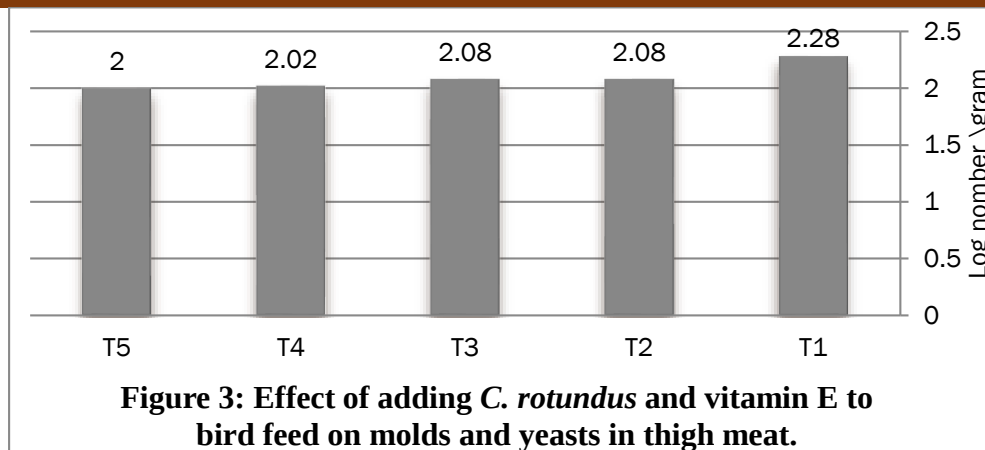
Effect of *C. rotundus* on total bacterial count: Figure 1 shows the impact of adding *C. rotundus* powder and vitamin E into broiler feed on total bacterial count in the thigh meat. A significant superiority in bacterial count appears in the control compared to other treatments, with the lowest counts recorded in the fourth and fifth treatments. The active compounds, including alkaloids and tannins, in the powder exerts inhibitory effects on microorganisms, leading to the suppression of microbial activity in treatments with elevated concentrations of *C. rotundus* (46).



Effect *C. rotundus* on coliform bacteria: Figure 2 demonstrates the impact of introducing *C. rotundus* powder in bird feed during breeding on the coliform count in thigh meat samples. A substantial reduction in bacterial count is observed in the fifth and fourth treatments relative to the others, whereas the control had the highest significant value for coliforms. The presence of phenols, tannins, and volatile oils in *C. rotundus* contributes to the inhibition of coliforms. These compounds enhance the digestive process and promote digestive health by augmenting beneficial bacteria and diminishing harmful bacteria, including those in the colon, thereby decreasing their transfer to the thigh meat (20). Beneficial bacteria were activated in the avian digestive system during the 42-day rearing period as a result of *C. rotundus*. These effectively eradicated pathogenic bacteria and inhibited their proliferation within the intestines. This leads to an increase in beneficial bacteria and a decrease in colon bacteria that enter the meat during the processing of the bird's carcasses, thereby preventing meat spoilage (23).



Effect *C. rotundus* on molds and yeast: Figure 3 shows how *C. rotundus* powder and vitamin E reduce yeast and mold growth. The fifth treatment had the fewest molds and yeasts, but the fourth, third, and second treatments did not differ significantly. *C. rotundus* contains phenols and tannins, which limit the enzymatic activity of fungi and promote digestive health, lowering yeasts and molds and improving meat quality. Its oils block ion and nutrition transfer across fungal cell membranes, killing them (37).



Sensory evaluation: Table 4 presents the sensory evaluation results of broiler thigh meat subjected to a 42-day diet with varying concentrations of *C. rotundus* powder and vitamin E. Significant differences ($P < 0.05$) were observed for all sensory characteristics in comparison to the control group that received no additives. The fourth and fifth treatments demonstrated significant superiority ($P < 0.05$) over the others, whereas the control had the lowest significant value for flavor. The third, fourth, and fifth treatments demonstrated a significant increase in juiciness relative to the control group. The fifth and fourth treatments had significantly better general acceptance and appearance compared to the others ($P < 0.05$). The superiority of meat treatments incorporating the two elements can be attributed to the antioxidants and microorganisms present in the powder. These components inhibit the oxidation of fats and oils in both feed and meat, a primary factor in meat spoilage, while also influencing the flavor profile of the meat. This process not only decreases the microbial load in the digestive system during feeding but also positively influences contamination, thereby enhancing the quality of chicken meat (6).

Table 3: Effect of *C. rotundus* powder and vitamin E in chicken feed on the sensory characteristics and overall acceptability of thigh meat.

Treatment	Sensory evaluation				
	Flavor	Juiciness	Tenderness	Public acceptance	Appearance
T1	5.50 c	5.54 a	5.28 d	5.40 c	5.32 b
T2	5.76 bc	5.76 b	5.96 c	6.02 b	5.48 b
T3	6.10 b	6.36 a	6.20 bc	6.40 ab	6.28 a
T4	6.70 a	6.62 a	6.54 ab	6.66 a	6.42 a
T5	6.86 a	6.76 a	6.68 a	6.84 a	6.62 a
Prob	<.0001	<.0001	<.0001	0.0005	<.0001
Total mean	6.18	6.20	6.13	6.26	6.02
Sem	0.129	0.114	0.115	0.122	0.124

**n.e.: no significant differences between the treatments at a significance level ($P \leq 0.05$).

a - d: Different letters within the same row indicate significant differences between the treatments at significance level ($P \leq 0.05$).

Conclusions

This study examined the impact of adding *Cyperus* tuber powder and vitamin E to broiler chicken feed on their carcass thigh meat slices. *Cyperus* powder preserved thigh meat pH and minimized cooking losses. It also decreased fat oxidation, MDA, PV, and FFA 8 levels compared to the control. Chickens fed the powder had better flavor and microbiology in their thighs. No effect was seen with vitamin E-fed chicken thigh slices or the control. *Cyperus* tuber powder increased chicken thigh flesh taste, juiciness, and acceptability.

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No Supplementary Materials.

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Both authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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