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Shelf life evaluation of functional restructured buffalo meat steaks fortified with *Mousambi* peel powder and *Amla* powder at refrigerated storage (4±1ºC)

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Abstract

This study was conducted to evaluate the shelf life of functional restructured buffalo meat steaks (FRBMS) fortified with *mousambi* peel powder (MPP) and *amla* powder (AP) separately, at refrigerated storage (4±1ºC). MPP (1:5 hydration, w/w) at 0.5% level and AP (1:5 hydration, w/w) at 0.5% level were incorporated by replacing the lean meat in pre-standardized formulation. Storage quality with respect to physico-chemical [pH and Thiobarbituric Acid Reacting Substances (TBARS) value], microbiological [Psychrophilic count (PC), Total plate count (TPC) and coliform count] and sensory properties of control and treatment products were studied. The products were aerobically packaged in low density polyethylene (LDPE) pouches and analysed at regular interval of 0, 5, 10, 15 and 20 days during refrigerated storage (4±1ºC). The storage period did not bring about any significant change in pH of the products up to 10 days of storage, but afterwards a significant increase ($P<0.05$) was noticed. The TBARS values, redness, yellowness, hue value, chroma value, PC, TPC and coli form showed linear increasing trend from 0 to 20th day of refrigerated storage in treatment products as well as control. Mesophilic and psychrotrophic counts did not exceed $\log_{10}$ 5.24 cfu/g and 3.46 cfu/g, $\log_{10}$ 4.46 cfu/g and 3.30 cfu/g, $\log_{10}$ 4.19 cfu/g and 3.27 cfu/g for control, MPP and AP treated FRBMS, respectively. The sensory scores of treatment and control samples for appearance, flavour, binding, texture, juiciness and overall acceptability showed progressive declines with increase in storage period but the scores were rated above good. Both the control and treated products retained good to very good acceptability scores throughout the storage period. These observations indicated that the product can be stored in aerobically packaged LDPE pouches for 20 days without much change in physico-chemical, microbiological and sensory properties at refrigerated storage.

Introduction

India has immense livestock wealth and claims a remarkable 58 per cent of the world's buffalo population (106.63 millions) (FAO, 2009). India produces 1.42 MT of buffalo meat, which accounts to 24.4% of total meat production of the country (FAO, 2009). In India, buffalo meat is primarily produced from aged/spent animals (about 12–15 years) after their productive/reproductive life is over. Meat from such aged/spent animals is tough, less juicy, and characterized by high amounts of connective tissue. This coarse textured meat needs to be subjected to special processing and cooking methods to improve tenderness (Hedrick *et al*., 1994).

One of the major causes of deterioration in colour and flavour of restructured meat product is lipid oxidation. Particle-size reduction and subsequent exposure to various microbial contaminants, combined with the pro-oxidant effect of salt, cause these products to have a relatively short shelf life. The oxidized or rancid flavour of cooked meat develops during refrigeration or freezing might be due to the development of warmed-over flavour (Pearson and Gray, 1983).

Restructuring technology can be effectively utilized for efficient incorporation of ingredients that can potentially enhance storage stability of convenience meat products. Natural antioxidants (NANT's) derived from plants are becoming increasingly popular as functional food and feed ingredients. NANT components in foods possess other important properties such as anti-radiation, anti-mutagenic, anti-inflammatory, anti-bacterial, and other beneficial effects (Sun *et al*., 2002; Belleville, 2002). Due to their high content of phenolic compounds, fruits and other plant materials are a good source of natural antioxidants and provide a good alternative to currently used conventional antioxidants (Nunez de Gonzales *et al*., 2008).

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Mousambi fruit (*Citrus limetta* Risso) is a citrus fruit variety known worldwide for its delicious taste and health promoting activities. Recent studies indicate that the peel yields thousand folds more phenolic compounds than pulp and possesses very high anti-oxidant, anti-microbial and anti-cancer properties. Further it is recommended for enrichment into value added nutraceutical product (Rather *et al*., 2010). Nogata *et al.* (2006) reported that flavonoids, hespiridin and naringin were found to be present in the peel and inner part of the fruit of *Citrus limetta* which are attributed to its anti-tumour, anti-infllamatory and anti-oxidant properties.

Amla (*Emblica officinalis* L.), an euphorbiaceous plant, is widely distributed in subtropical and tropical areas of China, India, Indonesia and Malay Peninsula, and used in many traditional medicinal systems, such as Chinese herbal medicine, Tibetan medicine and Ayurvedic medicine (Zhang *et al*., 2000). Emblica fruit is reported to have anti-oxidant, hypolipidemic and hypoglycemic activities, and acts as an important constituent of many hepatoprotective formulae available. It is also used as anti-microbial agent, anti-tumor or anti-inflammatory agent. Barthakur and Arnold, (1991) analyzed amla as potential food source and reported it to have considerably higher concentrations of most minerals, protein and amino acids like glutamic acid, proline, aspartic acid, alanine, cystine and lysine. Scartezzini *et al.* (2006) reported that fruits of *E. officinalis* contains higher amount of Vitamin C. The present study aims to study the shelf life of fortified restructured buffalo meat steaks at refrigerated storage (4±1ºC).

Materials and Methods

Source of materials

Buffalo meat free from external fat was obtained from rounds of spent adult female buffalo carcass within 5-6 hours after slaughter from meat market of Bareilly. All visible fascia and external fat were trimmed off and meat portions were made into cuts of approximately 0.5 kg. The cuts were then packaged separately in LDPE pouches and kept in refrigerator (4±1◦C) for conditioning for about 24 hours. Thereafter, the samples were shifted to deep freezer (Blue Star, FS345, Denmark) for storage at -18±2°C until further use. Analar and food grade chemicals were procured from Qualigens, Merck and BDH. Refined salt (Tata Chemicals Ltd., Mumbai), refined wheat flour (maida), AP, LDPE (200 gauges) bags, onion, garlic and ginger were procured from local market of Bareilly (U.P.). Mature and healthy fruits of mousambi were purchased from the local fruit market, washed and cut opened with a sharp knife to separate seeds, pulp and rind. The separated raw peels were then dried under shade till they attained constant weight. Dried pieces of fruit peel were powdered in a mixer (REMI MIXIE) and sieved using a 60 mesh sieve. The powder was packed in polypropylene bottles and stored at room temperature until use. To prepare condiment mix, onion, garlic and ginger in 3:1:1 ratio were peeled off, cut into small pieces and homogenized in a mixer to obtain a fine paste. Spice mix prepared in laboratory as per pre-standardized formulation. The curing solution was prepared by dissolving sodium chloride 120 g, cane sugar 60 g, sodium tripolyphosphates 25 g, monosodium glutamate 0.50 g and sodium nitrite 0.75 g in 1000 ml of water. The ingredients were dissolved, mixed well and then filtered. 200 ml curing solution was used for massaging of 1 kg of buffalo meat chunks. Readymade media from Hi-media Laboratories Pvt. Ltd., Mumbai were used for the enumeration of microbes.

Preparation of functional restructured buffalo meat steaks

Formulation consisted of lean meat (75%); curing solution (15%), condiment mix (5%), refined wheat flour (3%) and dry spice mix (2%). MPP (1:5 hydration, w/w at 0.5% level) and AP (1:5 hydration, w/w at 0.5% level) was incorporated by replacing the lean meat in pre-standardized formulation. The partially thawed meat was carefully trimmed free off adhering visible loose connective tissue and fascia sliced across the grain into 1cm thick slices. The sliced buffalo meat was then cut along and across to chunks of nearly 1 cm^3. Meat chunks in semi frozen state were placed in paddle mixture (HOBART, Model: N50G) and massaging was done initially at low speed with simultaneous addition of curing solution which facilitated the extraction of muscle proteins from meat and formed a tacky exudate to bind meat pieces. After the initial 7 minutes of mixing at low speed, refined wheat flour, spices, and condiments were added in order and concurrently mixed/blended for additional 3 minutes at medium speed for uniform mixing. After mixing, the meat was unloaded from the mixer, weighed and stuffed into stainless steel moulds. Moulds were squeezed with wooden press to remove air pockets. After that, the moulds were closed tightly and placed in pressure cooker filled 1/3rd with hot water and cooked by steam without pressure for 40 minutes. Slow heating rate was ensured by adjusting the flame regulating knob (Code: 637470, Regalia, Sun flame) to low, so that the required internal temperature 85°C of the

product was achieved. Meat blocks were removed from moulds after cooking and cut into slices of 10 mm thick with food slicer (Electrolux H 300). Pooled samples of each treatment were assigned for analysis.

Storage quality with respect to physico-chemical, microbiological and sensory properties of restructured buffalo meat steaks with 0.5% MPP (1:5 hydration, w/w) and 0.5% AP were studied. The products were aerobically packaged in LDPE pouches and analysed at regular interval of 0, 5, 10, 15 and 20 days during refrigerated storage at 4±1ºC.

Determination of pH

The pH of the cooked steak was determined by blending 10 g sample with 50 ml distilled water using pestle and mortar. The pH of the homogenate was recorded by immersing combined glass electrodes of digital pH meter EL 68 of ELICO pH meter (Model LI-120).

TBARS number

The TBARS Value of FRBMS was determined by using the distillation method described by Tarladgis *et al.* (1960).

Lovibond Tintometer Colour units (LTCU)

The colour of restructured buffalo meat steaks was measured using a Lovibond Tintometer (Model: F, Greenwich, U.K). Sample was taken in the sample holder and secured against the viewing aperture. The sample colour was matched by adjusting the red (a) and yellow (b) units, while keeping the blue unit fixed at 0. The corresponding colour units were recorded. The hue and chroma values were determined using the formula, $(\tan^{-1})$ b/a (Little, 1975) and $(a^2 + b^2)^{1/2}$ (Froehlich *et al*., 1983), respectively, where a = red unit, b = yellow unit.

Determination of microbiological quality

Total plate counts (TPC), psychrophilic counts (PC) and coliform counts in the samples during storage period were determined as per the method described by APHA (1984).

Sensory evaluation

Trained taste panel consisting of scientists and post graduate students of the LPT Division obliged in conducting the sensory evaluation of the product. They were requested to record their preferences on an 8 point hedonic scale (8 = like extremely, 1 = dislike extremely) for general appearance, flavour, juiciness, texture, binding and overall palatability. Plain water was provided to rinse the mouth in between the samples.

Statistical analysis

The experiment was replicated three times and the data generated from various trials under each experiment were pooled, processed and analyzed by statistical method of one way-ANOVA and Mean±S.E using SPSS software package developed as per the procedure of Snedecor and Cochran (1995) and means were compared by using Dunkan's multiple range test (Dunkan, 1955).

Results and Discussion

Physico-chemical parameters

The mean values for different physico-chemical parameters of restructured buffalo meat steaks with optimum level of MPP and AP and control products are presented in Table 1. The value of pH showed no significant change ($P>0.05$) up to 10 days of storage, but afterwards there was a significant increase ($P<0.05$) in pH value noticed in both control and treated products. The inconsistent behaviour of pH during the initial days of storage could be related to the utilization of sugar (0.8% in FRBMS) by the microbes for its metabolism and the acidic metabolites produced by carbohydrate metabolism may not be sufficient to lower the meat pH significantly. The increase in pH after 10 days could be due to the exhaustion of carbohydrate source and the action of microbes on meat proteins (a shift from saccharolytic to amino acid degrading metabolism) which in turn would increase their pH due to production of basic metabolites. On exhaustion of stored glucose, bacteria utilize amino acids and degradation of amino acids results in production of ammonia which ultimately increases the pH value of the product (Gill, 1983). Jose *et al*. (1984) also observed that pH values fell at the onset of spoilage but then increase as the spoilage develops. However, lower pH value for FRBMS incorporated with AP could be attributed to the low pH of AP. No significant ($P>0.05$) interaction between treatments and storage was observed for pH.

There was a significant ($P<0.05$) linear increase in TBARS values with increase in the storage period which remained well below threshold value of 1 mg malonaldehyde/kg of meat sample on 20^{th} day of storage. The concentrations of TBARS in treatment was considerably lower ($P<0.05$) than the control. Among the treatments, the value of AP treated product was significantly lower than MPP. This indicated a significant relation between phenolic content and antioxidant effect of fruit powders in protecting against lipid oxidation of FRBMS. No significant interaction between treatments and storage ($P>0.05$) was observed for TBARS. Rhee and Zipin (2001)

Table 1. Effect of refrigerated storage (4±1°C) on the pH and TBARS values of aerobically packaged functional restructured buffalo meat steaks (Mean±S.E.)

Treatments	Storage period (days)				
	0	5	10	15	20
pH					
Control	6.12^{b} ±0.05	6.09^{b} ±0.04	6.16^{b} ±0.04	6.30^{a} ±0.03	6.40^{a} ±0.0
MPP (0.5%)	6.14^{b} ±0.04	6.15^{b} ±0.04	6.14^{b} ±0.04	6.21^{ab} ±0.04	6.32^{a} ±0.0
AP (0.5%)	6.01^{d} ±0.07	6.07^{cd} ±0.06	6.15^{bc} ±0.01	6.23^{ab} ±0.03	6.31^{a} ±0.0
TBARS (mg Malonaldehyde /kg)					
Control	0.33^{dA} ±0.02	0.43^{dA} ±0.02	0.60^{cA} ±0.04	0.76^{bA} ±0.04	0.93^{aA} ±0.06
MPP (0.5%)	0.27^{cA} ±0.01	0.36^{cB} ±0.02	0.52^{bAB} ±0.02	0.63^{bAB} ±0.06	0.80^{aBC} ±0.06
AP (0.5%)	0.20^{cB} ±0.02	0.26^{cC} ±0.02	0.39^{bC} ±0.02	0.49^{abB} ±0.05	0.57^{aC} ±0.07

*Mean±SE bearing different superscripts row wise and column wise differ significantly (P<0.05) n=6 for each treatment at each storage period

reported a pro-oxidant effect of salt during storage of beef and chicken. Possible reasons for salt promoted lipid oxidation are the reduction in the activity of antioxidant enzymes like catalase and glutathione peroxidases, the stimulation of lipid oxidation via iron activation by chloride ions, the displacement of iron molecule from the myoglobin structure by sodium ions thereby providing free iron for the catalysis of lipid oxidation (O'Neill *et al*., 1990). The phenolic compounds of the antioxidant fruit powders might be the major functional material involved in the inhibition of lipid oxidation because they could inhibit free radical formation and the propagation of free radical reactions through the chelation of transition metal ions, particularly those of iron and copper (Brown and Mebine, 1969).

Lovibond Tintometer Colour units (LTCU)

Mean Lovibond tintometer colour unit scores of FRBMS fortified with optimum level of MPP, AP and control are presented in Table 2. There was no significant difference (P>0.05) in redness values up to 5th day of storage, afterwards it decreased significantly (P<0.05) with each storage period. However, the treatment with AP and MPP showed a relatively slower rate of decline in redness than

Table 2. Effect of refrigerated storage (4±1°C) on the Lovibond tintometer colour units (Mean±S.E.)

Treatments	Storage period (days)				
	0	5	10	15	20
Lovibond tintometer colour units (Red)					
Control	4.40^{a} ±0.09	4.27^{abB} ±0.09	4.05^{b} ±0.10	3.60^{cB} ±0.10	3.27^{d} ±0.08
MPP (0.5%)	4.47^{a} ±0.07	4.33^{aAB} ±0.07	4.15^{b} ±0.12	3.85^{bAB} ±0.12	3.43^{c} ±0.14
AP (0.5%)	4.62^{a} ±0.05	4.50^{aA} ±0.05	4.28^{ab} ±0.09	3.97^{bA} ±0.11	3.62^{c} ±0.20
Lovibond tintometer colour units (Yellow)					
Control	3.50^{a} ±0.14	3.38^{ab} ±0.13	3.13^{b} ±0.11	2.73^{c} ±0.10	2.43^{c} ±0.10
MPP (0.5%)	3.63^{a} ±0.10	3.52^{a} ±0.09	3.17^{b} ±0.11	2.80^{c} ±0.10	2.47^{d} ±0.08
AP (0.5%)	3.40^{a} ±0.07	3.28^{a} ±0.07	3.15^{a} ±0.09	2.75^{b} ±0.08	2.40^{c} ±0.12
Chroma					
Control	15.46^{a} ±0.90	14.49^{ab} ±0.84	12.75^{b} ±0.77	9.88^{c} ±0.61	7.99^{c} ±0.52
MPP (0.5%)	16.26^{a} ±0.69	15.27^{ab} ±0.66	13.21^{b} ±0.85	10.84^{c} ±0.72	8.52^{d} ±0.63
AP (0.5%)	15.71^{a} ±0.50	14.79^{ab} ±0.50	13.53^{b} ±0.69	10.95^{c} ±0.62	8.79^{d} ±0.86
Hue angle					
Control	38.43^{aA} ±0.58	38.35^{aA} ±0.58	37.68^{abA} ±0.42	37.19^{abA} ±0.46	36.63^{bA} ±0.42
MPP (0.5%)	39.09^{aA} ±0.41	39.03^{aA} ±0.37	37.32^{bAB} ±0.20	36.01^{cB} ±0.32	35.74^{cA} ±0.52
AP (0.5%)	36.35^{aB} ±0.33	36.09^{aB} ±0.34	36.31^{aB} ±0.32	34.72^{bC} ±0.38	33.62^{cB} ±0.34

*Mean±SE bearing different superscripts row wise and column wise differ significantly (P<0.05). n=6 for each treatment at each storage period

Table 3. Effect of refrigerated storage (4±1°C) on the microbiological quality of aerobically packaged functional restructured buffalo meat steaks (Mean±S.E.)*

Treatments	Storage period (days)				
	0	5	10	15	20
Total plate count (log_{10} cfu /g)					
Control	1.90^{e} ±0.15	2.34^{d} ±0.06	3.28^{cA} ±0.07	4.31^{bA} ±0.12	5.24^{aA} ±0.06
MPP (0.5%)	1.75^{d} ±0.17	2.13^{d} ±0.12	3.09^{cAB} ±0.11	3.88^{bB} ±0.08	4.46^{aB} ±0.19
AP (0.5%)	1.78^{d} ±0.14	2.13^{c} ±0.12	2.76^{bB} ±0.12	3.90^{aB} ±0.10	4.19^{aB} ±0.11
Psychrophillic count (log_{10} cfu /g)					
Control	ND	1.57^{d} ±0.08	2.28^{c} ±0.14	3.05^{b} ±0.06	3.46^{aA} ±0.03
MPP (0.5%)	ND	1.38^{d} ±0.05	2.12^{c} ±0.12	2.89^{b} ±0.08	3.30^{aAB} ±0.01
AP (0.5%)	ND	1.46^{d} ±0.06	2.22^{c} ±0.08	2.88^{b} ±0.06	3.27^{aAB} ±0.06
Coliform count (log_{10} cfu /g)					
Control	ND	1.48^{d} ±0.06	2.01^{c} ±0.05	2.61^{bA} ±0.03	2.88^{aA} ±0.02
MPP (0.5%)	ND	1.39^{d} ±0.04	1.98^{c} ±0.04	2.48^{bB} ±0.02	2.82^{aB} ±0.01
AP (0.5%)	ND	1.48^{d} ±0.07	1.95^{c} ±0.02	2.49^{bB} ±0.03	2.81^{aB} ±0.01

*Mean±SE bearing different superscripts row wise and column wise differ significantly (P<0.05). n=6 for each treatment at each storage period

the control which resulted in significant differences (P<0.05) in redness values at the end of storage among treatments. This indicated differences in the ability of natural antioxidants to retain the colour during storage which might be due to differences in antioxidant capacity among treatments. Naithani *et al.* (2006) reported that stability of antioxidant capacity of herbs declines with storage time and it is related to phenolic content. In general redness values were higher in treatments than in control. No significant interaction (P>0.05) between treatments and storage was observed for LTCU (redness). However, the AP treated steaks were slightly darker and showed a significantly higher (P<0.05) redness values than MPP treated steaks. This darkening of steaks treated with AP can be attributed to the low pH of AP. Thomas *et al*. (2008) reported that reduction in pH of pork sausages significantly increased the lovibond tintometer redness (a-values) while

Table 4. Effect of refrigerated storage (4±1°C) on the sensory quality of aerobically packed functional restructured buffalo meat steaks (Mean±S.E)*

Treatments	Storage period (days)				
	0	5	10	15	20
General appearance					
Control	7.15^{a} ±0.11	7.18^{a} ±0.09	7.04^{a} ±0.07	6.77^{b} ±0.07	6.65^{b} ±0.05
MPP (0.5%)	7.30^{a} ±0.08	7.35^{a} ±0.08^{a}	7.19^{a} ±0.07	6.92^{b} ±0.07	6.69^{c} ±0.09
AP (0.5%)	7.13^{a} ±0.07	7.15^{a} ±0.06	7.08^{a} ±0.07	7.00^{ab} ±0.06	6.89^{b} ±0.04
Flavour					
Control	7.33^{a} ±0.06	7.24^{a} ±0.06	7.14^{a} ±0.07	6.90^{b} ±0.07	6.56^{c} ±0.09
MPP (0.5%)	7.29^{a} ±0.09	7.20^{a} ±0.06	7.11^{a} ±0.05	6.79^{b} ±0.09	6.58^{b} ±0.09
AP (0.5%)	7.12^{a} ±0.08	7.09^{a} ±0.08	6.97^{ab} ±0.08	6.90^{ab} ±0.07	6.78^{b} ±0.05
Juiciness					
Control	7.10^{a} ±0.07	7.07^{a} ±0.07	7.07^{aAB} ±0.06	6.86^{bB} ±0.06	6.79^{bAB} ±0.06
MPP (0.5%)	7.23^{b} ±0.08	7.28^{b} ±0.08	7.28^{bA} ±0.08	7.13^{abA} ±0.07	6.97^{bA} ±0.05
AP (0.5%)	7.08^{a} ±0.08	7.08^{a} ±0.08	7.00^{aB} ±0.08	6.91^{abB} ±0.07	6.74^{bB} ±0.07
Texture					
Control	7.07^{a} ±0.06	7.01^{ab} ±0.09	6.97^{ab} ±0.08	6.85^{bc} ±0.05	6.69^{c} ±0.06
MPP (0.5%)	7.24^{a} ±0.09	7.21^{a} ±0.09	7.13^{ab} ±0.09	6.95^{bc} ±0.05	6.85^{c} ±0.08
AP (0.5%)	7.09^{ab} ±0.08	7.13^{a} ±0.10	7.08^{ab} ±0.09	6.99^{ab} ±0.07	6.86^{b} ±0.05
Binding					
Control	7.25^{a} ±0.09	7.30^{a} ±0.07	7.23^{a} ±0.05	7.14^{ab} ±0.07	6.97^{bA} ±0.05
MPP (0.5%)	7.33^{a} ±0.09	7.30^{a} ±0.09	7.25^{a} ±0.07	7.04^{b} ±0.07	6.86^{bAB} ±0.05
Overall acceptability					
Control	7.12^{a} ±0.05	7.10^{a} ±0.09	7.04^{a} ±0.06	6.78^{b} ±0.08	6.57^{bB} ±0.09
MPP (0.5%)	7.17 ±0.09	7.22 ±0.09	7.14 ±0.05	6.99 ±0.09	6.81^{A} ±0.06
AP (0.5%)	7.09^{a} ±0.04	7.04^{ab} ±0.05	6.99^{ab} ±0.07	6.86^{bc} ±0.04	6.72^{cAB} ±0.08

*Mean values are scores on 8-point descriptive scale where 1: extremely undesirable and 8: extremely desirable. Mean±SE bearing different superscripts row wise and coloumn wise differ significantly (P<0.05). n=21 for each treatment

decreased the yellowness (b-values). No significant interaction (P>0.05) between treatments and storage was observed for LTCU (redness).

LTCU (yellowness) value/ (b)

There was no significant difference (P>0.05) observed in yellowness values up to 5th day of storage, afterwards it decreased significantly (P<0.05) with each storage period. There was a higher rate of change in yellowness values of control steaks with storage as compared to treatments. There was a higher rate of change in yellowness values of control and MPP treated steaks with storage as compared to AP treated steaks. However, the higher yellowness values of steaks containing MPP could be due to the pale yellow colour of dried MPP which contains both flavedo (epicarp) and albedo (mesocarp). No significant interaction between treatments and storage (P>0.05) was observed for LTCU (yellowness).

The chroma value indicates the intensity of colour. Chroma value showed a significant decrease (P<0.05) with each increase in storage period. This indicated significant decline in the intensity of colour with storage period. No significant (P>0.05) interaction between treatments and storage was observed for chroma. The hue value was reported to be a more precise measure of colour. Hue value showed a declining trend with increase in storage period. Hue value on 15th day was significantly lower (P<0.05) than that of 10th day. The scores of 15th day were comparable to that of 20th day. In general, hue values of control samples were higher than all the treatments. Among treatments, the hue values were higher for treatment with MPP and it was comparable to control, whereas it decreased significantly (P<0.05) in AP treated products. The significantly higher (P<0.05) hue values for treatment with MPP could be due to higher yellowness values. The significantly lower (P<0.05) hue values for treatment with AP might be due to the higher redness and lower yellowness values.

Microbiological quality

The microbiological parameters of FRBMS fortified with optimum level of fruit additives (MPP and AP) and control are presented in Table 3. The results showed a significant increase (P<0.05) in TPC with increase in storage period. However, these values are very well within the permissible limits for cooked meat products prescribed by Jay (2005). In control, the TPC increased significantly (P<0.05) from 0 day to 20 day, whereas in case of treatment products with MPP the increase was not significant (P>0.05) up to 5th day but after that there was a significant increase (P<0.05) with increasing storage period. This relatively slow increase might be due to the profound antimicrobial effect of natural antimicrobial substances such as phenolics during the initial stages of storage which then reduced with elapse of storage. In case of treatment with AP, the stronger inhibitory effect might be due to high amount of phenolics and acidic pH of amla. Bahk *et al*. (1990) reported that microbial inhibition by plant extracts could be manifested by an extended lag phase, increased generation time, and decreased maximum growth or various combinations of these criteria. However, the treatments with AP and MPP showed no significant difference (P>0.05) between them. It could be due to significantly lower (P<0.05) pH and high phenolic content of treatment with AP and the presence of abundant amount of essential oils in the mousambi peel resulting in inhibition of microbial growth. Herent *et al.* (2007) isolated the essential oils with α-pinene, β-pinene, sabinene, β-myrcene, p-cymene, limonene, γ-terpinene, neryl acetate, β-bisabolene, α-bergamotene from the zests of *Citrus limetta* Risso. Shan *et al.* (2007) reported that partial hydrophobic nature of phenolic compounds may degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane, damage membrane proteins and interfere with membrane integrated enzymes, which may eventually lead to cell death. Significant interaction (P<0.01) was observed between treatments and storage for TPC. Psychrophiles were not detected on 0 day of storage in FRBMS with MPP and AP as well as control. Absence of psychrophiles at 0 day of storage might be associated with metabolic injury of cells due to environmental stress such as cooking. These counts were detected on 5th day of storage and thereafter increased significantly (P<0.05) in both control and treatments. PC showed significantly lower values (P<0.05) for treatments than control. However, no significant difference (P>0.05) was observed between treatments inspite of their differences in their antimicrobial activity. This might be due to the strong lipopolysaccharide layer present on the cell wall of Gram-negative psychrotrophs such as pseudomonas which may not be affected by any of treatments. Gram-positive bacteria have been reported to be more sensitive to plant extracts than Gram-negative bacteria (Ceylan and Fung, 2004; Shan *et al.*, 2007). Those Gram-negative organisms were less susceptible to the action of antibacterials, since they possessed an outer membrane surrounding the cell wall (Ratledge and Wilkinson, 1988), which restricted diffusion of hydrophobic compounds through its lipopolysaccharide covering (Vaara, 1992). The psychrotrophic count was well below the

limit of log 4 cfu/g, which causes bacterial spoilage of the food (Jay, 2005). Coliforms were not detected on 0 day of storage both in control and treatments. The absence of coliforms on 0 day might be due to thermal injury of cells during cooking. On 5th day storage coliforms were detected both in control as well as treatments and thereafter it increased significantly (P<0.05). Coliform count showed a significantly lower values (P<0.05) for treatments than control. However, no significant difference (P>0.05) was observed between treatments. The difference amongst control and treatments might be due to the presence of natural antimicrobial substances in the fruit powders. Higher coliform counts on 20th day could be because of contamination of samples while handling. However, coliforms were well below the standard log 3 cfu/g (Jay, 2005), that could cause microbiological spoilage of meat products.

Sensory quality

Mean sensory score of FRBMS fortified with optimum level of fruit additives (MPP and AP) and control are presented in Table 4. The general appearance scores of both control and treatments decreased gradually with increase in storage period. However, scores significantly decreased (P<0.05) on 15th day and further declined significantly with increase of storage period. The decrease in appearance scores could be due to some pigment and lipid oxidation resulting in non enzymatic browning as well as surface dehydration in aerobic packaging. The pigment oxidation may cause metmyoglobin accumulation during storage. Biswas *et al*., (2004) reported a decrease in general appearance of precooked pork patties during chilled and frozen storage. However, FRBMS treated with MPP showed a marginally higher general appearance scores than AP treated product. No significant interaction was observed between treatments and storage for general appearance scores. Flavour scores of both control and treatments at 0 day and 5th day were comparable but afterwards it declined significantly (P<0.05) with increase in the storage period. The decrease in flavour scores could be related to the microbial growth and oxidative rancidity as indicated by TBARS numbers. Tarladgis *et al*. (1960) reported that TBARS values are highly correlated with sensory scores of trained panelist. No significant interaction was observed between treatments and storage for flavour scores. Juiciness scores of both control and treatments were comparable at 0 and 5th day. The scores of the latter were comparable to that of 10th day. Thereafter, it showed a significant decline (P<0.05) on 15th day which was comparable with 20th day scores. Decrease in juiciness scores could be attributed to some moisture loss from the product during aerobic storage. Juiciness scores of FRBMS treated with MPP was significantly higher (P<0.05) than control and AP treated product. This could be related to the high water retention capacity (WRC) of MPP. No significant interaction was observed between treatments and storage for juiciness scores. Texture scores of FRBMS followed same pattern of juiciness but after 10th day, there was a significant decline with increase in the storage period. The decrease in texture scores might be due to protein oxidation and microbial action on proteins during the refrigerated storage. Texture scores were significantly higher (P<0.05) for all the treatments than control indicating the superiority of the treated products. No significant interaction was observed between treatments and storage for texture scores. Binding scores of both control and treatments were comparable at 0 and 5th day. The scores of the latter were comparable to that of 10th day. Thereafter, it showed a significant decline (P<0.05) with increase in storage period. The reduction in binding may be due to dehydration of the product during storage. Binding scores of FRBMS treated with AP showed a significant reduction (P<0.05) than control and other treatment. The reason for the reduction could be attributed to the low pH of AP that reduced the binding of meat proteins. Overall acceptability scores of both control and treatments were comparable at 0 and 5th day. The scores of the latter were comparable to that of 10th day. Thereafter, it showed a significant decline (P<0.05) with increase in storage period. This sensory attribute reflects the cumulative effect of all other attributes, which resulted in significant reduction of scores only after 10th day of storage period. Overall acceptability scores of control FRBMS and treatment with AP showed a significant reduction than MPP treated products.

These observations concluded that the product can be stored in aerobically packaged LDPE pouches for 20 days without much change in physico-chemical, microbiological and sensory properties at refrigerated storage.

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