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Subha Ganguly
Associate Professor,
Department of Veterinary
Microbiology, Arawali Veterinary
College (Affiliated to Rajasthan
University of Veterinary and
Animal Sciences, Bikaner), N.H. –
52 Jaipur Road, V.P.O. Bajor,
Sikar, Rajasthan, India

Parveez Ahmad Para
Assistant Professor,
Department of Livestock Products
Technology, Arawali Veterinary
College (Affiliated to Rajasthan
University of Veterinary and
Animal Sciences, Bikaner), N.H. –
52 Jaipur Road, V.P.O. Bajor,
Sikar, Rajasthan, India

Vikas Kumar
Assistant Professor,
Department of Veterinary
Anatomy and Histology, Arawali
Veterinary College (Affiliated to
Rajasthan University of
Veterinary and Animal Sciences,
Bikaner), N.H. – 52 Jaipur Road,
V.P.O. Bajor, Sikar, Rajasthan,
India

Nilesh Pagrut
Associate Professor,
Department of Veterinary
Pathology, Arawali Veterinary
College (Affiliated to Rajasthan
University of Veterinary and
Animal Sciences, Bikaner), N.H. –
52 Jaipur Road, V.P.O. Bajor,
Sikar, Rajasthan, India

Bhartendu
Assistant Professor,
Department of Veterinary
Physiology and Biochemistry,
Arawali Veterinary College
(Affiliated to Rajasthan University
of Veterinary and Animal Sciences,
Bikaner), N.H. – 52 Jaipur Road,
V.P.O. Bajor, Sikar, Rajasthan,
India

Correspondence
Subha Ganguly
Associate Professor,
Department of Veterinary
Microbiology, Arawali Veterinary
College (Affiliated to Rajasthan
University of Veterinary and
Animal Sciences, Bikaner), N.H. –
52 Jaipur Road, V.P.O. Bajor,
Sikar, Rajasthan, India

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

Subha Ganguly, Parveez Ahmad Para, Vikas Kumar, Nilesh Pagrut and Bhartendu

Abstract

The study was conducted to evaluate the physico-chemical, microbial and organoleptic qualities of cooked rohu fillets treated with, 1%, 1.5% and 2% levels of aqueous solution of crude extract of drumstick (*Moringa oleifera*) leaves. The fillets were 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 fillets samples.

Keywords: Cooking loss, *Moringa oleifera* leaves, Rohu fillets, Meat quality, Crude extract

Introduction

Fish is highly nutritious and protein rich food. Health benefits associated with fish consumption have resulted in consumers favoring fish products; as a result of this the fish eaters of the world have doubled during the last 50 years. India's seafood industry is one of our biggest foreign exchange earners. While considering fish as a source of food the main emphasis has been put on the protein [1]. About 85-90% of fish protein is easily digestible and contains all essential amino acids. Fish represents about 14% of all animal proteins and about 5% of total protein is eaten on a global basis. Worldwide fish consumption is on the rise due to its rich sources of high quality proteins, essential vitamins and healthful poly-unsaturated fatty acids [2]. Technology up gradation for enhancing shelf life of fresh fish has become necessity in fish processing sector to successfully marketing them in the urban domestic markets [3]. The increased demand for fresh fish has prompted the development of many new preservation techniques which can be adopted by the fish processing industry without sacrificing safety, quality, shelf life and satisfying the consumer demand [4].

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 fish samples

The fishes were brought from local fish market, dressed, deboned manually and made into fillets. Those fillets were chopped into cubes. Those were then transported to the laboratory in iced thermos flask. 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 fish meat cubes were manually mixing 1.0%, 1.5% and 2% of aqueous solution of crude extract of *Moringa* leaves and blended for 1 min.

The raw meat cubes were cooked thoroughly for 10 min at 160 °C. The cooked meat cubes were then loosely packed in Low Density Polyethylene (LDPE) bag for further analysis with head space air, so that oxidation could occur unless inhibited by the antioxidants [5].

Analytical methods

pH determination

For determination of pH, representative samples of 10 g 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 by immersing the electrode of pH meter into aliquot of the sample. [6] 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 [7]. 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 × 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 [8].

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%:

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

Protein

The protein content was determined by micro-Kjeldhal method as given in AOAC [9]. 2 g of sample was taken in a digestion flask followed by addition of 3 g of digestion mixture (K₂SO₄:CuSO₄:SeO₂ 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:

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

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

Fat (ether extract)

For estimation of fat, Soxhlet method [9] 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.* [10]. 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 washing the mortar and to it 2 ml of HCl solution (diluted 1:2 with distilled water) was added. The contents were transferred to Kjeldahl 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:

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

Total plate count (TPC)

It was determined by the APHA [11] method using plate count agar. 1 ml of appropriate dilution of sample was transferred aseptically to sterile petriplates in triplicate. The plates were then poured with 10–15 ml melted agar medium at 45 °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 where 8 denoted extremely desirable and 1 denoted extremely poor. [12] 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 [13] and means were compared by using Duncan's multiple range test [14].

Results and Discussion

The results depicted in Table 1 revealed a significant ($P<0.05$) rise in pH and WHC of cooked meat 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 meat treated with 1.5% can also be partly explained by the increased pH [15]. 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* [16], and Para *et al.* [17]. 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 [18] and also may be due to slight denaturation of sarcoplasmic proteins, which play an important role in determining WHC [17, 19]. The above results thus indicate that the treatment with crude extract of drumstick leaves improved the functional properties of muscle proteins.

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

Parameter	Control	1%	1.5%	2%
pH	6.20 \pm 0.05 ^a	6.40 \pm 0.05 ^b	6.60 \pm 0.05 ^c	6.60 \pm 0.05 ^c
WHC	12.50 \pm 0.05 ^a	13.0 \pm 0.57 ^a	15.50 \pm 0.28 ^b	15.83 \pm 0.46 ^b
CL %	36.70 \pm 0.05 ^b	35.0 \pm 0.57 ^a	34.0 \pm 0.57 ^a	34.0 \pm 0.57 ^a
Moisture	64.0 \pm 0.57	63.57 \pm 0.23	64.23 \pm 0.14	64.50 \pm 0.28
Protein	25.63 \pm 0.29 ^{bc}	24.63 \pm 0.29 ^b	25.23 \pm 0.14 ^{bc}	2.50 \pm 0.28 ^a
EE	9.50 \pm 0.28	9.40 \pm 0.30	9.50 \pm 0.28	9.66 \pm 0.22
TBA	0.48 \pm 0.55 ^b	0.25 \pm 0.02 ^a	0.23 \pm 0.08 ^a	0.25 \pm 0.02 ^a
TPC (log cfu/g)	4.0 \pm 0.57	3.50 \pm 0.28	3.00 \pm 0.57	3.23 \pm 0.14

*Mean \pm SE with different superscripts in a row differs significantly ($P<0.05$), n = 6 for each treatment

A significant ($P<0.05$) reduction in cooking loss (CL %) 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 [20]. Superoxide anion radical (O_2^-) is a precursor to active free radicals that has the potential of reacting with biological macromolecules and there by inducing tissue damage [21, 22]. The crude extract of *Moringa* leaf can actively scavenge free radicals and thus prevent cellular damage [23]. The increased meat pH by crude extract can also account for the observed decrease in cooking loss [24]. Here we can see the cooking loss is minimum in 1.5% treatment.

The results in Table 1 also showed that the moisture 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.

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* [25]. They act as chain-breaking peroxy-radical scavengers which lead to the inhibition of lipid peroxidation and also prevent low density lipoprotein (LDL) oxidation [5, 26]. As product stability (rancidity) was measured by TBA assay [27], 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 rohu 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 [27]. TPC in control sample was found to be log

2.96 \pm 0.22 CFU/g. After adding the leaf extract at 1%, 1.5% and 2% levels, TPC was found as log 2.95 \pm 0.29 CFU/g, 2.72 \pm 0.17 CFU/g and 2.65 \pm 0.19 CFU/g respectively. According to Baumann [1], the maximum limit of TPC for acceptability of a product is log 5.0 CFU/g. Hence, rohu meat 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 rohu meat 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 and partly by presence of aerobic bacteria [27-31]. The crude extracts of drumstick leaves can considerably scavenge free radicals [23] and thus retain the colour.

Table 2: Sensory attributes of cooked rohu fillet treated with crude extract of *Moringa oleifera* leaves (Mean \pm SE)

Parameter	Control	1%	1.5%	2%
Colour	6.60 \pm 0.05 ^a	6.80 \pm 0.11 ^{ab}	7.22 \pm 0.14 ^{bc}	7.30 \pm 0.18 ^c
Flavour	7.70 \pm 0.05	7.31 \pm 0.09	7.33 \pm 0.16	7.50 \pm 0.28
Juiciness	6.20 \pm 0.05 ^a	6.70 \pm 0.05 ^b	6.90 \pm 0.05 ^c	6.90 \pm 0.05 ^c
Tenderness	6.33 \pm 0.08 ^a	6.60 \pm 0.05 ^a	7.76 \pm 0.16 ^b	7.30 \pm 0.18

*Mean \pm SE with different superscripts in a row differs significantly ($P<0.05$). Mean values are scores on 8 point descriptive scale where 1- extremely poor and 8- extremely desirable. n = 21 for each treatment

The flavor score also showed a significant ($P<0.05$) improvement and meat 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 [24, 33-36].

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 rohu meat treated with 1.5% scored greater value than the other treated samples.

Conclusion

Based on the findings of the present study, 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 rohu meat cubes. They also improved the quality of the fish meat by enhancing the tenderness, juiciness and preventing discoloration. The study also indicated that the three different levels of crude extract of *Moringa* leaf can be successfully used as a food or fish meat additive due to its strong effect in preventing off-flavor formation and also in improving the organoleptic quality of cooked fish 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.

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