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Original Article

Lipids Oxidative Stability and Microbial Shelf Life Quality of Licorice (Glycyrrhiza glabra L.) Extract Supplemented Chicken Patties

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■Keywords

Glycyrrhiza glabra, Chicken processing, Natural antioxidants, quality attributes, microbial safety.

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ABSTRACT

The current study envisaged to evaluate the role of licorice (*Glycyrrhiza glabra* L.) extract on lipid oxidation, quality attributes, sensorial as well as microbial stability of chicken meat patties at refrigerated (4 ˚C) storage. Accordingly, 4 levels of licorice extract were added @0.25, 0.5, 1.0 and 1.25% in chicken patties development process along with positive (0.25% butylatedhydroxyanisole) and negative control (without antioxidant). The developed patties were cooked and subjected to thiobarbituric acid reactive substances (TBARS), total phenolics (TPC), ferric reducing antioxidant power (FRAP), color, pH, sensory evaluation, total plate count (TPC) and *Escherichia coli* analysis at 0, 3rd and 7th day of storage. The findings indicated that the addition of licorice extract decreased malonaldehyde (MDA) generation along with higher pH and redness of patties ($p \leq 0.05$). The lowest MDA reported in T_5 (1.25g LE/kg meat) was 0.63±0.04 whereas, the highest in T0 (control) was 0.90±0.02 that increased to 1.59±0.06 and 2.28±0.06 at the completion of the study. Also, the microbial load of chicken patties declined with treatments as indicated by the total plate count compared to the control. Conclusively, licorice extract incorporation in chicken patties is a pragmatic approach to improve oxidative stability, quality attributes and extended shelf life with preservation effect.

Practical Application

Meat processing industries look for additives to enhance oxidative stability, quality and microbial safety of processed chicken products. Accordingly, the present study was conducted to explore the best suited level of (Licorice extract) to increase stability, quality and microbial stability. Findings of the study are helpful for meat processing professionals especially for processed chicken meat industry to improve storability, quality as well as product shelf life by deploying licorice extract.

INTRODUCTION

Chicken meat is the most consumed meat around the globe owing to its availability, cheap price {Sohaib, 2017 #1}and processing convenience as well as its' high source of biological protein value, minerals and vitamins. Chicken meat and meat products are also a rich source of polyunsaturated fatty acids (PUFA) that can prompt oxidative degradation, leading towards decreased quality of meat and meat based products, ultimately leading to lower consumer acceptability of the meat products (Sohaib *et al.,* 2017). The mechanism of oxidation in meat involves production of reactive free oxygen species (ROS) and free radical formation, responsible for rancid flavor and poor quality of the meat products. Proteins in cooked meat products can also be degraded by ROS involving initiation, propagation and termination reactions

(Botsoglou *et al.,* 2014). The oxidation mechanism depends on factors like chemical composition of meat, presence of pro-oxidants, oxygen and temperature of processing and storage that generates compounds having toxic effects on human health. Apart from oxidation, microbial spoilage is also considered vital and can decrease the nutrient content as well as consumer acceptability of the meat based products. Meat being a rich source of nutrients provides an ideal medium for the growth of microbes especially of pathogenic nature. Thereby, shelf life of chicken meat and meat products can be increased by retarding the process of oxidation as well as inhibiting the microbial growth (Ferreira *et al.,* 2016).

Various herbal extracts and plant spices preparations are used in the meat-based products to improve organoleptic attributes, quality as well as safety of the product. Apparently, these formulations are rich in phenolic compounds that are shown to possess strong radical scavenging activity in processed products Kumar *et al.,* 2015a). Licorice is a herb used as food flavoring or medicinal additive to cure a wide range of diseases in humans for centuries. It is among popular therapeutic agents in Asian countries as well as around the world. Studies reported that licorice contains phenolic and terpenoid compounds that have potential to be used to increase oxidative stability as well as microbial safety of food products (Jiang *et al.,* 2013). Licorice exhibit biological properties mainly glycyrrhizin, triterpene, saponins and phenolics responsible for antioxidant and immune regulatory potential. Around more than 400 compounds reported in *Glycyrrhiza* species and mainly licorice have triterpene, saponins and flavonoids (Ko *et al.,* 2007; Zhang *et al.,* 2015). Similarly, a study was conducted to determine liquorice extract (LE) potential as a dietary supplement for sheep to improve the antioxidant level of meat. The sheep were given different levels of 0, 1000, 2000, 3000 and 4000 mg/kg feed and after 120 days, the longissimus thoracis muscle was sampled and stored at 4°C for analysis. The findings suggested that the addition of the extract increased free radical scavenging ability as well as decreased the TBARS levels in a dose response way indicating increased antioxidant potential of meat (Zargar *et al.,* 2014). Licorice is "generally recognized as a safe" (GRAS) food additive by the Food and Drug Administration and recently a study documented licorice as a strong inhibitor of lipid oxidation in chicken meat product. However, antimicrobial aspects in cooked meat products is yet needed to be explored (Zhang & Ye, 2009). Considering the scenario, the present study was planned to explicate the role of licorice extract to improve oxidative storability, quality attributes and microbial safety of chicken patties at refrigerated storage. The chicken meat patties were considered in the study because it is the most consumed meat product in Pakistan along with chicken meat nuggets.

MATERIALS AND METHODS

The study was divided into phases; first, the licorice extract was prepared for incorporation in chicken meat patties followed by product development, cooking and last storage at refrigeration temperature for analysis on the 1st, 3rd and 7th day of storage.

Licorice extract preparation

Licorice root obtained from a local market were subjected to washing followed by grinding to obtain a powder of homogenous consistency used for the withdrawal of the extract to be used in the meat patties. 50g of the powder was soaked in 500 mL water (to obtain the water extract) for 48 hrs at room temperature with occasional shaking. Afterwards, the mixture was filtered through filter paper followed by rotary evaporation to evaporate the moisture of the extract. The obtained extract was stored at (-40ºC) under vacuum to get hydroscopic powder for application in the meat product by following the guidelines of (Irani *et al.,* 2010).

Development of patties

The chicken meat obtained from the local market was grinded using a kitchen grinder to aid to develop uniform meat mince of homogenous consistency for the application of treatments. Considering the findings of the preliminary studies, 4 different concentrations of liquorice was applied along with positive and negative control. The extract was applied to the grounded meat followed by mixing for 2 min using a bowl mixer followed by the formation of the chicken meat patties (100 ± 2 g). After the preparation and application of the treatments, the meat patties were packaged in oxygen impermeable bags with O_2 permeability, 9.3 mL O_2/ m^2/ 24 hrs at 0ºC) followed by cooking at 95ºC using water bath until the internal temperature reached 75ºC. Afterwards, the cooked patties were cooled, repacked in new oxygen permeable bags and stored at 4ºC for analysis on the θ, 1st, 3rd and 7th day of storage. The treatment plan used for the application of various levels of LE along with positive and negative control as T_0: Control without antioxidants; T_1: Group containing 0.25mg/kg of butylated hydroxytoluene, T_2: Group containing 0.25mg LE/kg of meat; T_3: Group

containing 0.50mg LE/kg of meat; T_4: Group containing 1g LE/kg of meat; T_5: Group containing 1.25g LE/kg of meat.

Analysis of chicken meat patties

Lipid oxidation analysis

The oxidative stability of cooked meat patties having different levels of extract determined 2 thiobarbituric acid reactive substances (TBARS) analysed following the guidelines of (Liu *et al.,* 2010). Purposely, 5 g meat samples were weighed in a test tube of 50 mL and was homogenized with butylated hydroxytoluene (7.2%) 50 µL and deionized with distilled water using a homogenizer for 15 sec. A disposable test tube (13×100 mm) having 1 mL of homogenate and 2 mL of TBA/trichloroacetic acid (TCA; 15 mM TBA/15% TCA). After that boiling water bath is used for incubation for 15 min. Later, the samples were cooled in water bath for 10 min, vortexed again and centrifuged for 15 min at 2,000×g at 4°C. The absorbance of the resulting supernatant solution was determined at 531 nm against a blank containing 1 mL of deionized distilled water and 2 mL of TBA/TCA solution. The TBARS value was calculated as milligrams of malondialdehyde (MDA) per kilogram of meat.

Total phenolic contents (TPC) content of patties

The total phenolic contents of chicken meat patties enriched with LE at different storage days determined following the guidelines (Senevirathne *et al.,* 2006). The homogenized patties sample (100 µL) were mixed with 500 µL (95% ethanol), distilled water (2.5 mL) and 250 µL of 50% Folin Ciocalteu reagent. After 5 min, 250 µL of 5% Na_2CO_3 was added to the resultant mixture followed by vortex and then placed in the dark for 1 hr. Afterwards, the absorbance of the samples was measured at 725 nm using UV/Visible Spectrophotometer against positive and negative control. The total phenolic contents were estimated as gallic acid equivalent (mg gallic acid/g).

Ferric reducing antioxidant power (FRAP) Assay

The ferric reducing antioxidant power of chicken meat patties at various storage intervals was estimated using the procedure of (Rupasinghe *et al.,* 2010). The homogenized sample (200 µL) was mixed with 500 µL sodium phosphate buffer (0.2 M, pH 6.6) and 500 µL potassium ferric cyanide (1%) followed by incubation at 50°C in water bath for 20 min. After cooling, the sample was mixed with 2.5 mL (10% TCA), distilled water (1.25 mL) and 0.25 mL (0.1% ferric chloride) for 10 min. The absorbance of the sample measured at 700 nm. During the analysis, an increase in the absorbance of the reaction mixture indicated the higher reducing power of the samples.

pH measurement

pH values of chicken meat patties subjected to various levels of extract was determined following the protocol of (Choe *et al.,* 2010). The pH was determined by direct contact in sensitive diaphragm of electrode with the patties through diaphragm differences in electrical load between the meat and electrolyte solution (*e.g.* Potassium chloride KCl) inside glass electrode measured and directly indicated as the pH reading.

Color estimation

Surface color of chicken meat patties enriched with different levels of LE along with control at storage intervals was determined by using Minolta® CR-410 colorimeter (Hunter Laboratory Inc., Reston, VA) by following the guidelines of (Wyrwisz *et al.,* 2016). The color values of patties for parameters such as L*(lightness), a* (redness) and b*(yellowness) were determined.

Sensory evaluation

The descriptive organoleptic evaluation of cooked chicken meat enriched with extract was carried out by a panel of taste professionals using 9 point hedonic scale (9 = like extremely; 1 = dislike extremely) at storage considering the guidelines of (Meilguard *et al.,* 2007). In this context, meat patties were subjected to various quality attributes like appearance, taste, flavor, juiciness, tenderness and overall acceptability were determined. All measurements of sensorial evaluations for chicken meat patties were done by panelists in the Sensory Evaluation Laboratory Department of Food Science and Human Nutrition, University of Veterinary and Animal Sciences, Lahore. During the course of the evaluation, the panelists were given unsalted crackers, mineral water and expectorant cups to neutralize and rinse the taste receptors for cogent assessment. The descriptors rated using a scale, with "0" as the lowest score and "9" as the highest for the quality trait.

Microbial analysis of patties

Total Plate Count using nutrient agar

Total plate count of chicken meat patties treated with extract was determined at selected storage

intervals by following the guidelines of (Feng & Sun, 2013). For this purpose, 1 g meat sample was taken from the chicken meat patties dipped and soaked up with 100mL sterile distilled water with addition of 0.1% peptone water. After that 10 fold serial dilutions were prepared using 1mL meat sample. Afterwards, 1mL sample was transferred into a tube containing 9mL sterile normal saline solution followed by mixing well and then transferred 1mL to the next tube containing 9mL sterile normal saline. The procedure was repeated untill the 7th tube was reached. After doing this serial dilution, 1 mL sample was pipetted out from the 6th and 7th dilution and transferred to the sterile petri dishes containing solidified nutrient agar already autoclaved. The spreading of the meat samples was done using sterile bent glass stick and petri dishes were inverted and incubated at 37°C for 24 hours. After 24 hr incubation, colonies were observed, counted using a colony country and then results were documented.

Determination of E. coli

The *Escherichia coli* count of chicken meat patties having various concentrations of the extract at storage was calculated using the procedure of (Shimelis *et al.,* 2014). Accordingly, 1g meat sample was taken from the patties, dipped and soaked up with 100mL sterile distilled water with addition of 0.1% peptone water. Afterwards, 10 fold serial dilutions were prepared using 1mL of sample followed by transferred into a tube containing 9mL sterile normal saline. The samples were mixed well and then transferred 1mL to the next tube containing 9mL sterile normal saline and this method was repeated up to the 7th dilution. After completing serial dilutions, 1 mL sample was taken and transferred to sterile petri dishes followed by sample spreading on Mackonikey agar and then petri plates were incubated at 37°C for 24 hrs for observing growth of *E. coli.* Afterwards, the plates were removed and microbial colonies were counted using Colony counter for detection and enumeration of microbes.

Statistical analysis

The collected data was subjected to statistical analysis using analysis of variance and two way factor factorial design. Additionally, Tukey's multiple comparison test used as post hoc test for deciding the significance (p<0.05) of different parameters. All statistical analyses were performed using statistical package SPSS software version 20) by following the guidelines of (Steel *et al.,* 1997).

RESULTS AND DISCUSSION

Lipid oxidation of patties

The analysis of variance regarding thiobarbituric acid reactive substances (TBARS) of chicken meat patties showed significant differences due to treatments and storage. Means regarding TBARS in meat patties (Table 1) indicated the highest value of TBARS at day 0 reported in T_0 (control) as 0.90±0.02 whereas, the lowest in T_5 (Group containing 1.25 g LE/kg meat) 0.63±0.04 that were increased to 2.28±0.06 and 1.59±0.06, respectively at completion of storage. Overall, the addition of the extract has significant influence for the reduction of the TBARS however, the TBARS values increased with the progression of storage. The addition also influenced the rate of TBARS production in the patties and lower values were reported in the patties with a dose response effect. The findings of the present study are in accordance with (Muthukumar *et al.,* 2014) who reported lower TBARS in patties containing antioxidant in chicken meat. The observed antiradical activity of LE was consistent with Tohma & Gulçin, 2010 findings who also documented that TBARS production was significantly less (p<0.05) in patties treated with LE indicating the inhibition of lipid oxidation and licorice is an excellent source of phenolic compounds like Glabridin, an isoflavan present in *G. glabra* considered responsible for inhibition of $CuSO_4$ induced oxidation of low-density lipoproteins in living organism (Leopoldini *et al.,* 2011). Similarly, a study also stated that TBARS value of chicken meat patties decreased with licorice and increase with storage**.** They further suggested TBARS values in refrigerated precooked control patties (0.22 mg/kg) rose to 9.3 to 9.4 mg/kg after 14 d compared to 3.4 to 4.4 mg/kg in patties treated with 0.1% LE and attributed the presence of multiple phenolics (Jiang *et al.,* 2013).

Total phenolic contents of Chicken meat patties

The results (Table 1) indicated a significant effect by addition of various treatments of LE at total phenolic contents of the patties. At the start of the study trial, the lowest value for TPC was documented in the control (109.22±8.33) whereas the highest were documented in the T_5 (Group containing 1.25 g LE/kg of meat) as 182.28±8.74 that were further decreased to 96.63±8.77 and 168.11±6.58, respectively at the completion of the storage. Means for treatments indicated the highest value in T_5 as 175.01±5.21 followed by T_4 (163.41±6.25) and T_3 (150.14±6.78) whereas the

lowest in control as 102.75±8.54. The findings of the current study are supported by the Zhang *et al.* (2015) who reported an increase in total flavonoid content of the sheep meat by the supplementation of LE at levels of 0, 1000, 2000, 3000 and 4000 mg/kg feed. Similarly, another group of researchers also reported that licorice extract in combination with citric acid enhanced the preserving function of meat patties and significantly retard oxidation and inhibiting microbial growth (Qiu *et al.*, 2014). Similarly, another study reported that major bioactive components of liquorice extract obtained through supercritical CO2 extraction at elevated pressures using three solvents (ethanol, methanol, and ethyl acetate) at different ratios with water (25:75, 50:50, and 75:25) and supercritical fluid extracts (SFE) at varying pressures (3,500, 4,500, and 5,500 psi) are glycyrrhizin and glabridin. The study also concluded that the extraction of these compounds increased with increasing solvent concentration as well as 75% ethanolic extract showed the highest total phenolic content (TPC) among all extracts (Sohail *et al.*, 2018).

Ferric reducing antioxidant power of patties

The results denoted significant differences on the ferric reducing antioxidant power of the chicken meat patties by the addition of liquorice treatments (Table 1). At the initiation of the study, the highest value for FRAP documented in T_5 (Group containing 1.25 g LE/kg meat) as 566.63±14.85 followed by T4 (Group containing 1 g LE/kg meat) as 514.12±16.25, T3 (group containing 0.50 mg LE/kg meat) as 465.63±8.98 whereas lowest in control as 345.45±14.25 that were further decreased to 528.35±18.26, 484.66±15.25, 436.26±18.14 and 311.87±11.21, respectively at the completion of storage. The results of this study are in line with the findings of Zhang *et al.* (2015) who also reported an increase in antioxidant potential by the supplementation of LE at levels of 0, 1000, 2000, 3000 and 4000 mg/kg in the sheep meat via feed. They further documented that LE scavenged free radical in a dose response manner whereas, addition of LE via in animal diet increased antioxidant potential as well as decrease reactive oxygen species in meat. Similarly, Qiu *et al.* (2014) reported that licorice extract along with citric acid and chitosan increased can preserve the quality of fish fillets stored at 4°C by inhibiting lipid oxidation and microbial growth as well as increasing the antioxidant potential of the fillets which leads towards increased shelf life of the product.

Table 1 – Effect of liquorice extract on thiobarbituric acid reactive substances, total phenolics and ferric reducing antioxidant power in chicken meat patties at refrigerated storage.

Treatments	TBARS (mg/kg of meat)				TPC (mg GAE/100g meat)				FRAP ((µmol/Fe^{+2}/g meat))			
	Storage days				Storage days				Storage Days			
	0	3	7	Mean	0	3	7	Mean	0	3	7	Mean
T_0	0.90±0.02	1.25±0.04	2.28±0.06	1.47±0.04^{a}	109.22±8.33	102.41±5.49	96.63±8.77	102.75±8.54^{a}	345.45±14.25	326.78±12.21	311.87±11.21	328.03±12.11^{a}
T_1	0.81±0.05	1.11±0.03	1.47±0.03	1.13±0.03^{d}	116.21±7.66	105.34±6.25	100.65±8.65	107.40±4.25^{b}	360.21±11.02	344.39±17.18	334.66±13.35	346.42±11.23^{ab}
T_2	0.78±0.02	1.20±0.02	1.94±0.03	1.30±0.02^{b}	151.29±8.10	146.12±4.33	139.23±7.69	145.55±3.89^{c}	425.89±9.66	414.28±13.26	399.29±16.29	413.15±14.02^{c}
T_3	0.78±0.03	1.14±0.01	1.81±0.04	1.24±0.03^{bc}	159.45±5.21	149.36±6.22	141.61±5.46	150.14±6.78^{cd}	465.63±8.98	453.18±10.25	436.26±18.14	451.69±12.35^{d}
T_4	0.74±0.04	1.15±0.03	1.71±0.04	1.20±0.04^{c}	171.23±4.65	163.02±5.41	155.98±5.68	163.41±6.25^{d}	514.12±16.25	502.29±16.54	484.66±15.25	500.36±16.35^{e}
T_5	0.63±0.04	1.06±0.02	1.59±0.06	1.09±0.04^{e}	182.28±8.74	174.65±6.25	168.11±6.58	175.01±5.21^{e}	566.63±14.85	545.45±14.22	528.35±18.26	546.81±15.25^{f}

Values are Means±SD. Means sharing similar superscript differ non significantly (p>0.05).

T_0= Control

T_1= Group containing 0.25mg of BHT/kg meat

T_2= Group containing 0.25mg licorice extract/kg of meat

T_3= Group containing 0.50 mg licorice extract/kg of meat

T_4= Group containing 1 g licorice extract/kg of meat

T_5= Group containing 1.25 g licorice extract/kg of meat

Color of patties

Color is among the main aspect of meat quality attributes affecting consumers' buying choice for processed meat products. Consumers link meat products discoloration with its poor quality as well as acceptability. The rate of discoloration of meat products is believed to be related to the oxidation processes in controlling metmyoglobin levels in cooked meat products. Results (Figure 1) indicted that L* color values of chicken meat enriched with licorice extract at various storage intervals showed significant differences due to treatments and storage. Means reported the highest L* color values were reported in control as 52.40±0.86 whereas, the lowest as 47.35±1.22 in T_5 that were decrease to 47.35±1.22 and 42.58±0.78 at end of storage. Likewise, a* color values of chicken meat patties were significantly differing among treatments and storage intervals. a* color values among different treatments were 5.25±0.77, 5.18±0.04, 6.08±1.85, 7.09±0.16, 7.87±0.20 and 8.33±0.16 in T_0, T_1, T_2, T_4, T_5 and T_6 that were increased to 15.48±0.52, 14.71±0.42, 15.09±1.72,15.47±0.29, 18.42±0.84, and 18.76±0.38, respectively with the terminating of storage. The results also indicated the color significantly (p<0.05) become stable by addition of licorice extract. Similarly, analysis of variance for b* color values of meat patties also indicated momentous effects for the treatments. Means for b* values of patties indicated the highest value in T_2 as 12.86±0.18 whereas, the lowest was in T_5 as 11.20±0.81. Also, the mean values of yellowness at various storage intervals were found to be 12.782, 12.664, and 11.081 at 0, 3rd and 7th day of storage. Overall yellowness color values were decreased with storage progression.

The present study's results are supported by Valencia *et al.* (2008) who reported increase L* value in pork sausage containing herbs treatments relative to control. Similarly, chicken samples treated licorice extract exhibited intense red color resulted higher a* (redness) values. Similarly, Kumar *et al.* (2015) also documented the addition of natural extract enhanced a* value in the marinated chicken during 12 days storage (Tesoriere *et al.*, 2007). Similarly, Gibis and Weiss, (2012) observed discoloration in fried beef patties with natural antioxidant such as grape seed extracts addition. They also suggested lighter color of products at the start of the trail that becomes darker with storage.

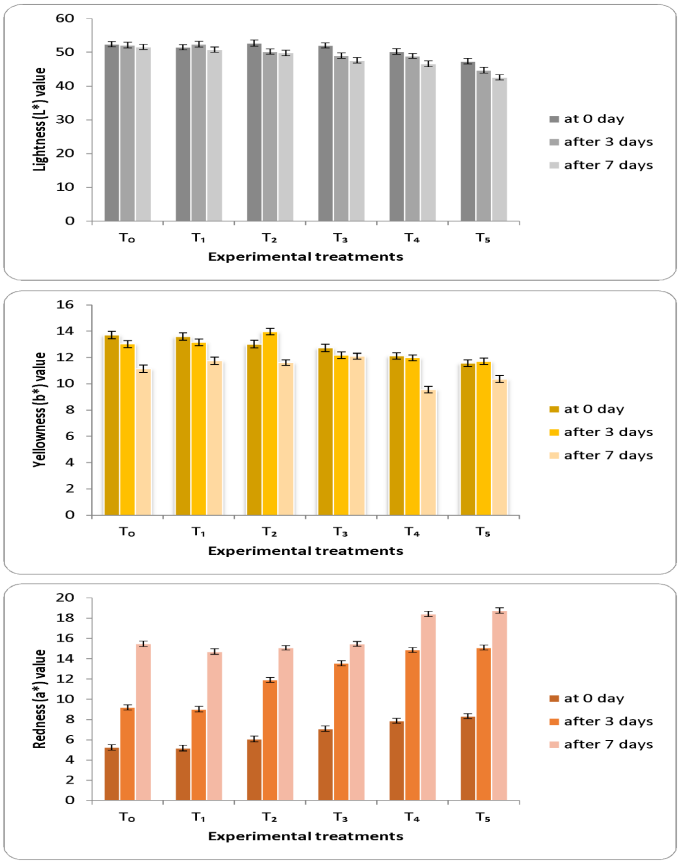

Figure 1 – Color (a*, b* and L* values) of chicken patties enriched with licorice extract stored at refrigeration temperature for various intervals.

pH of patties

The results (Figure 2) regarding pH of chicken meat patties enriched with licorice extracts indicated differences due to treatments and storage. At the start of the trial, the highest pH value (6.18±0.12) reported in control whereas, the lowest pH was observed in T_5 as 5.58±0.01 that were decreased to 6.13±0.13 and 5.33±0.18 at the end of storage. The addition of licorice extract in patties decreased pH to some extent during the storage period. The pH decrease (p<0.05) of samples attributed to by bacterial breakdown of amino acids as well as production of lactic acid

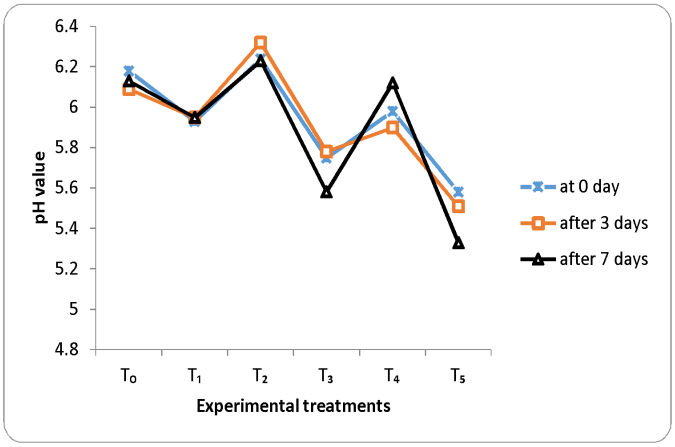

Figure 2 – Changes in pH of chicken patties enriched with licorice extract stored at refrigeration temperature at different storage intervals.

resulted glucose depletion leading towards breakdown of muscle proteins. Also, low pH values reported in T_3, T_4, T_5 meat patties are owing to inhibitory potential antimicrobials in licorice. These findings are supported by Kumar *et al.* (2015) who also suggested an increase in pH of meat product treated with antioxidants as well as meat proteins breakdown resulted higher pH for meat patties at storage.

Sensory evaluation of meat patties

Organoleptic evaluation of food products is a fundamental tool for accessing consumer acceptability. In this regard, chicken meat patties containing LE were tested by consumer panel to evaluate sensorial acceptability. The results (Figure 3) depicted for sensorial attributes such as appearance, flavor, color, juiciness, texture and overall acceptability with the addition of the treatments and storage. At day 0, the highest appearance score was 7.60±0.14 in T_0 group having no licorice extract whereas, the lowest appearance score was 7.37±0.10 in T_5 that decrease to 6.40±0.08 and 5.50±0.41, respectively at the completion of storage. Also, the sensory score for appearance for other treatments were 7.53±0.05, 7.50±0.41 and 7.43±0.33, 7.32±0.08 in T_1, T_2, T_3 and T_4, respectively. Similarly, the addition of licorice extract affected flavor attributes and the highest consumer acceptability was reported in the control and storage also results in the decreased score for the flavor. At initiation, the highest flavor score 6.67±0.47 was reported in T_0 and the lowest 5.33±0.24 in T_5 (group having the highest extract level) that further decreased to 6.10±0.29 and 5.00±0.15, correspondingly. Likewise, taste parameter exhibited a similar trend for treatments and the value of these attributes at various storage intervals 0, 3rd and 7th day were 6.96, 6.66 and 6.14, respectively. Additionally, the texture of the patties showed significant differences due to treatments and storage. Means reported the highest texture score as 7.33±0.47 in T_5 and the lowest 6.17±0.24 in T_0 (group without any antioxidant). However, the values of this attribute were 6.33±0.47, 6.17±0.24, 6.33±0.24, 7.27±0.33 in T_1, T_2, T_3 and T_4, respectively. Likewise, the juiciness of the meat patties showed similar trend like taste and its highest score was documented as 7.33±0.41 in T_5 and lowest 5.67±0.41 was in T_0 at the start of storage that further decreased to 6.50±0.41 and 5.00±0.41 at storage completion. Overall, the acceptability of the patties increased in terms of taste and juiciness and decreased for appearance and texture attributes. However, storage increment decreased the sensorial score for all the sensorial parameters of the study. The results of this study are in line with the findings of Shang & Xiong, (2010) who also reported patties were found to be juicier with the application of LE than that of the control. They also attributed the myofibrillar proteins extractions to form an adhesive gel as the major mechanism for the juiciness of the food product. Similarly, Jiang *et al.* (2013) reported that inhibitory potential of LE against the oxidation process byproducts mainly due to the presence of phenolics, attributed the mechanism for its antiradical power. The main reason for best sensory attributes of treated samples may be due to leaching of polyphenols, flavonoids, flavor and aroma components from the licorice extract and their interaction with different meat components. A previous study checked hydrophilic antioxidants (caffeic and carnosic acids) influence on consumer acceptability traits in sausages, and reported increase in sensory acceptability for antioxidant treatments (Capitani *et al.,* 2013).

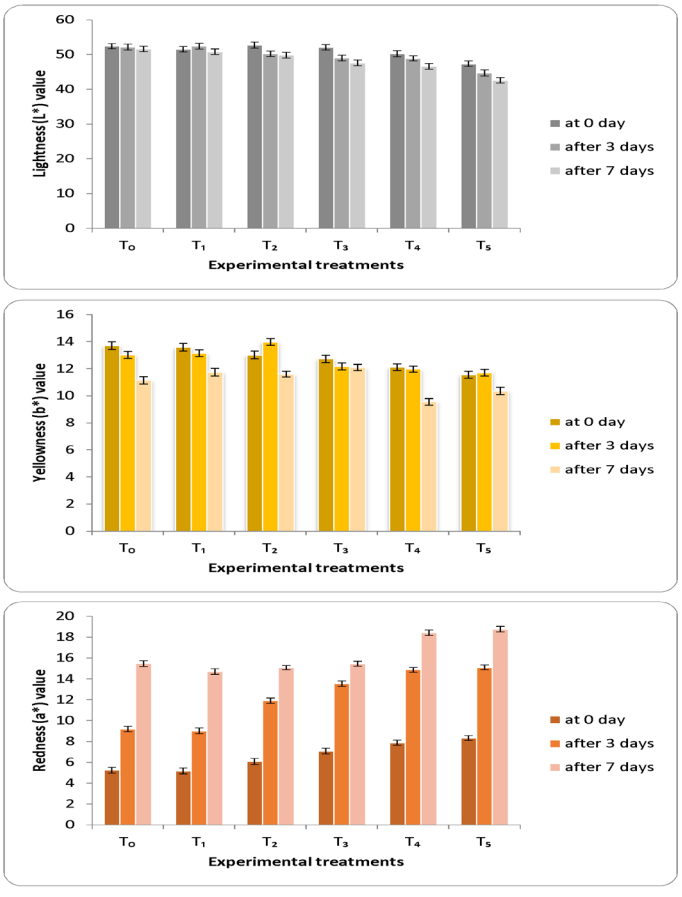

Figure 3 – Sensory evaluation (Hedonic Scale Response) of chicken patties enriched with licorice extract at refrigeration temperature at various storage intervals.

Microbiological parameters

Total plate count of patties

The results regarding total plate count (TPC) of chicken meat patties showed differences by treatments

as well as storage intervals (Table 2). At the initiation of the study, means for total plate count indicated the highest TPC value as 3.37±0.23in (control) whereas, the lowest as 2.02±0.16was reported in T_5 (Group 1.25 g LE /kg meat) that increased to 5.95±0.41and 3.41±0.19, respectively at the termination of storage. Overall, treatments addition positively influenced TPC in the patties in dose dependent manner. Flavonoids are widely distributed in plant-based extract and they are showed to possess biological activities with special focus on antimicrobial potential (Noumara & Akiyama, 2002). The present study's results correlate with the findings of Ceylan and Fung (2004) who reported flavonoids isolated from leguminous medicinal plants like licorice and Ku Shen (*Sophora* root) utilization in processed meat products resulted in the reduction of bacteria responsible for spoilage and quality deterioration. They further documented licorice enriched chicken meat patties have lower values for TPC (log10 CFU) under refrigerated conditions compared to the control. Moreover, results also suggested herbs extracts are more active against gram-positive than gram-negative. Likewise, Zargar *et al.* (2014) also reported results in agreement with findings obtained for chicken sausages during refrigerated storage.

Escherichia coli count

The results regarding *Escherichia coli* of chicken patties indicted differences due to treatments and storage (Table 2). The Means reported *E. coli* count as 1.98±0.11 in (control) whereas, lowest as 1.65±0.11 was reported in T_5 (Group 1.25 g LE/kg meat) at the start of storage that were deceased to 3.41±0.21 and 2.28±0.14, respectively at completion of the study trial. Overall, treatments addition positively influenced *Escherchia coli* in the patties in a dose dependent manner. The present study's findings supported by researches showed LE have potential in inhibiting activity of bacteria like *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Bacillus subtilis (*Wang *et al.,* 2015*)*. Likewise, Awandkar *et al.* (2012) conducted a trial to explore antibacterial potential of aqueous and ethanolic extract of roots of *Glycyrrhiza glabra* L. against pathogens *viz., Staphylococcus aureus* and *Escherichia coli*, isolated from milk samples of cows. Findings showed aqueous extract of *Glycyrrhiza glabra* L. roots is a potent antimicrobial agent against *S. aureus and E. coli* that supports the findings of the study.

CONCLUSION

Present study revealed licorice extract added in chicken patties improved oxidative degradation of lipids by reducing the level of production of malonaldehydes with storage and improved color stability of meat patties product. The addition of the liquorice extract decreased TBARS at storage days indicating potential against oxidation protection of meat product. Additionally, the extract improved the color and pH characteristics of the patties along with consumer acceptability of taste and juiciness. However, the native taste of licorice was reported at a higher dose (1.25% of extract in patties). Microbial stability was also improved indicted by the total plate count and *E. coli* reduction with treatments offering better protection against spoilage bacterium. Conclusively,

Table 2 – Effect of licorice extract on total plate count and *Escherichia coli* of chicken patties with progression of refrigerated storage and storage intervals.

Treatments	Total Plate Count (CFU/g of meat)				*Escherichia Coli* (CFU/g of meat)			
	Storage days				Storage days			
	0	3	7	Mean	0	3	7	Mean
T_0	3.37±0.23	3.91±0.28	5.95±0.41	4.41^{a}	1.98±0.11	2.41±0.11	3.41±0.21	2.60^{a}
T_1	3.19±0.30	3.67±0.21	5.37±0.26	4.08^{b}	1.78±0.06	2.43±0.14	3.43±0.16	2.55^{b}
T_2	3.02±0.21	3.24±0.17	5.01±0.34	3.76^{c}	1.79±0.08	2.41±0.21	3.41±0.19	2.53^{bc}
T_3	2.98±0.14	3.14±0.24	4.72±0.24	3.61^{cd}	1.76±0.09	2.36±0..08	3.36±0.06	2.50^{c}
T_4	2.51±0.11	3.01±0.26	3.84±0.26	3.12^{d}	1.74±0.14	2.16±0.13	2.45±0.08	2.12^{d}
T_5	2.02±0.16	2.55±0.15	3.41±0.19	2.66^{e}	1.65±0.11	1.95±0.14	2.28±0.14	1.96^{e}

Values are Means±SD. Means sharing similar superscript differ non significantly (p>0.05).

T_0= Control

T_1= Group containing 0.25mg of BHT/kg meat

T_2= Group containing 0.25mg licorice extract/kg meat

T_3= Group containing 0.50 mg licorice extract/kg meat

T_4= Group containing 1 g licorice extract/kg meat

T_5= Group containing 1.25 g licorice extract/kg meat

licorice formulation could be used to improve oxidative and microbial stability for developing processing meat product to preserve and increase shelf life with natural food additives.

ABBREVIATIONS

Licorice (*Glycyrrhiza glabra* L.) extract (LE); butylated hydroxyl anisole (BHT); thiobarbituric acid reactive substances (TBARS); total phenolics content (TPC); ferric reducing antioxidant power assay (FRAP); total plate count (TPC); polyunsaturated fatty acids (PUFA); reactive free oxygen species (ROS); generally recognized as safe" (GRAS); malondialdehyde (MDA); colony forming units (CFU).

DECLARATIONS

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not Applicable.

Ethics approval and consent to participate

Not Applicable.

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