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Research Article

SHELF LIFE ASSESSMENT OF COOKED GOAT MEAT PATTIES INCORPORATED WITH AMLA FRUIT AND AMLA SEED COAT EXTRACT AT REFRIGERATED STORAGE (4±1ºC)

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Abstract-In recent year demand for the production of high-quality and ready-to-eat products are increased. So keeping in mind about present national and international barriers regarding the use of chemical food additives in food processing and preservation, search for biological and plant derived food additives have also notably increased. So present study was envisaged with the purposes to check the shelf life of goat meat patties incorporating with Amla fruits extract and Amla seed coat extract as natural preservatives and to assess their effect on physico-chemical and sensory attributes of the product under refrigerated (4±1ºC) storage. The products incorporated with Amla fruit extract and Amla seed coat extract had lower Thiobarbituric reacting substances (TBARS) value, free fatty acid (FFA) value and pH value than the control. Total phenolic content was decreases as advancement of storage period. The sensory attributes such as colour and appearance, flavour, juiciness and overall acceptability were decreased significantly (p≤0.05) as storage day advances in both control as well as extract incorporated patties. Sensory evaluation scores showed that goat meat patties incorporated with Amla fruits extract and Amla seed coat extract were equally acceptable as reference product and rated good to very good for colour and appearance, flavour, juiciness and overall acceptability. Goat meat patties with Amla fruit and its seed coat extract can be stored safely without much loss in its quality even up to 15 days under refrigerated storage.

Keywords- Refrigerated storage,Amla fruits extract, Seed coat extract, Sensory evaluation, goat meat patties.

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Introduction

Many functional compounds beneficial to human health are developed by processing of meat and meat products [1,2]. New food products are often being introduced to the market that requires longer shelf life and larger guarantee of defence from microbial spoilage. Lipid oxidation and growth of undesirable microorganisms in food products leads the development of spoilage, off flavour, rancidity and deterioration, rendering such products unacceptable for human consumption [3-5]. In an effort to retard this process, several synthetic food additives have been widely used in the meat industry to overcome the undesirable changes.The synthetic anti-oxidants which are used in food industry have been found to exhibit various adverse health effects in consumers [6 and 7]. Therefore there is a increasing interest in natural antioxidants in meat products. Compounds obtained from natural sources such as grains, oil seeds, honey, fruits and vegetables have been investigated for their natural antioxidant and antimicrobial property in meat products. By products obtain after utilization of fruits and vegetables can offer a practical and economic source of strong antioxidants that could substitute synthetic preservatives [8]. Amla (*Emblica officinalis*) as a Euphorbiaceous plant and is widely distributed in subtropical and tropical areas of China, India, Indonesia and Malaysia [9]which is used as a main ingredient in numerous Ayurvedic preparations for promotion of healthiness and longevity. Amla is a good source of polyphenols, flavones, tannins and mixture of bioactive compounds which having strong antioxidants effect which contribute to the health effects. Several active compounds like gallic acid, ellagic acid, 1-O-galloyl-D glucose, chebulininc acid, quercetin, chebulagic acid, kaempferol, mucic acid 1, 4-lactone 3-O-gallate, isocorilagin, chebulanin, mallotusinin and acylated apigenin glucoside have been isolated from the aqueous extract of Amla [10]. Amla contain active ingredients that are effective against pathogenic strains of *Escherichia coli*, *Staphylococcus aureus, Klebsiella pneumonia, Pasteurella multocida, Streptococcus pyogenes, Vibrio cholerae, Pseudomona saeruginosa* [11-13]. The present study was conducted to assess the shelf life of goat meat patties added with amla fruit and its seed coat at refrigerated storage as natural preservatives (4±1ºC).

Materials and Methods

Materials

About 2 kg of fresh goat meat required for the experiments was procured from the meat shop located at Palanpur, Gujarat. Meat was brought in container covered with ice-bags and before processing it was stored at 4ºC in refrigerator. Food grade chemicals were procured from Qualigens and Merck. Standard gallic acid (SD Fine Chemicals, Mumbai, India), Thiobarbituric acid (MP Biomedicals Pvt. Ltd., Mumbai, India), 1, 1, 3, 3-tetraethoxypropane (Sigma Aldrich, New Delhi, India) and 1,1-diphenyl -2-picrylhydrazyl (Sigma Aldrich, New Delhi, India) used in the study were of analytical grade. Refined salt (Tata Chemicals Ltd., Mumbai), refined wheat flour, LDPE bags, onion, garlic and ginger were procured from local market of Palanpur. Amla fruits were procured from Sardarkrushi nagar Dantiwada Agricultural University, SDAU, Gujarat.

Preparation of powders and extracts

Tap water was used to clean Amla fruits and to remove adhering dust amla fruit were wiped with muslin cloth. The fleshy parts of Amla were nettled and seed was detached manually from adhering Amla. Preliminary trail was carried out to know the temperature and time combination for drying of Amla until it became gritty and about 40-60^{0}C for 48 hrs was required for drying of Amla shreds in hot air oven at. The seed coat was separated from the seed from each of the fragmented parts. The dried Amla fruit and seed coat were ground in laboratory grinder and passed through 60 mesh sieve and stored in LDPE pouches until used for the extraction. Amla fruit extract and seed coat extract were prepared by mixing 10 gm of powder in 100 ml boiled water for 1 hrsfor extraction. The extract obtained by filtration was analysedfor total phenolic content, DPPH radical scavenging activity and also incorporated at different concentration in goat meat patties. For each replication freshly prepared extract were used.

Preparation of goat meat patties

The Goat meat was washed thoroughly, deboned after trimming of fat and connective tissue. The Deboned meat was cut into small cubes and minced in Stadler meat mincer using 8 mm plates and used for preparation of patties. Sodium chloride (2 %), sodium tri-polyphosphate (0.5 %), spice mix (2 %), garlic paste (3 %), sunflower oil (3 %) and ice flakes (8 %) were used for preparation of patties. Amla fruit extract were prepared by mixing 5, 10 and 15 gm of powder in 100 ml boiled water whereas Seed coat extract were prepared by mixing 5, 10, 15 and 20 gm of powder in 100 ml boiled water and kept for 1 hr for extraction. 10 ml of each extract were used for the preparation of Goat meat patties. On the basis of preliminary trails of sensory evaluation of patties 10 gm extract of amla fruit and 15 gm extract of seed coat powder was optimize for preparation of patties. Selected raw as well as cooked patties along with control are present in [Plate-1 and 2]. About 70 g of emulsion moulded to form patties and were cooked in a preheated microwave oven at 180^{0}C for 15 minutes after which they were turned and allowed to get cooked for 10 more minutes till internal temperature reached 75-80^{0}C. Treatment with best sensory attributes was selected for further study for both amla fruit extract and seed coat extract incorporated patties. After cooling to room temperature the patties were aerobically packaged in low density polyethylene bags and stored at refrigeration temperature (4±1^{0}C) for 15 days andanalysed for total phenolic content, pH, Free fatty acid value, thiobarbituric acid reactive substances (TBARS) and sensory attributes at 3 days interval.

A- Control

B- 10 ml Amla fruit extract incorporated patties

C- 10 ml Seed coat extract incorporated patties

Plate-1 Final selected raw patties

A- Control

B- 10 ml Amla fruit extract incorporated patties

C- 10 ml Seed coat extract incorporated patties

Plate 2- Final selected cooked patties

Analysis of Amla fruit and seed coat samples

DPPH radical scavenging activity

The capacity to scavenge 2, 2-diphenyl -1- picryl hydrazyl (DPPH) radical by Amla fruit powder and seed coat powder was estimated [14]. 100 µl of approximate dilution of sample / trolox solution was mixed with 3.9 ml of freshly prepared DPPH working solution in 10 ml test tube; the contents were mixed with vortex

stirrer and incubated in dark for 120 min at 37˚C after covering the test tube with aluminium foil. The absorbance of the solution was measured at 515 nm against methanol using Thermo Scientific Multiskan Go. Spectrophotometer. For blank determination 100 µl methanol was taken in place of sample and absorbance was recorded immediately against methanol.

The result was expressed as:

% DPPH scavenging activity = [(A 515nm blank – A 515nm sample)/ A 515nm blank] × 100

Result were expressed as trolox equivalent antioxidant capacity (TEAC) values i.e. µmol of trolox equivalent / gram of fruit weight.

Total phenolics

Total phenolic content in the Amla fruit powder and seed coat powder extracts was determined by modified Folin-Ciocalteu method [15]. 400 µl of approximate diluted sample / gallic acid standard was taken in a test tube. To it added 2000 µl of diluted solution Folin-Ciocalteu's reagent and mixed with vortex mixer. After 3 minutes 1600 µl of Sodium carbonate solution was added and incubated in dark at room temperature for 30 min. For blank preparation 400 µl of distilled water was taken instead of sample. The absorbance of the sample was measured against blank at 765 nm using Thermo Scientific Multiskan Go. Spectrophotometer.

Analysis of meat patties samples

pH

The pH of meat samples was determined [16]. Meat sample (10g) was blended with 50 ml distilled water for 1 minute using pestle and mortar. The pH was recorded by dipping the electrodes of pH meter directly in suspension.

Total phenolics:

Total phenolic content in cooked goat meat patties was determined by modified Folin-Ciocalteu method [17 and 18]. 5 g of cooked patty was homogenized with 25 ml of 70% acetone and kept overnight for extraction in refrigeration condition. Appropriate aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml through distilled water followed by the addition of 0.25 ml F-C (1N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortex and the absorbance recorded at 725 nm after 40 min. The amount of total phenolics was determined as Gallic acid equivalent against the calibration curve using 0.1 mg/ml of standard gallic acid solution.

Thiobarbituric reacting substances (TBARS) value

Thiobarbituric acid reacting substances (TBARS) value was followed for determine the lipid oxidation [19]. Minced meat (5 g) was blended for 3 min with 25 ml 20% TCA. Slurry was kept for 10 min. it was filtered through Whatman No. 42 filter paper. % ml of TBA reagent was added to 5 ml of sample aliquot (filtrate). After mixing the contents, tubes were held for 35 min in a boiling water bath. Optical density was measured at 532 nm spectrophotometrically. Blank was run simultaneously for standard curve 1, 2, 3, 4, 5 ml of working standard solution were used.

Sensory evaluation

Semi trained taste panel, which includes professor and post graduate students of the LPT Division obliged in conducting the sensory evaluation of the product. They were requested to gave their desire on 9 point hedonic scale for attributes like colour and appearance, flavour, juiciness and overall acceptability.Where 9 = Like extremely, 8 =Like very Much , 7 = Like moderately, 6 = Like slightly, 5 = Neither like nor dislike, 4 = Dislike slightly, 3= Dislike moderately, 2 = Dislike very much, 1 = Dislike extremely. Patties were pre warmed before serving and water was served for rinsing the mouth between samples.

Statistical analysis

The results were statistically analysed [20]. The significant treatment effects, upon all profiles were tested using Duncan's multiple range test with p≤0.05 by SPSS software. Two-way analysis of variance was used to evaluate the results of the storage studies to determine the effect of treatment and storage period.

Results and discussion

Result of Total phenolics content and DPPH radical scavenging activity of Amla fruit and Seed coat extract are presented in [Table-1].

Table-1 *Total phenolics content and DPPH radical scavenging activity of Amla fruit and Seed coat extract*

Extract	DPPH radical scavenging activity (µmol TE/g)	Total phenolic content (mg GAE /100 g)
Amla fruit	21.18 ±0.30^{c}	1164.83 ± 1.77^{d}
Amla Seed coat	19.56 ±0.24^{c}	426.23 ±1.12^{f}

Mean ± S.E, n=3 GAE- gallic acid equivalent TE-Trolox equivalent

Mean ± S.E with different small letter superscripts in rows within each parameter differ significantly (p≤0.05); n=6.

DPPH radical scavenging activity of Amla fruit extracts and seed coat extracts were 21.18 (µmol TE/g) and 19.56 (µmol TE/g), respectively. There was no significant difference (p≥0.05) found for DPPH scavenging activity of both the extracts. The total phenolic content of Seed coat (426.23 mg GAE/100 g) was observed to be relatively low and highest was found for Amla fruit (1164.83 mg GAE/100 g). Highest antioxidant activity observed for Amla in the present study might be due to the high content of vitamin C and other compounds which have antioxidant activity. Estimation of total phenolic content of Amla fruit was done by different worker [21-23]. Ayub ali *et al.* [21] found 1285.63 total phenolic content (mg GAE /100 g) for Amla fruit which is similar to the polyphenol content of Amla fruit used in the present study. Estimation of total phenolic content of seed coat was done by Mishra and Mahanta [24] and found 593.06 mg GAE/100 g. The differences of total phenolic content of fruit and seed coat could be due to different preparations and extraction method. Mishra and Mahanta [24] also found that DPPH radical scavenging activity for Amla fruit and seed coat showed lower difference than the seed part of the Amla.

pH

The pH of aerobically packaged patties is presented in [Fig-1].

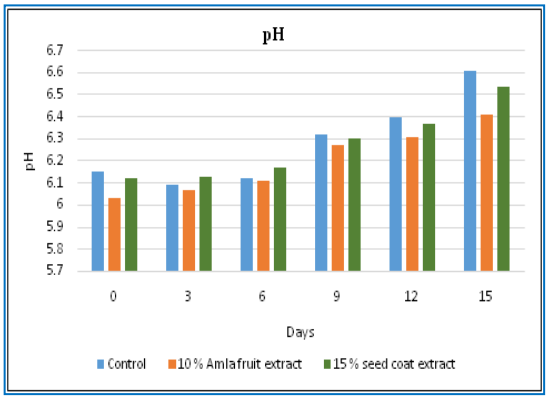

Fig-1 pH value of goat meat patties during storage

The pH value of all patties samples slightly decreased during the first 3 days after that value increased significantly (p≤0.05) as advancement in storage period. 10 % Amla fruit extract incorporated patties showed lowest value for pH during entire storage period as compared to the control and seed coat extract incorporated patties. The decrease in the pH of aerobically packaged control and extract incorporated patties during storage might be due to the action of the psychrophilic bacteria and that produce the acid from the fermentation of carbohydrates of meat, binders and spices and might be lead to reduction in pH[25] and the resultant increment of pH might be due to the release of alkaline metabolites from the action of bacteria. However, lower pH value for Amla fruit extract incorporated patties could be due to the low pH value of Amla fruit extract. Consistent with these data Karabagi as *et al.* [26]reported that the pH of air-packaged samples increased from 6.4 to 6.8 on day 4 of storage, Hur *et al.* [27]observed a significant increase in the pH of the air-packaged sample during storage, Giriprasad *et al.*

[28] also observed an increase in pH during storage.

Total phenolic content

Total phenolic content of control and both extract incorporated meat patties during storage at refrigeration temperature (4±1^0 C)are presented in [Table-3].

Table-3 *Total phenolic content (mg/100g) of aerobic packaged goat meat patties incorporated with selected level of Amla fruit extract and seed coat extract stored at refrigeration temperature (4±1^{0}C)*

Treatment	Days					
	0	3	6	9	12	15
Control	36.16± 0.90^{aA}	33.48± 0.30^{bA}	30.08± 0.62^{cA}	28.03± 0.35^{dA}	25.39± 0.52^{eA}	23.14± 0.95^{fA}
10 % Amla fruit extract	65.74± 0.28^{aB}	60.92± 0.64^{bB}	57.72± 0.69^{cB}	51.48± 0.57^{dB}	45.80± 0.83^{eB}	39.54± 0.81^{fB}
15 % seed coat extract	38.03± 0.76^{aA}	35.33± 0.77^{bA}	32.15± 0.65^{cC}	30.13± 0.78^{dC}	28.22± 0.61^{eA}	26.52± 0.67^{eC}

Mean ± S.E with different small letter superscripts in rows and capital letter superscript in columns within each parameter differ significantly (p≤0.05); n=6

There was a significantly (p≤0.05) decrease in total phenolic content in aerobically packaged both control and extract incorporated meat patties with advancement of storage. The total phenolic content was significantly (p≤0.05) higher in Amla fruit extract incorporated patties compared to control and seed coat extract incorporated patties at each evaluation period. Decrease in a phenolic content of patties might be due to the heating of the patties during the sensory evaluation at three days interval and heating leads to loss or denaturation of the some phenolic content in patties. Naveena *et al.* [8] in cooked chicken patties; Devatkal *et al.* [29]in goat meat patties; Verma *et al.* [30] in sheep meat nuggets and Serdaroglu *et al.* [31] in raw beef pattiesfound similar results with present findings. The higher level of phenolics may indicate patties is nutritionally enhanced due to the fruit extract and seed coat extract that was added [32].

TBA value

Thiobarbituric acid value of control and both extract incorporated meat patties during storage at refrigeration temperature (4±1^0 C)are presented in [Fig-2]. There was a significantly (p≤0.05) increase in TBA value in aerobically packaged both control and extract incorporated meat patties with advancement of storage period. At the 15th day of storage highest value was observed for control patties (0.93) than both extract incorporated meat patties (0.65 and 0.82).An increase in TBA value of aerobic packaged patties is an indication of development of oxidative rancidity due to formation of volatile compound as a result of oxidation of polyunsaturated fatty acids present in meat products. This is attributed due to oxygen present inside the packaging material [45]. The concentrations of TBA value in treatment was considerably lower than the control and seed coat extract incorporated patties and it indicated a significant relation between phenolic content and antioxidant effect of Amla fruit extract in protecting against lipid oxidation of patties.

Similar increase in TBA value during aerobic storage was noted by Zhou *et al.* [33] in pork sausage added with *Amaran thus* pigments extracted; Giriprasad *et al.* [28] in functional restructured buffalo meat steaks fortified with *amla* powder; Kumar *et al.* [34] in pork patties added with combination of natural antioxidants.

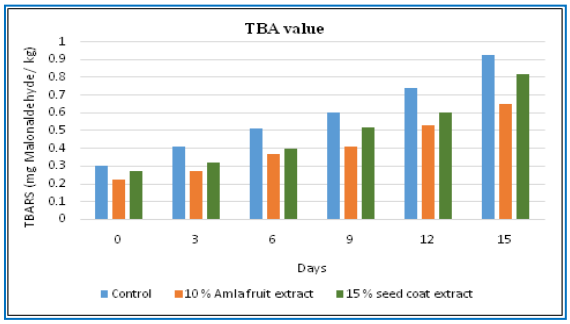

Fig-2 TBA value of goat meat patties during storage

Free fatty acid

Free fatty acid content (%) of control and both extract incorporated meat patties during storage at refrigeration temperature (4±1^0 C) are presented in [Table-5] and [Fig-1]. There was a gradual increase in free fatty acid contents in aerobically packaged both control and extract incorporated meat patties during storage. At the 15th day of storage control patties showed (0.286) higher free fatty acid content than both extract incorporated meat patties (0.144 and 0.183). The free fatty acid in meat decides the fat status and quality of the product. Free fatty acid content alone did not act as criteria for acceptability of products but support as a strong quality indicator for oxidative changes of fat during storage [35]. Free fatty acid content also showed increasing trend throughout storage. However, it was higher in control than all the treatments even on the first day of storage of patties. It might be due to the antioxidant effect of Amla fruit extract [36,37] and seed coat extract [24].

Table-5 *Free fatty acid value of aerobic packaged goat meat patties incorporated with selected level of Amla fruit extract and seed coat extract stored at refrigeration temperature (4±1^{0}C)*

Treatment	Days					
	0	3	6	9	12	15
Control	0.103± 0.013^{aA}	0.108±0. 012^{aB}	0.126 ± 0.016^{abC}	0.130 ± 0.013^{bD}	0.172 ± 0.014^{cD}	0.286±0 .015^{dE}
10 % Amla fruit extract	0.08± 0.015^{aA}	0.095±0. 012^{aB}	0.113 ± 0.010^{aC}	0.114 ± 0.010^{aD}	0.124 ± 0.009^{abE}	0.144±0 .012^{bF}
15 % seed coat extract	0.092± 0.011^{aA}	0.104±0. 010^{aB}	0.118±0. 011^{aC}	0.125 ± 0.014^{abD}	0.149 ± 0.014^{dF}	0.183± 0.016^{cG}

Mean ± S.E with different small letter superscripts in rows and capital letter superscripts in columns within each parameter differ significantly (p≤0.05); n=6

Sensory evaluation

Colour and appearance scores of meat patties

The colour and appearance score of aerobically packaged control and both extract incorporated goat meat patties are presented in [Fig-3]. As the storage day advanced decreasing trends in colour and appearance score were observed. Significant (p≤0.05) decline in colour and appearance score after 9 days of storage in control as well as both extracts incorporated patties were noticed. At the 15th day of storage colour and appearance, score of control as well as both extract incorporated patties ranged from 6.33 to 6.50. The decrease in colour and appearance score of aerobically packaged patties might be due to the oxidation of lipid and pigment which lead to the non-enzymatic browning [38]as well as surface dehydration. Similar decline in colour and appearance score during storage have been reported by Zargar *et al.* [39] in a chicken sausages; Najeeb *et al.* [40] in restructured chicken slices and Giriprasad *et al.* [28]in restructured buffalo meat steaks.

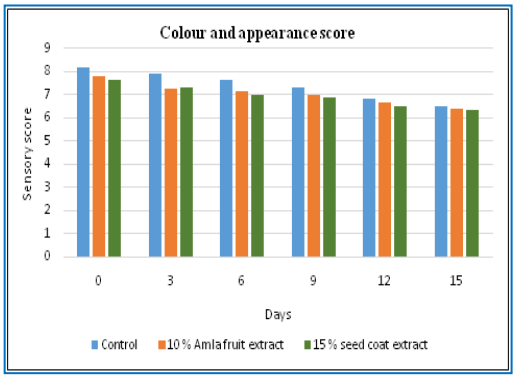

Fig-3 Colour and appearancescores of goat meat patties during storage

Flavour scores of meat patties

The flavour scores of aerobically packaged control and both extract incorporated

goat meat patties are presented in [Fig-4]. The flavour score of aerobically packaged control patties was higher than both extract incorporated patties. Up to 3 days there was no significant change in flavour score but after that liner decline in flavour was observed as storage period advanced. The decrease in flavour score in patties may be due to the microbial growth and oxidative spoilage as showed by TBARS numbers. Tarladg is *et al.* [46] described that TBARS values are highly correlated with sensory scores of trained panellist. Similar declining in flavour score during storage reported by Thomas *et al.* [47] in buffalo meat nuggets; Zargar *et al.* [39] in a chicken sausages; Najeeb *et al.* [40] in restructured chicken slices and Giriprasad *et al.* [28]in restructured buffalo meat steaks.

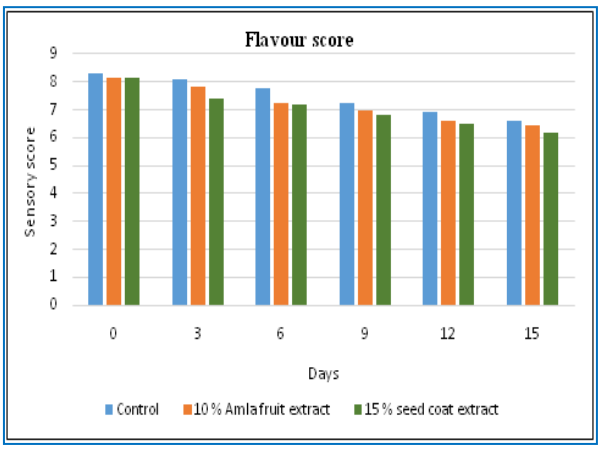

Fig-4 Flavourscores of goat meat patties during storage

Juiciness scores

The Juiciness score of aerobically packaged patties are presented in [Fig-5]. Juiciness score of aerobically packaged control goat meat patties were lower than the both extract incorporated patties and higher juiciness score are rated for 10% Amla fruit extract incorporated patties during entire storage period. There was a decline in juiciness score during storage as storage period advanced in control as well as both extract incorporated patties. At the 15th day of storage juiciness, score of control as well as both extract incorporated patties ranged from 6.42 to 6.75. Higher juiciness score for Amla fruit extract incorporated patties may be due to its water retention capacity. A decrease in juiciness during storage was observed in control as well as both extract incorporated patties. This might be due to loss of moisture from the product and presence of oxygen inside the packaging material [41, 42]. Bhuvana *et al.* [43] and Giriprasad *et al.* [28] also found that there is decrease in juiciness with advancement of storage period in pork fry and restructured buffalo meat steaks, respectively.

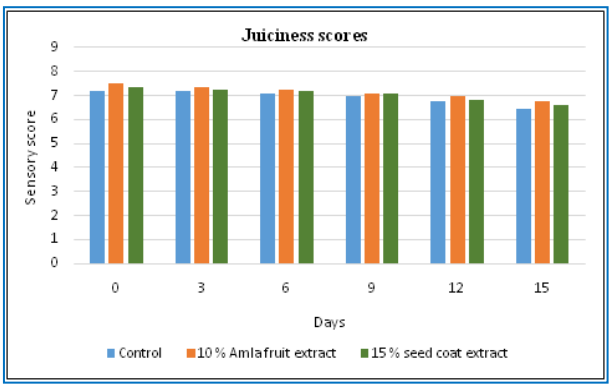

Fig-5 Juicinessscores of goat meat patties during storage

Overall acceptability scores of meat patties

Overall acceptability score of aerobically packaged patties are presented in [Fig-6]. A decrease in overall accepts ability during storage was observed in control as well as both extract incorporated patties as storage period advances. This might be due to decreased value of other sensory attributes, increased lipid oxidation, protein degradation and some bland flavour due to fat degradation. Similar trends of reduction in overall acceptability scores at the end of storage period have also been reported by Indu mathi and Obula Reddy [44] in Chicken meat nuggets added with three different anti-oxidant extracts (1% level) of curry leaf, guava leaf and green tea and Giriprasad *et al.* [28]in restructured buffalo meat steaks added with Amla powder.

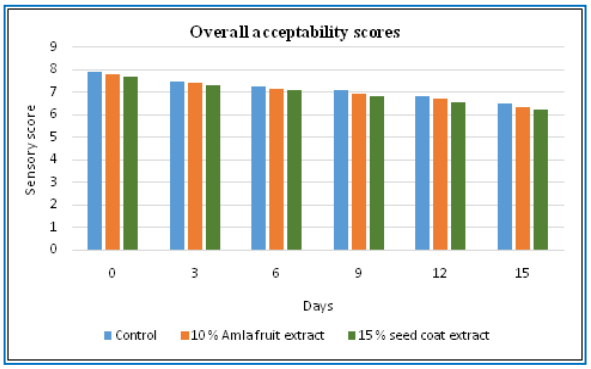

Fig-6 Overall acceptability scores of goat meat patties during storage

Conclusion

The sensory attributes such as colour, flavour, juiciness and overall acceptability decreased in as storage period advances. It was observed that slow increase in the free fatty acid (FFA), pH and Thiobarbituric acid (TBA) for both extract incorporated patties than control. Total phenolic content of patties decline during the storage and highest value found for Amla fruit extract incorporated patties. Result obtained for FFA, TBARS, pH and sensory were within an acceptable limit up to 15 days in aerobic packaging condition. So according to the finding of present study it is concluded that Amla fruit and its seed coat powder can be used as natural antioxidant in cooked goat meat patties.

Conflict of Interest: None declared

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