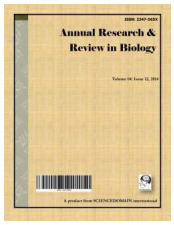

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Effect of Incorporation of *Tribulus terrestris* on Microbiology Characteristic of Cooked Chevon Sausages

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Authors' contributions

This work was carried out in collaboration between both authors. Author SN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ZFB managed the analyses of the study, literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To Study the effect of incorporation of *Tribulus terrestris*/Gokshura (a natural meat preservative) on microbiology characteristic of cooked chevon sausages (goat meat sausages).
Methodology: Meat emulsion for chevon sausages was prepared and was divided in four parts, as follows first part was kept as control and in which *Tribulus terrestris*/Gokshura was not added and rest three parts were incorporated with different levels of *Tribulus terrestris*/Gokshura viz. being T_1 (0.25%), T_2 (0.50%), T_3 (0.75%). All samples were vacuum packaged and assessed for microbiological characteristic under refrigerated (41℃) conditions at regular int ervals of 0, 14, 28, 42 and 56 days. Three independent experimental trials of the study were conducted and were carried out with duplicate sample analysis (n=6).
Results: Incorporation of *Tribulus terrestris* shows significant *(P<0.05)* effect on the microbiological characteristics of the products as treated products showed significantly lower values for microbial

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and yeast and mould counts. Based on microbiological parameters, the products incorporated up to 0.75% extract (T_3) of *Tribulus terrestris* were optimized as best and the products remained safe up to 42nd day of refrigerated storage.
Conclusion: *Tribulus terrestris* successfully improved the shelf life of the products by decreasing the microbiological growth during refrigerated (41℃) storage, thus it is suggested to use it commercially as a natural meat preservative in meat industry.

Keywords: Tribulus terrestris; chevon sausages; vacuum packaging; preservative; storage studies.

1. INTRODUCTION

Goat meat is one of the major sources of protein in many countries of the world including India. Goat is known as 'poor man's cow' and is very important component in dry land farming system. Production and consumption of goat meat is the highest in tropical and subtropical regions [1]. Goat meat cuts have protein levels comparable to similarly prepared beef, lamb, and veal but have lower fat content [2]. The percentage of saturated fat in goat meat is lower than in chicken, beef, pork, or lamb [3]. It is low in fat and saturated fatty acids, but high in unsaturated fatty acids, such as linoleic and oleic, which have been shown to possess hypocholestermic properties [4]. It is having high myoglobin content and provides a high level of bioavaiable iron. Because of low fat content, chevon is an ideal source for the preparation of healthy meat based products [5] and is preferred by many people these days and is widely used in many comminuted meat products including sausages. Sausages are convenient meat based products which are popular and widely consumed throughout the world. These are one of the oldest foods known to the mankind initially prepared by stuffing roasted intestines into stomach. A Chinese sausage namely '*lachang*' consisting of goat and lamb meat has been mentioned as early as 589 BC. Sausage making evolved as an effort to economize and preserve meat that could not be consumed fresh at slaughter. People living in particular areas developed their own types of sausages and those became associated with the area e.g. Bologna in northern Italy, Lyons sausages from Lyons in France, and Berliner sausages from Berlin in Germany. Today more than 2500 varieties are sold and many can be traced back to the town and country of origin. Although, highly popular and acceptable, sausages like other meat products are highly susceptible to the development of rancidity owing to their high fat content and, therefore, require certain interventions. The freshness of meat and meat products is affected by lipid oxidation which is considered as a major non-microbiological factor involved in quality deterioration of meat and meat products. It can seriously interfere with the efficiency of processing steps and therefore, leads to potential economic losses [6]. It reduces the organoleptic value of foods and imparts rancid and unpleasant flavours to the raw and end-use oil and fat products, thus making them unacceptable to consumers [6]. Lipid oxidation also produces reactive oxygen species (ROS), which have been implicated in carcinogenesis, inflammation, early ageing and cardiovascular diseases [7]. In order to inhibit the development of oxidative reactions in meat products, natural and synthetic antioxidants have been commonly used in meat industry [8].

Tribulus terrestris is an annual medicinal herb, commonly found throughout the India. It's popularly known as Gokshura or puncture vine and shows worldwide distribution [9]. *Tribulus terrestris* is known for its great medicinal value since ancient times. It has great antioxidant, antimicrobial, and antifungal properties in addition to its beneficial claims on various ailments such as urinary infection, kidney stones, kidney disorders, inflammation, odema, ascitis, skin disorders, heart and circulatory system problems, cancer, inflammation, rheumatism, leprosy, cough, headache dizziness, chronic fatigue syndrome and for stimulating appetite [10]. *Tribulus terrestris* leaf extract has been reported to contain different phyto-constituents like alkaloids, flavonids, steroids, triterpenoids, carbohydrates, aminoacids, tannins, saponin and glycosides. *Tribulus terrestris* leaf extract is a rich source of polyphenols and has strong free radical scavenging activity [11]. Therefore it is a possible new powerful source of antioxidant and could be useful in therapy of free radical pathologies, [12] has reported strong radical scavenging, antioxidant activity and lipid peroxidation inhibition of *Tribulus terrestris* compared to the control (BHT). The rate of growth of microorganism in meat system is determined by microbial contamination, chemical properties of meat viz: moisture, pH, salt content, availability

of O_2 (Packaging system) and storage temperature etc Therefore the aim of this investigation was to study the effect of incorporation of *Tribulus terrestris*/Gokshura on microbiology characteristic of cooked chevon sausages (goat meat sausages).

2. MATERIALS AND METHODS

Lean meat from goat was cut into smaller chunks and minced in a Sirman Mincer with 6 mm plate twice. The common salt, vegetable oil, refined wheat flour (maida), nitrite, sodium tripolyphosphate, spice mixture and condiment mixture were added to weighed meat according to formulation (mentioned in Table 1).

Meat emulsion for chevon sausages was prepared in Sirman Bowl Chopper. Minced meat was blended with salt, sodium tripolyphosphate and sodium nitrite for 1.5 minute. Water in the form of crushed ice was added and blending continued for 1 minute. This was followed by addition of refined soyabean oil and blended for another 1 to 2 minutes. This was followed by addition of condiments, spice mixture, and other ingredients and again mixed for 1.5 to 2 minutes to get the desired emulsion. And after emulsion preparation it was divided in four parts, first part was kept as control and in which Gokshuru was not added and rest three parts were incorporated with different levels of Gokshura viz. T_1 (0.25%), T_2 (0.50%), T_3 (0.75%).

2.1 Stuffing of Sausages

The emulsion was filled in to the Sirman sausage filler and the artificial casings were applied on the nozzle of sausage filler. Pressure was applied in such a way so that the emulsion was filled in to the casings.

Table 1. Formulation of chevon sausages

Ingredients	Percent (w/w)
Lean meat	67.4
Ice flakes	10
Vegetable oil	9.0
Condiment mixture	5.0
Refined wheat flour	4.0
Spice mixture	2.0
Monosodium glutamate	0.5
Sodium tri polyphosphate	0.3
Sodium nitrite	150 ppm
Sodium chloride	1.75

2.2 Cooking of Sausages

Hot air oven method was used for cooking of sausages. Raw sausages were hanged vertically with the help of skewers in a preheated hot air oven at 140±5℃ for a total time of about 30 minutes. The internal temperature of sausages was monitored by a thermometer and were cooked to an internal temperature of 80±2℃. The sausages were removed, cooled to room temperature and weighed. Pooled sample of each treatment was assigned for analysis and vacuum packaged, microbiological assay was done as mentioned below.

2.3 Microbiological Profile

The total plate count, psychrophillic count, coliform count and yeast and mould count in the sample were determined by method described [13].

2.4 Sample Preparation

10 gm of meat sample was taken aseptically and blended with 90 ml of 0.1 percent sterile peptone water with a pre-sterilized blend. Serial ten-fold dilution was made in pre-sterilized tubes containing 9 ml volume of 0.1 percent peptone water. The sample preparation was done near flame under laminar flow.

2.5 Total Plate Count

The plate count agar of amount 23.5 g obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai was suspended in 1000 ml distilled water. It was boiled to dissolve the suspension completely and sterilized by autoclaving at 15 lb pressure (121℃) for 15 minutes. Final pH was adjusted to 7.0±0.2 at 25℃. Duplicate sets of sterilized petri plates were inoculated aseptically with 1 ml of aliquots from appropriate dilution. About 10-15 ml of plate count agar melted and maintained at 45±2℃ was poured gently and rotated the disc clockwise and anticlockwise to mix the media uniformly. The plates were incubated at 35±2℃ for 24 hours. Following incubation plates showing 30-300 colonies were preferably counted and expressed as $\log_{10}$ cfu/g of sample.

2.6 Psychrophillic Count

The sample procedure as for total plate counts was followed for media preparation and plating. The plates were incubated at 4±1℃ for 10-14 days and colonies were counted and expressed as $\log_{10}$ cfu/g of sample.

2.7 Coliform Count

41.5 g of Violet Red Bile Agar obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai was suspended in 1000 ml of distilled water, boiled to dissolve the medium completely and cooled to 45℃. The final pH was adjusted to 7.4±0.2 at 25℃. Precautions were taken not to autoclave the media. 1 ml of suitable dilutions in duplicate was introduced into the sterile petriplates and about 20 ml of molten media was poured into the petriplates. The plates were incubated at 35±2℃ for 24 hrs. The numbers of red or purple colonies with about 0.5 mm diameter surrounded by a zone of precipitated bile were counted. Then the average number of colonies were multiplied with reciprocal of the respective dilutions and expressed as $\log_{10}$ cfu/g of sample.

2.8 Yeast and Mould Count

39 g of Potato Dextrose Agar obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai was suspended in one liter of distilled water, boiled to dissolve the medium completely and sterilized by autoclaving at 25 lb pressure (121℃) for 15 min. The final pH was adjusted to 3.5 at 25℃. Pour plate with overlay technique was followed for inoculation of suitable sample dilution and the plates were incubated at 35±2℃ for 5 days. The colonies were counted and results were expressed as $\log_{10}$ cfu/g of sample.

2.9 Statistical Analysis

Three independent experimental trials of the study were conducted and were carried out with duplicate sample analysis (n=6). The data generated by repeating the experiments for different quality characteristics were compiled and analyzed using Statistical Package for the Social Sciences version 16.0 software program (SPSS Inc., Chicago, IL, USA). Means for various parameters were analyzed using two-way analysis of variance (ANOVA) with Duncan *post-hoc* multiple comparisons test to determine significant differences among treatments at each storage time and also among storage times at each treatment at 5% level of significance [14].

3. RESULTS AND DISCUSSION

Under microbiological investigation, parameters (total plate count, psychrophillic count, coliform count, yeast and mould count) for control as well as chevon sausages treated with *Tribulus terrestris* were studied on 0, 14, 28, 42 and 56 days of refrigerated storage (4±1℃) under vacuum packaging and are depicted in Table 2.

3.1 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 [15] and exceeded the limit on 56th day of storage for control and T_1. Cremer and Chipley [16] reported that 5.33 log cfu/g could be considered as indicative of unacceptability of cooked meat products. [17] observed similar results with a significant ($P<0.05$) increase in the TPC values of vacuum packed mutton patties for control as well as treatment products at each subsequent storage interval. A significant increasing trend was also reported by [18] in TPC of vacuum packed goat meat nuggets both in control and treatment nuggets. Similar findings were also observed by [19] in chicken sausages and [20] in chicken *seekh kababs* who also observed an increasing trend in the TPC during storage.

Although, the total plate counts of the products incorporated with *Tribulus terrestris* (T_1, T_2 and T3) increased significantly ($P<0.05$) with storage period, however, the values were significantly ($P<0.05$) lower than control on all intervals of storage which may be attributed to the bioactive compounds and other constituents of *Tribulus terrestris* which are said to have antimicrobial properties [21]. [17] reported a significant decrease in the TPC of vacuum packed mutton patties incorporated with cabbage powder as compare to control. [22] recorded a slower increase in the TPC of *Tabaq-Maz* treated with oleuropein in comparison to control. [23] also reported significantly lower values for TPC of the beef sausages containing different concentrations of pomegranate peel powder during refrigerated storage. Similar findings were also reported by [24] who also reported a significant decline in the total plate count of lamb patties with added olive leaf extract. Similar findings were also reported by [25] in chevon cutlets who also observed a similar decrease in the total plate count of the products treated with clove oil.

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

Treatments	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}
T_1 (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}
T_2 (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}
T_3 (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}
Psychrophillic count (log cfu/g)					
Control	Not detected	Not detected	1.30±0.004^{Ac}	2.83±0.006^{Ab}	3.50±0.006^{Aa}
T_1 (0.25%)	Not detected	Not detected	1.26±0.006^{Bc}	2.7±0.004^{Bb}	3.32±0.006^{Ba}
T_2 (0.50%)	Not detected	Not detected	1.21±0.006^{Cc}	2.64±0.005^{Cb}	3.22±0.006^{Ca}
T_3 (0.75%)	Not detected	Not detected	1.15±0.006^{Dc}	2.56±0.005^{Db}	3.13±0.005^{Da}
Coliform count (log cfu/g)					
Control	Not detected	Not detected	Not detected	Not detected	Not detected
T_1 (0.25%)	Not detected	Not detected	Not detected	Not detected	Not detected
T_2 (0.50%)	Not detected	Not detected	Not detected	Not detected	Not detected
T_3 (0.75%)	Not detected	Not detected	Not detected	Not detected	Not detected
Yeast and mould count (log cfu/g)					
Control	Not detected	Not detected	1.96±0.005^{Ac}	2.74±0.008^{Ab}	3.81±0.008^{Aa}
T_1 (0.25%)	Not detected	Not detected	1.82±0.004^{Bc}	2.67±0.039^{ABb}	3.73±0.035^{Ba}
T_2 (0.50%)	Not detected	Not detected	1.78±0.004^{Cc}	2.64±0.048^{ABb}	3.68±0.007^{BCa}
T_3 (0.75%)	Not detected	Not detected	1.75±0.010^{Dc}	2.59±0.048^{Bb}	3.65±0.006^{Ca}

**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

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

3.2 Psychrophillic Count (Log cfu/g)

Psychrophillic counts were not detected on day 0 and 14 of storage in control as well as treated (T_1, T_2 and T_3) sausages. Counts were observed on day 28 of storage in all treated products as well as control 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. [16] described permissible level of psychrophillic count as 4.6 log cfu/g in cooked meat products. A detectable count from 28th day onwards while nil on preceding observations might be attributed to the fact that bacteria generally need some lag phase before active multiplication is initiated [15]. The psychrophilles undergo metabolic injuries due to environmental stress such as cooking etc. Initially, these injured cells could not form the colonies. However, with the advancement of storage, these injured cells could get repaired and might have started forming colonies after day 14th of storage. Similar findings were also reported by [26] in chicken meat nuggets, [22] in *Tabaq-Maz*, [19] in chicken sausages and [20] in chicken *seekh kababs* during refrigerated storage.

Although, psychrophilles followed a significant (P<0.05) increasing trend in all the products with storage, however, counts of the treated products (T_1, T_2 and T3) were significantly (P<0.05) lower as compared to control. Significantly (P<0.05) lower counts of the treated products may be attributed to the bioactive compounds and other constituents of *Tribulus terrestris* which are said to have antimicrobial properties [21]. [26] reported significantly lower psychrophillic counts for the chicken nuggets treated with pomegranate seed powder, grape seed extract and tomato powder in comparison to control during storage at refrigeration temperature. A similar observation was also reported by [17] in vacuum packed mutton patties incorporated with cabbage powder. Similar findings were also presented by [22] who also recorded significantly lower counts for the products treated with oleuropein in comparison to control.

3.3 Coliform Count (Log cfu/g)

No coliforms were detected in any of the products on any interval of storage period. It could be due to destruction of these bacteria during cooking at 140℃ much above their thermal death point of 57℃. [27] reported that coliform species were sensitive to heat treatment with a decimal reduction time under 2 minutes at 60ºC. Further, hygienic practices followed during the preparation and vacuum packaging of chevon sausages could also be the contributing reasons for the absence of coliforms. The absence of coliforms during storage depicts that the heat processing and subsequent hygienic handling and vacuum packaging were effective to control coliform growth. Presence of coliforms in the cooked products is an indication of feacal or soil contamination. Similar results were reported by [17] who observed no coliforms in vacuum packed pork patties during entire period of storage. Similar results were also recorded by [25,28] who also reported zero counts for the meat products heated to such a high temperature.

3.4 Yeast and Mould Count (Log cfu/g)

The yeast and mould counts were not detected on day 0 and 14 of storage in case of control as well as treated (T_1, T_2 and T_3) samples. Counts were observed on day 28^{th} and thereafter followed a significant ($P<0.05$) increasing trend in case of control as well as treated samples. This appearance of yeast and mould counts could possibly be due to post processing contamination. Similar findings were also observed by [19,29] who reported similar increasing trend in the yeast and mould counts of chevon cutlets, chicken sausages, chicken nuggets and chicken snacks, respectively.

Although, yeast and mould counts followed a significant ($P<0.05$) increasing trend in all the products with storage, however, counts of the treated products (T_1, T_2 and T3) were significantly ($P<0.05$) lower as compared to control. Significantly ($P<0.05$) lower counts of the treated products may be attributed to the bioactive compounds and other constituents of *Tribulus terrestris* which are said to have antifungal properties [26,30] reported significantly lower yeast and mould counts for the chicken nuggets incorporated with pomegranate seed powder, grape seed extract and tomato powder in comparison to control during storage at refrigeration temperature. [31] recorded a similar observation in *Tabaq-Maz* treated with lemon peel extract. Similar findings were also observed by [32,33] who reported similar results in chicken meat rings, *Tabak-Maz*, chevon cutlets, chicken nuggets and chicken snacks, respectively.

4. CONCLUSION

Tribulus terrestris is an annual medicinal herb, commonly found throughout the India. It was used as a natural antimicrobial up to level of 0.75% shows a significant effect on microbiological characteristics of the products, as treated products showed a significant lower values for total plate count, yeast and mould count as compared to other treatments. Thus selected levels of *Tribulus terrestris* were found to be optimum for making functional chevon sausages. As per results *Tribulus terrestris* successfully improved the shelf life of the products by decreasing the microbiological growth during refrigerated (41℃) storage and thus may be commercially exploited by the meat industry as efficient alternatives to synthetic preservatives.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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