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The effect of the natural antioxidants extracted from Glycyrrhiza glabra (licorice) on the cryopreservation of chicken breast meat

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ABSTRACT

Background: Chicken meat is highly nutritious, valued for its unique properties and versatility in culinary applications. Also, preparation methods and natural additives to its diet play an important role in estimating the nutritional value of its meat by increasing consumer acceptance of it from health, nutritional, and material aspects.

Objective: This study was conducted to determine the effect of licorice as a natural antioxidant on cold-preserved chicken meat.

Methods: Phenolic compounds were quantified using the colorimetric method with Folin's reagent, measuring absorbance at 765 nm, and results were expressed in terms of gallic acid equivalents per gram of dry weight. Gallic acid was used as a reference solution to prepare the standard curve with concentrations ranging between 20-120 µg/mL to determine the quantity of phenolic compounds. Determination of antioxidant activity was done using the DPPH method by taking 0.5 mL of the extract solution at concentrations ranging from 50-300 µg/mL and mixing with 3 mL of methanolic DPPH solution. The mixture was mixed for 1 min with a vortex mixer, and samples were measured by spectrophotometer at 517 nm. Moisture, ash, fat, protein, and pH were determined according to the methods mentioned by AOAC. Thiobarbituric acid-reactive substances (TBARS) were estimated by mixing 0.5 g of the sample with 2.5 mL of TBA solution. The mixture was heated in a boiling water bath for 10 min to orange color, chilled by adding 1 mL of chloroform

and centrifuged at 5000 rpm for 25 min, and the absorbance was read at 532 nm wavelength. Total microorganisms, molds, and yeast were counted.

Results: Protein content increased proportionally with higher concentrations of ethanolic licorice root extract. The gradual increase in protein content in samples is attributed to their antimicrobial effect, which leads to the preservation of meat from microbial growth and the action of proteolytic enzymes. The increase in protein during refrigerated storage is due to the decrease in moisture and increase in dry matter reflected in the increase in protein. The reduced acidity in chicken meat treated with licorice root ethanolic extract is attributed to its antimicrobial effects, which limit the growth of microorganisms responsible for metabolizing nitrogenous compounds.

Licorice extracts, rich in phenolic compounds, reduce lipid oxidation. The observed decrease in thiobarbituric acid-reactive substances with increasing extract concentration is due to the antioxidant properties of these phenolics with the hydroxyl group attached to the aromatic ring capable of giving hydrogen atoms and inhibiting free radicals. This study found that the growth of microorganisms could be lowered by licorice extract containing phenolic compounds, flavonoids, tannins, and alkaloids, which showed a significant antimicrobial effect.

Conclusion: This study examined how natural antioxidants, like licorice Glycyrrhiza glabra, affected the cryopreservation of chicken breast.

Keywords: Licorice root extract, chicken breast, antioxidants, total phenol, Meat science

Graphical Abstract: The effect of the natural antioxidants extracted from Glycyrrhiza glabra (licorice) on the cryopreservation of chicken breast meat

INTRODUCTION

Meat and meat products are essential in maintaining a healthy, balanced diet because they provide energy, high-quality digestible protein with all the essential amino acids, and absorbable nutrients necessary for growth [1]. Poultry meat is preferred for its nutritional properties, low-fat content, and high concentration of unsaturated fatty acids. Fresh beef is preserved at 2-5°C and is susceptible to oxidation and microbial spoilage [2]. Meat oxidation affects food packaging and organoleptic qualities [3]. It leads to the formation of aldehydes, ketones, carbonyls, and hydroxides that occur when unsaturated fatty acids react with oxygen, producing toxic compounds such as malondialdehyde [4-5]. To eliminate or minimize these changes, manufacturers have turned to using chemical and synthetic antioxidants, including PG, BHT, and BHA; even these chemicals lead to health risks that have a carcinogenic effect [6].

Researchers have been replacing synthetic antioxidants with natural substances [7]. These substances were obtained from the plant kingdom, one of the most abundant sources of natural antioxidants [8,9]. Natural antioxidants can be incorporated into diets or used as extracts to donate hydrogen ions, thereby inhibiting oxidation and preventing the formation of lipid and protein radicals in food products [10]. They also have a role in preventing the formation of free radicals, retarding microbial activity, and improving meat quality because they contain chemical nutrients and are considered food and pharmaceutical products [11-12].

Licorice, or sweet wood, is an antioxidant perennial herbal plant with active ingredients, including terpenoids, flavonoids, alkaloids, and phenolic compounds [13-14]. It contains antioxidant activity, whereas licorice roots are dietary supplements and possess anti-inflammatory, anti-ulcer, anti-inflammatory, anti-fungal, and anti-allergic properties [15-16].

This study aimed to determine the effect of ethanolic extract of licorice root as an antioxidant and antimicrobial agent for chicken breast meat during refrigerated storage at 4°C.

METHODS

This study was conducted in the laboratories of the College of the Agriculture / University of Baghdad, where Licorice samples were collected from the local markets of Baghdad (Shurja) and kept at room temperature until use and fresh chicken breasts were obtained after slaughter directly from the local markets of Baghdad.

Preparation of licorice root extract: 25 g of licorice root powder was added to 250 ml of 99.5% ethanol solvent, mixed well on a magnetic shaker, and left for 24 hours at room temperature, filtered, and evaporated using a rotary evaporator at 40 ml, then transferred to a convection oven until dry at 40 ml, and the dry powder was collected in containers and kept in the refrigerator until use.

Preparation of chicken meat samples: The chicken breast meats were cut into cubes and divided into five groups. The first group included the control sample, and the rest of the treatments presented the pieces, which were immersed into 2, 4, 6, and 8% ethanolic licorice root extract, then placed in polyethylene bags and sealed tightly and stored in the refrigerator at 4°C for 21 days and were subjected to chemical and microbial tests.

Chemical tests of ethanolic licorice root extract:

Quantification of phenolic compounds:

The colorimetric method using Folin's reagent was adopted according to the method followed by [17]. The extract solution was prepared at a concentration of 1 mg/ml, and the reaction mixture was prepared by mixing 0.5 ml of the extract solution with 2.5 ml of Folin's reagent diluted with distilled water at a concentration of

10%. The mixture was mixed and kept in the dark for 2 hours at room temperature, then read at a wavelength of 765 nm and the results were expressed as gallic acid/g dry weight, and gallic acid was used as a reference solution to prepare the standard curve with a concentration range of 20-120 µg/mL.

Determination of DPPH antioxidant activity: 0.5 mL of the extract solution at 50-300 µg/mL concentrations was mixed with 3 mL of methanolic DPPH solution. The mixture was mixed for 1 min with a vortex mixer, the reaction was incubated in the dark at room temperature for 30 min, and the optical absorbance was measured by spectrophotometer at 517 nm wavelength [18].

%inhibition = A0-A1/A0 *100%

A0 = absorbance of the standard solution, A1 absorbance of the sample

Chemical tests of chicken breast pieces: Chemical tests were carried out on chicken breast pieces stored refrigerated at 4°C for 21 days at 0, 5, 10, 15, and 21 days. Moisture, ash, fat, protein, and pH were determined according to the methods mentioned by AOAC [19].

Number of reactants with thiobarbituric acid: 0.5 g of the sample was mixed with 2.5 mL of TBA prepared from 0.375 g thiobarbituric acid, 0.25 g trichloroacetic acid, 0.25 g titrated acetic acid. The mixture was heated in a boiling water bath for 10 min to orange color and chilled by adding 1 mL of chloroform, placed in a centrifuge at 5000 rpm for 25 min, and read the absorbance at 532 nm wavelength [20].

Microbial investigations of chicken breast pieces: Total bacterial count and molds and yeasts were counted according to the methods of [21]

RESULTS AND DISCUSSION

Estimation of phenolic content in licorice root ethanolic extract and antioxidant activity: Gallic acid was used as a standard for determination of the total phenolic compound content of licorice root extract that was calculated based on the gallic acid standard curve as shown in Figure (1), the results here were expressed as gallic acid equivalent/g dry weight, the content was 114. The free radical scavenging activity of licorice root extract is shown in Table (1). The ethanolic extract showed an activity of 78.01 at 300 µg/ mL concentration. The antioxidant BHT reached to 87.02 µg /ml. These results agreed with [22], which found that licorice roots act more effectively than synthetic chemical antioxidants.

Table1. Antioxidant activity of licorice root extract compared with natural antioxidant BHT.

No	50	100	150	200	250	3
Ethanolic extract	29.21	40.64	53.10	63.90	70.20	78.01
BHT	49.01	59.94	67.10	74.00	82.02	88.20

Chemical tests during cold storage:

Moisture: Table 2 shows significant differences ($p < 0.05$) in the moisture percentage between the control sample and the treatments, as the results showed a decrease during refrigerated storage for all treatments. The percentage of moisture in the control sample reached 74.94% compared to 70.72, 70.84, 70.94, and 70.98% for samples treated with ethanolic licorice roots at concentrations of 0.20, 0.40, 0.60, and 0.80% at the beginning of the storage period. Still, at the end of storage, the moisture in the control sample decreased compared to the treated samples as it reached 70.63%

compared with 68.82, 68.96, 68.98, and 68. 40% for the treatments, respectively. The reason for this decrease is due to the ability of the protein to bind moisture, which leads to its losses, but from the results, we noticed that the control sample lost moisture, unlike the treatments, which were less if we see the effect of licorice on it. It is good if the plant materials have an effective role in preserving cellular membranes from oxidative damage that occurs in the lipids of these membranes and thus maintain the moisture content and nutritional value [23-24].

Table 2. The moisture content of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	74.94	70.72	70.84	70.94	70.98
2	5	73.83	70.54	70.66	70.64	70.78
3	10	72.96	69.90	69.94	69.98	70.54
4	15	71.74	69.44	69.85	69.88	68.98
5	21	70.63	68.82	68.96	68.98	68.40

Ash: The percentage of ash in the control sample and treatments treated with ethanolic extract of licorice roots showed that there were a few differences; if it presents, in the form of an increase in the control sample during storage as shown in Table 3 due to the increase in dry matter and decreased in moisture content, the results agreed with what was mentioned earlier [25].

Table 3. Ash content of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	1.03	1.10	1.17	1.23	1.26
2	5	1.05	1.15	1.22	1.25	1.28
3	10	1.09	1.21	1.30	1.35	1.38
4	15	1.17	1.28	1.35	1.39	1.40
5	21	1.21	1.39	1.56	1.52	1.50

Fat: The results in Table 4 showed differences during the storage period between the control and the treatments; the control sample reached 0.85% compared to 1.28, 1.25, 1.21, and 1.19%, respectively. This means there was a slight increase in the control sample during storage, reaching 0.90%, while the other treatments were 1.40, 1.45, 1.48, 1.56, and 1.60, respectively. These results are due to the increase in the dry matter associated with the decrease in the moisture content of the samples. Results agreed with what was mentioned elsewhere (26).

Table 4. The fat content of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	0.85	1.28	1.25	1.21	1.19
2	5	0.86	1.39	1.43	1.28	1.25
3	10	0.87	1.45	1.42	1.24	1.29
4	15	0.88	1.56	1.50	1.49	1.35
5	21	0.90	1.60	1.56	1.48	1.40

Protein: The results indicated in Table 5 showed that there were differences between the control and treatment samples in protein content; the control sample reached 32.12% while the treatments reached 25.01, 25.12, 25.18 and 25.24%, for each of the concentrations 0.2, 0.4, 0.6 and 0.8%, respectively, during the cold storage period. The results showed that the protein percentage increased directly with the high concentrations of the ethanolic extract of licorice roots. The gradual increase in protein content in the samples compared to the control was attributed to their antimicrobial effect, which leads to the preservation of meat from microbial growth and the action of proteolytic enzymes [27]. The increase in protein during refrigerated storage is due to the decrease in moisture and increase in dry matter reflected in the increase in protein. The results are consistent with what was reported [28].

Table 5. Protein content of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	23.12	25.01	25.12	25.18	25.24
2	5	23.24	25.22	25.34	25.38	25.48
3	10	24.38	26.01	25.90	25.14	25.98
4	15	24.82	26.32	26.52	26.58	26.66
5	21	25.52	26.48	27.02	27.10	27.28

pH: The results of Table (6) showed no significant differences between the treatment samples, but there is a difference between the control sample during refrigerated storage, which is due to the accumulation of receptors because of the bacterial activity in meat, to protein degradation. Bacteria metabolize amino acids released through protein degradation, resulting in ammonia formation and high pH values [29]. The decrease in acidity in chicken meat samples treated with licorice root ethanolic extract was due to the antimicrobial effect in limiting the growth and proliferation of microorganisms that metabolize essential nitrogen compounds [30,31].

Table 6. pH Values of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	5.82	5.86	5.80	5.84	5.87
2	5	5.94	5.82	5.76	5.82	5.80
3	10	6.40	5.78	5.73	5.72	5.68
4	15	6.68	5.74	5.68	5.65	5.46
5	21	6.72	5.69	5.64	5.60	5.58

Thiobarbituric acid reactants: The results of Table 7 indicated that values of thiobarbituric acid were increased from the first day until the last day of cold storage; the control sample was 0.204 mg malonaldehyde /g at the beginning of storage, then increased to 0.998 mg malonaldehyde /g at the end of the storage phase. The same trend was noted for the samples treated with licorice extract. In a concentration of 0.2%, 0.196 mg malonaldehyde /g at the beginning increased to 0.305 mg malonaldehyde /g at the end of the storage phase, and it was the same for all samples with increasing concentrations. This gradual increase is due to the spontaneous oxidation of fats, which is an inevitable reaction during cold storage [32]. Licorice

extracts are rich in phenolic content that reduces lipid oxidation, so the value of thiobarbituric with increasing concentration of the extract is related to the antioxidant phenolic compounds with the hydroxyl group attached to the aromatic ring capable of giving hydrogen atoms and inhibiting free radicals. The results agreed with what was reported by other authors [33].

Table 7. Thiobarbituric acid reactants content (malonaldehyde/ g) of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	0.204	0.196	0.096	0.098	0.092
2	5	0.238	0.200	0.198	0.102	0.098
3	10	0.558	0.244	0.148	0.156	0.110
4	15	0.780	0.288	0.190	0.192	0.182
5	21	0.998	0.305	0.298	0.300	0.202

Microbial tests: Table (8) results indicated differences between the control sample and the samples treated with licorice root extract. The number of microorganisms for the control sample increased steadily during the storage period, but the increase was slower for treated samples, especially at higher concentrations. The lower the growth of microorganisms was because licorice extract contains phenolic compounds, flavonoids, tannins, and alkaloids, which showed a significant antimicrobial effect [34-35].

Table 8. Bacterial counts (log cfu/ g) of chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	8.01	5.58	5.01	5.00	0.8%
2	5	8.21	5.66	5.18	5.12	-
3	10	8.38	5.90	5.24	5.18	-
4	15	8.40	5.98	5.38	5.20	-
5	21	8.68	6.06	5.45	5.22	5.14

Total number of molds and yeasts: The results of Table 9 showed differences between the control sample and the samples treated with ethanolic extract of licorice roots. The count of molds and yeasts decreased as the concentration of the extract increased; the highest value for the control sample was 4.00 cfu/ g at the beginning of cold storage, and it increased to 5.01 cfu/ g at the end. It was (1.48 – 2.24 cfu/g) for the samples treated with the extract at the beginning and (2.20 – 3.18 cfu/ g) at the end of the storage period. These results agreed with what was mentioned by others [36].

Table 9. Counts of mold and yeasts (cfu/g) for chicken breast samples immersed in ethanolic extract of licorice extract

	Storage days	control	0.2%	0.4%	0.6%	0.8%
1	0	4.00	2.24	2.01	1.84	1.48
2	5	4.20	2.46	2.10	2.03	1.56
3	10	4.58	2.88	2.34	2.12	2.00
4	15	4.80	3.01	2.48	2.22	2.08
5	21	5.01	3.18	2.52	2.34	2.20

CONCLUSION

Research on the longevity and quality of meat products with added antioxidants is scarce. This study examined how natural antioxidants like licorice *Glycyrrhiza glabra* affected the cryopreservation of chicken breast. Antioxidant-treated chicken breast meats and other foods with health-promoting qualities are more acceptable to most consumers. When selecting a meal, choosing these products is always in progress because of their natural source. Chicken breast may treat with many herbs, and there are many natural substances to preserve it.

List of Abbreviations: mm: measuring unit, gm: gram, mL: milliliter, °C: Celsius degree.

Authors' Contributions: Omar Almuharib: formal analysis; methodology; project administration; funding acquisition; validation; writing the original draft. Firas Riyadh Jameel: data duration; formal analysis; methodology. Marina Yousif Yaakop: project administration, supervision, resources, validation.

Competing Interests: The authors have no conflict of interest.

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