



# *Terminalia arjuna*: A novel natural preservative for improved lipid oxidative stability and storage quality of muscle foods

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## Abstract

The study was conducted to explore the possibility of utilization of *Terminalia arjuna* as a novel natural preservative in meat products by using chevon sausages as a model system. Chevron sausages were prepared by incorporating different levels of *T. arjuna* viz. T<sub>1</sub> (0.25%), T<sub>2</sub> (0.50%) and T<sub>3</sub> (0.75%) and were assessed for various lipid oxidative stability and storage quality parameters under refrigerated (4 ± 1 °C) conditions. *T. arjuna* showed a significant ( $p < 0.05$ ) effect on the lipid oxidative stability as the treated products exhibited significantly ( $p < 0.05$ ) lower TBARS (mg malonaldehyde/kg) values in comparison to control. A significant ( $p < 0.05$ ) effect was also observed on the microbial stability as *T. arjuna* incorporated products showed significantly ( $p < 0.05$ ) lower values for total plate count (log cfu/g), psychrophilic count (log cfu/g), yeast and mould count (log cfu/g) and FFA (% oleic acid) values. Significantly ( $p < 0.05$ ) higher scores were observed for various sensory parameters of the products incorporated with *T. arjuna* during refrigerated storage. *T. arjuna* successfully improved the lipid oxidative stability and storage quality of the model meat product and may be commercially exploited as a novel preservative in muscle foods.

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**Keywords:** *Terminalia arjuna*; Chevron sausages; Natural preservative; Lipid oxidation; Storage quality

## 1. Introduction

Due to abundance of lipids, meat and meat products are highly susceptible to oxidation which is the major cause of quality loss during their storage [1] and a huge challenge for food industry and food scientists [2]. The lipid oxidation products could adversely affect sensory traits, nutritive value and safety of meat and meat products [1]. The lipid oxidation products can react

with proteins, peptides and amino acids and contribute to oxidation of proteins causing changes in their structure which leads to loss of their biological function [3]. Thus avoiding or reducing oxidative changes is an important concern for the meat industry. Lipid oxidation in meat and meat products can be reduced by the presence of antioxidants which are naturally present in elevated levels in some plants. Several plants have been reported to have significant bioactive compounds and free radicals scavengers such as phenols, flavonoids and terpenoids [4] which give them interesting antioxidant properties to act as potential natural preservatives in foods. *Terminalia arjuna*, that contains many pharmacologically active principles like alkaloids, tannins, flavonoids, triterpenoids, saponins and reducing sugars [5], may be explored for its potential as a natural preservative in food industry.

*Terminalia arjuna*, commonly named as *Arjuna*, is a deciduous and ever green tree belonging to *Combretaceae* family [5]. Its stem, bark and leaves possess glycosides, large quantities of

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flavonoids, polyphenols, tannins and minerals. *T. arjuna* bark extract has been reported to have an antioxidant activity comparable to standard antioxidants like butylated hydroxyanisole and ascorbic acid due to the presence of phenolic compounds like  $\beta$ -sitosterol, catechin, rutin and tannic acid [6]. Javed et al. [7] reported moderate antifungal properties of *T. arjuna* leaves extract against *Microsporum canis*. The fruit extract was observed to have good antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [7]. It has been reported to possess multiple medicinal properties, such as antioxidant, antidiabetic, antimicrobial, cardioprotective, antiarthritic, cytotoxic, antidiarrheal, antidysenteric and hepatoprotective activity [8]. It has been reported to decrease the level of serum triglycerides and cholesterol, recover the level of high density lipoproteins, act as an anti-ischemic agent, relieve myocardial necrosis, modulate platelet aggregation and also act as an effective antioxidant [9]. It is traditionally renowned as a cardiac stimulant and also recommended by many indigenous systems of medicine to address miscellaneous problems. The ethnomedical relevance of this plant with reference to cardiovascular ailments has been widely investigated. Experiments revealed that *T. arjuna* bark exerted significant hypolipidaemic activity, produced inotropic and hypotensive effects, and increased coronary artery flow to protect myocardium against ischemic damage [5].

Although, *T. arjuna* has been reported to have strong antioxidant, antimicrobial and other health beneficial properties, however, its application as a natural preservative or as a functional ingredient in the food industry is still an unexplored area and needs immediate scientific attention. Keeping in view all the above facts, the present study was envisaged to explore the possibility of utilization of *T. arjuna* as a functional ingredient and as a natural preservative in muscle foods. A study was designed to evaluate the effect of different concentrations of *T. arjuna* on the lipid oxidative stability and storage quality of chevon sausages.

## 2. Materials and methods

### 2.1. Chevon meat

The goat meat was procured from a local market and utilized in various experiments. The meat was deboned manually after trimming the fat. All tendons and separable connective tissue were also removed. The lean meat was packed in polythene bags, frozen at  $-18 \pm 2^\circ\text{C}$  and was thawed at refrigeration temperature ( $4 \pm 1^\circ\text{C}$ ) before use.

### 2.2. Spice mixture

The spice mix formula used for preparation of the products was standardized in the laboratory and contained coriander (*Coriandrum sativum*) 20%, cumin seed (*Cuminum cyminum*) 15%, aniseed (*Pimpinella anisum*) 12%, black pepper (*Piper nigrum*) 10%, Red chilli (*Capsicum frutescense*) 8%, green cardamom (*Elettaria cardamomum*) 6%, cinnamon (*Cinnamomum zeylanicum*) 6%, white pepper (*Piper nigrum*) 5%, black cardamom (*Amomum subulatum*) 5%, degi mirch (*Capsicum*

*annuum*) 5%, bay leaves (*Laurus nobilis*) 2%, cloves (*Syzygium aromaticum*) 2%, mace (*Myristica fragrans*) 2% and nutmeg (*Myristica fragrans*) 2%.

### 2.3. Condiment mixture

Condiments used in the study were onion, garlic and ginger in a ratio of 3:2:1 and ground to the consistency of a fine paste.

### 2.4. Fat

Refined Soyabean oil of brand name “Mahakosh” was purchased from local market and used. It approximately contained 900 kcal of energy, 0 g of carbohydrate, 0 g of proteins, 0 g of cholesterol, 14 g of saturated fatty acids, 23 g of mono-unsaturated fatty acids, 63 g poly-unsaturated fatty acids and <1 g of *trans*-fatty acids per 100 g.

### 2.5. Terminalia arjuna

Commercially available *T. arjuna* in the capsular form was procured from “The Himalaya Drug Company”. The products were prepared by incorporating different concentrations of *T. arjuna* viz. 0.25% (T<sub>1</sub>, 2500 mg per Kg of meat emulsion), 0.50% (T<sub>2</sub>, 5000 mg per Kg of meat emulsion) and 0.75% (T<sub>3</sub>, 7500 mg per Kg of meat emulsion).

### 2.6. Method of preparation of chevon sausages

Lean meat was cut into smaller chunks and minced in a Sirman mincer (MOD-TC 32 R10 U.P. INOX, Marsango, Italy) with 6 mm plate twice. Meat emulsion was prepared in Sirman Bowl Chopper [MOD C 15 2.8G 4.0 HP, Marsango, Italy]. Minced meat (67.4%) was blended with salt (1.75%), sodium tripolyphosphate (0.3%) and sodium nitrite (150 ppm) for 1.5 min. Water in the form of crushed ice (10%) was added and blending continued for 1 min. This was followed by the addition of refined vegetable oil (9%) and blended for another 1–2 min. This was followed by addition of spice mixture (2%), condiments (5%) and other ingredients and again mixed for 1–2 min to get the desired emulsion. The emulsion was filled into the artificial polyamide casings 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  $140 \pm 5^\circ\text{C}$  for a time of about 30 min in a hot air oven. The products were cooled and packaged under vacuum in laminate pouches and stored under refrigerated conditions ( $4 \pm 1^\circ\text{C}$ ).

### 2.7. Analytical procedures

#### 2.7.1. pH

The pH of the product was determined by the method of [10] using a digital pH meter.

### 2.7.2. Thiobarbituric acid reacting substances (TBARS) value and free fatty acid (FFA)

Thiobarbituric acid reactive substances (mg malonaldehyde/kg) value was determined as per the method described by [11]. FFA (% oleic acid) was determined by the method described by [12].

### 2.7.3. Moisture content and cooking yield

Moisture content of the products was determined by using standard AOAC procedure [13] using hot air oven. The weight of each sausage was recorded before and after cooking. The cooking yield was calculated and expressed as percentage by a formula:

$$\text{Cooking yield(\%)} = \frac{\text{Weight of cooked sausages}}{\text{Weight of raw sausages}} \times 100$$

### 2.7.4. Microbiological profile

Total plate count, psychrophilic count, coliform count, anaerobic count and yeast and mould count were determined by the methods of APHA [14]. Readymade media (Hi-Media) were used for the analysis.

### 2.8. Sensory evaluation

The sensory evaluation of fresh and stored samples was carried for various attributes namely colour and appearance, flavour, juiciness, texture and overall acceptability by a panel of ten trained members composed of scientists and research scholars of the division based on a 8-point hedonic scale [15]. Ten members of the panel were trained according to the guidelines of American meat science association [16] and were well acquainted with product descriptions and terminology and replicated the experiment thrice (n = 30). The panels were trained for four basic tastes i.e. recognition and threshold test and hedonic tests routinely performed in the division. Three digit coded samples were served to the panellists in random order. The nature of experiments was explained to the panellists without disclosing the identity of samples. Samples were heated in hot air oven and served warm (40 °C) to panellists. Each panellist received control and treatment samples in random order and water was provided for oral rinsing between the samples. Panellists scored each sample on the basis of 8-point scales for colour and appearance, flavour, juiciness, texture and overall acceptability where 8 = excellent, extra desirable, extra juicy, extra desirable, and extra acceptable and 1 = extremely poor, extremely undesirable, extremely dry, extremely undesirable, and extremely unacceptable.

### 2.9. Statistical analysis

Three independent experimental trials of the study were conducted and all experiments 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 [17].

## 3. Results and discussion

The mean values of various physicochemical parameters of chevon sausages containing different levels of *T. arjuna* viz. T<sub>1</sub> (0.25%), T<sub>2</sub> (0.50%) and T<sub>3</sub> (0.75%) are presented in Table 1.

### 3.1. Physicochemical parameters

#### 3.1.1. pH

A significant ( $p < 0.05$ ) decline was observed in the mean pH values of control and treated sausages from day 0 to day 56 of the storage. The decline in pH may be attributed to the accumulation of acids produced by the bacteria, predominantly anaerobic and lactic acid bacteria, during storage and depends on the available carbohydrates in the meat products [18–20]. The results were in agreement with the findings of [21] who also reported an overall decline in pH values of *Capoeta umbla* sausages during storage at refrigeration temperature. El-Nashi et al. [22] also reported a significant ( $p < 0.05$ ) decline in the pH values of beef sausages containing different concentrations of pomegranate peel powder during refrigerated storage. Similar results were also presented by [23] who also observed a decrease in the pH values of pork sausages incorporated with tea polyphenols during refrigerated storage.

#### 3.1.2. Thiobarbituric acid reacting substances (TBARS) value

The TBARS values followed a significant ( $p < 0.05$ ) increasing trend from day 0 to day 56 in case of control as well as treated sausages. The values were well below the acceptable limit of 1 mg malonaldehyde per kilogram [11] till 42nd day of storage for control as well as the products incorporated with extract. Products exceeded the limit on 56th day of storage. Significantly increasing TBARS values with storage may be attributed to lipid hydrolysis, oxidative rancidity and secondary products formation at refrigeration temperature [24]. Rajkumar et al. [25] recorded a significant increase in the TBARS values of the nuggets incorporated with *Aloe vera* gel throughout the storage period. Similar increase in TBARS values was also reported by [26] in chicken nuggets and [27] in restructured goat meat as the days of storage advanced.

Although, TBARS values followed an increasing trend with storage, however, values of the products treated with extract (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) were significantly ( $p < 0.05$ ) lower than control on all intervals of storage except on day 0. This may be attributed to antioxidant properties of *T. arjuna* which contains high amounts of polyphenols, flavonoids and tannins [28]. The antioxidant activity of phenolic compounds is mainly due to their redox properties which can play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen, and decomposing peroxides [29]. Arjunolic acid, a triterpenoid saponin isolated from *T. arjuna*, has multifunctional medicinal applica-

Table 1  
Effect of *Terminalia arjuna* on the physico-chemical properties of chevon sausages during refrigerated storage (Mean  $\pm$  SE) \*.

Treatments	Storage Period (days)				
	0	14	28	42	56
<b>pH</b>					
Control	6.30 $\pm$ 0.108 <sup>a</sup>	6.19 $\pm$ 0.114 <sup>ab</sup>	6.08 $\pm$ 0.127 <sup>ab</sup>	5.92 $\pm$ 0.144 <sup>ab</sup>	5.78 $\pm$ 0.150 <sup>b</sup>
T <sub>1</sub> (0.25%)	6.26 $\pm$ 0.112 <sup>a</sup>	6.13 $\pm$ 0.113 <sup>ab</sup>	5.92 $\pm$ 0.150 <sup>ab</sup>	5.86 $\pm$ 0.20 <sup>ab</sup>	5.71 $\pm$ 0.154 <sup>b</sup>
T <sub>2</sub> (0.50%)	6.21 $\pm$ 0.102 <sup>a</sup>	6.08 $\pm$ 0.099 <sup>ab</sup>	5.85 $\pm$ 0.139 <sup>abc</sup>	5.79 $\pm$ 0.165 <sup>bc</sup>	5.67 $\pm$ 0.125 <sup>c</sup>
T <sub>3</sub> (0.75%)	6.17 $\pm$ 0.113 <sup>a</sup>	6.01 $\pm$ 0.096 <sup>ab</sup>	5.78 $\pm$ 0.193 <sup>ab</sup>	5.71 $\pm$ 0.199 <sup>ab</sup>	5.59 $\pm$ 0.150 <sup>b</sup>
<b>TBARS (mg malonaldehyde/kg)</b>					
Control	0.38 $\pm$ 0.035 <sup>e</sup>	0.56 $\pm$ 0.055 <sup>Ad</sup>	0.72 $\pm$ 0.036 <sup>Ac</sup>	0.87 $\pm$ 0.035 <sup>Ab</sup>	1.49 $\pm$ 0.061 <sup>Aa</sup>
T <sub>1</sub> (0.25%)	0.35 $\pm$ 0.033 <sup>c</sup>	0.39 $\pm$ 0.030 <sup>Bc</sup>	0.59 $\pm$ 0.053 <sup>Bb</sup>	0.65 $\pm$ 0.090 <sup>Bb</sup>	1.28 $\pm$ 0.057 <sup>Ba</sup>
T <sub>2</sub> (0.50%)	0.31 $\pm$ 0.035 <sup>c</sup>	0.35 $\pm$ 0.032 <sup>Bc</sup>	0.41 $\pm$ 0.034 <sup>Cbc</sup>	0.51 $\pm$ 0.073 <sup>Bb</sup>	1.19 $\pm$ 0.029 <sup>BCa</sup>
T <sub>3</sub> (0.75%)	0.28 $\pm$ 0.040 <sup>c</sup>	0.31 $\pm$ 0.037 <sup>Bc</sup>	0.39 $\pm$ 0.048 <sup>Cbc</sup>	0.47 $\pm$ 0.55 <sup>Bb</sup>	1.09 $\pm$ 0.027 <sup>Ca</sup>
<b>FFA (% Oleic acid)</b>					
Control	0.125 $\pm$ 0.005 <sup>e</sup>	0.159 $\pm$ 0.004 <sup>Ad</sup>	0.275 $\pm$ 0.002 <sup>Ac</sup>	0.393 $\pm$ 0.004 <sup>Ab</sup>	0.493 $\pm$ 0.013 <sup>Aa</sup>
T <sub>1</sub> (0.25%)	0.121 $\pm$ 0.003 <sup>d</sup>	0.136 $\pm$ 0.004 <sup>Bd</sup>	0.251 $\pm$ 0.002 <sup>Bc</sup>	0.334 $\pm$ 0.016 <sup>Bb</sup>	0.454 $\pm$ 0.006 <sup>Ba</sup>
T <sub>2</sub> (0.50%)	0.117 $\pm$ 0.012 <sup>d</sup>	0.125 $\pm$ 0.004 <sup>BCd</sup>	0.242 $\pm$ 0.003 <sup>Cc</sup>	0.328 $\pm$ 0.024 <sup>Bb</sup>	0.441 $\pm$ 0.006 <sup>BCa</sup>
T <sub>3</sub> (0.75%)	0.113 $\pm$ 0.012 <sup>d</sup>	0.119 $\pm$ 0.005 <sup>Cd</sup>	0.221 $\pm$ 0.002 <sup>Dc</sup>	0.322 $\pm$ 0.018 <sup>Bb</sup>	0.425 $\pm$ 0.003 <sup>Ca</sup>
<b>Moisture (%)</b>					
Control	62.52 $\pm$ 0.099 <sup>a</sup>	62.25 $\pm$ 0.039 <sup>b</sup>	61.59 $\pm$ 0.043 <sup>c</sup>	61.28 $\pm$ 0.052 <sup>d</sup>	60.32 $\pm$ 0.044 <sup>e</sup>
T <sub>1</sub> (0.25%)	62.47 $\pm$ 0.055 <sup>a</sup>	62.22 $\pm$ 0.036 <sup>b</sup>	61.56 $\pm$ 0.056 <sup>c</sup>	61.24 $\pm$ 0.043 <sup>d</sup>	60.29 $\pm$ 0.057 <sup>e</sup>
T <sub>2</sub> (0.50%)	62.41 $\pm$ 0.070 <sup>a</sup>	62.19 $\pm$ 0.021 <sup>b</sup>	61.53 $\pm$ 0.024 <sup>c</sup>	61.19 $\pm$ 0.034 <sup>d</sup>	60.25 $\pm$ 0.031 <sup>e</sup>
T <sub>3</sub> (0.75%)	62.36 $\pm$ 0.072 <sup>a</sup>	62.16 $\pm$ 0.015 <sup>b</sup>	61.49 $\pm$ 0.020 <sup>c</sup>	61.15 $\pm$ 0.027 <sup>d</sup>	60.21 $\pm$ 0.020 <sup>e</sup>
<b>Cooking yield (%)</b>					
Control	91.85 $\pm$ 0.033	–	–	–	–
T <sub>1</sub> (0.25%)	91.81 $\pm$ 0.094	–	–	–	–
T <sub>2</sub> (0.50%)	91.77 $\pm$ 0.159	–	–	–	–
T <sub>3</sub> (0.75%)	91.74 $\pm$ 0.156	–	–	–	–

n = 6 for each treatment.

T<sub>1</sub> (0.25%) = Sausages with 0.25% of *Terminalia arjuna*.

T<sub>2</sub> (0.50%) = Sausages with 0.50% of *Terminalia arjuna*.

T<sub>3</sub> (0.75%) = Sausages with 0.75% of *Terminalia arjuna*.

\* Mean  $\pm$  SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly ( $p < 0.05$ ).

tions including strong antioxidant and free radical scavenging activity [30]. Dua et al. [31] recorded a similar observation in the TBARS values of *Tabaq-Maz* treated with lemon peel extract. Wenjiao et al. [23] also observed a significant ( $p < 0.05$ ) decrease in TBARS values of pork sausages incorporated with tea polyphenols.

### 3.1.3. Free fatty acids (% oleic acid)

Free fatty acid (FFA) values followed a significant ( $p < 0.05$ ) increasing trend from day 0–56 with increasing days of storage. There was a positive correlation between TBARS values and free fatty acid values. FFA content of the treated products was well below the threshold value i.e. 1.8% [32]. Free fatty acids are the products of enzymatic or microbial degradation of lipids and determination of FFA gives information about the stability of fat during storage. The significant ( $p < 0.05$ ) increase in FFA content of the products during storage might be due to the growth of lipolytic microorganisms [33]. Lipolytic enzymes could either be endogenous of the food products or derived from psychrotrophic microorganisms [34]. During lipolysis, lipases split the glycerides and form free fatty acids which are responsible for the development of off-flavour [35]. Malav et al. [36] reported a significant increasing trend in the FFA values of mut-

ton patties during refrigerated storage. A significant increasing trend was also observed by [37] in the FFA values of pork frankfurters incorporated with sea buckthorn, grape seed, green tea, fenugreek seed and *Acacia catechu*.

Although, FFA followed a significant ( $p < 0.05$ ) increasing trend from day 0–56, however, the values were significantly ( $p < 0.05$ ) lower in the products incorporated with *T. arjuna* extract (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) on all intervals of storage except day 0. Significantly ( $p < 0.05$ ) lower FFA values of the treated products may be attributed to antimicrobial properties of *T. arjuna* [5,28]. Malav et al. [36] observed significantly lower FFA values for mutton patties incorporated with cabbage powder in comparison to control. Indumathi et al. [26] also reported a significant decrease in FFA values of chicken nuggets incorporated with curry leaf, guava leaf and green tea as compared to control.

### 3.1.4. Moisture (%)

A significant ( $p < 0.05$ ) decrease was observed in the moisture content of all the products with storage from 0 day to 56th day. No significant ( $p > 0.05$ ) difference was observed between the treated sausages and control. The decrease in the moisture content of the products may be attributed to the evaporative losses. Similar observations were reported by [38] who also recorded

a similar decline in the moisture content of *Tabaq-Maz* treated with oleuropein during refrigerated storage. El-Nashi et al. [22] also reported a significant ( $p < 0.05$ ) decrease in the moisture content of beef sausages incorporated with pomegranate peel powder during storage.

### 3.1.5. 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 also reported by [39] who also observed no significant ( $p > 0.05$ ) change in the cooking yield of chevon cutlets incorporated with sorghum bicolour and clove oil. Singh et al. [40] and Singh et al. [41] also presented similar findings in the meat products.

## 3.2. Microbiological characters

The mean values of various microbiological characteristics of chevon sausages containing different levels of *T. arjuna* viz.  $T_1$  (0.25%),  $T_2$  (0.50%) and  $T_3$  (0.75%) are presented in Table 2.

### 3.2.1. Total plate count (log cfu/g)

A significant ( $p < 0.05$ ) increasing trend was observed in total plate count (TPC) from day 0 to day 56 in control as well as treated products. The counts were well below the permissible limits up to 42nd day of storage for all the products [42] and exceeded the limit on 56th day of storage for control and  $T_1$ . Cremer and Chipley [43] reported that 5.33 log cfu/g could be considered as indicative of unacceptability of cooked meat products. Malav et al. [36] observed similar results with a significant ( $p < 0.05$ ) increase in the TPC values of mutton patties for control as well as treatment products. A significant increasing trend was also reported by [44] in TPC of goat meat nuggets in control and treatment nuggets.

Although, the TPC of the products treated with *T. arjuna* ( $T_1$ ,  $T_2$  and  $T_3$ ) increased significantly ( $p < 0.05$ ) with storage, however, the values were significantly ( $p < 0.05$ ) lower than control on all intervals of storage which may be attributed to the phenolic compounds and other constituents of *T. arjuna* which are said to have antimicrobial properties [5,28]. Antimicrobial activity of *T. arjuna* leaves and bark extracts has been reported against various pathogenic and spoilage bacteria like *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Proteus vulgaris*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli*, *Acinetobacter* sp., and *Pseudomonas aeruginosa* [5,45]. Dua et al. [38] recorded a slower increase in the TPC values of *Tabaq-Maz* treated with oleuropein in comparison to control. El-Nashi et al. [22] also reported significantly lower values for TPC of the beef sausages containing different concentrations of pomegranate peel powder during refrigerated storage.

### 3.2.2. Psychrophillic count (log cfu/g)

Psychrophillics were not detected up to 14th day of storage in control as well as *T. arjuna* treated products ( $T_1$ ,  $T_2$  and  $T_3$ ) and thereafter followed a significant ( $p < 0.05$ ) increasing trend throughout the storage period. The counts were well below the

permissible limits up to 56th day of storage for all the products. Cremer and Chipley [43] described permissible level of psychrophillic count as 4.6 log cfu/g in cooked meat products. A detectable count from 14th 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 [42]. The psychrophillics may undergo metabolic injuries due to cooking and other environmental stress and get repaired with the advancement of storage time and start forming colonies after day 14 of storage. Similar findings were also reported by [31,38] in *Tabaq-Maz* and [46] in chicken sausages during refrigerated storage.

Although, psychrophillics 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  $T_3$ ) 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 antimicrobial properties of *T. arjuna* [5,28]. Kaur et al. [47] 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 [36] in mutton patties incorporated with cabbage powder.

### 3.2.3. 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 °C much above their thermal death point of 57 °C. The absence of coliforms during storage depicts that the heat processing and subsequent hygienic handling and packaging were effective to control coliform growth. Similar results were reported by [36] who observed no coliforms in pork patties during entire period of storage. Similar results were recorded by [39] and [48] who also reported zero counts for the meat products heated to such a high temperature.

### 3.2.4. Yeast and mould count (log cfu/g)

Yeast and mould counts were not detected up to 14th day of storage in control as well as treated samples. The yeast and mould counts were detected from day 28th of the storage and thereafter followed a significant ( $p < 0.05$ ) increasing trend throughout the storage period. However, counts of the treated products ( $T_1$ ,  $T_2$  and  $T_3$ ) 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 phenolic compounds and other constituents of *T. arjuna* which may have antifungal properties [5,49]. Kaur et al. [47] 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. Dua et al. [31] recorded a similar observation in *Tabaq-Maz* treated with lemon peel extract.

Table 2  
Effect of *Terminalia arjuna* on the microbiological characteristics of chevon sausages during refrigerated storage (Mean  $\pm$  SE) \*.

Treatments	Storage Period (days)				
	0	14	28	42	56
<b>Total plate count (log cfu/g)</b>					
Control	1.52 $\pm$ 0.036 <sup>Ae</sup>	2.82 $\pm$ 0.038 <sup>Ad</sup>	3.95 $\pm$ 0.097 <sup>Ac</sup>	4.87 $\pm$ 0.068 <sup>Ab</sup>	5.93 $\pm$ 0.178 <sup>Aa</sup>
T <sub>1</sub> (0.25%)	1.49 $\pm$ 0.021 <sup>ABe</sup>	2.34 $\pm$ 0.046 <sup>Bd</sup>	3.46 $\pm$ 0.066 <sup>Bc</sup>	4.31 $\pm$ 0.027 <sup>Bb</sup>	5.42 $\pm$ 0.053 <sup>Ba</sup>
T <sub>2</sub> (0.50%)	1.45 $\pm$ 0.033 <sup>ABe</sup>	2.29 $\pm$ 0.033 <sup>BCd</sup>	3.37 $\pm$ 0.067 <sup>Bc</sup>	4.27 $\pm$ 0.041 <sup>Bb</sup>	5.35 $\pm$ 0.035 <sup>Ba</sup>
T <sub>3</sub> (0.75%)	1.41 $\pm$ 0.033 <sup>Be</sup>	2.19 $\pm$ 0.028 <sup>Cd</sup>	3.28 $\pm$ 0.044 <sup>Bc</sup>	4.21 $\pm$ 0.045 <sup>Bb</sup>	5.29 $\pm$ 0.034 <sup>Ba</sup>
<b>Psychrophillic count (log cfu/g)</b>					
Control	Not detected	Not detected	1.79 $\pm$ 0.046 <sup>Ac</sup>	2.69 $\pm$ 0.054 <sup>Ab</sup>	3.87 $\pm$ 0.038 <sup>Aa</sup>
T <sub>1</sub> (0.25%)	Not detected	Not detected	1.32 $\pm$ 0.057 <sup>Bc</sup>	2.25 $\pm$ 0.038 <sup>Bb</sup>	3.42 $\pm$ 0.075 <sup>Ba</sup>
T <sub>2</sub> (0.50%)	Not detected	Not detected	1.27 $\pm$ 0.051 <sup>Bc</sup>	2.19 $\pm$ 0.028 <sup>BCb</sup>	3.35 $\pm$ 0.038 <sup>Ba</sup>
T <sub>3</sub> (0.75%)	Not detected	Not detected	1.21 $\pm$ 0.046 <sup>Bc</sup>	2.11 $\pm$ 0.030 <sup>Cb</sup>	3.28 $\pm$ 0.050 <sup>Ba</sup>
<b>Coliform count (log cfu/g)</b>					
Control	Not detected	Not detected	Not detected	Not detected	Not detected
T <sub>1</sub> (0.25%)	Not detected	Not detected	Not detected	Not detected	Not detected
T <sub>2</sub> (0.50%)	Not detected	Not detected	Not detected	Not detected	Not detected
T <sub>3</sub> (0.75%)	Not detected	Not detected	Not detected	Not detected	Not detected
<b>Yeast and mould count (log cfu/g)</b>					
Control	Not detected	Not detected	1.85 $\pm$ 0.201 <sup>Ac</sup>	2.69 $\pm$ 0.042 <sup>Ab</sup>	3.83 $\pm$ 0.178 <sup>Aa</sup>
T <sub>1</sub> (0.25%)	Not detected	Not detected	1.35 $\pm$ 0.050 <sup>Bc</sup>	2.42 $\pm$ 0.026 <sup>Bb</sup>	3.39 $\pm$ 0.046 <sup>Ba</sup>
T <sub>2</sub> (0.50%)	Not detected	Not detected	1.29 $\pm$ 0.039 <sup>Bc</sup>	2.31 $\pm$ 0.058 <sup>BCb</sup>	3.27 $\pm$ 0.024 <sup>Ba</sup>
T <sub>3</sub> (0.75%)	Not detected	Not detected	1.21 $\pm$ 0.042 <sup>Bc</sup>	2.27 $\pm$ 0.028 <sup>Cb</sup>	3.21 $\pm$ 0.033 <sup>Ba</sup>
<b>Anaerobic plate count (log cfu/g)</b>					
Control	Not detected	Not detected	1.95 <sup>Ac</sup> $\pm$ 0.067	2.24 <sup>Ab</sup> $\pm$ 0.035	2.89 <sup>Aa</sup> $\pm$ 0.042
T <sub>1</sub> (0.25%)	Not detected	Not detected	1.71 <sup>Bb</sup> $\pm$ 0.081	1.82 <sup>Bb</sup> $\pm$ 0.041	2.53 <sup>Ba</sup> $\pm$ 0.046
T <sub>2</sub> (0.50%)	Not detected	Not detected	1.43 <sup>Cb</sup> $\pm$ 0.073	1.54 <sup>Cb</sup> $\pm$ 0.061	2.32 <sup>Ca</sup> $\pm$ 0.053
T <sub>3</sub> (0.75%)	Not detected	Not detected	1.21 <sup>Db</sup> $\pm$ 0.067	1.35 <sup>Db</sup> $\pm$ 0.059	2.15 <sup>Da</sup> $\pm$ 0.024

n = 6 for each treatment.

T<sub>1</sub> (0.25%) = Sausages with 0.25% of *Terminalia arjuna*.

T<sub>2</sub> (0.50%) = Sausages with 0.50% of *Terminalia arjuna*.

T<sub>3</sub> (0.75%) = Sausages with 0.75% of *Terminalia arjuna*.

\* Mean  $\pm$  SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly ( $p < 0.05$ ).

### 3.3. Sensory parameters

The mean values of various sensory parameters of chevon sausages containing different levels of *T. arjuna* viz. T<sub>1</sub> (0.25%), T<sub>2</sub> (0.50%) and T<sub>3</sub> (0.75%) are presented in Table 3.

#### 3.3.1. Colour and appearance

A significant ( $p < 0.05$ ) decreasing trend was observed in the scores of colour and appearance throughout the period of storage which might be due to pigment and lipid oxidation and non-enzymatic browning resulting from Millard's reaction. Lipid oxidation produces secondary reaction products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde as well as other oxygenated compounds such as aldehydes, acids and ketones which can cause loss of colour [35]. A decrease in appearance and colour scores of meat products with storage period was also reported by [47,50,51].

The scores for appearance and colour were significantly ( $p < 0.05$ ) higher for the treated products (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>) in comparison to control on all days of storage with highest values observed for T<sub>3</sub>. This might be attributed to the colour pigments and phenolic compounds and other constituents of *T. arjuna* which are said to have antioxidant and antimicrobial proper-

ties [5,49]. Bhat et al. [51] also reported the higher scores for the chicken sausages incorporated with *Ocimum sanctum* Linn (*Tulsi*) in comparison to control during storage. Similar observations were also reported by [41] in chevon cutlets treated with clove oil during refrigerated storage.

#### 3.3.2. Flavour

The scores for flavour decreased significantly ( $p < 0.05$ ) with the advancement of storage period for all treated chevon sausages as well as for control. Increased lipid oxidation, liberation of fatty acids, increased microbial load, and loss of volatile flavour components could be the contributing factors responsible for the decline in scores [52]. Production of some bitter compounds during lipid oxidation, lipolysis and proteolysis affects the flavour and may be responsible for decline in the flavour scores of the products with storage [53]. Decline in flavour scores during refrigerated storage was also reported by [46] in chicken sausages and [36] in restructured chicken meat blocks.

Significantly ( $p < 0.05$ ) lower scores were observed for the treated samples (T<sub>3</sub>) on day 0 and 14 in comparison to control, however, the mean flavour scores of the sausages containing 0.25% extract (T<sub>1</sub>) and 0.50% extract (T<sub>2</sub>) were comparable ( $p > 0.05$ ) with control. Higher concentration of *T. arjuna* (T<sub>3</sub>)

Table 3

Effect of *Terminalia arjuna* on the sensory characteristics of chevon sausages during refrigerated storage (Mean  $\pm$  SE)\*.

Treatments	Storage Period (days)				
	0	14	28	42	56
<b>Colour and Appearance</b>					
Control	6.34 $\pm$ 0.031 <sup>Ca</sup>	6.06 $\pm$ 0.027 <sup>Db</sup>	5.25 $\pm$ 0.019 <sup>Dc</sup>	4.28 $\pm$ 0.026 <sup>Cd</sup>	4.15 $\pm$ 0.096 <sup>Cd</sup>
T <sub>1</sub> (0.25%)	6.89 $\pm$ 0.022 <sup>Ba</sup>	6.36 $\pm$ 0.028 <sup>Cb</sup>	5.72 $\pm$ 0.044 <sup>Cc</sup>	4.78 $\pm$ 0.045 <sup>Bd</sup>	4.46 $\pm$ 0.080 <sup>Be</sup>
T <sub>2</sub> (0.50%)	7.07 $\pm$ 0.039 <sup>Aa</sup>	6.73 $\pm$ 0.029 <sup>Bb</sup>	5.82 $\pm$ 0.019 <sup>Bc</sup>	5.29 $\pm$ 0.021 <sup>Ad</sup>	4.61 $\pm$ 0.061 <sup>Be</sup>
T <sub>3</sub> (0.75%)	7.08 $\pm$ 0.040 <sup>Aa</sup>	6.85 $\pm$ 0.017 <sup>Ab</sup>	5.91 $\pm$ 0.011 <sup>Ac</sup>	5.35 $\pm$ 0.018 <sup>Ad</sup>	4.85 $\pm$ 0.059 <sup>Ae</sup>
<b>Flavour</b>					
Control	7.39 $\pm$ 0.023 <sup>Aa</sup>	6.20 $\pm$ 0.048 <sup>Ab</sup>	5.49 $\pm$ 0.029 <sup>Bc</sup>	4.34 $\pm$ 0.028 <sup>Bd</sup>	Not evaluated
T <sub>1</sub> (0.25%)	7.37 $\pm$ 0.021 <sup>Aa</sup>	6.24 $\pm$ 0.021 <sup>Ab</sup>	6.18 $\pm$ 0.022 <sup>Ab</sup>	5.69 $\pm$ 0.031 <sup>Ac</sup>	Not evaluated
T <sub>2</sub> (0.50%)	7.34 $\pm$ 0.023 <sup>Aa</sup>	6.27 $\pm$ 0.021 <sup>Ab</sup>	6.21 $\pm$ 0.021 <sup>Ab</sup>	5.72 $\pm$ 0.034 <sup>Ac</sup>	Not evaluated
T <sub>3</sub> (0.75%)	7.03 $\pm$ 0.097 <sup>Ba</sup>	6.05 $\pm$ 0.059 <sup>Bb</sup>	5.41 $\pm$ 0.045 <sup>Bc</sup>	4.29 $\pm$ 0.042 <sup>Bd</sup>	Not evaluated
<b>Juiciness</b>					
Control	7.28 $\pm$ 0.120 <sup>a</sup>	6.45 $\pm$ 0.033 <sup>b</sup>	5.56 $\pm$ 0.031 <sup>c</sup>	5.20 $\pm$ 0.131 <sup>d</sup>	Not evaluated
T <sub>1</sub> (0.25%)	7.24 $\pm$ 0.143 <sup>a</sup>	6.43 $\pm$ 0.032 <sup>b</sup>	5.53 $\pm$ 0.028 <sup>c</sup>	5.16 $\pm$ 0.143 <sup>d</sup>	Not evaluated
T <sub>2</sub> (0.50%)	7.21 $\pm$ 0.143 <sup>a</sup>	6.39 $\pm$ 0.032 <sup>b</sup>	5.49 $\pm$ 0.040 <sup>c</sup>	5.12 $\pm$ 0.132 <sup>d</sup>	Not evaluated
T <sub>3</sub> (0.75%)	7.19 $\pm$ 0.152 <sup>a</sup>	6.36 $\pm$ 0.023 <sup>b</sup>	5.46 $\pm$ 0.130 <sup>c</sup>	5.08 $\pm$ 0.127 <sup>d</sup>	Not evaluated
<b>Texture</b>					
Control	7.25 $\pm$ 0.153 <sup>a</sup>	6.20 $\pm$ 0.051 <sup>Cb</sup>	5.21 $\pm$ 0.019 <sup>Dc</sup>	4.15 $\pm$ 0.022 <sup>Cd</sup>	3.80 $\pm$ 0.024 <sup>De</sup>
T <sub>1</sub> (0.25%)	7.19 $\pm$ 0.100 <sup>a</sup>	6.24 $\pm$ 0.111 <sup>B<sup>Cb</sup></sup>	6.22 $\pm$ 0.023 <sup>Cb</sup>	5.17 $\pm$ 0.048 <sup>Bc</sup>	4.69 $\pm$ 0.029 <sup>Cd</sup>
T <sub>2</sub> (0.50%)	7.13 $\pm$ 0.165 <sup>a</sup>	6.42 $\pm$ 0.065 <sup>Bb</sup>	6.34 $\pm$ 0.031 <sup>Bb</sup>	5.27 $\pm$ 0.023 <sup>A<sup>Bc</sup></sup>	4.78 $\pm$ 0.026 <sup>Bd</sup>
T <sub>3</sub> (0.75%)	7.06 $\pm$ 0.092 <sup>a</sup>	6.76 $\pm$ 0.041 <sup>Ab</sup>	6.45 $\pm$ 0.054 <sup>Ac</sup>	5.36 $\pm$ 0.066 <sup>Ad</sup>	4.86 $\pm$ 0.032 <sup>Ae</sup>
<b>Overall Acceptability</b>					
Control	7.36 $\pm$ 0.107 <sup>Aa</sup>	6.23 $\pm$ 0.072 <sup>Ab</sup>	5.65 $\pm$ 0.033 <sup>Bc</sup>	4.51 $\pm$ 0.052 <sup>Bd</sup>	Not evaluated
T <sub>1</sub> (0.25%)	7.33 $\pm$ 0.100 <sup>Aa</sup>	6.29 $\pm$ 0.018 <sup>Ab</sup>	6.27 $\pm$ 0.016 <sup>Ab</sup>	5.59 $\pm$ 0.033 <sup>Ac</sup>	Not evaluated
T <sub>2</sub> (0.50%)	7.29 $\pm$ 0.123 <sup>Aa</sup>	6.35 $\pm$ 0.029 <sup>Ab</sup>	6.31 $\pm$ 0.023 <sup>Ab</sup>	5.62 $\pm$ 0.033 <sup>Ac</sup>	Not evaluated
T <sub>3</sub> (0.75%)	6.75 $\pm$ 0.103 <sup>Ba</sup>	6.03 $\pm$ 0.074 <sup>Bb</sup>	5.76 $\pm$ 0.080 <sup>Bc</sup>	4.62 $\pm$ 0.034 <sup>Bd</sup>	Not evaluated

n = 30 for each treatment.

T<sub>1</sub> (0.25%) = Sausages with 0.25% of *Terminalia arjuna*.T<sub>2</sub> (0.50%) = Sausages with 0.50% of *Terminalia arjuna*.T<sub>3</sub> (0.75%) = Sausages with 0.75% of *Terminalia arjuna*.\* Mean  $\pm$  SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly ( $p < 0.05$ ).

was perceived as slightly bitter by the panellists and obtained lower scores on day 0 and 14 in comparison to control. The sausages containing 0.25% extract (T<sub>1</sub>) and 0.50% extract (T<sub>2</sub>) showed significantly ( $p < 0.05$ ) higher scores on day 28 and 42 with highest scores for 0.50% extract (T<sub>2</sub>) in comparison to all other products. Higher scores for products containing 0.25% extract (T<sub>1</sub>) and 0.50% extract (T<sub>2</sub>) may be correlated with comparatively lower TBARS values, FFA values and microbial counts due to the antioxidant, antimicrobial and antifungal properties of *T. arjuna* [5,28,49]. Several studies [47,48,54] have reported significantly ( $p < 0.05$ ) higher scores for meat products incorporated with different natural antioxidant sources.

### 3.3.3. Juiciness

The mean juiciness scores decreased significantly ( $p < 0.05$ ) from day 0 to 56th day of storage for all the products. This may be attributed to the gradual loss of moisture from the products. Verma et al. [55] also reported a decreasing trend in the juiciness scores of chevon patties incorporated with jack fruit during refrigerated storage. Jin et al. [56] and Baker et al. [57] also observed a decrease in juiciness scores of the pork sausages and lamb patties, respectively, during storage.

### 3.3.4. Texture

Texture scores decreased significantly ( $p < 0.05$ ) with the advancement of storage period in all the products. The probable reasons may be due to loss of moisture leading to hardening of the texture, breakdown of fat, and degradation of muscle proteins by bacterial action [42]. Similar results were presented by [48] in chicken patties and [46] in chicken sausages who also observed a significant decline in the texture scores with storage period. The sausages incorporated with *T. arjuna* showed significantly ( $p < 0.05$ ) higher scores for texture as compared to control. The higher scores for the treated products could be attributed to the antimicrobial and antioxidant properties of *T. arjuna* [5,28,49] that may have resulted into lower degradation of proteins and breakdown of fats. An increase in the texture scores of meat products with addition of natural antioxidants was also reported by [47,48,54].

### 3.3.5. Overall acceptability

The mean scores for overall acceptability decreased significantly ( $p < 0.05$ ) with the advancement of storage period in all treated chevon sausages as well as in control samples. Continuous decrease in overall acceptability scores might be reflective of the decline in scores of colour and appearance, flavour, juici-

ness and texture. Kumar et al. [58] also reported a decreasing trend in overall acceptability scores of pork patties incorporated with sea buckthorn and grape seed extract with the advancement of storage. [47,48] also observed similar decline in the overall acceptability scores of meat products with storage period. Significantly ( $p < 0.05$ ) lower scores were observed for the treated samples containing 0.75% extract ( $T_3$ ) on day 0 and 14 in comparison to control, however, the mean scores for the sausages containing 0.25% extract ( $T_1$ ) and 0.50% extract ( $T_2$ ) were comparable ( $p > 0.05$ ) with control. The sausages containing 0.25% extract ( $T_1$ ) and 0.50% extract ( $T_2$ ) showed significantly ( $p < 0.05$ ) higher scores on day 28 and 42 with highest scores for 0.50% extract ( $T_2$ ) in comparison to all other products. Significantly higher scores for products containing 0.25% extract ( $T_1$ ) and 0.50% extract ( $T_2$ ) may be due to significantly ( $p < 0.05$ ) higher flavour and colour scores. Several studies [47,48,39] have reported an increase in the overall acceptability scores of meat products with addition of some natural antioxidants.

#### 4. Conclusions

The present study showed successful utilization of *T. arjuna* as a novel natural preservative in meat products. The results indicated that incorporation of chevon sausages with *T. arjuna* improved the lipid oxidative stability and storage quality and the products remained fit for consumption up to 42nd day at  $4 \pm 1^\circ\text{C}$ . Significantly ( $p < 0.05$ ) higher sensory scores for treated products might be correlated with comparatively lower FFA values, TBARS values and microbial counts. *T. arjuna* may be commercially exploited as a natural preservative in muscle foods.

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