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Tribulus terrestris Linn.: A Novel Natural Preservative for Improved Lipid Stability and Storage Quality of Meat Products

Sabahu Noor, Z. F. Bhat*, Sunil Kumar and Insha Kousar Kalem

Division of Livestock Products Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R. S. Pura, Jammu-181102, India

[1]*College of Veterinary Pharmacy, Abhilashi University, Chail Chowk Mandi, Himachal Pradesh, India, 175045.*

ABSTRACT

A study was conducted to evaluate the potential of *Tribulus terrestris* as a novel natural preservative in meat products. Chevon sausages were used as a model system and incorporated with different levels of *T. terrestris viz.* T_1 (0.25%), T_2 (0.50%) and T_3 (0.75%) and were vacuum packaged and assessed for various lipid stability and storage quality parameters under refrigerated (4±1^{O}C) conditions. Significantly ($p<0.05$) lower TBARS (mg malonaldehyde/kg) values were observed for the products containing *T. terrestris*. A significant ($P<0.05$) effect was also observed on the microbial stability as *T. terrestris* containing products showed lower total plate count (log cfu/g), psychrophilic count (log cfu/g), yeast and mould count (log cfu/g), anaerobic count (log cfu/g) and FFA (% oleic acid) values.Significantly ($p<0.05$) higher scores were observed for various sensory parameters of the products containing *T. terrestris* (0.25%). T. terrestris successfully improved the lipid stability and storage quality of the model meat product under vacuum packaging conditions and might be commercially utilized as a novel natural preservative for muscle foods.

Keywords: *Tribulus terrestris, Chevon Sausages, natural preservative, Lipid oxidation, Storage quality*

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INTRODUCTION

Majority of diseases or disorders in humans are mainly linked to oxidative stress due to free radicals and reactive oxygen species (ROS) (Rahman *et al.* 2012). The ROS induced oxidation can result incell membrane disintegration, membrane proteindamage and DNA mutation (Hifnawy *et al.* 2015), which can further initiate or propagate the development of manydiseases, such as cancer, Alzheimer's disease, Parkinson's disease, neuronal disorders, asthma, cardiovascular disease, ulcerative colitis, acute respiratory distress syndrome etc. (Lokhande *et al.* 2014). An important role is played by antioxidants in inhibiting and scavenging free radicals, thus providing protection to humans against various infections and degenerative diseases. Plant derived polyphenolic compounds, like flavonoids and phenolic acids, have been reported to have multiple biological effects, including antioxidant activities (Hifnawy *et al.* 2015), and as such have been used for the prevention and cure of conditions and diseases which are mainly associated with free radicals and reactive oxygen species (Lokhande *et al.* 2014). This explains the increasing trend of incorporating plant based polyphenolic compounds and extracts in the functional foods. These compounds not only benefit the consumer but also improve the lipid oxidative stability and storage quality of the foods. While substantial literature is available in favour of the use of bioactive compounds from numerous plant extracts, interest in the polyphenol and flavonoid related properties of Tribulus terrestris has recently emerged.

Tribulus terrestris Linn. (Zygophyllaceae) is an annual herb and is found throughout the world (Lokhande *et al.* 2014).The plant contains numerous phytochemicals like saponins, flavonoids, alkaloids, steroids, carbohydrates, tannins, glycosides, lignin, and amino acids (Abubakar *et al.* 2016; Hifnawy *et al.* 2015). The plant is a rich source of bioactive compounds responsible for its strong antibacterial, antioxidant and free radical scavenging activity (Abubakar *et al.* 2016; Mohammed *et al.* 2016; Ram *et al.*2015; Hifnawy *et al.*2015; Eagappan *et al.*2015).Hifnawy *et al.*(2015) reported that *T. terrestris* fruits contain thirty one phenolic compounds: 9 flavonoids (hesperidin, rutin, quercitrin, quercetin, naringin, naringenin, hesperitin, apigenin and kaempferol) and 22 phenolic acids (pyrogallic, gallic, p-hydroxy benzoic, protocatechuic, catechol, caffeic, vanillic, syringic, chlorogenic, ferulic, pcoumaric, ellagic, coumarin salicylic, cinnamic, 3,4,5 methoxy cinnamic, 4-amino benzoic acid, catechin, epicatechin, isoferulic, resveratrol and coumaric).The plant also possessesantifungal activity, antidiabetic activity, antidiuretic and contractile activity, antihypertensive and vasodilator activity (Abubakar *et al.* 2016; Ram *et al.*2015; Hifnawy *et al.*2015).

The present study was envisaged to attempt the still inconclusive studies on utilization of *T. terrestrisas* a novel natural preservative in muscle foods. The study was designed with chevon sausages as a model system to analyse the effect of *T. terrestris* on the lipid stability and storage quality.

MATERIALS AND METHODS

Chevon meat: The goat meat wasobtained from a local market and was manually deboned after removing all tendons and separable connective tissue and trimming the fat. The lean meat was frozen at -18±2^{o}C within the transparent low-density polythene pouches (250 gauge) and thawed at refrigeration temperature (4±1^{o}C) before use.

Spice mixture: The spice mixture contained coriander (*Coriandrum sativum*) 20%, cumin seed (*Cuminum cyminum*) 15%, aniseed (*Pimpinalla anisum*) 12%, black pepper (*Piper nigrum*) 10%, red chilli (*Capsicum frustescence*) 8%, green cardamom (*Elettaria*

*Corresponding author Email address: Zuhaib.Bhat@lincolnuni.ac.nz
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cardamomum) 6%, cinnamon (*Cinnamomum zeylanicum*) 6%, white pepper (*Piper nigrum*) 5%, black cardamom (*Amomum subulatum*) 5%, degi mirch (*Capsicum annum*) 5%, bay leaves (*Laurus nobilis*) 2%, cloves (*Syzygium aromaticum*) 2%, mace (*Myristica fragrans*) 2% and nutmeg (*Myristica fragrans*) 2%.

Tribulus terrestris: Commercially available *Tribulus terrestris* was procured from "The Himalaya Drug Company". The products were prepared by incorporating different concentrations of *T. terrestris* viz. 0.25% (T_1, 250 mg per Kg of meat emulsion), 0.50% (T_2, 500 mg per Kg of meat emulsion) and 0.75% (T_3, 750 mg per Kg of meat emulsion).

Method of preparation of chevon sausages: Small chunks of chevon were minced in a Sirman meat mincer (MOD-TC 32 R10 U.P. INOX, Marsango, Italy) with 6mm plate twice and blended with salt, sodium nitrite and sodium tripolyphosphate for 1.5 minute in Sirman bowl chopper (MOD C 15 2.8G 4.0 HP, Marsango, Italy). Water was added in the form of crushed ice and blending continued for 1 minute which was followed by the addition of refined soyabean oil and continued for another 1 to 2 minutes. Thereafter, condiments, spice mixture and other ingredients were added and further continued for 1 to 2 minutes to get the desired emulsion.The emulsion was filled into the artificial rapid peel casing (No.C 25* 60) procured from M/S Euromate Food Tech. Pvt. Ltd, New Delhi with the help of Sirman sausage filler (Model-1S-V15-IRDA-VERT, S. NO. 07L01410. Marsango, Italy). The raw sausages were cooked at a temperature of 140±5°C for a time of about 30 minutes in a hot air oven (Yorco sales Pvt. Ltd. India, Model-YS1-431, S.No. 02B2843). The products were cooled and packaged under vacuum (Model DZ-500/2ES) in laminate pouches (polyethylene/aluminium/polyethylene) and stored at refrigeration temperature (4±1°C).

Analytical Procedures

pH: The pH was measured as per the method of Keller *et al.*(1974) using a digital pH meter.

Thiobarbituric acid reacting substances (TBARS) value and free fatty acid (FFA): TBARS (mg malonaldehyde/kg) value was estimated according to Witte *et al.*(1970) whereas the method of Koniecko (1979) was following for FFA (% oleic acid) estimation.

Moisture content: Procedure described by AOAC (2000) was used to measure the moisture content of the products using a hot air oven.

Microbiological characteristics: Various microbiological characteristics were determined viz. total plate count, psychrophilic count, coliform count, anaerobic count, and yeast and mould count as per the methods described by APHA (1984).

Sensory evaluation: A semi-trained sensory panel of ten members evaluated the products for various sensory parameters. The panel members were scientists and postgraduate scholars of the division and conducted the sensory analysis based on an 8-point hedonic scale (Keeton 1983).

Statistical analysis: The study was conducted in three independent experimental trials and all the samples were analysed in duplicate (n=6). SPSS version 16.0 software program was used to analyse the data generated by repeating the experiments for all quality parameters. Two-way ANOVA was used to analyse the means of different parameters. Duncan post-hoc multiple comparisons test was used to see significant differences among storage times at each treatment and among treatments at each storage time at 5% level of significance (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The mean values of different physicochemical parameters of vacuum packaged chevon sausages containing different levels of Tribulus terrestrisviz. T_1 (0.25%), T_2 (0.50%) and T_3 (0.75%) are presented in Table 1.

Physicochemical parameters

pH: The mean pH values showed a significant ($p<0.05$) decreasing trend from day 0 to day 56 in case of control as well as treated samples (T_1, T_2 and T_3). The decline in pH may be attributed to the accumulation of acids produced by the bacteria, predominantly anaerobic and lactic acid bacteria, during vacuum storage. Ozpolat *et al.* (2014) also reported an overall decline in pH of vacuum packed sausages during refrigerated storage. The mean pH values of the products incorporated with *T. terrestris* (T_2 and T_3) were significantly ($p<0.05$) lower than control on day 42 and 56 of storage. Dua *et al.* (2015a) recorded a similar decline in the pH values of *Tabaq-Maz* treated with oleuropein and the authors attributed the decline in pH to the acidic and polyphenolic compounds present in the extract.

Thiobarbituric acid reacting substances (TBARS) value: TBARS values followed a significant ($p<0.05$) increasing trend with storage in all the products. This might be attributed to lipid hydrolysis, oxidative rancidity and secondary products formation at refrigeration temperature. Unsaturated fatty acids might have undergone a process of oxidative changes causing increase of TBARS values. Rajkumar *et al.* (2016) recorded a significant increase in the TBARS values of the nuggets incorporated with Aloe vera gel throughout the storage period. TBARS values of the products incorporated with T. terrestris (T_1, T_2 and T_3) were significantly ($P<0.05$) lower than control on all intervals of storage except on day 0. This might be attributed to antioxidant properties of T. terrestris which contains high amounts of bioactive phytochemicals, polyphenols and flavonoids (Abubakar *et al.*2016; Mohammed *et al.* 2016; Ram *et al.*2015; Eagappan *et al.*2015; Lokhande *et al.* 2014). *T. terrestris* extract can directly neutralize radicals by providing hydroxyl groups and cause an inhibition of the chain reactions during lipid oxidation. Dimitrova *et al.* (2012) reported a strong FRAP activity of *T. Terrestris* compared to BHT

Table1: Effect of *Tribulus terrestris* on the physicochemical properties of vacuum packaged chevon sausages during refrigerated storage (Mean ± SE)*

Treatment	Storage period (Days)				
	0	14	28	42	56
			pH		
Control	6.37±0.027^{a}	6.28±0.046^{a}	6.18±0.019^{b}	6.10±0.228^{Ab}	5.84±0.008^{Ac}
T_1 (0.25%)	6.30±0.006^{a}	6.25±0.045^{a}	6.12±0.017^{b}	6.08±0.016^{ABb}	5.81±0.007^{ABc}
T_2 (0.50%)	6.28±0.006^{a}	6.23±0.065^{a}	6.15±0.044b^{c}	6.05±0.013^{Bc}	5.78±0.021^{BCd}
T_3 (0.75%)	6.35±0.067^{a}	6.30±0.043^{a}	6.11± 0.045^{b}	6.04±0.016^{Bb}	5.75±0.016^{Cc}
			TBARS (mg malonaldehyde/kg)		
Control	0.35±0.036^{e}	0.46±0.006^{Ad}	0.67±0.006^{Ac}	0.83±0.007^{Ab}	1.28±0.006^{Aa}
T_1 (0.25%)	0.31±0.035^{e}	0.38±0.007^{Bd}	0.60±0.007^{Bc}	0.72±0.004^{Bb}	1.20±0.008B^{a}
T_2 (0.50%)	0.28±0.024^{e}	0.30±0.005^{Cd}	0.52±0.009^{Cc}	0.64±0.006^{Cb}	1.16±0.008^{Ca}
T_3 (0.75%)	0.25±0.031^{e}	0.21±0.007^{Dd}	0.43±0.009^{Dc}	0.55±0.007^{Db}	1.02±0.013^{Da}
			FFA (% oleic acid)		
Control	0.128±0.005^{e}	0.155±0.0008^{Ad}	0.263±0.0007^{Ac}	0.352±0.001^{Ab}	0.465±0.001^{Aa}
T_1 (0.25%)	0.116±0.004^{e}	0.126±0.0006^{Bd}	0.252±0.0008^{Bc}	0.264±0.002^{Bb}	0.386±0.001^{Ba}
T_2 (0.50%)	0.114±0.004^{e}	0.119±0.0011^{Cd}	0.224±0.0019^{Cc}	0.253±0.007^{Cb}	0.352±0.001^{Ca}
T_3 (0.75%)	0.110±0.008^{e}	0.114±0.0004^{Dd}	0.215±0.0013^{Dc}	0.322±0.001^{Db}	0.324±0.001^{Da}
			Moisture (%)		
Control	64.63±0.059^{a}	64.54± 0.176^{a}	63.72±0.311^{b}	63.62±0.064^{b}	62.53±0.135^{c}
(0.25%)	64.59±0.056^{a}	64.51± 0.147^{a}	63.68±0.065^{b}	63.59±0.056^{b}	62.50±0.053^{c}
(0.50%)	64.55±0.047^{a}	64.48± 0.102^{a}	63.63±0.063^{b}	63.55±0.051^{b}	62.47±0.100^{c}
(0.75%)	64.51±0.048^{a}	64.44± 0.208^{a}	63.59±0.075^{b}	62.50±0.039^{b}	62.44±0.090^{c}
			Cooking yield (%)		
Control	91.26±0.199	-	-	-	-
(0.25%)	91.24±0.220	-	-	-	-
(0.50%)	91.21±0.182	-	-	-	-
(0.75%)	91.18±0.161	-	-	-	-

*Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) in a subgroup differ significantly (P<0.05), n = 6 for each treatment

T1 (0.25%) = Sausages with 0.25% of *Tribulus terrestris*

T2 (0.50%) = Sausages with 0.50% of *Tribulus terrestris*

T3 (0.75%) = Sausages with 0.75% of *Tribulus terrestris*

and observed an inhibition of lipid peroxidation by *T. terrestris* in linoleic acid system. Dua *et al.* (2015b) recorded a similar observation in the TBARS values of *Tabaq-Maz* treated with lemon peel extract rich in polyphenols and flavonoids.

Free fatty acids (% oleic acid): A significant (p<0.05) inclining trend was recorded with storage in case of control and treated sausages. FFA content of the products was well below the threshold value i.e. 1.8% (Pearson and Gillet 1983). Growth of lipolytic microorganisms might be the reason for this significant (p<0.05) increase in FFA content of the products during storage (Das *et al.* 2008). Malav *et al.* (2015) reported a significant increasing trend in the FFA values of vacuum packaged mutton patties during refrigerated storage. The mean FFA values were significantly (p<0.05) lower in the products incorporated with T. terrestris (T_1, T_2 and T_3) on all intervals of storage except day 0. Significantly (p<0.05)lower FFA values of the treated products might be attributed to the antimicrobial and antioxidant properties of T. terrestris (Abubakar *et al.* 2016; Mohammed *et al.* 2016; Ram *et al.*2015; Eagappan *et al.* (2015). Malav *et al.* (2015) observed significantly lower FFA values for vacuum packaged mutton patties incorporated with cabbage powder in comparison to control.

Moisture (%): A significant (p<0.05) decrease was observed in the moisture content of all the products from 0thday to 56th day of storage. This might be attributed to the evaporative losses. El-Nashi *et al.* (2015) also recorded a significant (p<0.05) decrease in the moisture content of beef sausages incorporated with pomegranate peel powder during storage.

Cooking yield (%): No significant (p>0.05) difference was observed between the cooking yield of treatments (T_1, T_2 and T_3) and control. Similar findings were reported by Singh *et al.* (2015a) who also observed no significant (p>0.05) change in the cooking

yield of chevon cutlets incorporated with sorghum bicolour and clove oil.

Microbiological characters: The mean values of various microbiological characteristics of vacuum packaged chevon sausages containing different levels of *T.terrestris* viz. T_1 (0.25%), T_2 (0.50%) and T_3 (0.75%) are presented in Table-2.

Total plate count (log cfu/g): The total plate counts increased significantly (p<0.05) from day 0 to 56 of storage in treated products (T_1, T_2 and T_3) as well as in control. The counts were well below the permissible limits up to 42nd day of storage for all the products (Jay 1996) and exceeded the limit on 56th day of storage for control and T1. Cremer and Chipley (1977) reported that 5.33 log cfu/g could be considered as indicative of unacceptability of cooked meat products. Malav *et al.* (2015) observed similar results with a significant (p<0.05) increase in the TPC values of vacuum packed mutton patties for control as well as treated products. The TPC of the products incorporated with *T. terrestris* (T_1, T_2 and T_3) were significantly (P<0.05) lower than control on all intervals of storage which might be attributed to the bioactive compounds and other constituents of *T. terrestris* which are reported to have antimicrobial properties (Kianbakht and Jahaniani 2003). Senthil kumaran *et al.* (2014) reported the invitro antibacterial properties of *T. terrestris* against *E.coli*, *Proteus vulgaris, Enterobacter aerogens, Bacillus cereus, Streptococcus faecalis, Klebsiella pneumoniae, Salmonella typhi, Serratia marcescensm and Pseudomonas aeruginosa.*

Table 2: Effect of *Tribulus terrestris* on the microbiological quality of vacuum packaged chevon sausages during refrigerated storage (Mean ± SE)*

Treatment	Storage period (Days)				
	0	14	28	42	56
			Total plate count (log cfu/g)		
Control	1.37±0.007^{Ae}	2.51±0.005^{Ad}	3.87±0.005^{Ac}	4.88±0.005^{Ab}	5.51±0.030^{Aa}
(0.25%)	1.31±0.008^{Be}	2.35±0.006^{Bd}	3.53±0.004^{Bc}	4.72±0.006^{Bb}	5.42±0.007^{Ba}
(0.50%)	1.23±0.008^{Ce}	2.27±0.007^{Cd}	3.35±0.003^{Cc}	4.53±0.006^{Cb}	5.33±0.006^{Ca}
(0.75%)	1.17±0.009^{De}	2.06±0.004^{Dd}	3.23±0.003^{Dc}	4.40±0.016^{Db}	5.19±0.007^{Da}
			Psychrophilic count (log cfu/g)		
Control	ND	ND	1.30±0.004^{Ac}	2.83±0.006^{Ab}	3.50±0.006^{Aa}
(0.25%)	ND	ND	1.26±0.006^{Bc}	2.7±0.004^{Bb}	3.32±0.006^{Ba}
(0.50%)	ND	ND	1.21±0.006^{Cc}	2.64±0.005^{Cb}	3.22±0.006^{Ca}
(0.75%)	ND	ND	1.15±0.006^{Dc}	2.56±0.005^{Db}	3.13±0.005^{Da}
			Coliform count (log cfu/g)		
Control	ND	ND	ND	ND	ND
T_1 (0.25%)	ND	ND	ND	ND	ND
T_2 (0.50%)	ND	ND	ND	ND	ND
T_3 (0.75%)	ND	ND	ND	ND	ND
			Yeast and mould count (log cfu/g)		
Control	ND	ND	1.96±0.005^{Ac}	2.74±0.008^{Ab}	3.81±0.008^{Aa}
T_1 (0.25%)	ND	ND	1.82±0.004^{Bc}	2.67±0.039^{ABb}	3.73±0.035^{Ba}
T_2 (0.50%)	ND	ND	1.78±0.004^{Cc}	2.64±0.048^{ABb}	3.68±0.007^{BCa}
T_3 (0.75%)	ND	ND	1.75±0.010^{Dc}	2.59±0.048^{Bb}	3.65±0.006^{Ca}
			Anaerobic count (log cfu/g)		
Control	ND	ND	1.46±0.007^{Ac}	1.68±0.008^{Ab}	2.79±0.006^{Aa}
T_1 (0.25%)	ND	ND	1.31±0.008^{Bc}	1.54±0.006^{Bb}	2.62±0.007^{Ba}
T_2 (0.50%)	ND	ND	1.25±0.006^{Cc}	1.44±0.007^{Cb}	2.46±0.007^{Ca}
T_3 (0.75%)	ND	ND	1.18±0.021^{Dc}	1.36±0.005^{Db}	2.32±0.006^{Da}

*Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05)

n = 6 for each treatment; T1 (0.25%) = Sausages with 0.25% of *Tribulus terrestris*

T2 (0.50%) = Sausages with 0.50% of *Tribulus terrestris*;

T3 (0.75%) = Sausages with 0.75% of *Tribulus terrestris*; ND = Not detected

Psychrophilic count (log cfu/g): Counts were observed on day 28 of storage in all products and thereafter followed a significant (P<0.05) increasing trend. The counts were well below the permissible limits up to 56th day of storage for all the products. Cremer and Chipley (1977) described permissible level of psychrophilic count as 4.6 log cfu/g in cooked meat products. Similar findings were also reported by Kaur *et al.* (2015) in chicken meat nuggets. The psychrophilic counts of the treated products (T_1, T_2 and T_3) were significantly (p<0.05) lower as compared to control which might be attributed to the bioactive compounds of *T. terrestris* which are said to have antimicrobial properties. Kaur *et al.* (2015) reported significantly lower psychrophilic counts for the chicken nuggets treated with grape seed extract.

Coliform count (log cfu/g): No coliforms were detected in any of the products on any interval of the storage period. Absence of coliforms might be attributed to their destruction during cooking at 140°C which is much above their thermal death point of 57°C. Similar findings were reported by Malav *et al.* (2015) who also observed no coliforms in vacuum packed pork patties during entire period of storage.

Yeast and mould count (log cfu/g): Counts were observed on day 28th and thereafter followed a significant (p<0.05) increasing trend in case of control as well as treated samples. Similar findings were also observed by Singh *et al.* (2014) who reported similar increasing trend in the yeast and mould counts of chevon cutlets. Counts of the treated products (T_1, T_2 and T_3) were significantly (p<0.05) lower as compared to control which might be attributed to the bioactive compounds of *Tribulus terrestris* having antifungal properties (Abubakar *et al.* 2016; Ram *et al.* 2015; Al-Bayati and Al-Mola 2008). Senthil kumaran *et al.*(2014) reported invitro antifungal properties of *T. terrestris* against *Aspergillus niger, A. flavus, A. fumigatus,Fusarium oxysporum, F. solani, Trichophytonmentagrophytes, T. rubrum, Trichoderma viridae, Microsporum gypseum* and *Candida albicans.*

Anaerobic count (log cfu/g): Counts were observed on day 28th and thereafter followed a significant (p<0.05) increasing trend in case of control as well as the products incorporated with *T. terrestris*. Non-detection of anaerobes in cooked products up to 28th day could be due to sufficient heat treatment and antimicrobial effect of nitrite used in the formulation. Conducive anaerobic environment could have favoured the growth of lactic acid bacteria with the depletion of nitrite at a later stage. However, at this low count, lactic acid production would not have been enough to cause any fermentative repugnant odour and flavour in the products. Rodriguez *et al.* (2003) reported a critical limit of 10^7-10^8 for LAB. Anaerobic counts of the treated products (T_1, T_2 and T_3) were significantly (P<0.05) lower as compared to control which might be attributed to the antimicrobial properties of *T. terrestris*. Malav *et al.*(2015) also reported a significant decrease in the counts of vacuum packed mutton patties incorporated with cabbage powder in comparison to control.

Sensory parameters: The mean values of various sensory parameters of vacuum packaged chevon sausages containing different levels of *T.terrestris viz.* T_1 (0.25%), T_2 (0.50%) and T_3 (0.75%) are presented in Table 3.

Colour and appearance: The colour and appearance scores showed a significant (p<0.05) decreasing trend throughout the period of storage for all products. This decreasing trend might be due to pigment and lipid oxidation and non-enzymatic browning resulting from Millard's reaction (Chandralekha *et al.* 2012). Lipid oxidation produces secondary reaction products such as pentanal, 4-hydroxynonenal, hexanal and malondialdehyde as well as other oxygenated compounds such as ketones, acids and aldehydes which can cause loss of colour (Dave and Ghaly 2011). Significantly (p<0.05) higher scores were recorded for the treated products (T_1, T_2 and T_3) in comparison to control on all days of storage. This might be attributed to the colour pigments and phenolic compounds of *T. Terrestris* which are said to have antioxidant and antimicrobial properties. Bhat *et al.* (2015b) reported significantly (p<0.05) higher scores for the products treated with *Ocimum sanctum Linn* in comparison to control.

Flavour: The scores for flavour decreased significantly (p<0.05) for all products as the period of storage progressed. Increased oxidation of lipids, liberation of free fatty acids, higher microbial load and loss of volatile flavour components from spices and condiments could be the contributing factors responsible for the decline (Chandralekha *et al.*2012). Production of some bitter compounds during lipid oxidation, lipolysis and proteolysis affects the flavour. Significantly (P<0.05) higher scores were observed for the treated samples (T_1, T_2 and T_3) on day 28 and 42 in comparison to control. Bhat *et al.* (2015a) also observed significantly higher flavour scores for chicken nuggets incorporated with *Aloe vera*.

Juiciness: The scores for juiciness decreased significantly (p<0.05) for control as well as treated products (T_1, T_2 and T_3) with storage. This might be attributed to the gradual loss of moisture from the products. Jin *et al.* (2015) also observed a decrease in juiciness scores of the pork sausages during refrigerated storage.

Texture: A significant (p<0.05) decreasing trend was observed with storage for control as well as treated products (T_1, T_2 and T_3) which might be due to loss of moisture; breakdown of fat and degradation of proteins by bacterial action (Jay 1996). The sausages incorporated with *T. terrestris* showed significantly (P<0.05) higher scores for texture on day 14 and onwards. The higher scores of treated products could be attributed to the antimicrobial and antioxidant properties of the extract. An increase in the texture scores of meat products with addition of natural antioxidants was also reported by Kaur *et al.* (2015)and Singh *et al.* (2015a).

Overall acceptability: The mean scores decreased significantly (p<0.05) with the advancement of storage period for all products. This might be reflective of the decline in scores for colour and appearance, flavour, juiciness and texture. Significantly (p<0.05)

Table 3: Effect of *Tribulus terrestris* on the sensory attributes of vacuum packaged chevon sausages during refrigerated storage (Mean ± SE)*

Treatment	Storage period (Days)				
	0	14	28	42	56
Colour and appearance					
Control	7.18±0.061^{Ba}	6.18±0.051^{b}	5.42±0.048^{Bc}	4.61±0.133^{Bd}	4.47±0.045^{Bd}
T_1 (0.25%)	7.20±0.029^{Ba}	6.23±0.079^{b}	5.54±0.047^{Bc}	4.83±0.094^{ABd}	4.52±0.063^{Be}
T_2 (0.5%)	7.28±0.021^{ABa}	6.26±0.079^{b}	5.62±0.103^{Bc}	4.89±0.088^{ABd}	4.69±0.036^{Ad}
T_3 (0.75%)	7.36±0.041^{Aa}	6.32±0.085^{b}	5.86±0.109^{Ac}	4.95±0.118^{Ad}	4.72±0.055^{Ad}
Flavour					
Control	7.26±0.016^{a}	6.43±0.054^{b}	5.52±0.030^{Bc}	4.48±0.055^{Bd}	NE
T_1 (0.25%)	7.24±0.020^{a}	6.36±0.096^{b}	6.28±0.092^{Ab}	5.43±0.070^{Ac}	NE
T_2 (0.50%)	7.21±0.072^{a}	6.38±0.072^{b}	6.30±0.086^{Ab}	5.49±0.081^{Ac}	NE
T_3 (0.75%)	7.19±0.099^{a}	6.41±0.050^{b}	6.36±0.086^{Ab}	5.53±0.009^{Ac}	NE
Juiciness					
Control	ND	ND	ND	ND	ND
T_1 (0.25%)	ND	ND	ND	ND	ND
T_2 (0.50%)	ND	ND	ND	ND	ND
T (0.75%)	ND	ND	ND	ND	ND
Yeast and mould count (log cfu/g)					
Control	7.38±0.047^{a}	6.54±0.133^{b}	5.69±0.094^{c}	5.41±0.076^{d}	NE
(0.25%)	7.36±0.036^{a}	6.50±0.076^{b}	5.76±0.080^{c}	5.37±0.080^{d}	NE
(0.50%)	7.32±0.032^{a}	6.46±0.085^{b}	5.72±0.094^{c}	5.34±0.071^{d}	NE
(0.75%)	7.29±0.038^{a}	6.42±0.086^{b}	5.69±0.095^{c}	5.31±0.076^{d}	NE
Texture					
Control	7.23±0.050^{a}	6.56±0.004^{Db}	5.32±0.004^{Dc}	4.38±0.005Dd	4.26±0.048^{Ce}
(0.25%)	7.25±0.075^{a}	6.59±0.005^{Cb}	6.42±0.004^{Cc}	5.63±0.005Cd	4.83±0.004^{Be}
(0.50%)	7.27±0.087^{a}	6.64±0.004^{Bb}	6.49±0.003^{Bc}	5.75±0.008Bd	4.88±0.026^{ABe}
(0.75%)	7.30±0.096^{a}	6.68±0.006^{Ab}	6.53±0.004^{Ac}	5.82±0.003Ad	4.92±0.032^{Ae}
Overall acceptability					
Control	7.32±0.029^{a}	6.56±0.049^{b}	5.43±0.033^{Ce}	4.26±0.038^{Cd}	NE
T_1 (0.25%)	7.29±0.040^{a}	6.45±0.037^{b}	6.36±0.006^{Bc}	5.63±0.004^{Bd}	NE
T_2 (0.50%)	7.26±0.040^{a}	6.48±0.077^{b}	6.38±0.007^{Bb}	5.67±0.004^{Bc}	NE
T_3 (0.75%)	7.23±0.092^{a}	6.52±0.057^{b}	6.45±0.004^{Ab}	5.74±0.003^{Ac}	NE

*Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05)

n = 30 for each treatment, NE = Not evaluated; T_1 (0.25%) = Sausages with 0.25% of *Tribulus terrestris*

T_2 (0.50%) = Sausages with 0.50% of *Tribulus terrestris*; T_3 (0.75%) = Sausages with 0.75% of *Tribulus terrestris*

higher scores were observed for the treated samples (T_1, T_2 and T_3) on day 28 and 42. Kaur *et al.* (2015) and Singh *et al.* (2015b) also observed an increase in the overall acceptability scores of meat products with addition of some natural antioxidants.

CONCLUSION

The present study showed successful utilization of *T. terrestrisas* a novel natural preservative in meat products. Treatment of chevon sausages with *T. terrestris* improved the lipid stability and storage quality of the products under vacuum at 4±1°C. Significantly (p<0.05) higher sensory scores for treated products might be correlated with lower FFA and TBARS values and microbial counts. *T.terrestris* could be commercially exploited as a natural preservative for muscle foods.

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