



EFFECT OF LONGEVITY SPINACH LEAVES POWDER (LSP) AS A BROILER FEED ADDITIVE ON SOME PHYSICAL AND CHEMICAL PARAMETERS OF FROZEN STORED THIGH MEAT

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ABSTRACT

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This investigation aimed to ascertain the impact of various concentrations of Longevity Spinach leaf powder (LSP) as broiler feed additives on chemical composition, some physical traits (each pH, Water holding capacity, and cooking loss), chemical and oxidative traits (Thiobarbituric acid, Free fatty acids, Total volatile nitrogen, Myoglobin and Met-Myoglobin) and validity period of the thigh meat which preserved for different freezing periods. Ninety broiler chickens at the age of one day- (Ross-308) were distributed into six treatments randomly. A commercial concentrated diet was provided for all treatments. As a control, the T1 without additives. T2: 0.75 kg LSP per ton of feed; T3: 1.5 kg LSP per ton of feed; and T4: 2.25 kg LSP per ton of feed; T5: 3 kg LSP per ton of feed while T6 offers 3.75 kg of LSP per feed ton. After 35 days of rearing, 6 broilers were randomly slaughtered for each treatment. Carcasses were washed with cooled water and dried with gauze then kept at refrigeration for 8 hrs. Carcasses thigh cut were placed in polyethylene bags then stored for 0, 30, and 90 days at -18 °C and required measurements were collected at each period's end. Results indicated that LSP treatments, especially T5 (adding 3 kg LSP / ton of feed), had a positive effect on all studied traits for most storage periods, and suggested that using LSP powder as natural additives to broiler feed enhances the quality attributes of meat and prolongs its validity period in freezer storage.

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INTRODUCTION

The need for animal protein is largely met by the broiler chicken sector, primarily because the cost of broiler chicken meat is lower than that of other sources of animal protein, and is favored by most people, as an option for healthier meat (Saewan *et al.*, 2021). In fact, dietary and lifestyle changes have been the mainstay of recommendations to reduce the risk of cardiovascular disease. One such alteration has been to increase the intake of chicken meat and decrease red meat consumption (Altaieb and Batkowska, 2023).

Poultry meat has health benefits due to its high nutritional value, highly digestible proteins, unsaturated lipids, fat-soluble and B-complex vitamins, and

minerals. Broiler meat also has a variable and moderate energy content (Kralik and Kralik, 2017, Mustafa, *et al.*, 2022). Spoilage is one of the important problems that accompany the meat industry, from slaughtering to consumption, because of the properties that meat possesses. The high content of long-chain polyunsaturated fats in broiler meats could benefit the health of customers (Mawlood and Khidhir, 2018a), also meat from broilers is especially vulnerable to oxidative damage (Mawlood and Khidhir, 2018b), nevertheless, the oxidative stability of meats may be harmed by the presence of such fatty acids (Mir *et al.*, 2017). This process also forms toxic substances at advanced stages and reduces sensory qualities and consumer desire. (Domínguez *et al.*, 2019). Moreover, a variety of dangerous chemicals that are harmful to human health can be produced by lipid oxidation like free radicals (Mir *et al.*, 2017; Domínguez *et al.*, 2019). The growing consumer demand for safe animal food has been a problem for animal scientists, articulating an interest in the use of natural feed additives (Christaki *et al.*, 2020). Antioxidants are required in broiler meats to reduce lipid oxidation. In this instance, antioxidants may counteract free radicals or other catalysts that cause lipid oxidation in meats (Domínguez *et al.*, 2019).

In the production of broilers, artificial antioxidants have long been used as feed additives. However, prolonged use of synthetic antioxidants may be harmful to human health due to the chemical residue found in broiler meats (Petcu, *et al.*, 2023). Supplements containing natural antioxidants have been utilized to lessen the negative impacts on the physiological and health conditions of broilers, as well as their shelf life and meat quality.

The traditional use of longevity spinach (*Gynura procumbens* L.) has been validated by the finding of active chemical ingredients such as flavonoids (kaempferol, rutin, and quercetin), chlorogenic acids, phenolics, and steroids, which functions as natural antioxidants (Timotius and Rahayu, 2020). Longevity spinach is also known by this name. Tropical Asian nations like China, Thailand, Indonesia, Malaysia, and Vietnam are frequent places to find this plant (Ashraf, 2019). Due to its chemical bioactive contents, which include chlorogenic acid, essential oils, caffeic acid, para-fumaric acid, vanillic acid, para-hydroxy benzoic acid, unsaturated sterol, flavonoids, triterpenoid, polyphenol, steroids, and saponin, it is frequently used as medicine. The purpose of this study was to examine the effects of various dosages of powdered Longevity Spinach (LSP) Leaves on the oxidative, chemical, and physical characteristics as well as the shelf life of the produced thigh meat that was frozen for various lengths of time.

MATERIALS AND METHODS

Birds, feeding, and treatments

The 90 (one-day old) Ross-308 broiler chicks were housed in 18 cages; Cages floor is covered with sawdust. The chicks were randomly assigned to six treatments, with three replicates of each treatment and five birds per replicate. The replicates were randomly assigned and treatments started on the first day. LSP leaf powder was purchased from the Malaysian University of Putra. Table (1) shows the chemical composition of LSP leaves (Puangpronpitag *et al.*, 2010).

According to the Ross Broiler Management Guide, the aforementioned meals' components, chemical composition, and amounts were fed to the chicks. LSP leaves powder was added to the treatments in the following ways: T1: had no additives (control treatment), in T2, 0.75 kg LSP is added per ton of feed; in T3, 1.5 kg LSP is added per ton of feed; and in T4, 2.25 kg LSP is added per ton of feed. Add 3 kg of LSP per ton of feed in T5, and 3.75 kilograms of LSP per ton of feed in T6.

Throughout the 35-day breeding period, water was freely available. The Ross Broiler Management Guide's guidelines were generally followed for immunization schedules, lighting programs, ventilation and humidity levels, and temperature control

Table (1): Chemical composition of LSP leaves powder

Ingredients	% of LSP leaves powder extract
Moisture	19.17
Dry matter	80.83
Ash	18.11
Carbohydrates	0.0537-0.1968µg
Protein	4.51
Fat	0.023

Slaughter, samples, and preservation

Upon the end of the study, the fodder was removed, and six birds were chosen at random from each treatment group and slaughtered. After cleaning, the carcasses were placed in the refrigerator for eight hours. The meat from the thigh pieces was stored in polyethylene bags after the carcasses had been cut up. The following were the preservation periods: P1 was frozen for 0 days, P2 for 30 days, and P3 for 90 days at -18 degrees Celsius.

Measurements

Following the completion of each preservation period, examinations of chemical, physical, and oxidative indicators were carried out, and meat samples were measured for the following parameters: Chemical composition was determined using the techniques outlined in Association of Official Analytical Chemists and Helrich. (1990), Moisture content must determine as weight loss after the samples were dried in a convection oven at 105°C for 16hr. Protein content was determined by using micro Kjeldahl and was calculated as follows: Protein %= Nitrogen ×6.25. The percentage of fat in fish meat samples was estimated by taking a known weight of dried samples and extracted with diethyl ether using the Soxhlet apparatus. Ash content was determined by taking a known weight of flesh and placing it in a muffle furnace at 550 °C for 16 hrs. The ash percent was determined as follows: Ash %=W₁/W₂ ×100. Where W₁ = weight of ash, and W₂ = initial weight. pH of muscle sample measure according to the method described by Purchas and Barton (2012). Muscle samples (10gm) homogenize with 100 ml distilled water for 1 min, the pH then measure by a pH meter.

Water holding capacity (WHC) determine according to Wardlaw *et al.*, (1973). 20gm of minced muscle sample place in centrifuge tube containing 30ml of 0.6M NaCl and stirrer with glass rod for 1 min. The tube keeps at refrigeration temperature (4°C) for 15 min, stirrer again and centrifuge at 2806.1 xg (4°C) for 15 min. The

supernatant measure and amount of water retain by samples and express in percentage. The WHC report as ml of 0.6 M NaCl per 100g of muscle according to the following formula:

$$WHC\% = (Initial\ solution\ weight - final\ solution\ weight/sample\ weight(gm) \times 100$$

Cooking loss determine according to Murphy and Zerby (2004). Muscle samples (20gm) place in an open aluminum box and cook for 8.5 min in oven pre-heated to 176°C to an internal temperature of 70°C. After cooking, the samples must dry with a paper towel. Each sample cool for 30 min, cooking weight measure. The cooking loss calculates by the following formula:

$$Cooking\ loss\ \% = Raw\ sample\ weight - cooked\ sample\ weight \times 100$$

The TBA was determined according to the method described by Witte *et al.* (1970). 20 g of the muscle was blended with 50 ml of cold solution containing 20% trichloroacetic acid (TCA) in 2M phosphoric acid. The resulting slurry was transferred to a 100 ml volumetric flask with 40 ml distilled water. The sample was diluted to 100 ml with distilled water and homogenized by shaking. A 50 ml protein was filtered through Whatman No.1 filter paper. 5 ml of filtrate was transferred to a test tube followed by 5 ml of fresh thiobarbituric acid (TBA) (0.005M in distilled water). The blank was prepared by mixing 5 ml of distilled water with 5 ml of TBA. The solution mixture was kept in the dark for 15-17 h at room temperature to develop the colour reaction. The absorbance was read at 530 nm by using spectrophotometer (Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde (MDA)/kg muscle, which was calculated by multiplying the absorbance (A) by 5.2 factors as follows:

$$TBA\ value\ (mg\ MDA/kg\ muscle) = A_{530} \times 5.2$$

Free fatty acids were determined as reported by Egan *et al.* (1981). Weights of 10 gm of sample were placed in a mechanical blender and 25ml of chloroform was added, the mixture was blended for 2-3 min and filtered immediately through a large filter paper. This was then re-filtered through a paper containing a small amount of anhydrous sodium sulfate. Portions of the filtrate were used for the determination of free fatty acids as follows: Five ml of 95% ethanol neutralized with drops of 0.1 N NaOH after adding phenolphthalein. The solution was added to 5 ml of the filtered and the mixture titrated with 0.1 N NaOH until the pink colour persists for 15 seconds. The FFA calculates as oleic acid as percentage of the sample:

$$Free\ fatty\ acid\ (FFA.)\ \% = \frac{ml\ of\ 0.1\ NaOH \times 0.0282 \times dilution\ factor}{sample\ weight} \times 100$$

Total volatile nitrogen (TVB-N) (Malle and Poumeyrol, 1989), A 100 g of the minced samples were mixed for 1 min with 200 ml of 7.5% Trichloroacetic acid (TCA) in the blender, the mixture was filtered, 25 ml of the filtrate were transferred to macro-kjeldahl distillation apparatus of 250 ml capacity, then 5 ml of 10 % NaOH solution were added to the distillation which was carried out, and the distillate was collected in 15 ml of 4% boric acid. The distillate was titrated with 0.05 N H₂ SO₄, using methyl red –bromocresol green as an indicator. The blank was carried out using 25 ml of 7.5% Trichloroacetic acid instead of the meat sample, the T.V.N. value was estimated as following:

$$TVB.N.(mg\ N/100gm) = (V \times 14 \times (200 + M/100 \times 100))/25 \times 100$$

Where: V= ml of 0.05 of H₂SO₄, M=moisture content

Myoglobin and Met-myoglobin were determinate according to Krzywicki (1982). Five gm of minced meat was used to determine MMb concentration in each sample. Myoglobin was extracted with cold 0.04 M phosphate buffer, pH 6.8, with a sample to buffer ratio of 1:10. Samples were homogenized for 15 second with an Ultraturrax homogenizer T20 Standard (IKA, Staufen, Alemania) at speed 13500 rpm. The homogenates were then centrifuged for 30 min at 5000 rpm. The absorbance of the filtered supernatant was read at 525, 572 and 700 nm. Mb was determining using the formula of Krzywicki (1982).

$$Mb\ (mg/kg) = (A_{525} - A_{700}) \times 2,303 \times 5\ (dilution\ factor) \times 1000$$

and percentage MMb was determining using the formula of Krzywicki (1982).

$$MMb\ (\%) = 1.395 - ((A_{572} - A_{700})/(A_{525} - A_{700})) \times 100$$

Samples were kept on ice at all points during the assay.

Statistical analysis

To examine the impact of each treatment and period on various parameters, a simple factorial experiment with a complete randomized design (CRD) was conducted. The statistical analysis program SAS (2010) and Duncan's (1955) multinomial test were used to analyze the significant differences between the means.

RESULTS AND DISCUSSION

The results show that adding of LSP powder effect significantly on chemical composition of broiler chicks' thigh meat Table (2). Adding LSP powder to feed of chick's effect significantly ($p \leq 0.05$) on moisture percentages, the highest moisture percentages recorded in T5(3 kg LSP / ton of feed) meat which were 76.257, 76.038 and 75.619% for the 0, 30, and 90-days storage periods respectively, while the lowest moisture percentages were in T1(control) meat which were 74.483, 74.375 and 72.633% for the same storage periods respectively. About the effect of storage periods, only T1(control) meat showed a significant decrease ($p \leq 0.05$) in the moisture content, 90-day storage period recorded the lowest percentage (72.633%) compared to 0- and 30 days storage periods (74.483 and 74.375%) respectively.

The protein content of thigh meat increased significantly($p \leq 0.05$) with adding LSP powder to chicks' feed table (2), for each storage period, all treatments surprised. Meat of T1(control), T5 (3 kg LSP / ton of feed) treatments, recorded the highest protein percentage (21.935, 20.809 and 19.879%) for 0, 30 and 90 days storage periods respectively, and the lowest percentage (21.141, 20.108 and 19.141%) noticed in T1(control) meat for the same storage periods respectively. With the extension of storage periods, there was a significant ($p \leq 0.05$) decrease in protein content for all treatments.

Lipid content increased significantly ($p \leq 0.05$) for all storage periods with additive treatments and surpassed T1(control) meat which recorded the lowest percentage (3.469, 3.440, and 3.339%) for the 0, 30, and 90 days storage periods

respectively, for all treatments with increasing storage period there was a significantly ($p \leq 0.05$) decrease in lipid percentage especially for 90 days as compared with 0 and 30 days storage.

The significantly ($p \leq 0.05$) highest ash content recorded in thigh meat from broiler of T5(3 kg LSP / ton of feed) (0.596%) at 0 days' storage which surpassed the other treatments, also thigh meat of T5(3 kg LSP / ton of feed) showed the highest ash content (0.588 and 0.579%) at 30 and 90 days storage periods respectively, while T1(control) recorded the lowest percentage (0.499 and 0.491%) for the same storage periods respectively. There was a significant ($p \leq 0.05$) decrease in ash content with an expanding storage period for T5(3 kg LSP / ton of feed) meat. The increase in the chemical composition of the additive treatments and surpassed the control may be due to the role of the LSP compounds with strong antioxidant activity that protects cell membranes and the components from the oxidative damage process and free radicals, thus preserving their composition content (Arora *et al.*, 2000).

Table (2): Effect of different LSP powder concentrations and freeze-preservation periods on chemical composition of broiler thigh meat (mean \pm std error).

Treat	Moisture %			Protein %			Lipid %			Ash %		
	Storage period (days)											
	0	30	90	0	30	90	0	30	90	0	30	90
T1	b A	b A	c B	d A	c B	c C	a A	a A	b B	b A	c A	c A
	74.483	74.375	72.633	21.141	20.108	19.141	3.469	3.440	3.339	0.505	0.499	0.491
	±0.489	±0.395	±1.892	±0.022	±0.152	±0.155	±0.044	±0.013	±0.013	±0.007	±0.011	±0.017
T2	ab A	ab A	b A	c A	b B	b C	a A	a A	a B	b A	bc A	bc A
	75.204	74.996	74.127	21.355	20.443	19.400	3.471	3.470	3.359	0.512	0.505	0.497
	±0.182	±0.006	±0.201	±0.118	±0.096	±0.053	±0.041	±0.042	±0.055	±0.004	±0.007	±0.011
T3	ab A	ab A	ab A	b A	a B	b C	a A	a A	a B	b A	bc A	bc A
	75.267	75.118	74.403	21.663	20.639	19.479	3.499	3.493	3.418	0.515	0.513	0.505
	±0.093	±0.152	±0.216	±0.053	±0.105	±0.048	±0.017	±0.011	±0.043	±0.004	±0.004	±0.004
T4	ab A	ab A	ab A	ab A	a B	a C	a A	a A	a B	b A	b A	b A
	75.635	75.399	75.003	21.827	20.743	19.747	3.502	3.495	3.395	0.523	0.519	0.510
	±0.151	±0.037	±0.159	±0.024	±0.078	±0.040	±0.018	±0.021	±0.020	±0.004	±0.004	±0.003
T5	a A	a A	a A	a A	a B	a C	a A	a A	a B	a A	a AB	a B
	76.257	76.038	75.619	21.935	20.809	19.879	3.509	3.492	3.395	0.596	0.588	0.579
	±0.921	±0.654	±0.540	±0.049	±0.012	±0.030	±0.013	±0.033	±0.036	±0.001	±0.001	±0.001
T6	ab A	ab A	ab A	ab A	a B	a C	a A	a A	a B	b A	bc A	b A
	75.816	75.484	74.986	21.752	20.745	19.731	3.500	3.497	3.401	0.523	0.515	0.510
	±0.089	±0.281	±0.021	±0.178	±0.064	±0.043	±0.017	±0.017	±0.013	±0.011	±0.007	±0.006

- Different lowercase letters indicate significant differences ($p \leq 0.05$) between treatments for each trait.

- Different uppercase letters indicate significant differences ($p \leq 0.05$) between periods for each trait. T1: had no additives (control treatment), T2: 0.75 kg LSP is added per ton of feed; T3: 1.5 kg LSP is added per ton of feed; T4: 2.25 kg LSP is added per ton of feed. T5: Add 3 kg of LSP per ton of feed, T6: 3.75 kilograms of LSP per ton of feed.

Results in Table (3) revealed that adding LSP powder as a feed additive affect significantly ($p \leq 0.05$) on physical traits of thigh meat of broiler chicks. pH value results revealed that the highest pH values were in the thigh meat of broiler chicks

from T5(3 kg LSP / ton of feed) which were 6.250 and 6.250 for 0 and 30 days' storage respectively and surpassed the other treatments, also at 90 days storage thigh meat of broiler chicks from T5 (3 kg LSP / ton of feed) recorded the highest pH (6.350), while the lowest value (5.750) was in T1(control) for the same storage period. No significant ($p \leq 0.05$) differences were detected in pH value between storage periods.

WHC content of thigh meat increased significantly($p \leq 0.05$) with adding LSP powder to chick's feed table (3), the highest WHC percentages recorded in thigh meat of broiler chicks from T5(3 kg LSP / ton of feed) (69.500 and 68.500%) at 0 and 30 days' storage respectively in comparison control group, which recorded the lowest percentage (65.500 and 64.500%) for the same storage periods respectively. WHC values in thigh meat of broiler chicks from T3 (1.5 kg LSP / ton of feed), thigh meat of broiler chicks from T4 (2.25 kg LSP / ton of feed), thigh meat of broiler chicks from T5 (3 kg LSP / ton of feed) and thigh meat of broiler chicks from T6 (3.75 kg LSP / ton of feed) decreased with the increase in the preservation period.

As shown in Table (3), Cooking loss percentages reduced significantly ($p \leq 0.05$) in thigh meat of broiler chicks from T5(3 kg LSP / ton of feed) which recorded the lowest percentages (26.906, 28.950 and 32.483%) at 0, 30 and 90 days storage respectively, while the highest cooking loss percentages were in thigh meat of broiler chicks from T1(control) (37.874, 34.732 and 39.098%) for the same storage periods respectively, with the expanding of storage periods there were significant ($p \leq 0.05$) increased in cooking loss value for all treatments.

The relationship between pH and meat quality, and thus validity period, is what gives pH its ultimate significance. A high pH promotes water-binding and reduces cooking loss because it affects the contractile fibers' tendency to shrink. pH is correlated with the WHC value, which is a measure of the quality of meat (Balcha and Hagos, 2018). The use of natural antioxidants as feed additives with broiler diets increased pH and WHC. Feed additives containing antioxidants (flavonoids) also increased WHC may have reduced water release by protecting the protein-water contact. preventing oxidative damage to muscle tissue and improved cooking yield could be attributed to meat's capacity to retain water (Ma *et al.*, 2019, Majid, *et al.*, 2020a and Majid *et al.*, 2020b,). Arora *et al.* (2000) found that protecting muscle fiber's cellular membranes intact maintains the cellular components, lowering drip loss and raising WHC during storage. The use of antioxidants sources may have significant effects on keeping the pH and WHC of meat samples at optimum levels (Al-Obaidi *et al.* 2022). The effects of meat lipolysis enzymes and microbes, which promote the release of amine groups, may be the cause of an increase in meat pH with longer storage times (Verma *et al.*, 2008, Abdullah and Qudsieh, 2009 and Muela *et al.*, 2010,). Conversely, a longer storage duration may cause a drop in WHC because of increased protein hydrolysis, which reduces the meat's capacity to hold water (Muela *et al.*, 2015).

As a result of cellular membranes being damaged by the physical impact of ice crystals, extending the storage period decreases WHC, which increases thaw loss and cooking loss (Lu *et al.*, 2019). Additionally, meat water loss caused by proteins denaturation negatively impact the product's texture, making it unacceptable to

consumers (Khalid *et al.*, 2023). As the storage time goes on, the ice crystals' width grows, and their damage increases (Fernández *et al.*, 2007).

Table (3): Effect of different LSP powder concentrations and freeze-preservation periods on some physical traits of broiler thigh meat (mean±std error)

Treat	pH			WHC%			Cooking loss%		
	Storage period (days)								
	0	30	90	0	30	90	0	30	90
T1	b A 5.750 ±0.071	b A 5.700 ±0.141	b A 5.750 ±0.071	b A 65.500 ±0.707	b A 64.500 ±0.707	a A 62.500 ±2.121	a A 34.732 ±0.437	a B 37.874 ±0.313	a C 39.098 ±1.044
T2	b A 5.750 ±0.212	ab A 5.850 ±0.212	ab A 6.000 ±0.283	ab A 66.500 ±0.707	ab A 65.500 ±0.707	a A 63.500 ±0.707	ab A 34.488 ±0.615	ab B 36.674 ±0.030	b B 37.093 ±0.077
T3	b A 5.700 ±0.283	b A 5.750 ±0.212	ab A 6.000 ±0.141	ab A 67.500 ±0.707	ab AB 66.500 ±0.707	a B 64.000 ±1.414	c A 33.006 ±0.016	bc B 35.840 ±0.184	c B 35.757 ±0.564
T4	ab A 5.950 ±0.071	ab A 6.050 ±0.071	a A 6.250 ±0.071	ab A 68.500 ±2.121	ab AB 67.500 ±0.707	a B 64.500 ±0.707	c A 32.905 ±0.063	c B 35.146 ±0.067	bc B 36.057 ±0.082
T5	a A 6.250 ±0.071	a A 6.250 ±0.071	a A 6.350 ±0.495	a A 69.500 ±2.121	a AB 68.500 ±0.707	a B 65.500 ±0.707	e A 26.906 ±1.585	d B 28.950 ±0.073	d C 32.483 ±0.416
T6	b A 5.700 ±0.141	b A 5.750 ±0.071	ab A 5.950 ±0.071	a A 69.000 ±2.828	ab AB 67.000 ±0.000	a B 64.000 ±1.414	bc A 33.446 ±0.607	c B 35.095 ±0.149	c B 35.711 ±0.409

- Different lowercase letters indicate significant differences ($p \leq 0.05$) between treatments for each trait.

- Different uppercase letters indicate significant differences ($p \leq 0.05$) between periods for each trait. T1: had no additives (control treatment), T2: 0.75 kg LSP is added per ton of feed; T3: 1.5 kg LSP is added per ton of feed; T4: 2.25 kg LSP is added per ton of feed. T5: Add 3 kg of LSP per ton of feed, T6: 3.75 kilograms of LSP per ton of feed.

Results present in Table (4) show significant differences ($p \leq 0.05$) among treatments for both TBA and FFA percentages during the storage period. Using LSP powder as a feed additive led to decreased TBA value in comparison to T1(control) which recorded the highest TBA (1.424 and 1.846 mg MDA/kg meat) for 30 and 90 days of storage respectively. The thigh meat of broiler chicks from treatments T1(control) and T2(0.75 kg LSP / ton of feed) recorded a significant ($p \leq 0.05$) increase for the values of TBA trait with the increase in the preservation period. The lowest FFA percentages were in the thigh meat of broiler chicks from T5(3 kg LSP / ton of feed) (0.035, 0.091, and 0.600%) for 0, 30, and 90 days' storage respectively, while the lowest FFA values recorded in thigh meat of broiler chicks from T1(control) (0.210, 0.522 and 0.955%) for the same storage periods respectively. Also, FFA value increased in with increasing preservation periods. The concentration of malondialdehyde (MDA) determines the TBA value, one of the markers of lipid oxidation. If the value of meat does not exceed 2 mg of malondialdehyde per kilogram of meat, it is regarded as normal (Greene and Cumuze,1982), Due to their ability to

block the chain reaction involved in fat oxidation, certain feed additives including antioxidants that transfer to meat decreased MDA production and lipid oxidation (Florou-Paneri, *et al.*,2005). LSP like other natural feed additives contains chemical bioactive compounds, such as flavonoids (Stoev, 2024). Which reduced meat MDA and made the tissue content of total phenols increase when these feed additives were used (Sohaib, *et al.*2015). Flavonoids can improve the antioxidant capacity in animal tissues by reducing oxygen concentrations and quenching oxygen, thus preventing peroxide production while activating antioxidant enzymes such as superoxide dismutase and catalase (Procházková, *et al.*,2011).

FFA is a measure that quantifies the stability of fats and their resistance to breakdown brought on by the presence of lipolytic enzymes and microorganisms. FFA is allowed up to 0.5–1.5 oleic acid (Mawlood and Khidhir, 2018a). Therefore, for all storage periods and additional treatments, the FFA value significantly decreases when natural antioxidants are present. This decrease is caused by active substances such as flavonoids, which limit the growth of bacteria that secrete lipolytic enzymes (Al-Rubeii, *et al.*,2009 and Maqsood, *et al.*,2015).

Table (4): Effect of different LSP powder concentrations and freeze-preservation periods on some chemical traits of broiler thigh meat (mean \pm std error)

Treat.	TBA (mg malonaldehyde/kg Muscle)			FFA %		
	Storage period (days)					
	0	30	90	0	30	90
T1	a 0.658±0.068	a 1.424±0.641	a 1.846±0.048	a 0.210±0.013	a 0.522±0.030	a 0.955±0.064
T2	a 0.432±0.008	b 0.817±0.022	b 0.830±0.004	b 0.106±0.006	b 0.319±0.012	b 0.856±0.035
T3	a 0.393±0.003	b 0.685±0.001	b 0.734±0.015	bc 0.078±0.006	c 0.170±0.006	c 0.744±0.008
T4	a 0.380±0.008	b 0.653±0.004	b 0.671±0.015	bc 0.066±0.004	cd 0.144±0.006	c 0.720±0.030
T5	a 0.333±0.007	b 0.515±0.007	b 0.608±0.001	c 0.035±0.005	d 0.091±0.010	d 0.600±0.001
T6	a 0.369±0.001	b 0.577±0.007	b 0.671±0.013	c 0.044±0.001	cd 0.133±0.046	c 0.714±0.020

- Different lowercase letters indicate significant differences ($p \leq 0.05$) between treatments for each trait.

- Different uppercase letters indicate significant differences ($p \leq 0.05$) between periods for each trait. T1: had no additives (control treatment), T2: 0.75 kg LSP is added per ton of feed; T3: 1.5 kg LSP is added per ton of feed; T4: 2.25 kg LSP is added per ton of feed. T5: Add 3 kg of LSP per ton of feed, T6: 3.75 kilograms of LSP per ton of feed.

Table (5) shows the presence of a significant decrease ($p \leq 0.05$) in TVN value for LSP additive treatments for all periods of preservation, thigh meat of broiler chicks from T5(3 kg LSP / ton of feed) had the lowest TVN values, which were 3.760, 7.639 and 12.330 mg N/100 gm meat) for 0, 30, and 90 days storage respectively, and thigh meat of broiler chicks from T1(control) had the highest values (10.760, 14.000, and 17.993 mg N/100 gm meat) for the same storage periods respectively. Extending storage periods resulted in a significant ($p \leq 0.05$) rise in TVN levels for all treatments.

TVN is a common biomarker for nitrogenous compound degradation. Meat deterioration happens when metabolic processes or microbial activity modify its physical and chemical qualities to an unacceptable amount or limit (Alaa El-Din, *et al.*, 2021). The frozen chicken TVN Value was set by the Iraqi Central Organization for Standardization and Quality Control (IQS 1179) to not exceed 20 mg N/100g meat (Mawlood and Khidhir, 2018a), meat from all our treatments not exceed this. Proteins are protected by natural antioxidants against oxidative stress and the ensuing degradation (Pogorzelska, *et al.*, 2018). Reducing lipid oxidation may improve the stability of proteins because it influences the breakdown of proteins in freeze-preserved products and because the radicals produced by lipid oxidation are expected to intensify protein oxidation (Estévez, *et al.*, 2008; Moroney, *et al.*, 2013). Many of the most common bacterial species found in meat, including *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are inhibited by flavonoids (Guzzo *et al.*, 2020).

Table (5): Effect of different LSP powder concentrations and freeze-preservation periods on TVN value of broiler thigh meat (mean±std error)

Treat.	TVN (mg N/100 gm muscle)		
	Storage period (days)		
	0	30	90
T1	a A 10.760±0.125	a B 14.000±0.129	a C 17.993±0.052
T2	b A 8.375±0.661	b B 12.395±0.803	a C 17.982±0.184
T3	c A 6.160±0.478	b B 11.424±0.696	b C 16.430±0.776
T4	c A 5.621±0.381	c B 8.371±0.797	c C 14.216±0.775
T5	d A 3.760±0.123	c B 7.639±0.284	d C 12.330±0.614
T6	c A 5.758±0.307	c B 8.086±0.485	c C 13.807±0.192

- Different lowercase letters indicate significant differences ($p \leq 0.05$) between treatments.

- Different uppercase letters indicate significant differences ($p \leq 0.05$) between periods.

T1: had no additives (control treatment), T2: 0.75 kg LSP is added per ton of feed;

T3: 1.5 kg LSP is added per ton of feed; T4: 2.25 kg LSP is added per ton of feed.

T5: Add 3 kg of LSP per ton of feed, T6: 3.75 kilograms of LSP per ton of feed.

The results of thigh meat color represented in Table (6), results revealed that adding LSP powder as a feed additive affected significantly ($p \leq 0.05$) both Mb and Met-Mb values and protected the color, while the storage period was not affected significantly ($p \leq 0.05$) on both color traits, Mb value was higher in thigh meat of broiler chicks treated with additive LSP powder and T5(3 kg LSP / ton of feed) showed the highest Mb values, which were 0.301, 0.296 and 0.294 mg/gm meat for 0, 30 and 90 days storage periods respectively, in contrast the lowest values recorded in thigh meat of broiler chicks from T1(control) which were 0.197, 0.192 and 0.188

mg/gm meat for the same storage periods respectively. The lowest Met-Mb percentage was in the thigh meat of broiler chicks from T5(3 kg LSP / ton of feed) (45.726 and 48.550%) for 0 and 90 days' storage respectively, while the highest percentages were in the thigh meat of broiler chicks from T1(control) which were 50.021 and 51.990 % for the same storage periods respectively. Color is one of the most important quality attributes of meat and effects on consumer acceptance of a product. Meat color is influenced by the quantity and chemical composition of the pigment myoglobin; oxidation led to unattractive colored metmyoglobin appears (Ramanathan *et al.*,2020). Since Mb oxidation can be influenced by fat oxidation, additional antioxidants that reduce fat oxidation also reduce Mb oxidation, Met-Mb formation, and brown color formation. They achieve this by providing hydrogen, which creates a reduction state, protecting pigment and saturated fatty acids from oxidation, and lowering the generation of free radicals (Mitsumoto *et al.*, 2005; Faustman *et al.*, 2010).

Table (6): Effect of different LSP powder concentrations and freeze-preservation periods on some color traits of broiler thigh meat (mean \pm std error)

Treat.	Mb (mg/gm Meat)			Met-Mb %		
	Storage period (days)					
	0	30	90	0	30	90
T1	b A 0.197±0.002	c A 0.192±0.001	c A 0.188±0.001	a A 50.021±0.182	a A 50.614±0.543	a A 51.990±0.014
T2	b A 0.211±0.014	b A 0.210±0.014	b A 0.208±0.014	a A 49.070±1.696	a A 49.636±2.455	ab A 50.390±0.863
T3	a A 0.294±0.005	a A 0.290±0.003	a A 0.288±0.004	ab A 48.427±0.662	a A 48.999±1.413	ab A 50.299±0.568
T4	a A 0.294±0.001	a A 0.291±0.001	a A 0.288±0.003	ab A 48.529±1.596	a A 49.077±2.371	ab A 49.849±0.391
T5	a A 0.301±0.002	a A 0.296±0.006	a A 0.294±0.006	b A 45.726±0.998	a A 47.829±0.412	b A 48.550±0.778
T6	a A 0.291±0.001	a A 0.289±0.002	a A 0.286±0.001	ab A 48.465±1.648	a A 49.110±0.863	ab A 49.350±0.354

- Different lowercase letters indicate significant differences ($p \leq 0.05$) between treatments for each trait.

- Different uppercase letters indicate significant differences ($p \leq 0.05$) between periods for each trait. T1: had no additives (control treatment), T2: 0.75 kg LSP is added per ton of feed; T3: 1.5 kg LSP is added per ton of feed; T4: 2.25 kg LSP is added per ton of feed. T5: Add 3 kg of LSP per ton of feed, T6: 3.75 kilograms of LSP per ton of feed.

CONCLUSIONS

When LSP leaf powder is added to poultry feed, it works on the synthetic and actual qualities, that lead to improves the chemical and physical characteristics, decrease the fat and color oxidation, and extends the meat's shelf life under freezing, so we recommended to use LSP leaf in poultry feed to improve meat quality.

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CONFLICT OF INTEREST

We declare that we don't have affiliation or entity with any organization regarding the financial a non- financial interest in this subject matter discussed in this article.

تأثير مسحوق أوراق السبانخ الطويلة كإضافات علفية لفروج اللحم في الصفات الفيزيائية والكيميائية للحم الفخذ المجمد

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الخلاصة

هدف هذا البحث إلى التحقق من تأثير تراكيز مختلفة من مسحوق أوراق السبانخ طويلة العمر كإضافات علفية لفروج اللحم على التركيب الكيميائي، وبعض الصفات الفيزيائية (الاس الهيدروجيني، قابلية مسك الماء، وفقدان اثناء الطبخ)، والصفات الكيميائية والأكسدة (حامض الثايوبايتريك، الاحماض الدهنية الحرة، النتروجين الكلي المتطاير، الميوكلوبين والميتوكلوبين) ومدة صلاحية لحم الفخذ الذي تم حفظه لفترات تجميد مختلفة. تم توزيع 90 دجاجة لحم بعمر يوم واحد (روس 308) على ست معاملات عشوائياً، تم توفير نظام غذائي تجاري مركز لجميع المعاملات. كمعاملة سيطرة (T1) بدون أي إضافات، المعاملة الثانية (T2) اضافة 0.75 كغم مسحوق اوراق السبانخ لكل طن من العلف، المعاملة الثالثة 1.5 (T3) كغم مسحوق اوراق السبانخ لكل طن من العلف، المعاملة الرابعة 2.25 (T4) كغم مسحوق اوراق السبانخ لكل طن من العلف، المعاملة الخامسة 3 (T5) كغم مسحوق اوراق السبانخ لكل طن من العلف والمعاملة السادسة 3.75 (T6) كغم مسحوق اوراق السبانخ لكل طن من العلف. وبعد 35 يوماً من التربية، تم ذبح 6 افراخ اختيرت عشوائياً من كل معاملة. غسلت الجثث بالماء البارد وجففت بالشاش ثم حفظت في الثلاجة لمدة 8 ساعات. تم وضع قطع أفخاذ الذبائح في أكياس من البولي إيثيلين، ثم تم تخزينها لمدة 0، 30، و90 يوماً عند -18 درجة مئوية وتم جمع القياسات المطلوبة في نهاية كل فترة. أشارت النتائج إلى أن معاملات اوراق السبانخ وخاصة T5 (إضافة 3 كغم مسحوق اوراق السبانخ لكل طن من العلف) كان لها تأثير إيجابي على جميع الصفات المدروسة لمعظم فترات التخزين، ونقترح أن استخدام مسحوق اوراق السبانخ كإضافات طبيعية لأعلاف فروج اللحم يعزز الصفات النوعية للحوم. ويطيل مدة صلاحيته في التخزين بالتجميد، خاصة عند تصنيع اللحوم.

الكلمات المفتاحية: واحد، اثنان.

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