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Effect of natural antioxidants on the lipid stability of fluidised bed-dried mutton

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Abstract

Studies were carried out to develop a shelf stable meat product using natural antioxidants [Maillard reaction products (MRP's) (60 mM/2 h), ascorbic acid (500 ppm) and spices (250 mg%)] using a combination of freeze-thaw and fluidised bed-drying (FT/FBD) processes. Products prepared with addition of varied combinations of additives, were evaluated for changes in chemical and organoleptic characteristics during storage at ambient temperature (25 ± 2 °C), to assess the shelf stability. Oxidative rancidity was evaluated by determining thiobarbituric acid reactive substance (TBARS), total carbonyls and non-haem iron values during storage. The results revealed an individual, as well as synergistic, antioxidant potential of natural antioxidants in enhancing the shelf life of FT/FBD mutton samples. A combination of natural antioxidants showed the minimum increase ($p > 0.01$) in rancidity values after 6 months of storage. Non-linear correlation regression analysis was performed between non-haem iron, TBARS and total carbonyls and a reciprocal logarithmic fit equation was established with standard errors of 0.0988, 0.7802 and correlation coefficients of 0.93, 0.931 for TBARS and total carbonyls, respectively. The product exhibited excellent organoleptic characteristics on a 9 point hedonic scale (8.2 ± 0.68) and required 8–10 min for reconstitution. It exhibited a shelf stability of 6 months under ambient temperature conditions. The critical water content and water activity were found to be 7.8 g/100 g and 0.47, respectively.

Keywords: Natural antioxidants; Fluidised bed drying; Maillard reaction products; Lipid oxidation; Ascorbic acid; TBARS; Non-haem iron

1. Introduction

Lipid oxidation is one of the primary causes of deterioration in quality of meat during storage, leading to development of off-flavour, as well as reduced shelf stability and acceptability (Rhee, Anderson, & Sams, 1996). Lipid oxidation is initiated during cooking which then continues during storage. Various factors affect the extent of oxidation, e.g., availability of oxygen, temperature of cooking, method of cooking, species of the meat, packing and storage conditions and presence of various pro and anti-oxidants (Buckley, Morrissey, & Gray, 1996; Ladikos & Langovois, 1990). Oxidation of lipids in vivo and in muscle foods is believed to be initiated in the highly unsaturated phospholipid fraction in sub-cellular membranes (Morrissey, Buckley, & Galvin, 2000). Lipid peroxidation can occur through enzymatic and/or non-enzymatic mechanisms. Lipoxygenases, the non haem enzymes that contain iron in the active state, can catalyse lipid peroxidation to produce hydroperoxides (Gordon, 2001; Tomchick, Phuc, Cymborowski, Minor, & Holman, 2001) but only if the enzyme is activated by preformed peroxides and the fatty acids are in the free form. The lipid oxidation in muscle foods is generally non-enzymatic and mainly involves either free radicals and/or reactive oxygen species such as singlet oxygen (Gray, Gomma, & Buckley, 1996) to react with substrates such as unsaturated fatty acids. Cooked meat will develop off flavours faster than its uncooked counterpart during refrigerated storage (Shahidi, 2005). Cations, such as iron and copper, present in meat, are known to act as pro-oxidant catalysts and quenching of such a property is considered to be a key factor for the oxidative stability of meats (Zanardi, Novelli, Ghiretti, &

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Chizzolini, 2000). The increase in non-haem iron with temperature and time of cooking and decrease in haem iron was reported by Feng-Sheng-Wang and Chin-Wen-Lin (1994). Igene, King, Pearson, and Gray (1979) concluded that level of ferrous iron greatly increased during cooking and accelerated lipid oxidation in cooked meat. Heme pigments serve as a source of free iron, being readily broken down during the cooking process and catalyse auto oxidation leading to rancidity in cooked or dehydrated meat more so if the meat has a high degree of unsaturation. Processing operations which disrupt the oxidative balance of skeletal muscle include: particle size reduction, which mixes oxidation catalysts with lipids and introduces oxygen into previously an aerobic tissue, cooking, which causes disruption of cellular organization in the skeletal muscle tissues, resulting in protein denaturation and loss of antioxidant enzyme activity, the release of protein-bound iron and salting, which increase the catalytic activity of iron and reduce antioxidant enzyme activity (Decker & Crum, 1993). These processing operations can markedly increase the lipid oxidation in muscle foods. Therefore, during processing of meat, control of these catalytic systems is very important, to minimize lipid oxidation.

In foods, including those made from muscle, there is a need to extend the shelf life of the product until it is consumed. Many of the naturally-occurring antioxidants are destroyed during the processing of the raw product. (Allen J. St. Angelo, 1996). The shelf life of processed meat can be extended by using antioxidants and proper packaging materials. Because of the growing consensus on the potential health hazard caused by synthetic antioxidants (Jones, 1992), there is a renewed interest in the increased use of naturally occurring antioxidants. Spice and herb extracts have been reported to contain compounds with antioxidant activity, when used in food systems (Fereidoon Shahidi & Champaign, 1998). Antioxidant properties of natural plant extracts in meat were reported by Ahn, Gruen, and Ferrnando (2002). Besides spices, MRP's formed from the condensation of sugar and amino acids have been reported to possess promising antioxidant activity (Arosha & David, 1998; Bailey, Shin Lee, & Dupy, 1987; Chuyen, 1998; Lingnert & Ericksson, 1981). One of the naturally occurring antioxidants most widely used in the food industries is ascorbic acid, with a varied chemistry. It has complex multi functional effects. Depending on conditions, ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or an oxygen scavenger, ascorbic acid and its esters function as antioxidants by protecting double bonds and scavenging oxygen (Allen J. St. Angelo, 1996; Verma & Sahoo, 2000).

The main problems that were encountered in the conventional air-drying of meat are case-hardening, oxidative breakdown of lipids leading to rancidity development and lower reconstitution characteristics and overall acceptability values. There is no literature available on the application of the fluidised bed-drying process in the development of meat products; however, some reports are available on cereals, pulses and vegetables. Patki, Srihari, and Arya (2002) reported the application of freezing prior to fluidised bed drying (FT/FBD) in enhancing the overall product quality of whole legumes. Somchart, Thanit, Somboon, and Wivat (2001), studied fluidised bed-drying of soybean and the production of quick-cooking potato cubes by osmotic pre-concentration and fluidised bed-drying was reported by Ravindra and Chattopadhyay (2000).

The present study was undertaken to develop a shelf stable processed meat product using the FT/FBD process and to evaluate the feasibility of using natural antioxidants such as like MRP's, ascorbic acid and spices, for the lipid stability of the product. As of now, not many shelf-stable meat products are available in the market, so development of a shelf-stable easily rehydratable, tasty, convenient and nutritious meat product will be of much significance to civil as well as service sectors.

2. Materials and methods

2.1. Materials/chemicals

2.1.1. Meat samples

Fresh mutton (leg portion, 2–3 h post mortem) was purchased from the local market, washed thoroughly under running water, deboned and cut into small pieces (1.5 cm × 1 cm) and used for processing.

2.1.2. Spices

Various green spices, e.g., onion, garlic, ginger, green chillies and turmeric, pepper, cloves, cinnamon, cardamom and cumin, were obtained from the local market.

2.1.3. Reagents and chemicals

All the chemicals and reagents used for the study were of Analar® grade and procured from M/s Sigma Chemicals Corporation USA and M/s BDH company.

2.2. Preparation of MRPs

MRPs were prepared by refluxing 60 mM concentrations of glucose and lysine in 100 ml of water for 2 h over a sand bath maintained at 100–110 °C. Losses in water content were periodically restored by adding fresh water to original volume.

2.3. Sample preparation

Wet masala paste was prepared by using onion, garlic, ginger, green chillies and turmeric powder. Acidity and salt were adjusted. Masala was fried in vegetable oil and divided into five equal parts.

One portion of mutton sample was cooked with the above prepared masala (sample A). Another portion was cooked with the preformed MRPs and masala (sample B). Third portion was cooked with the masala and spices (cloves, cinnamon, cardamom and pepper) (sample C). A

fourth portion was cooked with masala and ascorbic acid (sample D) and the last and fifth portion was cooked with masala, spices, MRPs and ascorbic acid (sample E).

2.4. Freezing and fluidised bed drying

All the five samples were separately frozen in a blast freezer (Hull Corp, USA) at −35 °C for 3 h. The samples, after uniform freezing, were subjected to drying in a fluidised bed drier (M/s Chemech Industries, Mumbai) at 75 °C for 6 h. The products after FT/FBD drying were packed in PFP (45 GSM paper/20 μ Al. foil/37.5 μ LDPE) and stored to assess the shelf stability at ambient temperature (25 ± 2 °C).

2.5. Chemical and sensory evaluation

Proximate composition of the product was determined by the (AOAC (1984)) procedure. Lipid extraction of the samples was carried out by the method of Folch, Lees, and Sloane Stanley (1957).

TBARS values, expressed as malonaldehyde contents, were estimated colorimetrically in reaction with 2-thiobarbituric acid (Taraldigis, Watts, Younathan, & Dugan, 1960) using a chemito UV–visible spectrophotometer model 160, chemito instruments, India. Total carbonyl estimation, to assess the lipid oxidative changes, was monitored by the method of Henick Benca and Mitchela (1954). The catalytic activity of non-haem iron was estimated by the method of Igene, Yamauchi, Pearson, and Gray (1985).

The sensory characteristics of the FT/FBD mutton samples treated with antioxidant combinations (samples A, B, C, D and E) were evaluated during storage by subjecting these samples to an overall acceptability score on a 9 point hedonic scale by a panel of judges, using the procedure of Murray, Delahunty, and Baxter (2001).

2.6. Critical water content and water activity of the product

Sorption characteristics of the dehydrated FT/FBD samples were determined by the method of Resnik, Fasetto, Chirife, and Feirofoantam (1984). Material (2–5 g) were placed in a 3″ dia Petri dish and exposed to different relative humidity conditions, ranging from 0% to 85 %, using various salt solutions in desiccators. Changes in weight of the samples were recorded at hourly interval in the first 24 h and later, at one day intervals, up to seven days, followed by weekly intervals until equilibrium was achieved. These data were used to evaluate the equilibrium moisture content and water activity of the product (Brunauer, Emmett, & Teller, 1938).

2.7. Statistical analysis

Data obtained were subjected to Duncan's multiple range test to evaluate the statistical significance of the treatments using analysis of variance (ANOVA) and the significance was established at $p < 0.01$. Regression analysis for the correlation of parameters was performed using the software curve Expert 1.3.

3. Results and discussion

3.1. Evaluation of the oxidative deterioration of lipids in the presence of natural antioxidants

Data on the changes in TBARS and total carbonyl content as a measure of the extent of lipid oxidation for all the five samples, A, B, C, D and E are presented in Tables 1 and 2, respectively. From the data, it could be seen that the natural antioxidants, MRPs, spices and ascorbic acid, exhibited individual as well as synergistic antioxidant effects and these could be utilized to control the lipid oxidation and extend the shelf life of meat products. The TBARS and total carbonyls, in the case of sample A (control), increased significantly ($p < 0.01$) from 0.186 to 1.192 mg/kg and 3.25 to 10.2 mg of *n*-hexanal/100 g fat, respectively, rendering the product unacceptable. During storage at ambient temperature (25 ± 2 °C) all samples showed increased TBARS and total carbonyls; however, the extent of increase was maximum in sample A and least in sample E, indicating the synergistic antioxidant potential of MRPs, ascorbic acid and spices in retarding the lipid oxidative changes. Among the five different treatments, the values of TBARS and total carbonyls varied significantly ($p < 0.01$) in the case of samples B and E, while the level of significance in the case of samples C and D was $p < 0.05$ in relation to sample A, after 6 months of storage at ambient temperature, thus revealing the good antioxidative properties of these treatments. The order of antioxidant potential was MRPs > spices > ascorbic acid. TBA and total carbonyl estimation in meat products constitute an important tool to understand the extent of oxidative rancidity in cooked, stored muscle foods (Melton, 1983). The antioxygenic characteristics of MRPs, in controlling the lipid oxidation, have been reported earlier (Chuyen, 1998; Lingnert & Ericksson, 1981). There are reports about the complexing of MRPs with metals, which act as catalyst in lipid oxidation (Arosha & David, 1998;

Table 1
TBARS values (mg/kg)[a,b] for FT/FBD mutton samples stored under ambient temperature (25 ± 2 °C) conditions

Sample	Storage period (months)			
	0	2	4	6
A	0.186 ± 0.013[a]	0.201 ± 0.014[a]	0.608 ± 0.019[a]	1.192 ± 0.028[a]
B	0.133 ± 0.009[b]	0.153 ± 0.010[b]	0.213 ± 0.011[b]	0.403 ± 0.019[b]
C	0.164 ± 0.008[a]	0.185 ± 0.013[a]	0.332 ± 0.016[b]	0.504 ± 0.020[b]
D	0.172 ± 0.010[a]	0.194 ± 0.012[a]	0.384 ± 0.018[b]	0.596 ± 0.017[b]
E	0.104 ± 0.006[b]	0.115 ± 0.009[b]	0.182 ± 0.008[b]	0.303 ± 0.014[b]

[a] Values are shown as means ± standard deviation ($n = 5$).
[b] Within the column, values superscripted with different letters are significantly different. ab ($p < 0.01$) ac and bc ($p < 0.05$). Values with same letters are not significantly different ($p > 0.05$).

Table 2
Total carbonyls (mg of *n*-hexanal/100 g fat)[a,b] for FT/FBD mutton samples stored under ambient temperature (25 ± 2 °C) conditions

Sample	Storage period (months)			
	0	2	4	6
A	3.25 ± 0.18[a]	4.55 ± 0.16[a]	8.25 ± 0.34[a]	10.2 ± 0.42[a]
B	2.54 ± 0.13[b]	3.40 ± 0.24[b]	3.92 ± 0.26[b]	4.86 ± 0.31[b]
C	2.85 ± 0.11[a]	3.62 ± 0.20[b]	5.16 ± 0.26[b]	6.18 ± 0.42[b]
D	2.93 ± 0.18[a]	3.75 ± 0.13[b]	5.25 ± 0.33[b]	6.45 ± 0.29[b]
E	1.93 ± 0.18[b]	2.27 ± 0.16[b]	3.03 ± 0.21[b]	3.72 ± 0.26[b]

[a] Values are shown as means ± standard deviation ($n = 5$).
[b] Within the column, values superscripted with different letters are significantly different. ab ($p < 0.01$) ac and bc ($p < 0.05$). Values with same letters are not significantly different ($p > 0.05$).

Gomino & Horikoshi, 1976; Kajimoto & Yoshick, 1975). The data obtained in Tables 1 and 2 are well supported by the earlier findings on the ability of spices and herb extracts (Ahn et al., 2002; Fereidoon Shahidi & Champaign, 1998; Jayathilakan, Kumudavally, & Vasundhara, 1997) and ascorbic acid (Allen J. St. Angelo, 1996; Verma & Sahoo, 2000) to control lipid oxidation. Evaluation of the data in Tables 1 and 2 clearly indicated the individual and synergistic effect of these natural antioxidants in inhibiting the rancidity development in FT/FBD mutton samples during storage under ambient temperature conditions thus enhancing the shelf life of products.

3.2. Evaluation of the catalytic activity of non-haem iron in the presence of natural antioxidants

Fig. 1 depicts the data on non haem iron for all the five treatments, A (control), B, C, D and E during storage under ambient temperature conditions for 6 months. In meat, it has been generally accepted that the iron, in some form, promotes the oxidation of meat lipids. Out of the different forms of iron, non-haem iron has been reported to play a major role in accelerating lipid oxidation in cooked meats (Chen, Pearson, Gray, Fooladi, & Ku, 1984; Liu & Watts, 1970; Sato & Hegarty, 1971). The release of non-haem iron in the presence of salt and its role in enhancing lipid oxidation in pork was studied by Hsing-Feng-Liu (1997). Therefore, the release of non-haem iron during storage in the presence of natural antioxidants has been evaluated and presented in Fig. 1. The data depicted in Fig. 1 reveals that the values of non-haem iron for sample A, increased significantly ($p < 0.01$) from 1.16 to 3.49 mg/100 g during storage under ambient temperature conditions. However, in the case of sample E due to the synergistic effect of the various antioxidants, the non-haem iron released during storage was inhibited and was non significant ($p > 0.05$), in turn restricting the catalytic activity of non-haem iron in enhancing the oxidative deterioration of lipids in the fluidised bed dried mutton samples as reflected in Tables 1 and 2 in terms of oxidative rancidity. The samples B, C and D also showed a moderately significant increase ($p < 0.05$), indicating the capabilities of these antioxidants individually to restrict the non-haem iron release.

3.3. Correlation between non-haem iron values and the oxidative rancidity parameters (TBARS and total carbonyls)

A non-linear correlation between non-haem iron and the oxidative rancidity parameters was observed while establishing the best fit non linear equation (Figs. 2 and 3). Regression of the data in Fig. 1 was carried out in relation to the data in Tables 1 and 2 using the software curve Expert 1.3., reciprocal logarithmic fit equation, $y = 1/$

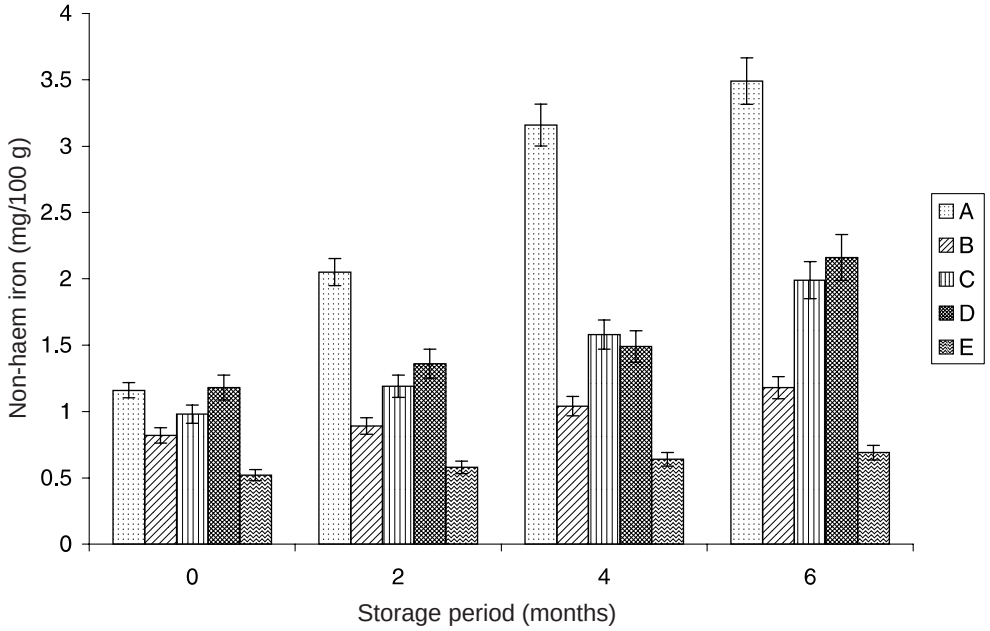

Fig. 1. Non-haem iron values of FT/FBD mutton samples stored under ambient temperature (25 ± 2 °C) conditions.

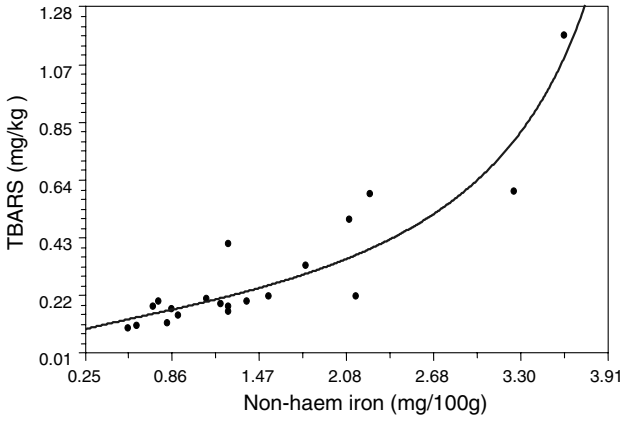

Fig. 2. Correlation between non-haem iron and TBARS values of FT/FBD mutton.

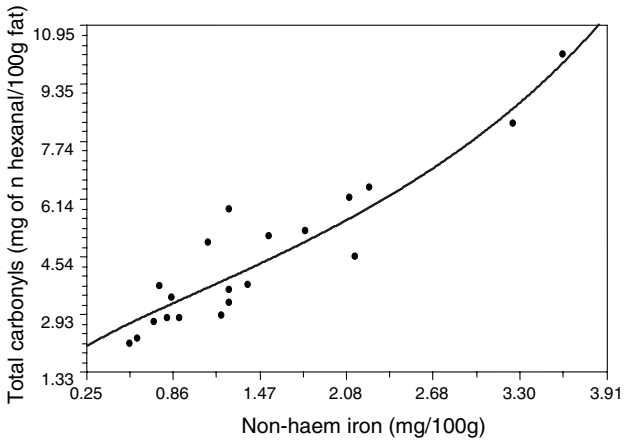

Fig. 3. Correlation between non-haem iron and total carbonyls in FT/FBD mutton.

$(a + b*\ln(x))$ was established for both TBARS and total carbonyls with non-haem iron having a standard error of 0.0988 and 0.780 and correlation co-efficients of 0.926 and 0.931 for TBARS and total carbonyls, respectively, where $x =$ non-haem iron values and $y =$ TBARS or total carbonyl values. The values for a and b were found to be, $a = 5.32$ and 0.285, $b = -3.44$ and -0.144 for TBARS and total carbonyls, respectively. Residuals were obtained on the basis of the difference between actual and predicted values using the above equations and it was found that prediction error varied between -0.19 and 0.20 and -1.44 and 2.04 for TBARS and total carbonyls, respectively. From the regression analysis, it could be ascertained that positive correlation existed between non-haem iron values and TBARS as well as total carbonyls. Evaluation of these parameters could help in gauging the lipid oxidation profile of the products.

3.4. Critical water content and water activity

Final moisture content and water activity play a major role in assessing the shelf stability of the product. Chemical, microbiological and sensory deterioration of the product has a direct correlation with the moisture content and water activity. Hence, the determination of critical moisture content and water activity was carried out and is represented in Fig. 4. The equilibrium moisture content of the product was determined by exposing the product to various relative humidity conditions and has been plotted in Fig. 4. From the figure, water activity of the product, corresponding to a moisture content of 7.8/100 g, was found to be 0.47. The product exhibited maximum shelf stability in the presence of natural antioxidants at these moisture content and water activity. Lipid oxidation and other deteriorative changes were minimum as reported in the earlier part of the discussion. In the case of hot air dehydration and freeze-drying meat lipid oxidation has been reported to be the major problem limiting the acceptability of the products (Radhakrishna, Vijaya Rao, Jayathilakan, D' Souza, & Sharma, 1988). Since the product under discussion has a moisture content of 7.8/100 g, which is near to the monomolecular layer value of meat (Attrey & Sharma, 1979) the deteriorative changes are expected to be minimum.

3.5. Proximate composition and organoleptic characteristics

Table 3 illustrates the proximate composition of the FT/FBD mutton prepared using natural antioxidants. Moisture was found to be 7.8/100 g, which is close to the monomolecular layer value for meat products at which the

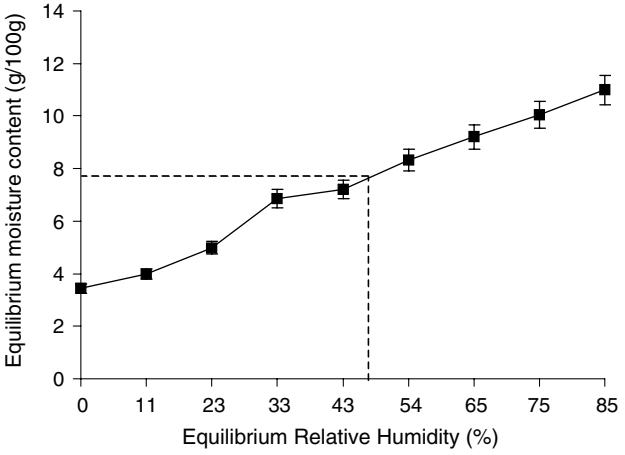

Fig. 4. Evaluation of critical water content and water activity of FT/FBD mutton.

Table 3
Proximate compostion[a] of fluidised bed dried mutton

Parameter	% Composition
Moisture	7.80 ± 0.98
Protein	63.9 ± 2.18
Fat	21.4 ± 1.06
Total Ash	5.59 ± 0.69

[a] Values are shown as means ± standard deviation ($n = 5$).

Table 4
Organoleptic characteristics of FT/FBD mutton samples expressed as overall acceptability score[a,b] during storage under ambient temperature (25 ± 2 °C) conditions

Sample	Storage period (months)			
	0	2	4	6
A	7.90 ± 0.46[a]	7.08 ± 0.23[a]	5.34 ± 0.49[a]	4.8 ± 0.39[a]
B	8.01 ± 0.61[a]	8.05 ± 0.56[a]	6.93 ± 0.43[b]	6.38 ± 0.52[b]
C	8.21 ± 0.49[a]	8.04 ± 0.49[a]	7.16 ± 0.39[b]	6.83 ± 0.42[b]
D	7.90 ± 0.46[a]	7.25 ± 0.62[a]	6.82 ± 0.26[b]	6.13 ± 0.56[b]
E	8.82 ± 0.12[a]	8.43 ± 0.42[a]	8.3 ± 0.29[b]	8.2 ± 0.68[b]

[a] Values are shown as means ± standard deviation ($n = 8$).
[b] Within the column, values superscripted with different letters are significantly different. ab ($p < 0.01$) ac and bc ($p < 0.05$). Values with same letters are not significantly different ($p > 0.05$).

product shows maximum stability. The protein content was 63.8%, fat 21.4% and total ash was found to be 5.69%. The sample had a moderate percentage of fat, considerable amount of protein and a calorific value of 455 kcal/100 g.

Organoleptic characteristics of the FT/FBD mutton samples treated with different antioxidant combinations (samples A, B, C, D and E) were evaluated during storage for 6 months under ambient temperature conditions and the overall acceptability score is shown in Table 4. The score was calculated on the basis of evaluating the texture, taste, flavour, chewability, reconstitution profile, colour and other related aspects on a 9 point hedonic scale. Initially, all the samples had good overall acceptability scores while after 2 months of storage, sample A exhibited a significant ($p < 0.01$) reduction in organoleptic score as compared to sample E, indicating the positive effect of natural antioxidants in maintaining the sensory attributes of the product during storage. Other treatments (B, C and D) resulted a score of 6–7 during storage showing moderately significant ($p < 0.05$) differences in relation to samples A and E. Sample A was not acceptable after 2 months of storage, but all the other samples were acceptable up to 6 months, sample E being the best. These values positively correlated with other chemical parameters.

4. Conclusion

From the above studies, it could be concluded that the FT/FBD processing technique could be adopted successfully for the development of shelf stable meat and poultry products. Natural antioxidants, e.g., MRPs, spices and ascorbic acid, acted as promising alternative to synthetic antioxidants in controlling the lipid oxidation and thus could enhance the stability of lipids and, in turn, products. Individual and synergistic antioxidant potential of these antioxidants have been established in this study. These antioxidants played a significant role in controlling the release of non-haem iron, thus inhibiting its catalytic activity. There existed a correlation between non-haem iron values and rancidity parameters and this could be utilized to assess/evaluate the antioxygenic capabilities of antioxidants. Judicious application of antioxidants in the FT/FBD process can facilitate the development of meat products shelf stable under ambient temperature conditions and can meet the requirements of services as well as civilian sectors.

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