Quality of cooked ground buffalo meat treated with the crude extracts of *Moringa oleifera* (Lam.) leaves

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Abstract The study was conducted to evaluate the physico-chemical, microbial and organoleptic qualities of cooked ground buffalo meat (GBM), treated with, 1, 1.5 and 2% levels of aqueous solution of crude extract of drumstick (*Moringa oleifera*) leaves. The meat samples treated with 1.5% crude extract of drumstick leaves significantly ($P < 0.05$) improved meat pH and water holding capacity (WHC) and lowered cooking loss and thiobarbituric acid (TBA) value as compared to control and other treated samples. Microbial load in terms of Total Plate Count (TPC) was found to be decreased significantly ($P < 0.05$) in treated samples. No significant ($P > 0.05$) difference was observed in juiciness, tenderness and overall acceptability scores between the treated meat samples.

Keywords *Moringa oleifera* · Ground buffalo meat · Meat quality · Crude extract

Introduction

India has largest livestock population in the world. It has about 98 million buffalos, which is 57% of total population in the world. They contribute to 1.48 million metric tonnes of meat, amounting 24.54% of the total meat produced in the country (FAO 2008). Buffalo meat

is relatively coarse (tough and not fine in texture) as it is produced from aged to spent animals (animals above 12 years of age which are no longer productive and commercially beneficial). As comminution breaks down the structure of tough meat and reduces the energy requirement of cooking, so development of further processed comminuted meat products is, therefore a solution to the problem of utilizing such tough meat (Anjaneyulu et al. 1990). Considering the need of extending the shelf life of buffalo meat in the perspective of its export potentiality, a various items in terms of antioxidant, preservatives and anti bacterial activity have been tried with varying degree of success. In vogue of present national and international barriers regarding use of chemical food additives to food processing and preservation, the biological and plant origin food additives have got better significance and require emphasis for attention and study.

As per Gray and Pearson (1987), one of the major problems in the development of new convenient meat products and processes is the deterioration of flavor, texture and nutritive value of buffalo meat due to oxidation of lipid and browning of pigment. Though a variety of substances i.e. chemical (Butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, phosphate, nitrite, etc.), nutritive (α -tocopherol, β -carotene, vitamin C etc.) and spice extracts (Chan et al. 1994) have already been investigated as potential anti-oxidant but in the recent years the interest in the application of naturally occurring antioxidant in muscle foods has amplified, since the use of synthetic antioxidants have become less acceptable. *Moringa oleifera* is not only an important source of naturally occurring antioxidant (Dillard and German 2000); it is also an important Indian medicinal plant and an important ingredient of the Indian cuisine. It

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contains high concentrations of ascorbic acid, estrogenic substances and beta-sitosterol, iron, calcium, phosphorus, copper, vitamins A, B and C, alpha-tocopherol, riboflavin, nicotinic acid, pyridoxine, beta-carotene, protein, and in particular essential amino acids such as methionine, cystine, tryptophan and lysine. The presence of these substances in *Moringa* leaves which attribute to the importance of drumstick leaves an important dietary supplement (Makkar and Becker 1996). It is also having antibacterial (Rahman et al. 2009), anticancer (Makonnen et al. 1997) and purgative effect (Makonnen et al. 1997). Crude extracts showed antioxidant properties as revealed by the following determinations: the Total Antioxidant Activity (TAA), 2,2-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity and reducing power (Sreelatha and Padma 2009), antimicrobial activity and purgative action. Nitrile mustard oil glycosides and thiocarbamate glycosides have been isolated from *Moringa* leaves. These compounds are found to have blood pressure lowering effect (Faizi et al. 1994a, b, 1995).

Hence, the present study was conducted to compare the physico-chemical properties, sensory qualities and storage stability of cooked ground buffalo meat incorporated with different levels of crude extract of *Moringa oleifera* leaves.

Materials and methods

Plant materials The *Moringa oleifera* leaves were purchased from the local market.

Preparation of the extract The leaves were thoroughly washed and chopped into small pieces. They were then dried in shade for about 30 min. The dried leaves were powdered. The powder was passed through sieve no. 20 and extracted (100 g) successively with 500 ml of water in a soxhlet extractor for 20–22 h. The extracts were concentrated to a dry mass by using reduced pressure and controlled temperature (40–45 °C). The yield (w/w) of the extract from fresh leaves was 12–15%.

Source of meat samples The samples were obtained from biceps femoris to quadriceps muscles of spent, adult, male (around 13 years of age) of Murrah breed of water buffalos (*Bubalus bubalis*). Carcasses slaughtered according to traditional halal method at Kolkata Municipal Slaughter House. The slaughtering, transportation and

other invasive procedures on animals were carried out by standard humane methods and following jurisdiction of Local Ethical Committee. The samples were collected within 2 h of slaughter, packed in low density polyethylene (LDPE) bags and brought to the Dept. of Livestock Products Technology (LPT), West Bengal University of Animal and Fishery Sciences. The samples were placed within 15 min in a refrigerator at 4 ± 10 °C for about 24 h for conditioning.

Sample preparation After conditioning, the meat chunks were cut into small cubes and minced twice with a meat mincer (Sirman®, Italy; Model Buffalo TC 32) fitted with 6 mm plate. The samples were then prepared by manually mixing 1.0%, 1.5% and 2% of aqueous solution of crude extract of *Moringa* leaves to 200 g of ground buffalo meat (GBM) and blended for 1 min. The raw meat mixtures were cooked thoroughly for 10 min at 1,600 °C. The cooked meat was then loosely packed in Low Density Polyethylene bag for further analysis with head space air so that oxidation could occur unless inhibited by antioxidants.

Analytical methods

pH determination

For determination of pH, representative samples of 10 g of patties from each treatment were homogenized for 30 s with 100 ml distilled water using a blender. The pH of prepared homogenates was recorded by using a digital pH meter (WTW®, Germany, Model 330i fitted with Sen Tix sp electrode) by immersing the electrode of pH meter into aliquot of the sample (Egbert et al. 1992). The pH meter was calibrated with known buffers of pH 7 and 4.01 before use every time.

Water holding capacity (WHC)

Water Holding Capacity (WHC) was determined by modifying the method of Hughes et al. (1997) as outlined by Cengiz and Gokoglu (2007). 1 g of sample was placed in polycarbonate centrifuge tubes and heated to 90 °C in a water bath for 1 h. Samples were then removed from water bath, cooled to centrifuged at $4,000 \times g$ for 1 h (4 °C). After centrifugation, samples were dried on a blotting paper and then re-weighed. WHC was calculated from the following formula as percent expressible moisture.

$$WHC = \{1 - (\text{Initial weight} - \text{Final weight}) / \text{Percent Moisture in sample}\} \times 100$$

Cooking loss%

Cooking loss% was determined by weighing the sample before and after cooking (Chamul 2007).

Moisture

Moisture was determined as per AOAC (1984) method. 10 g of sample was transferred to weighed metallic dish which was then transferred to a hot air oven at 1,000 °C and dried till a constant weight was obtained. The dish was kept in a desiccator for cooling. After cooling, the loss in weight was determined to calculate moisture content and expressed as%:

$$\text{Moisture (\%)} = \{(Fresh\ weight - Dry\ weight) / Fresh\ weight\} \times 100$$

$$\%N = \frac{\text{Sample Titre} - \text{Blank Titre} \times \text{Normality of HCl} \times 14 \times \text{Volume made up}}{\text{Aliquot of digest taken} \times \text{Weight of sample taken}}$$

% Nitrogen was converted to% protein by multiplying with 6.25.

Fat (ether extract)

For estimation of fat, Soxhlet method (AOAC 1984) was used. 1 g of dried sample was transferred to a thimble. Petroleum ether (B. Pt. 400 °C–600 °C) was used as solvent which was subsequently evaporated and the extracted fat was weighed after complete drying in an oven at 600 °C. Percent fat in dried sample was calculated as follows, which was converted into wet basis by multiplying by a moisture factor:

$$\% \text{ Fat} = \frac{\text{Weight of fat(g)}}{\text{Weight of sample(g)}} \times 100$$

Thiobarbituric acid (TBA) value

Thiobarbituric acid value (TBA) was estimated as per procedure given by Tarladgis et al. (1960). 10 g of sample was taken and added to 49 ml of distilled water and 1 ml of sulphanilamide reagent (1 g of sulphanilamide dissolved in solution containing 40 ml of conc. HCl and 160 ml of distilled water) and blended with the help of pestle and mortar. After this 48 ml of distilled water was used for

Protein

The protein content was determined by micro-Kjeldhal method as given in AOAC (1984). 2 g of sample was taken in a digestion flask followed by addition of 3 g of digestion mixture (K_2SO_4 : $CuSO_4$: SeO_2 in 100:20:2.5 ratio) and 25 ml of conc. sulphuric acid. The contents were then digested till a blue/green transparent liquid was obtained. The volume of digested mixture was made up to 100 ml with distilled water. 20 ml aliquot of digested mixture was distilled with excess of 40% NaOH solution and liberated ammonia was collected in 20 ml of 2% boric acid solution containing 2 to 3 drops of mixed indicator (10 ml of 0.1% Bromocresol green + 2 ml of 0.1% methyl red indicator in 95% alcohol). The entrapped ammonia was titrated against 0.1 N HCl. A reagent blank was similarly digested and distilled. Nitrogen content in sample was calculated as follows:

washing the mortar and to it 2 ml of HCl solution (diluted 1:2 with distilled water) was added. The contents were transferred to Kjeldhal flask after adding several glass beads. These were heated at high temperature and 50 ml of distillate was collected in a graduated cylinder. After mixing the distillate well, 5 ml of it was taken into a 50 ml glass stopper flask and 5 ml of TBA reagent (1.442 g of TBA dissolved in 450 ml of glacial acetic acid and made up to 500 ml with distilled water) was added. The contents were mixed and the flask was immersed in boiling water bath for exactly 35 min. A blank was also prepared consisting of 5 ml of distilled water and 5 ml of TBA reagent. The flasks were then cooled under tap water for 10 min. The optical density (O.D.) of the distillate was then recorded at 538 nm against blank. The TBA value as mg of malonaldehyde per 1,000 g of sample was calculated using following formula:

$$\begin{aligned} \text{TBA value (mg of malonaldehyde/1000g of sample)} \\ = \text{O.D. of sample} \times 7.8 \end{aligned}$$

Total plate count (TPC)

It was determined by the APHA (1992) method using plate count agar. 1 ml of appropriate dilution of sample was transferred aseptically to sterile petri-plates in triplicate. The

plates were then poured with 10–15 ml melted agar medium at 450 °C. After solidification the petri-plates were incubated at 37 °C for 24–28 h. The colonies were counted by using colony counter. The average number of colonies was multiplied with dilution factor to obtain total count as colony forming unit (CFU) per g of the sample. This count was then converted to total plate count of log CFU/g of sample.

Sensory evaluation

The sensory qualities of samples were evaluated by meat descriptive analysis method. The sensory quality of samples was evaluated using 8 point descriptive scale (Keeton et al. 1984) where 8 denoted extremely desirable and 1 denoted extremely poor. A sensory panel (semi trained) of seven judges were requested to evaluate the product for different quality attributes such as: colour, flavor, juiciness, tenderness and overall acceptability.

Statistical analysis

A total number of 4 replications were conducted and each replication was carried out in duplicate. One total experiment was replicated thrice and the data were analyzed by statistical method of one way ANOVA as per the procedure of Snedecor and Cochran (1989) and means were compared by using Duncan's multiple range test (Duncan 1955).

Results and discussion

The results depicted in Table 1 revealed a significant ($P < 0.05$) rise in pH and WHC of cooked GBM treated with 1%, 1.5% and 2% crude extract of drumstick leaves respectively. However, pH and WHC of the meat samples treated with 1.5% crude extract were significantly ($P < 0.05$) higher than those of other samples. The higher WHC values

in cooked GBM treated with 1.5% can also be partly explained by the increased pH (Bernthal et al. 1991). As pH value is increased above the isoelectric pH of proteins, there is an increase in WHC. This is also in agreement with the report of Bouton et al. (1973). The relatively lower WHC value of the control group in our experiment might be due to meat from very old animals that have lower WHC (Syedziauddin 1994) and also may be due to slight denaturation of sarcoplasmic proteins, which play an important role in determining WHC (Joo et al. 1999). The above results thus indicate that the treatment with crude extract of drumstick leaves improved the functional properties of muscle proteins.

A significant ($P < 0.05$) reduction in cooking loss% was observed in 1.5% and 2% treated samples compared to others. The degradation of sarcoplasmic and myofibrillar proteins during storage may be responsible for the increase in cooking loss (Pawar et al. 2007). Superoxide anion radical (O_2^-) is a precursor to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage (Pardini 1995). The crude extract of *Moringa* leaf can actively scavenge free radicals and thus prevent cellular damage (Sreelatha and Padma 2009). The increased meat pH by crude extract can also account for the observed decrease in cooking loss (Thomsen and Zeuthen 1988). Here we can see the cooking loss is minimum in 1.5% treatment.

The results (Table 1) also showed that the moisture, total protein and the ether extract content did not differ significantly ($P > 0.05$) between control and treated samples. However, the samples treated with 2% crude extract have scored higher for protein and ether extract values in comparison to other treated samples. This may be due to increase in solubility of the total protein and a higher of moisture from the 2% treated cooked samples has resulted in increasing the concentration of other ingredients.

Table 1 Physico-chemical characteristics and microbial profile of cooked ground buffalo meat treated with crude extract of *Moringa oleifera* leaves (Mean $\pm$ SE)

Parameters	Control	Crude extract of <i>Moringa oleifera</i> leaves		
		1%	1.5%	2%
pH	5.5 ^a $\pm$ 0.05	6.0 ^b $\pm$ 0.03	6.0 ^c $\pm$ 0.06	6.0 ^c $\pm$ 0.04
WHC	12.3 ^a $\pm$ 0.30	13.9 ^b $\pm$ 0.43	15.1 ^c $\pm$ 0.37	15.0 ^c $\pm$ 0.26
Cooking loss%	36.7 ^a $\pm$ 0.20	35.8 ^b $\pm$ 0.93	34.8 ^c $\pm$ 0.42	34.8 ^c $\pm$ 0.43
Moisture	54.2 $\pm$ 0.28	54.4 $\pm$ 0.36	54.7 $\pm$ 0.38	54.8 $\pm$ 0.40
Protein	20.1 $\pm$ 0.12	19.9 $\pm$ 0.23	20.1 $\pm$ 0.18	20.2 $\pm$ 0.22
Ether extract	9.1 $\pm$ 0.16	9.2 $\pm$ 0.19	9.4 $\pm$ 0.21	9.6 $\pm$ 0.12
TBA value (mg malonaldehyde/kg)	0.42 ^a $\pm$ 0.09	0.27 ^b $\pm$ 0.05	0.29 ^c $\pm$ 0.05	0.28 ^c $\pm$ 0.05
TPC (log CFU/g)	3.0 ^a $\pm$ 0.22	3.0 ^b $\pm$ 0.29	2.7 ^c $\pm$ 0.17	2.7 ^c $\pm$ 0.19

Means bearing different superscripts between columns (a, b, c) differ significantly ($P < 0.05$)

Number of observations (n) = 8

The present study also showed (Table 1) that the meat samples treated with 1%, 1.5% and 2% had significantly ($P<0.05$) lower TBA values than the control. Among the treated samples, the 1.5% treated meat showed a significantly ($P<0.05$) lowest TBA value. This may be due to inhibition of lipid peroxidation by the crude extract treated samples. The *Moringa* leaf extract contains polyphenols that have antioxidant effects. Polyphenols, but not vitamin E, are known to produce strong antioxidant effect in vitro. They act as chain-breaking peroxy-radical scavengers which lead to the inhibition of lipid peroxidation and also prevent low density lipoprotein (LDL) oxidation (O'Byrne et al. 2002). As product stability (rancidity) was measured by TBA assay (Buege and Aust 1978), so the present study indicated that the sample treated with 1.5% extract was more stable in comparison to others.

The results (Table 1) also showed that the total plate count (TPC) of ground buffalo meat were significantly ($P<0.05$) affected by *Moringa* leaf extract treatment. Microbial load significantly decreased on treated samples. The fresh leaf juice has been found to prevent the growth of microorganisms (Caceres et al. 1991). TPC in control sample was found to be $\log 2.96\pm0.22$ CFU/g. After adding the leaf extract at 1%, 1.5% and 2% levels, TPC was found as $\log 2.95\pm0.29$ CFU/g, 2.72 ± 0.17 CFU/g and 2.65 ± 0.19 CFU/g respectively. According to Bauemann (1979), the maximum limit of TPC for acceptability of a product is $\log 5.0$ CFU/g. Hence, GBM treated with *Moringa* leaf extract at 1%, 1.5% and 2% pass the TPC acceptability test as all the values of control and treated samples remained much less than the maximum limit. This may be due to the surrounding environment being free from contamination to the sterility of the procedure adopted for microbiological analysis, in addition to the anti-microbial activity of *Moringa* leaves.

The results depicted (Table 2) showed a significant ($P<0.05$) improvement in colour of GBM treated with 1.5% extract in comparison to other treated meat. The colour of meat changes depending on the state of myoglobin. The formation of metmyoglobin leads to unfavorable colour change by the action of free radicals predominantly (Renner and Labas 1987) and partly by presence of aerobic bacteria (Robach and Costilow 1962; Stanbridge and

Davies 1998). The crude extracts of drumstick leaves can considerably scavenge free radicals (Sreelatha and Padma 2009) and thus retain the colour.

The flavor score also showed a significant ($P<0.05$) improvement and GBM treated with 1.5% crude extract has scored highest in comparison to other treated samples. This may be due to more effective inhibition of lipid peroxidation (O'Byrne et al. 2002).

As the juiciness and tenderness are interrelated, scores for juiciness and tenderness also showed a similar trend. The treated samples differed significantly ($P<0.05$) from the control but there were no significant ($P>0.05$) difference between themselves.

The scores for overall acceptability also showed a significant ($P<0.05$) improvement but there were no significant difference between the treated samples. However, the GBM treated with 1.5% scored greater value than the other treated samples.

Conclusion

Based on the above findings, it was concluded that use of crude extract of *Moringa oleifera* leaves @ 1%, 1.5% and 2%, each had significant antioxidant and antimicrobial effects in cooked GBM. They also improved the quality of the meat by enhancing the tenderness, juiciness and preventing discoloration. The study also clearly indicates that these three different levels of crude extract of *Moringa* leaf can be successfully used as a food or meat additive due to its strong effect in preventing off-flavor formation and also in improving the organoleptic quality of cooked meat. Although the result variations between 1.5% and 2% are very less but 1.5% may be preferred over 2%, considering the lower amount.

However, further research is required to find the shelf-life of the cooked GBM treated with this natural antioxidant as well as the other beneficial aspects of the uses of *Moringa oleifera* need to be further studied by augmenting proper plan of work. The experiment can be tried with extract of *Moringa* leaves at different stages of maturity. The work may also be extended for other species meats.

Table 2 Sensory attributes of cooked ground buffalo meat treated with crude extract of *Moringa oleifera* leaves (Mean $\pm$ SE)

Means bearing different superscripts between columns (a, b, c) differ significantly ($P<0.05$)
Number of observations (n) = 8

Sensory attributes	Control	1%	1.5%	2%
Colour	6.7 ^a $\pm$ 0.15	6.9 ^b $\pm$ 0.06	7.2 ^c $\pm$ 0.11	7.2 ^c $\pm$ 0.09
Flavor	6.2 ^a $\pm$ 0.22	6.9 ^b $\pm$ 0.09	7.3 ^c $\pm$ 0.04	7.1 ^c $\pm$ 0.12
Juiciness	6.1 ^a $\pm$ 0.18	6.8 ^b $\pm$ 0.05	6.9 ^b $\pm$ 0.11	6.9 ^b $\pm$ 0.07
Tenderness	5.7 ^a $\pm$ 0.34	6.9 ^b $\pm$ 0.06	7.1 ^b $\pm$ 0.09	7.1 ^b $\pm$ 0.09
Overall acceptability	5.9 ^a $\pm$ 0.08	7.0 ^b $\pm$ 0.05	7.2 ^b $\pm$ 0.09	7.1 ^b $\pm$ 0.06

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