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Effect of Poppy (*Papaver somniferum*) seed extract on the Physico-chemical, microbial and sensory Characteristics of Chevon patties

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Abstract
The present study was carried out to evaluate the antioxidant potential of poppy seed extract (PSE) and its effect on the Physico-chemical, microbiological and sensory properties of chevon patties during frozen storage. Antioxidant potential by various biochemical tests (Antioxidant ability assays, total phenol content, total flavonoid content) were evaluated. The result showed that PSE exhibit high antioxidant ability assays (224.56±1.27 µg ascorbic acid), total phenol content (275.5±3.26 mg PSE/g), total flavonoid content (68.79±3.16 mg rutin/g). The chevon patties treated with PSE had significantly (p<0.01) lower thiobarbituric acid (TBA) and free fatty acid % (FFA) compared to control during frozen storage. The PSE treated chevon patties recorded significantly (p<0.05) superior score of flavor, juiciness and overall acceptability than control. It can be concluded that PSE has excellent antioxidant properties, could be used as an antioxidant to meat products without affecting its quality and acceptability.

Keywords: Antioxidant, chevon patties, poppy seed extract, microbiological

Introduction
Chevon is a rich source of nutrients and micronutrients that are needed for good health throughout life. The percentage of saturated fat in goat meat is lower than chicken, beef, pork or lamb (Banskalieva *et al*., 2000) [2]. Chevon is almost universally acceptable and free from culture, tradition, social and economic conditions (Webb *et al*., 2005; Xazela *et al*., 2011) [27, 30]. This unique feature of chevon categorized it as "National meat animal of India" (Sharma, 1995) [24]. According to Kadim and Mahgoub (2012) [11], chevon and its product are considered as high-quality meat products on sensory basis by the trained panellists. Chevon has also been reported to contain higher collagen and has lower solubility and its intramuscular connective tissues remain unchanged during post-mortem ageing (Kannan *et al*., 2006) [13]. The tenderness of meat is affected by the solubility and the amount of collagen, while the juiciness in cooked meat is closely related to the intramuscular fat content. However, chevon is found to be less tender with higher shear force values and collagen content than lamb (McMillan and Brock, 2005) [17]. To alleviate toughness problems, meat from older (spent) animals is generally processed as ground or comminuted meat products. The process of comminution was proved to alter the relatively tough and less juicy meat into a product with desirable sensory characteristics.
Poppy Seeds (*Papaver somniferum*) with good taste, are nutritious oilseeds used in Indian cuisine in the form of Khus Khus (white small size granules) and historically, it has been utilized for the treatment of several diseases like asthma, stomach disorder and eyesight improvement. The functional potentials of poppy seeds its less studied and only a few reports are available in this regards. In some studies poppy seed have been demonstrated to have lower total plate count as compair to other very common extract like clove buds and cinnamon bark. The aqueous extracts from spices exhibit antioxidant activity due to their high phenolic contents (Chan *et al*., 2011) [4].

Materials and Methods

Meat and other ingredients

Deboned goat meat was obtained from local market, packed in low density polyethylene bags and kept under frozen storage at -18±2 °C for the subsequent use. Frozen meat was thawed at 4±1 °C for 24 hrs before use and cut into small pieces before grinding. Refined salt (Tata Chemicals Ltd., Mumbai), refined wheat flour, spice mix ingredients, flaxseed powder, skim milk powder (Anikspray, Nutrica) and poppy seed were procured from local market of Jaipur. Fine pastes of onion, garlic and ginger in the ratio 3:1:1 were used for preparation of chevon patties. Chemicals and media used for analysis of product were procured from standard firms like Sigma, Mark, SRL and Hi-media *etc*.

Preparation of Chevon patties

Frozen chevon was partially thawed overnight, cut into small cubes and double minced with meat mincer. Meat emulsion was prepared in a bowl chopper (Hakimi, India). Pre-weighed quantity of minced chevon, salt, sodium tripolyphosphate, and sodium nitrite were added and chopped for about 2-3 minutes. It was chopped again for 2 minutes after the addition of ice flakes. Animal fat was slowly incorporated while chopping till it was completely dispersed in the batter. Condiment paste, dry spice mix, and other ingredients *viz*: poppy seed extracts were added. Chopping was continued till uniform dispersion of all the ingredients and desired consistency of the emulsion was achieved. Weighed quantity of emulsion was taken, patties moulded in shape and cooked in hot air oven at 180 °C for 40 minutes. Core temperature of cooked blocks was recorded by using probe thermometer that should reach to 73 °C. Chevon meat block obtained was sliced and cut into pieces to get patties and stored at -18±2 °C. At 15 days intervals sample were removed for analysis of physico-chemical, microbiological and sensory properties. The analysis was continued for 30, 45 and 60 day in frozen storage.

Analytical procedure

Extract preparation

The dried seed of poppy seed were air-dried in hot air oven at 50 °C for 2 hrs, followed by grinding in grinder (Uno (mx-140), Groupe SEB India Pvt. Ltd) and sieving. The powder was weighed in the electronic weighing balance. Extracts from pre weighed, dried powdered seed were extracted with 70% ethanol in ether extraction assembly. The color of the extracts was dark brown and light green for poppy seed respectively. The extract was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Labconco corporation, USA) until semi solid consistency was obtained. The semisolid mass was air-dried to obtain solid mass. Powdered substances were reconstituted with the same solvent as used for extraction to make 5% stock solutions (0.5g of dried extract /10 ml) and stored under refrigeration at 4 °C for further use.

In vitro antioxidant assays

The antioxidant activity of the extracts were evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999) [22]. Briefly, 0.3 ml of extracts were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing reaction solution were incubated at 95 °C for 90 min and cooled to room temperature. The absorbance of the solution was measured at 695 nm using spectrophotometer against the blank. Ascorbic acid was used as reference standard. The antioxidant activity is expressed as the number of equivalents of ascorbic acid (AscAE).

Physico-chemical parameters

The determination of TBA value was based on the procedure of Witte *et al.* (1970) [29] and Free Fatty Acid was estimated as per Koniecko (1979) [14].

Microbiological examinations

Total plate count, psychrophilic count, coliform count and yeast and mold count in the samples were determined following the methods described by APHA (1984) [1].

Sensory Evaluation

There were 7 experienced taste panel members consisting of faculty members and postgraduate students of the department and the institute, evaluated the sensory attributes *viz*. appearance, flavor, texture, juiciness and overall acceptability of treatments and control using 9 point hedonic scale (Wichchukita and O'Mahonyc, 2014) [28].

Statistical analysis

Data hence obtained through the experiments were analyzed as per Snedecor and Cochran (1994) [25] using Statistical Software Packages (SPSS 16.0).

Results and Discussion

Assessment of *in vitro* antioxidant activity of poppy seed extract

Antioxidant ability assays, total phenolic and flavonoid content

The total antioxidant activity of the herb extract was evaluated by the phosphomolybdenum method and followed by comparing with the standard solution of ascorbic acid. The standard curve of ascorbic acid was made by using ascorbic acid concentrations ranging from 50 to300 µg/ml (Deepa *et al.*, 2013) [7]. Total antioxidant capacity of poppy seed extract has also been previously determined by various workers. Md *et al*. (2013) [18]. found total antioxidant capacity of poppy seed extract was 224.56 µg/ml, depending on the concentrations and solvent medium. The total phenolic content for the ethanolic extracts of poppy seed, determined by the Folin-Ciocalteu method, were found to be106.36±1.20 mg of gallic acid (GAE) per g for poppy seed extracts respectively. The total phenolic content of poppy seed extract has been also previously determined by different workers. Yucel Sengun *et al*. (2020) [31] and Palamutoglu *et al*. (2020) [20] reported total phenolic content of poppy seed extract was 275.5 mg GAE/kg and 2612 µg/ml respectively, depending on concentrations and solvent medium. The total flavonoid content of poppy seed extracts were found to be 68.79±0.36 mg rutin/g.

Physico-chemical properties

TBA value

The Result of ANOVA revealed a highly significant (P<0.01) effect of poppy seed on incorporation TBA values of the product. At the end of storage period all the treatments T_3 (0.71±0.02) mg malonaldehyde/kg, T_2 (0.80±0.01) mg malonaldehyde/kg and T_1 (0.91±0.01) mg malonaldehyde/kg have significantly lower TBA value than the control (0.99±0.01) mg malonaldehyde/kg (Table 1). Throughout the storage period TBA value of T_3 was lower than other treatments and control. the highest antioxidant activity of T_3

among all product groups, may be due to higher levels of poppy seeds in product, poppy seed have a high antioxidant potential which was also reflected in the results of different *in-vitro* antioxidant activity assay. However, the TBA value of T_2 was significantly lower than the T_1 and control. After the end of the study, the TBA value of T_3 was significantly lower than the control and all treatments on the 60th day. It may be attributed to the fact that poppy seed contains linoleic acid and other poly unsaturated fatty acids and mono unsaturated fatty acids which delayed lipid peroxidation and lipolysis in ground poppy seed paste incorporated chevon patties. During the storage period consistence increase in TBA value of all treatments and control groups were observed. This might be due to auto-oxidation of lipids over a period time and increased microbial population (Talukder *et al.*, 2016) [26]. Increased lipid oxidation during storage was also reported for chicken nuggets by (Lal *et al.*, 1995) [16]. Similar results were also reported by Ozcan and Cigdem (2006) [19]. and Ernic *et al.*, (2009) [9] that ground poppy seed paste fortification had lower TBA value as compared to control meat burgers. Similar results were also reported in chevon nuggets with addition of *papaver somniferum* (Kamal *et al.*, 2017) [12]. Pandey *et al.* (2016) [21] observed that samples treated with antioxidant had lower TBARS value.

Table 1: Effect of poppy seed extracts incorporation on TBA value of chevon patties during frozen storage at 18±2 °C (Mean ± SE)

Days/Group	0	15	30	45	60	Treatment Mean ± SE
C	0.29 ± 0.01^{aA}	0.64 ± 0.02^{aB}	0.79 ± 0.01^{aB}	0.81 ± 0.1^{bB}	0.99 ± 0.01^{bC}	0.70 ± 0.06^{b}
T_1	0.29 ± 0.01^{aA}	0.44 ± 0.01^{aA}	0.64 ± 0.01^{aB}	0.75 ± 0.01^{aBC}	0.91 ± 0.01^{bC}	0.61 ± 0.05^{a}
T_2	0.27 ± 0.01^{aA}	0.43 ± 0.02^{aA}	0.60 ± 0.01^{aB}	0.70 ± 0.01^{aB}	0.80 ± 0.01^{aC}	0.55 ± 0.05^{a}
T_3	0.27 ± 0.01^{aA}	0.40 ± 0.08^{aA}	0.55 ± 0.01^{aB}	0.65 ± 0.01^{aB}	0.71 ± 0.02^{aB}	0.52 ± 0.05^{a}
Days Mean ± SE	0.28 ± 0.0^{A}	0.48 ± 0.05^{B}	0.61 ± 0.01^{C}	0.72 ± 0.01^{D}	0.83 ± 0.06^{E}	

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differed highly significantly ($P<0.01$), significantly ($P<0.05$) C=control, T_1=2% poppy seed extract, T_2= 3%poppy seed extract and T_3= 4% poppy seed extract n=6 for each treatment.

Free fatty acid value

The result of ANOVA revealed a highly significant ($p<0.01$) effect of poppy seed on the free fatty acid value of the chevon patties. At the end of storage period all the treatments T_3 (0.33±0.01 g of oleic acid/100 g), T_2 (0.39±0.01 g of oleic acid/100 g) and T_1 (0.40±0.01 g of oleic acid/100 g) have significantly lower FFA value than the control (0.45±0.01 g of oleic acid/100 g) (Table 2).

Table 2: Effect of poppy seed extracts incorporation on free fatty acid value of chevon patties during frozen storage at 18±2 °C (Mean ± SE)

Days/Group	0	15	30	45	60	Treatment Mean ± SE
C	0.18 ± 0.04^{bA}	0.34 ± 0.13^{cB}	0.35 ± 0.01^{bB}	0.40 ± 0.05^{cB}	0.45 ± 0.01^{Cb}	0.34 ± 0.06^{c}
T_1	0.15 ± 0.01^{bA}	0.21 ± 0.01^{bBC}	0.29 ± 0.11^{bBC}	0.33 ± 0.01^{bCD}	0.40 ± 0.01^{bcD}	0.27 ± 0.03^{b}
T_2	0.09 ± 0.01^{aA}	0.13 ± 0.02^{aA}	0.17 ± 0.01^{aAB}	0.31 ± 0.01^{bBC}	0.39 ± 0.01^{bC}	0.22 ± 0.03^{ab}
T_3	0.09 ± 0.01^{aA}	0.10 ± 0.01^{aA}	0.15 ± 0.01^{aBC}	0.27 ± 0.01^{aBC}	0.33 ± 0.01^{aC}	0.19 ± 0.02^{a}
Days Mean ± SE	0.13 ± 0.05^{A}	0.19 ± 0.03^{A}	0.24 ± 0.01^{B}	0.33 ± 0.01^{C}	0.39 ± 0.01^{D}	

*Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differed highly significantly ($P<0.01$), significantly ($P<0.05$) C=control, T_1=2% poppy seed extract, T_2= 3% poppy seed extract and T_3= 4% poppy seed extract n=6 for each treatment.

Microbiological Properties

Total plate count: The Result of ANOVA revealed a significant ($p<0.05$) effect of poppy seed incorporation on total plate count (TPC) of the chevon patties. At the end of storage period all the treatments T_3 (4.33±0.0 log cfu/g 1), T_2 (4.49±0.01 log cfu/g) and T_1 (4.78±0.01 log cfu/g) have significantly lower TPC than the control (5.21±0.01 log cfu/g) (Table 3). Throughout the storage period, TPC of T_3 was lower than other treatments and control. However, TPC of T_2 was significantly lower than the T_1 and control. The results of treatment and storage interaction exhibit a highly significant ($p<0.01$) effect on the TPC of the products. After the 60 days of storage, the TPC of T_3 was significantly lower than the control and all other treatments.

Table 3: Effect of poppy seed extracts incorporation on total plate count of chevon patties during frozen storage at -18±2 °C (Mean ± SE)

Days/Group	0	15	30	45	60	Treatment Mean ± SE
C	2.68 ± 0.15^{aA}	3.32 ± 0.23^{bB}	3.53 ± 0.01^{cB}	4.22 ± 0.01^{dC}	5.21 ± 0.01^{cD}	3.79 ± 0.23^{c}
T_1	2.32 ± 0.01^{abA}	2.88 ± 0.01^{aB}	3.18 ± 0.01^{bB}	3.73 ± 0.01^{cC}	4.78 ± 0.01^{bD}	3.37 ± 0.22^{b}
T_2	2.32 ± 0.28^{aA}	2.76 ± 0.01^{aB}	2.99 ± 0.01^{abB}	3.41 ± 0.02^{bC}	4.49 ± 0.01^{aD}	3.19 ± 0.20^{a}
T_3	2.22 ± 0.01^{aA}	2.63 ± 0.03^{aA}	2.72 ± 0.11^{aA}	3.16 ± 0.08^{aB}	4.33 ± 0.03^{aC}	3.01 ± 0.19^{a}
Days Mean ± SE	2.39 ± 0.08^{A}	2.9 ± 0.09^{B}	3.10 ± 0.09^{C}	3.63 ± 0.12^{D}	4.70 ± 0.10^{E}	

*Mean ± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differed highly significantly ($P<0.01$), significantly ($P<0.05$) C=control, T_1=2% poppy seed extract, T_2= 3%poppy seed extract and T_3= 4% poppy seed extract n=6 for each treatment

Antimicrobial activity of poppy seed is might be due to higher MUFA, PUFA, alpha-tocopherol and gama-tocopherol content. Which is a major compound of the poppy seed extract reported by Erinic *et al.* (2009) [9] and Gok *et al.* (2011) [10]. The poppy seed oils contained an appreciable amount of β-tocopherol (309.5 ppm-567.3 ppm). Stearic, palmitic, oleic, linoleic and linolenic were determined as the main fatty acids. Poppy seed and oil having high nutritive value were recommended for processing as healthy food products. Linoleic acid was established as the dominant fatty acid in all varieties reported by Ozcan, M.M. and Atalay, C., (2006) [19]. The result of the present study was in agreement with reports of Saniya *et al.* (2017), who observed a reduction in TPC in chevon nuggets with poppy seed extract during

refrigerated storage.

Similar findings were observed by Devatkal *et al*. (2014) [8], Bhat *et al*. (2016) [3], Kamal *et al*. (2017) [12] and Rathour *et al*. (2017) [23] who also found a similar increase in TPC during storage in ground meat nuggets, chevon nuggets, fortified chevon nuggets and chevon rolls respectively.

Sensory Characteristics

The sensory properties like appearance and texture of chevon patties treated with poppy seed extract and under refrigerated storage at 4±1 °C were analyzed on the day 0, 7, 14, 21 and 28 by panelists.

Results of ANOVA revealed that there was no significant effect ($p>0.05$) of poppy seed treatment on the appearance scores of the product. Within the treatments, the scores ranged from 6.87 to 6.94 and during storage period the appearance scores ranged from 6.80 to 7.04. The panelists rated the color of product as good to very good (Table 4).

Table 4: Effect of poppy seed extracts incorporation on appearance of chevon patties during refrigeration storage at 4±1 °C (Mean ± SE)

Days/Group	0	7	14	21	28	Treatment Mean ± SE
C	6.98±0.04	6.91±0.05	6.88±0.18	6.80±0.10	6.78±0.13	$6.87±0.05^{a}$
T_1	7.00±0.15	6.91±0.12	6.88±0.13	6.85±0.10	6.80±0.11	$6.89±0.05^{a}$
T_2	7.11±0.06	6.95±0.12	6.93±0.04	6.88±0.11	6.81±0.18	$6.94±0.05^{a}$
T_3	7.08±0.10	6.91±0.07	6.90±0.11	6.86±0.13	6.81±0.12	$6.91±0.05^{a}$
Days Mean ± SE	$7.04±0.06^{C}$	$6.92±0.06^{B}$	$6.90±0.06^{B}$	$6.85±0.06^{A}$	$6.80±0.06^{A}$	

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differed highly significantly ($p<0.01$), significantly ($p<0.05$) C=control, T_1=2% poppy seed extract, T_2= 3% poppy seed extract and T_3= 4% poppy seed extract n=6 for each treatment

The result of ANOVA revealed a highly significant ($p<0.01$) effect of poppy seed treatment on the flavor of the chevon patties. At the end of storage period all the treatments T3 (5.73±0.02), T2 (6.03±0.03) and T1 (5.60±0.02) have significantly higher flavor score than the control (5.36±0.02). Throughout the storage period flavor score of T2 was higher than other treatments and control (Table 5). However, Flavor score of T_3 was significantly higher than the T1 and control. During the storage period consistence decrease in flavor score of all treatments and control group were observed. Due to the high antioxidant activity of all treatments, all the treatments fetched higher flavor scores than the control.

Table 5: Effect of poppy seed extracts incorporation on flavor of chevon patties during refrigeration storage at 4±1 °C (Mean ± SE)

Days/Group	0	7	14	21	28	Treatment Mean ± SE
C	$6.98±0.06^{aE}$	$6.70±0.02^{aD}$	$6.26±0.02^{aC}$	$5.91±0.03^{aB}$	$5.36±0.02^{aA}$	$6.24±0.01^{a}$
T_1	$7.35±0.02^{bE}$	$7.08±0.02^{bD}$	$6.76±0.03^{bC}$	$6.18±0.04^{bB}$	$5.60±0.02^{bA}$	$6.59±0.01^{b}$
T_2	$7.66±0.03^{dE}$	$7.35±0.04^{dD}$	$6.95±0.02^{dC}$	$6.71±0.02^{dB}$	$6.03±0.03^{dA}$	$6.88±0.01^{d}$
T_3	$7.36±0.02^{cE}$	$7.18±0.05^{cD}$	$6.85±0.03^{cC}$	$6.45±0.02^{cB}$	$5.73±0.02^{cA}$	$6.77±0.01^{c}$
Days Mean ± SE	$7.34±0.01^{T}$	$7.07±0.01^{D}$	$6.70±0.01^{C}$	$6.31±0.01^{B}$	$5.68±0.01^{A}$	

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differed highly significantly ($p<0.01$), significantly ($p<0.05$) C=control, T_1=2% poppy seed extract, T_2= 3% poppy seed extract and T_3= 4% poppy seed extract n=6 for each treatment

The progressive decrease in flavor scores could be correlated with an increase in TBA value and free fatty acids in the chevon patties. Succeeding storage days favor oxidative rancidity, thereby increasing the TBA value, which might be attributed to a decrease in flavor scores. Kamal *et al*. (2017) [12] and Gok *et al*., (2011) [10] reported similar decrease flavor scores in chevon nuggets and meat burgers respectively. A decrease in flavour scores during storage was also reported by Kumar and Sharma (2004) [15], Das *et al*. (2008) [6] and Chandralekha *et al*. (2012) [5] in different meat products.

Conclusion

The results clearly demonstrate the antioxidant effect of poppy seed extract in chevon patties during frozen storage. The extract effectively improved physio-chemical and microbiological quality had superior sensory score than control. Thus, Industrial waste like poppy seed could be successfully used to extend the shelf life of frozen meat products.

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