









# Study on the antioxidant properties of *Moringa oleifera* leaf aqueous extract and its effect on the sensory attributes of raw and cooked Japanese quail meat<sup>#</sup>

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## Abstract

The study was conducted to standardise the concentration of *Moringa* leaf aqueous extract (MLE) as a natural antioxidant for applying on Japanese quail carcasses and its effect on the sensory attribute using semi-trained panellists. The concentration of the extract was selected initially on the basis of phytochemical and antioxidant activities of MLE. The total phenolics and flavonoid content of MLE was  $72.16 \pm 1.80$  mg GAE/mL and  $12.65 \pm 0.83$  mg QE/mL of MLE. Analysis of the antioxidant activity of MLE was expressed in terms of IC<sub>50</sub> (half maximal inhibitory concentration) value and four best concentrations having antioxidant activity greater than that of the IC<sub>50</sub> concentration were selected. IC<sub>50</sub> concentration obtained for MLE was  $4.60 \pm 0.20$  per cent and 5, 10, 15 and 20 per cents of MLE were selected, having antioxidant activities of  $52.22 \pm 1.09$ ,  $71.50 \pm 2.23$ ,  $82.53 \pm 1.84$  and  $87.30 \pm 1.46$  per cents, respectively. Freshly prepared cold MLE solutions were used for dipping of quail carcass for five minutes against cold distilled water as negative control and cold water with synthetic antioxidants (BHA and BHT) as positive control. Sensory evaluation of the raw and cooked quail meat was conducted. No significant difference ( $p > 0.05$ ) was observed. between treatments and controls in the sensory parameters of raw quail carcasses.

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But there was a significant ( $p < 0.05$ ) difference in sensory attributes of cooked quail carcasses. Carcasses treated with 20 per cent MLE had lower flavour values and overall acceptability scores compared to other treatments and controls. The results indicated that dipping of quail carcasses in MLE (upto 15 per cent) had higher sensory scores and it can be used as a natural antioxidant without affecting the sensory quality.

**Keywords:** *Moringa leaf aqueous extract, antioxidant activity, quail meat, sensory attributes*

Meat consumption in India is increasing, and poultry meat is the most popular meat due to its affordability, small size of birds and freedom from religious taboos. Japanese quail (*Coturnix coturnix japonica*) meat is one of the alternatives to poultry meat, having low fatty acid composition, low cholesterol, high polyunsaturated fatty acids and essential amino acid content, good source of oleic acid and high biological value, preferred by people of all age groups (Swain *et al.*, 2006). Nowadays, consumers prefer fresh and chilled meat over frozen meat. Higher unsaturated fatty acid content makes poultry meat more prone to oxidative rancidity (Moawad *et al.*, 2018). Oxidative rancidity causes retardation of the quality of meat by changes in meat colour, texture, flavour, nutritional losses and poor shelf life. Thus, quail meat is highly perishable having relatively short shelf life of three to four days under chiller conditions of  $4 \pm 1$  °C (Lin *et al.*, 2004).

Many commercial producers try to extend the shelf life using synthetically available antioxidants without concern about human health. Synthetic antioxidants, including BHT and BHA, have been reported to have carcinogenic, tumour promotion and cytotoxic effects by long-term use (Castro *et al.*, 2017). So, consumers prefer natural ingredients than others. *Moringa oleifera* leaf is cheaply available in Kerala and is a rich source of ascorbic acid, phenolics, flavonoids and other phytochemicals and acts as a good antioxidant (Chakraborty *et al.*, 2017). Therefore, this present study focuses on quantifying the

major phytochemicals in moringa leaf aqueous extract, their antioxidant activity and finding optimised extract concentration for the dipping of quail carcasses with good sensory attributes from consumers.

Fresh mature *M. oleifera* leaves were collected from the Mannuthy college campus and moringa leaf powder was prepared as per the method suggested by Mashau *et al.* (2021). Fine powder was put in a polyethene bags until extraction, which was closed and stored in a cool dry place. Moringa leaf extract was prepared by mixing about 20g of dried powder with 100mL distilled water as per the method of Prabakaran *et al.* (2018) and the resulting extract was stored in an air-tight container for 24 hours at 4°C.

Phyto-chemical analysis and antioxidant activity of moringa leaf extract were conducted initially and each day before application on quail carcass. The total phenolic and flavonoid contents in the *M. oleifera* leaf extract were determined by the Folin-Ciocalteu method as described by Mashau *et al.* (2021) and aluminium chloride ( $AlCl_3$ ) method described by Sankhalkar and Vernekar (2016) respectively, with slight modifications. The total phenolic and flavonoid amounts were calculated as gallic acid equivalents (mg GAE/mL) of plant extract and quercetin equivalents (mg quercetin QE/mL) from the standard curve using different concentrations of gallic acid and quercetin as standards, respectively. The DPPH free radical scavenging activity of each sample of different dilutions of moringa leaf extract was determined using UV-VIS spectrophotometer (Model No: Systronics-119, UV-visible spectrophotometer, Ahmadabad, India) according to the method described by Wong *et al.* (2006) with slight modifications and IC50 (half -maximal inhibitory concentration) was calculated from the different concentrations of the extract.

Standardisation of concentration of moringa leaf extract (MLE) for application on quail carcass was conducted by a pilot study using different concentrations of MLE and different time combinations. Different concentrations of aqueous MLE were prepared and kept in chiller to cool it to 4 °C (Pre – cold at

4±1°C). Quails were slaughtered scientifically and dipped immediately after slaughter in selected concentrations of moringa leaf extract for 5 minutes. Carcass to extract solution ratio was 1:3 by weight and they were then removed from the solutions and allowed to drain on a stainless steel wire mesh screen for 3 minutes. Carcasses were then packed aerobically in polyethylene bags and kept refrigerated for 2 hours for myoglobin oxygenation, then subjected to sensory evaluation. Colour and odour attributes of raw and cooked quail meat (appearance, flavour, juiciness and tenderness) were evaluated using semi-trained scientific panel using 6- and 9-point hedonic scales, respectively at Meat Technology Unit, Mannuthy. The experiment was repeated six times. The best-accepted concentration of MLE was compared with positive control (quail carcass treated with a combination of 0.01 per cent BHT and 0.01 per cent of BHA) and negative control (quail carcass with no antioxidant). The most accepted concentration was selected based on the sensory attributes of raw and cooked quail meat, and the experiments were then repeated six times.

The total phenolic content of MLE ranged from 66.11 to 84.92mg GAE/mL of extract with a mean value of 72.16 ± 1.8mg GAE/mL. Iqbal and Bhanger (2006) reported that total phenolic content of moringa leaf powder was 88.2 to 127.9 mg /g. According to Das *et al.* (2011) and Muthukumar *et al.* (2014), total phenolic acid content was 45.81mg/g and 60.78 to 70.27 mg of tannic acid equivalents/g, respectively. Total flavonoid content of prepared extract ranged from 8.43 to 15.91mg QE/mL of extract with a mean value of 12.65 ± 0.83 mg QE/mL of MLE. Abo El-Fadl *et al.* (2020) studied different species of moringa plants

and it was found that the flavonoid content of MLE extracted using different solvents ranged between 6.28 to 7.21g QE/100g. There were variations in the phytochemical contents of different moringa leaf extracts and it might be due to the variation in the geographical location, stage of maturity, drying process and solvent used for the extraction.

Antioxidant activity is expressed in terms of IC50 value and the mean IC50 value was 4.60±0.20 per cent of MLE. The best four concentrations with antioxidant activity higher than IC50 value were selected for application on quail carcasses. The concentrations of 5, 10, 15 and 20 per cent of MLE having mean antioxidant activity value of 52.22 ± 1.09, 71.50 ± 2.23, 82.53 ± 1.84 and 87.30 ± 1.46 per cent, respectively were selected. The results obtained were similar to the findings of Sreelatha and Padma (2009), Das *et al.* (2011) and Aravind *et al.* (2022) who observed direct relationship between the concentration and phenolic content of the extract and the antioxidant activity of the MLE. Sreelatha and Padma (2009) observed an IC50 value of 18.54µg/mL from the Soxhlet extracted water extract of moringa leaves. The mean values of phenolics and flavonoid and DPPH activity of four different extract concentrations were studied and are presented in Table 1. Data was statically analysed. There was a significant difference observed among the treatments with different concentrations of phenolics and flavonoids. However, there was no significant difference observed in the antioxidant activity between the 15% and 20% extract concentrations. There was a direct relationship between phenolic content and antioxidant activity of the MLE. This result was similar to the results obtained for Muthukumar *et al.* (2014).

**Table 1.** The Mean (±) SE values of total phenolics content, total flavonoid content of moringa leaf extract (MLE) and total antioxidant activity of selected concentrations of MLE.

Concentration of MLE	Concentration of phenolics content (mg GAE/mL)	Concentration of flavonoid content (mg QE/mL)	Antioxidant activity (%)	p-Value
4.6 ± 0.20 per cent	37.04	5.96	IC50	
5 per cent	45.15 ± 3.47 <sup>a</sup>	8.19 ± 0.10 <sup>a</sup>	52.22 ± 1.09 <sup>a</sup>	<0.001
10 per cent	82.95 ± 6.23 <sup>b</sup>	14.90 ± 0.10 <sup>b</sup>	71.50 ± 2.23 <sup>b</sup>	<0.001
15 per cent	109 ± 4.43 <sup>c</sup>	20.40 ± 0.87 <sup>c</sup>	82.53 ± 1.84 <sup>c</sup>	<0.001
20 per cent	128.10 ± 1.85 <sup>d</sup>	25.24 ± 1.33 <sup>d</sup>	87.30 ± 1.46 <sup>c</sup>	<0.001

Standardisation of concentration of extract for application on quail carcass was using the selected concentrations of extract viz., 20 (M<sub>4</sub>), 15 (M<sub>3</sub>), 10 (M<sub>2</sub>) and 5 (M<sub>1</sub>) per cents, and they were used directly for dipping quail carcasses against negative (C<sub>1</sub>) and positive control (C<sub>2</sub>). There was no significant difference between treatments and controls in all the raw sensory parameters viz., appearance and colour, odour and overall acceptability (Table 2). From the raw sensory evaluation of quail carcasses, all treatments had higher sensory scores than negative and positive controls and overall acceptability scores were higher for M<sub>3</sub> i.e., dipping 15 per cent of MLE.

There was significant difference in the cooked quail meat sensory scores except tenderness scores of controls and treatments (Table 3). Treatment 3 was significantly ( $p < 0.001$ ) different from M<sub>4</sub>, M<sub>1</sub>, C<sub>2</sub> and C<sub>1</sub>. There was a significant difference in overall acceptability scores of cooked quail meat between C<sub>1</sub> and

M<sub>1</sub>, C<sub>1</sub> and M<sub>2</sub>, C<sub>1</sub> and M<sub>3</sub>, M<sub>3</sub> and M<sub>4</sub>, C<sub>2</sub> and M<sub>3</sub> and M<sub>1</sub> and M<sub>3</sub>. Carcasses treated with 20 per cent MLE had lower flavour values and overall acceptability scores compared to other treatments and controls. M<sub>3</sub> had higher values for all the sensory attributes except juiciness scores and significantly higher values ( $p < 0.05$ ) for appearance, flavour and overall acceptability scores. Hence, dipping of quail carcass in 15 per cent moringa leaves aqueous extract was suggested to reduce oxidative rancidity of quail carcasses after hygienic slaughter.

The present findings were in accordance with the results obtained for Mashau *et al.* (2020), who found significant decrease ( $p < 0.05$ ) in the sensory attributes viz. colour, springiness, taste and overall acceptability of ground beef with incorporation of moringa leaf powder (MOLP). No decrease was reported for juiciness and tenderness scores. Contrary to present findings, the authors observed higher overall acceptability scores for control than the

**Table 2.** Effect of different levels of moringa leaf aqueous extract concentrations on raw sensory attributes of quail carcass

Parameters	C <sub>1</sub>	C <sub>2</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	p-Value
Appearance and colour	5.00 ± 0.10	5.06 ± 0.09	5.06 ± 0.09	5.08 ± 0.07	5.24 ± 0.08	5.17 ± 0.08	0.171 <sup>ns</sup>
Odour	5.37 ± 0.07	5.14 ± 0.12	5.30 ± 0.09	5.20 ± 0.10	5.32 ± 0.09	5.26 ± 0.09	0.154 <sup>ns</sup>
Overall acceptability	5.31 ± 0.06	5.30 ± 0.08	5.33 ± 0.06	5.34 ± 0.07	5.39 ± 0.07	5.36 ± 0.06	0.708 <sup>ns</sup>

ns – non significant. The values are expressed as their Mean ± standard error. (Number of observations = 42) Based on six – point hedonic scales. 1 – very acceptable and 6 – extremely unacceptable.

(C<sub>1</sub> = Quail carcass with no antioxidants, C<sub>2</sub> = Quail carcass with combination of 0.01 per cent BHA and 0.01 per cent BHT, M<sub>1</sub> = Quail carcass treated with 5 per cent of MLE, M<sub>2</sub> = Quail carcass treated with 10 per cent of MLE, M<sub>3</sub> = Quail carcass treated with 15 per cent of MLE, M<sub>4</sub> = Quail carcass treated with 20 per cent of MLE)

**Table 3.** Effect of different levels of moringa leaf aqueous extract concentrations on cooked sensory attributes of quail meat

Parameters	C <sub>1</sub>	C <sub>2</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	p-Value
Appearance	6.98 ± 0.12 <sup>ab</sup>	6.98 ± 0.11 <sup>a</sup>	7.13 ± 0.09 <sup>ab</sup>	7.17 ± 0.07 <sup>ab</sup>	7.2 ± 0.07 <sup>b</sup>	7.08 ± 0.07 <sup>ab</sup>	0.005 <sup>*</sup>
Flavour	6.97 ± 0.08 <sup>bc</sup>	6.83 ± 0.11 <sup>ab</sup>	6.98 ± 0.13 <sup>bc</sup>	7 ± 0.12 <sup>bc</sup>	7.16 ± 0.08 <sup>c</sup>	6.55 ± 0.07 <sup>a</sup>	< 0.001 <sup>**</sup>
Juiciness	6.66 ± 0.08 <sup>a</sup>	6.86 ± 0.11 <sup>ab</sup>	6.95 ± 0.10 <sup>b</sup>	6.95 ± 0.07 <sup>b</sup>	6.89 ± 0.06 <sup>ab</sup>	6.89 ± 0.09 <sup>ab</sup>	0.012 <sup>*</sup>
Tenderness	6.97 ± 0.05	6.98 ± 0.06	6.91 ± 0.12	6.98 ± 0.1	7.11 ± 0.05	7.02 ± 0.06	0.359 <sup>ns</sup>
Overall acceptability	6.67 ± 0.10 <sup>a</sup>	6.83 ± 0.11 <sup>ab</sup>	6.93 ± 0.09 <sup>b</sup>	7.05 ± 0.09 <sup>bc</sup>	7.2 ± 0.06 <sup>c</sup>	6.88 ± 0.08 <sup>ab</sup>	< 0.001 <sup>**</sup>

\*\* - significant at 1% level, \* - significant at 5% level, ns – non significant, means with same superscript between columns have no significant difference between them. The values are expressed as their Mean ± Standard error. (Number of observations = 42) Based on eight – point hedonic scales. 1 – extremely acceptable and 8 – extremely unacceptable.

MOLP treated ground beef samples. Similar to the present findings, they reported unfamiliar odour scores in higher MOLP incorporated ground beef than in controls. Hussain *et al.* (2022) observed increased appearance scores in Ladakhi *churpe* with the addition of apricot and spinach powder. In accordance with the present findings, Das *et al.* (2011) and Abd El-Rahman *et al.* (2019), reported that MLE had no adverse effect on sensory attributes scores of fresh goat meat patties.

### Summary

Moringa leaf aqueous extract is a good antioxidant ingredient and can replace synthetic antioxidants during the decontamination process of quail carcasses. Prepared MLE had 72.16 mg GAE/mL and 12.65 mg QE/mL of phenolic and flavonoid contents, respectively. The selected four concentrations of MLE based on IC50 values *viz.*, 5, 10, 15 and 20 per cents showed antioxidant activity of 52.71, 71.50, 82.55 and 87.55 per cent, respectively in the study. Results of the study revealed that moringa leaf aqueous extract had a substantial amount of phytochemicals with good antioxidant properties and dipping of quail carcass in moringa leaf aqueous extract up to 15 per cent level had no adverse effect on the raw and cooked sensory attributes.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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