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Role of Dried Fenugreek (*Trigonella Foenum-graecum* L.) Leaves as Antioxidant and Antimicrobial in Quality Preservation in Burgers Made of Mutton and Beef Cattle Meat During Refrigerator Storage

ABSTRACT

The effect of different levels of crud fenugreek leaves on lipid oxidation and microbial spoilage of mutton and beef cattle meat postmortem refrigerated storage was examined. Samples meat of mutton and beef cattle were incorporated with crud fenugreek leaves at four different levels 0, 0.5, 1.0 and 1.5 %. Mutton and beef meat in burgers form were subjected to 7 and 10 d postmortem refrigerated storage. Mutton and beef cattle meat marinated with crud fenugreek leaves had significantly lower content of malondialdehyde (MDA) at day 7 and 10 postmortem, respectively. Bacterial counts was lower ($p<0.05$) in mutton and beef cattle meat contenting crud fenugreek leaves compared to control at 3, 5, 7 and 10 postmortem. Regardless of treatment, postmortem refrigerated storage influenced oxidative and microbial stability of meat. Adding crud fenugreek leaves into mutton and beef cattle meat exhibited effective antimicrobial properties and high antioxidant activity.

INTRODUCTION:

Meat is the most important nutrients needed by humans because it is a good source of protein, contains the necessary elements needed by the body and minerals. However, the quality of meat characteristic is affected by ante and postmortem factors. The ante mortem factors involve sex, age, animal species, gene regulation, muscle groups and nutritional type, while postmortem factor comprises basically the refrigerated storage of meat, often termed ageing (Sabow et al., 2016). Development of microbial spoilage is identified as primary mechanisms of quality deterioration in meat and meat products during cold storage (Choi et al., 2010). Therefore, muscle tissue or meat becomes unhealthy for consumption of human through the microbial growth, and the occurrence of chemical and physical changes. According to Insausti et al. (2001) bacteria levels between 6 to 7 log CFU cm^{-2} or g-1 during refrigerated storage are significant levels for meat and meat product spoilage. Additionally, lipid oxidation is also considered as the major source of technological characteristics, palatability and wholesomeness quality deterioration in meat (Dai et al., 2014). Metabolism of muscle and other processes cause development of reactive oxidative species such as superoxide, peroxide, hydroxyl as well as nitric oxide radicals that are able to link with lipid tissues during maturation and storage of meat (Falowo et al., 2014). The changes of lipid oxidation lead to unpleasant flavor and aroma, degradation of protein, discoloration, shelf life reduction and the toxic compounds accumulation that may perhaps influence consumers' health (Falowo et al., 2014; Sabow et al., 2015).

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Insausti et al. (2001) showed that the range of thiobarbituric acid reactive substances (TBARS) value higher than 5 mg malondialdehyde/kg meat is threshold value and becomes unfit for human consumption. Spices are parts of plants that due to their properties are used as preservatives or medicine. The uses of natural herbal plants have been introduced for many years ago, and the interest in the potential of herbal plants is noteworthy due to their chemical compounds which include phenylpropanoids, terpenes, flavonoids and anthocyanins. The nature herbal plants, such fenugreek (*Trigonella Foenum-graecum* L.) among others, are identified and investigated for their antioxidant and antimicrobial characteristics due to their main chemical composition. Although fenugreek seed has been examined as preservation method in many foods specifically in meat during its process to replace chemical preservatives, there is hardly any study about the antimicrobial and antioxidant activity in crude leaves fenugreek. A study on the quantification of phytochemicals in different parts of fenugreek indicates that leaves are rich sources of phytochemicals which have a wide range of biological effects including antioxidant and antimicrobial properties. Thus, the objective of the current study was to determine microbial spoilage and lipid oxidation in marinated mutton and beef cattle meat with different levels of crude leave fenugreek during refrigerated storage.

MATERIALS AND METHODS

Preparation of crude fenugreek leaves (CFL)

Trigonella foenum graecum fresh and healthy leaves were obtained from local market of Baghdad during September 2018. The leaves of *T. foenum graecum* were washed thoroughly in distilled to remove the undesired particles and the most important thing to do with freshly collected material to dry it as fast as possible to prevent fungal infection and preserve color. Therefore, leaves had been dried in ventilated an oven at 40 °C for two days. The dried leaves were differently crushed into fine powder by using an electric grinder and stored at 4 °C in dark containers. The chemical compositions of leaves were as following: 86.1% moisture, 4.4% protein, 0.9% fat, 1.5% minerals, 1.1% fiber and 6% carbohydrates.

Meat sample collection

Samples meat (leg meat) of mutton and beef cattle were gathered from slaughterhouse in Erbil governorate, Iraq in the day before conducting the experiment. All samples were collected in polyethylene bags as per their category and transported to the lab. Fresh samples of burger were prepared following procedure described by Ramadan et al. (2016). The gathered meat were washed with refined water to evacuate any contaminant particles and minced twice. The burger patties were formulated to contain 0 (Control), 0.5, 1.0 and 1.5 % crude fenugreek leaves (CFL). The mixture was shaped manually using a patty marker (stainless steel model) to obtain round discs of 10 cm diameter and 0.5 cm thickness with average weight 50 g. Beef and mutton burgers were packaged in polyethylene bags and stored in cooling up to 7 and 10 days at 4 ˚C, respectively.

Lipid oxidation determination

The oxidation of lipid in burger samples was measured as 2-thiobarbituric acid reactive substances (TBARS) using method described by Aminzade et al. (2012) with slight modification. Approximately 5 g of homogenized samples were distilled with 50 ml of water and 1.25 ml of 4N HCl then the mixture was distilled until 25 ml was obtained. About 2.5 ml of the distillated combination and 2.5 ml of TBA reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid) were boiled using a heating water bath for 35 min. The absorbance was measured at 538 nm against a blank after cooling samples under running tap water for 10 min. The values of TBARS concentration were calculated by multiplying optical density by 7.843. Lipid oxidation was presented as malondialdehyde equivalents (mg MDA per kg meat).

Microbiological analysis

At 1, 3, and 7 or 10 d reregister storage, 1 g of meat samples from mutton and beef cattle was drawn aseptically and transferred to a test tube containing 9 ml of distil water. 0.1 ml samples of serial dilutions (1:10 diluent, and distil water) of burger homogenates were spread on the surface of dry media in order to determine the microbial counts. In order to enumerations total aerobic counts

(TAC), Ten-fold dilutions were spread on Petri dishes in duplicate on Standard Methods Agar (Neogen®, Lansing, Michigan, United States) following 3 days incubation at 32 °C whereas counts of pseudomonas spp. were investigated after 2 days incubation at 25 ºC using Pseudomonas Isolation Agar (Neogen®, Lansing, Michigan, United States) following the method described by Ghollasi Mood et al. (2017). The growth counts (data) were transformed to log10 values.

Statistical analysis

The experimental results were presented as means ± standard error. Variables were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis System package (SAS) Version 9.1.3 software (SAS Institute Inc., Cary, NC, USA). Duncan's test was carried out to determine the differences among means with a p value of 0.05.

RESULTS AND DISCUSSION

Lipid oxidation

The major non-microbial causes of quality deterioration during processing meat and meat product are lipid oxidation (Falowo et al., 2014). Generally, lipid is subjected to oxidative damages due to the fast reduction of endogenous antioxidants immediately post-slaughter through chilling and freezing storage (Xiao et al., 2013). The mean values of oxidative stability of mutton and beef cattle meat marinated with 0, 0.5, 1.0 and 1.5 % of crud fenugreek leaves are presented in Table 1.

Table 1: The malondialdehyde content (mg/kg meat) of mutton and beef cattle meat marinated with different levels of crud fenugreek leaves during postmortem aging

Storage time (days)	Treatment				P value	
	C	0.5CFL	1.0CFL	1.5CFL	Treatment	Treatment × Storage
			Mutton			
1	1.91 ± 0.12^{w}	1.83 ± 0.18	1.85 ± 0.17	1.83 ± 0.06	0.385	0.195
3	2.22 ± 0.25^{x}	1.87 ± 0.31	1.89 ± 0.06	1.96 ± 0.22	0.171	
5	2.93 ± 0.17^{y}	2.39 ± 0.18	2.44 ± 0.20	2.36 ± 0.34	0.299	
7	3.88 ± 0.16^{az}	3.19 ± 0.26^{b}	3.06 ± 0.24^{b}	3.36 ± 0.11^{b}	0.018	
P value	0.001	0.044	0.036	0.032		
			Beef cattle			
1	1.94 ± 0.05^{v}	1.90 ± 0.14^{v}	1.91 ± 0.16^{v}	1.90 ± 0.11^{v}	0.452	0.142
3	2.31 ± 0.11^{w}	2.23 ±0.08^{w}	2.13 ±0.33^{w}	2.25 ± 0.24^{w}	0.121	
5	2.72 ± 0.18^{x}	2.88 ± 0.78^{x}	2.57 ± 0.35^{x}	2.56 ± 0.23x	0.455	
7	3.17 ± 0.06^{y}	2.83 ± 0.22^{y}	2.79 ± 0.21^{y}	2.91 ± 0.31y	0.059	
10	5.71 ± 0.33^{az}	4.66 ± 0.38^{bz}	4.88 ± 0.15^{bz}	4.39 ± 0.22^{bz}	0.039	
P value	0.039	0.018	0.017	0.008		

^{a,b} means within the same row with different superscripts are significantly different ($p<0.05$).

^{w,x,y,z} means within the same column with different superscripts are significantly different ($p<0.05$).

C- 0% crude fenugreek leaves, 0.5CFL- 0.5% crude fenugreek leaves, 1.0CFL - 1.0% crude fenugreek leaves, 1.5CFL- 1.5% crude fenugreek leaves.

Fenugreek leaves was not a source of variation influencing thiobarbituric acid reactive substances (TBARS) value in mutton meat on day 1, 3 and 5 postmortem. Nonetheless, at 7 d postmortem, a significant ($p< 0.05$) decrease in TBARS value was observed in meat with increase in the level of crud fenugreek leaves. From the same table, a gradual decrease was also observed in lipid oxidation concentrations of beef cattle samples incorporated with 0.5, 1.0 and 1.5 percent levels of fenugreek leaves at 10 days postmortem refrigerated storage. This observation could be due to flavonoid or chelator compound which prevents the formation of the free radicals and therefore reduce the value of thiobarbituric acid in meat. The current findings are in agreement with those of Al-Hegazy (2011) who observed lower counts of TBARS in beef cattle burger marinated with different concentrations of fenugreek seeds powder during postmortem aging. Similarly, Baker (2012) reported that lipid oxidation in lamb meat was affected significantly by adding some natural plants such as ginger and rosemary. Regardless of treatment, the TBARS value increased ($p<0.05$)

over storage. Due to chemically unstable of lipids, they are vulnerable to oxidation, particularly during refrigerator storage (Sabow et al., 2016). In fact, oxidation of lipid is a result of oxy or lipid free radical generation and leads to the toxic compounds formation like the cholesterol oxidation and malondialdehyde products. Lipid oxidation is negatively affected the characteristics quality of meat and meat products such as the formation of rancid odors, discoloration, deterioration of flavor and potentially toxic compounds production that can influence humans' health (Falowo et al., 2014). Except for beef cattle meat at 10 days postmortem, the range of lipid oxidation value obtained in the present study was lower than 5 mg MDA /kg meat, which is the value responsible for the detection of off-taste and off-odors for consumer (Insausti et al., 2001).

Microbiological changes

In meat and meat productions, the quality of microbiological is influenced by a number of factors such as the contamination during slaughter, animal physiological condition at slaughter time and storage conditions (Sabow et al., 2016). Furthermore, Ghollasi-Mood et al. (2017) reported that storage in refrigerator affected the microorganisms growth in meat and meat products. Table 2 shows the microbial spoilage growth in mutton and beef cattle meat marinated with 0, 0.5, 1.0 and 1.5 % of crud fenugreek leaves during postmortem. The microbial counts in mutton meat at d 1 were not influenced ($p>0.05$) by fenugreek leaves. However, at d 3, 5 and 7 postmortem, a lower ($p<0.05$) total aerobic counts and pseudomonas spp. counts were present in samples of meat marinated with crud fenugreek leaves. Likewise, the fenugreek leaves on aerobic count of beef cattle meat at 1 d postmortem. However, at 3, 5, 7 and 10 d postmortem, the beef cattle samples contenting fenugreek leaves had lower ($p<0.05$) total aerobic counts and pseudomonas spp. population compared to those subjected to the control. This observation could be due to the influence of the inhibitory effect of fenugreek for the growth of bacteria, which had an important role of reducing and stabilizing the growth of microorganisms. A similar explanation was given by Ajeena et al. (2012) who attributed the lower bacterial growth exhibited by the in fenugreek leaves groups over storage to antimicrobial compounds in crude fenugreek leaves which could be due to degrade the wall of cell, damage the cytoplasmic membrane, disrupt membrane proteins and interfere with membrane–integrated enzymes, and consequently lead to death of cell. Additionally, crude fenugreek leaves could influence pH of meat as well as its content of steroids compound and volatile oil such as tannins and flavonoids that reduce or inhibition of microbial activity and biochemical reactions that cause deteriorative changes and spoilage through enzymatic, chemical and physical activities. The results of the present study are in tandem with those of Hegazi (2011) in beef burger and Baker (2012) in lamb meat who found lower counts of bacteria in meat contenting some natural herbal plants like fenugreek and rosemary. Regardless of treatment, bacterial counts enhanced ($p<0.05$) with increase in aging time in both type of meat. In the present study, except for control samples of beef cattle at 10 day postmortem refrigerated storage, all microbe levels detected throughout the storage were acceptable as claimed by Insausti et al. (2001). According to Insausti et al. (2001), meat becomes spoilage when the value of total counts of bacteria reaches 7-8 log cfu/g.

Table 2: The Microbiological quality of mutton and beef cattle meat marinated with different levels of crud fenugreek leaves during postmortem aging

Parameter	Storage time (days)	Treatment				P value	
		C	0.5FL	1.0FL	1.5FL	Treatment	Treatment × Storage
				Mutton			
Total aerobic count (Log10 CFU/g)	1	3.97 ± 0.05^{w}	3.88 ± 0.06^{x}	3.73 ± 0.02^{x}	3.88 ± 0.05^{w}	0.058	0.351
	3	5.03 ± 0.11^{ax}	4.87 ± 0.16^{by}	4.84 ± 0.37^{by}	2.65 ± 0.07^{cx}	0.016	
	5	7.09 ± 0.36^{y}	6.96 ± 0.11^{z}	6.96 ± 0.31z	6.89 ± 0.29^{y}	0.156	
	7	7.81± 0.22^{az}	7.16 ± 0.14^{bz}	7.21 ± 0.42^{bz}	7.19 ± 0.33^{bz}	0.001	
	P value	<0.0001	0.0004	0.0016	0.0011		
Pseudomonas spp. (Log10 CFU/g)	1	2.41 ± 0.01^{w}	2.65 ± 0.18^{x}	2.73 ± 0.17^{x}	2.65 ± 0.08^{w}	0.471	0.222
	3	3.79 ± 0.14^{ax}	3.13 ± 0.14^{by}	3.22 ± 0.17^{by}	3.17 ± 0.09^{bx}	0.001	
	5	4.55 ± 0.36^{ay}	4.12 ± 0.22^{bz}	4.15 ± 0.38^{bz}	4.06 ± 0.29^{by}	0.004	
	7	5.39 ± 0.14^{az}	4.76 ± 0.20^{bz}	4.86 ± 0.18^{bx}	4.92 ± 0.11^{bz}	0.025	
	P value	<0.0001	0.0083	0.0025	0.0004		
				Beef cattle			
Total aerobic count (Log10 CFU/g)	1	3.91 ± 0.03^{v}	3.85 ± 0.01^{v}	3.88 ± 0.01^{v}	3.94 ± 0.05^{v}	0.993	0.652
	3	4.55 ± 0.11^{w}	4.46 ± 0.02^{w}	4.41 ± 0.04^{w}	4.22 ± 0.06^{w}	0.062	
	5	6.06 ± 0.23^{ax}	5.88 ± 0.08^{bx}	5.96 ± 0.09^{aby}	5.68 ± 0.24^{bx}	0.001	
	7	7.08 ± 0.14^{ay}	6.43 ± 0.17^{by}	6.38 ± 0.13^{by}	6.19 ± 0.29^{by}	0.001	
	10	8.27 ± 0.30^{ax}	7.68 ± 0.22^{bz}	7.60 ± 0.15^{bx}	7.25 ± 0.24^{bz}	0.001	
	P value	<0.0001	<0.0001	<0.0001	<0.0001		
Pseudomonas spp. (Log10 CFU/g)	1	Absent	Absent	absent	absent		0.193
	3	2.38 ± 0.11^{aw}	1.05 ± 0.01^{bx}	1.90 ± 0.16^{aby}	0.41 ± 0.03^{cx}	0.001	
	5	3.67 ± 0.06^{ax}	1.53 ± 0.02^{by}	2.19 ± 0.33^{by}	0.52 ± 0.01^{cx}	0.001	
	7	4.17 ± 0.13^{ay}	1.68 ± 0.07^{by}	2.57 ± 0.15^{bz}	0.73 ± 0.01^{cy}	0.004	
	10	5.03 ± 0.09^{cz}	2.49 ± 0.12^{bz}	2.48 ± 0.20^{bz}	1.07 ± 0.06^{cz}	0.005	
	P value	<0.0001	0.0011	0.0033	0.0071		

a,b means within the same row with different superscripts are significantly different (p<0.05).
^{w,x,y,z} means within the same column with different superscripts are significantly different (p<0.05).
C- 0% crude fenugreek leaves, 0.5CFL- 0.5% crude fenugreek leaves, 1.0CFL - 1.0% crude fenugreek leaves, 1.5CFL- 1.5% crude fenugreek leaves.

CONCLUSION:

The results of current study show that lipid oxidation and microbiological changes of mutton and beef cattle meat marinated with crude fenugreek leaves is comparable to that of control at d 1 postmortem storage. However, at 3, 5, 7 postmortem, crude fenugreek leaves had negative influences on the shelf life of mutton and beef cattle. Crude fenugreek leaves can be used for better shelf life during postmortem aging.

Disclosure statement

The authors declare that they have no conflicts of interest.

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دور أوراق الحلبة المجففة كمضاد أكسدة ومضاد مايكروبى في حفظ البركر المنتج من لحم الغنم والبقر المخزنة بالتبريد

بيستون حسن أحمد وشونم جبار صالح وأزاد بهنان سبو

قسم الثروة الحيوانية– كلية الزراعة/ جامعة صلاح الدين– أربيل أقليم كوردستان– العراق

المستخلص

هدفت هذه الدراسة لمعرفة تأثير مستويات مختلفة من مسحوق أوراق الحلبة على أكسدة الدهون والنمو المايكروبي للحوم الأغنام والأبقار المخزون عند درجة حرارة 4 مئوي. ثم تحضير العينات من شرائح اللحم الخالص لقطعيات الفخذ من ذبائح الأغنام والأبقار وبعد ذلك تم مزجها مع تراكيز مختلفة من مسحوق أوراق الحلبة 0 , 0.5 , 1 , 1.5 % . خزنت عينات لحم الاغنام والأبقار على شكل برغر في التبريد لمدة 7 و 10 يوم. اظهرت عينات لحم الأغنام والأبقار المتبلة بمسحوق أوراق الحلبة انخفاض معنوي في قيمة حامض الثايو باربيوتريك(TBA) الحلبة في اليوم السابع والعاشرة من فترة الخزن علي التوالي. لوحظ انخفاض معنوي في تعداد البكتيري أيضا لعينات لحم الغنم والبقر المعاملة مع مسحوق أوراق الحلبة مقارنة بمعاملة السيطرة بعد 3 , 5 ,7 و 10 أيام من الخزن المبرد. بغرض النظر عن المعاملة، فترة الخزن أثرت معنويا على استقرار التأكسدي والميكروبي. يمكن الأستنتاج بأن اضافة مسحوق أوراق الحلبة المجفف الي لحم الغنم والبقر كان تأثيره فعالا كمضاد لأكسدة الدهون ومثبط للنمو البكتيري.

الكلمات المفتاحية: لحم البقر، أوراق الحلبة، اكسدة الدهون، تعداد البكتيري، لحم الغنم، خزن بالتبريد.