Oxidative stability and quality attributes of emu meat nuggets incorporated with selected levels of broccoli (Brassica oleracea) powder

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Abstract

The present study was conducted to develop emu meat nuggets with better oxidative stability by incorporating broccoli powder. Four different levels 0%, 4%, 6% and 8% were incorporated with the replacement of lean meat and optimum level was selected on the basis of various physico-chemical (pH, water activity and moisture), instrumental colour and sensory parameters. Oxidative stability was evaluated on the basis of total phenolics, ABTS and 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Water activity and moisture content decreased (p ≥ 0.05), whereas pH increased (p ≥ 0.05) with the increase in the level of incorporation of broccoli powder in the formulation. The total phenolics and radical scavenging activity was also concentration dependent correlated with the level of incorporation of broccoli powder. The sensory panelists observed poor juiciness and texture in emu meat nuggets incorporated with broccoli powder and sensory scores decreased with level of incorporation of broccoli powder. Results concluded that oxidative stability and total fiber content of emu meat nuggets has improved significantly (p<0.05) with some loss in sensory attributes.

Keywords: Emu meat, broccoli powder, functional products, oxidative stability and sensory parameters.

Introduction

Emu (Dromaius novaehollandiae) is flightless bird, native of Australia. It is very well adapted to different climatic conditions and immune to the diseases. The bird is reared for meat, oil, skin, feathers, eggs and toes. Emu meat has dark red appearance and resembles more with the red meat. Thus emu meat has low-fat, low-cholesterol and high in iron, zinc, phosphorus, magnesium, vitamin B₂, B₆ and B₁₂. On an average, a typical muscle of emu contains 73.80% moisture, 22.86% protein, 0.84% fat and 1.81% ash (Naveena et al., 2013). However, the utilization and consumption of emu meat products is limited due to high cost, tougher texture and bland flavor. The marketing and popularization of emu meat products is essential for the sustainability of emu industry. Chevon meat is a choice meat for various communities in India, which is a red meat, high in fat content and tougher texture. Emu meat can be a suitable alternative to it.

Emu meat like other meat is inherently low in antioxidant capacity. The lack of antioxidant capacity as well as availability of high quality nutrients lead to the problem of perishability of these products (Chan et al., 1993). Oxidative stability can be improved with the addition of natural as well as synthetic antioxidants. However, the incorporation of synthetic antioxidants in meat products may leads to health hazards if used injudiciously. Hence, this lead to a challenge for scientific fraternity to explore newer, novel antioxidant compounds from plant source including fruits and vegetables. Recently, various studies reported the incorporation of the vegetables in comminuted meat products with improved quality and health benefits (Eim et al., 2008; Bhosale et al., 2011; Mendiratta et al., 2013).

Brassica, cruciferous family (cauliflower, broccoli, cabbage, and brussels sprouts) is known to be a rich sources of antioxidants (Kurilich et al., 1999; Mukherjee et al., 2008; Kim et al., 2013). Broccoli (Brassica oleracea) is also good source of valuable nutrients such as Vitamin C and E. Broccoli has anti-cancer properties due to presence of 3,3-di-indolylmethane, a potent modulator of the innate immune response system. Broccoli powder and extract are rich source of various phenolic compounds (Kim et
al., 2013) and thus can be incorporated into meat products as a source of natural antioxidants to prolong quality and stability. In the present study, the oxidative stability and quality attributes of emu meat nuggets incorporated with selected level of broccoli (Brassica oleracea) powder were studied.

Materials and Methods

Fresh emu meat was procured from local emu farm (Sidhu Emu Farms, Raikot, Punjab). The carcass were hot deboned and meat was chilled overnight in refrigerator and packed in LDPE bags and stored under frozen condition (-18°C) till further use. Fresh broccoli were procured from local market, washed with clean water and sliced into small pieces. The sliced broccoli was heated in microwave oven at low frequency (914 Hz) for 12 minutes and then further dried in hot-air oven to a moisture level of <8%. The dried broccoli was ground in a grinder (Inalsa, India) to make fine powder. All other ingredients including spice and condiments (onion, garlic, ginger: 3:1:1) mixture used in the study were procured from the local market.

Preparation of nuggets: Emu meat nuggets were prepared as per the formulation given in Table 1. Emulsions were prepared in four groups as control (C) and treatments viz. T1, T2 and T3 with varying level of broccoli powder as 4, 6 and 8%, respectively. The deboned frozen emu meat was cut into small batches and minced twice in a meat mincer (Mado Eskimo MEW-714, Germany) through 6 mm and 4 mm plates and emulsion was prepared in a bowl chopper (Scharfen, Germany). The meat emulsion obtained was filled in aluminium mould and was cooked under pressure (15 lb) for 15 min. The cooked products were cooled and sliced into nugget of uniform size (10 x 15 mm) and analysed for various quality parameters.

Proximate analysis: Moisture (oven drying), protein (kjeldahl distillation), fat (soxhlet method) and ash (muffle furnace) content of both control and treated emu meat nuggets were determined by using standard procedure described by AOAC (2000).

Physico-chemical analysis: Emulsion stability (ES) was determined using the method described by Townsend et al. (1968) with some modifications. About 25 g emulsion samples were placed in polyethylene bags, sealed and heated at 80°C in a thermostatically controlled water bath for 20 min. After draining out the exudates, the cooked mass was cooled, weighed and the yield was expressed as percent emulsion stability.

Cooking yield was determined by measuring the difference in the sample weight before and after cooking.

\[
\text{Cooking yield} = \frac{\text{weight of cooked emu meat nugget}}{\text{weight of uncooked emu meat nugget}} \times 100
\]

The pH of emu meat nuggets was measured as per the procedure of Trout et al. (1992) using combined glass electrode of Elico pH meter (Model; LI 127, India).

Total phenolics: The polyphenol content was quantified by Folin-Ciocalteau’s reagent assay and expressed as Gallic acid equivalents (mg/g) (Yuan et al., 2005). For preparation of sample extracts the same procedure for DPPH was followed. About 100 µl of extract (250 µM concentration) was mixed with 2 ml of 2.0% Na2CO3 buffer and incubated at room temperature for 2 min. Total volume was made to 2.4 ml adding distilled water. After addition of 100 µl of 1 N Folin-Ciocalteau’s reagents, the reaction tubes were further incubated for 30 min at room temperature, and finally absorbance was read at 720 nm. The amount of total phenolics was determined by using standard calibration curve (y = 0.015x + 0.006; R2 = 0.9949; Where, y = absorbance, x = gallic acid concentration, and R2 = correlation coefficient) in which standard gallic acid solutions of 0.25-5.0 mg/ml concentration was used.

ABTS radical scavenging activity: The spectrophotometric analysis of ABTS\(^+\) radical scavenging activity was determined according to method of Shirwaikar et al. (2006). This method is based on the ability of antioxidants to quench the long-lived ABTS\(^+\) radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of standard antioxidants. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS\(^+\)) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowed the mixture to stand in the dark at room temperature for 16 h before use. Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.70 at t= 0 (t = 0 min) and equilibrated at 3°C exactly 6 min after initial mixing. About 4.9 ml of ABTS working standard solution was mixed with 100 µl of sample extract/standard and absorbance was measured after 20 min (t=20) at 734 nm. Gallic acid (50-400 µM/ml) was used as a standard antioxidant. The ABTS\(^+\) activity was calculated by using following formula:

\[
\text{ABTS activity} (\%) = \left[ \frac{(A_{t=0} - A_{t=20})}{A_{t=0}} \right] \times 100
\]
Table 1: Formulation for preparation of emu meat nuggets incorporated with broccoli powder

<table>
<thead>
<tr>
<th>Ingredients (% w/w)</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>T1</td>
</tr>
<tr>
<td>Emu Meat</td>
<td>70.0</td>
<td>66.0</td>
</tr>
<tr>
<td>Broccoli powder</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>Refined Oil</td>
<td>8.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Water</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Condiments</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Salt</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Sodium tetra pyro-phosphate</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Refined wheat flour</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Spices mix.</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Whole egg liquid</td>
<td>2.75</td>
<td>2.75</td>
</tr>
<tr>
<td>Sodium Nitrite</td>
<td>150 ppm</td>
<td>150 ppm</td>
</tr>
</tbody>
</table>

**DPPH radical scavenging activity**: The ability to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical by added antioxidants in the product was estimated following the method of Kato et al. (1988) with slight modifications. Fresh DPPH solution was prepared in ethanol before every measurement. In this method, about 5 g of sample was triturated with 20 ml of ethanol for 2 min. The contents were quantitatively transferred into a beaker and filtered through Whatman filter paper No 42. Then, 1 ml of the filtrate was mixed with 1 ml of 0.1M Tris-HCl buffer (pH 7.4) and 1 ml of DPPH reagent (250 µM) in test tubes. The content was gently mixed and then the absorbance in time, t= 0 min \( (t_0) \) was measured at 517 nm. The sample tubes were also incubated at room temperature (27±1°C) under dark for measurement of absorbance in time \( t=20 \text{ min} \) \( (t_{20}) \). Ethanol was used as blank sample. Gallic acid (200-600 µM/ml) was used as a standard. The free radical scavenging activity was calculated as a decrease of absorbance from the equation:

\[
\text{Scavenging activity (\%)} = 100 - \left( \frac{A_{t_0}}{A_{t_{20}}} \right) \times 100
\]

**Lovibond tintometer colour**: Colour profile was measured using Lovibond Tintometer (Model: RT-300, UK) set at 2 of cool white light (D65) and known as \( L^* \), \( a^* \), and \( b^* \) values. \( L^* \) value denotes (brightness 100) or lightness (0), \( a^* \) (redness/greenness), \( b^* \) (yellowness/blueness) values. The instrument was calibrated using a light trap (black hole) and white tile provided with the instrument. Then the above colour parameters were selected. The instrument was directly put on the surface of emu meat nuggets at three different points. Mean and standard error for each parameter were calculated.

**Sensory evaluation**: A seven member trained panel comprising of scientists and postgraduate students of the Department evaluated the samples for different sensory quality attributes viz. appearance and colour, flavour, tenderness, juiciness and overall acceptability using 8 point descriptive scale (Keeton, 1983), where 8=extremely desirable and 1=extremely undesirable. Three sittings \( (n=21) \) were conducted for each replicate. The panelists were seated in a room free of noise and odours and suitably illuminated with natural light. Coded samples at a temperature of 37°C were presented to the panelists. The potable water was provided in between samples to cleanse the mouth palate.

**Statistical analysis**: The data obtained from various trials under each experiment was subjected to statistical analysis (Snedecor and Cochran, 1994) for Analysis of Variance (ANOVA) and Duncan’s multiple range test (DMRT) to compare the means by using SPSS16 software package. Each experiment was replicated thrice and the samples were analysed in duplicate leading to total observation 6 \( (n=6) \). Sensory evaluation was performed by a panel of six member judges three times, \( (n=18) \). The statistical significance was expressed at \( p<0.05 \).

**Results and Discussion**: The results of different physico-chemical, proximate and processing parameters of emu meat nuggets incorporated with different levels of broccoli powder are presented in Table 2. Cooking yield was significantly \( (p<0.05) \) higher for nuggets incorporated with 8% broccoli powder than other treatments as well as control. The increase in the cooking yield might be due to higher water retention properties of the fiber in
broccoli powder. Garcia et al. (2002) also reported an increase in cooking yield and moisture retention in dry fermented sausage upon incorporation of fruit fiber and cereals. Mehta et al. (2013) and Mendiratta et al. (2013) also reported an increase in cooking yield of meat products on incorporation of fruit and vegetable fibers.

Emulsion stability (ES) was significantly (p<0.05) higher for T2 and T3 than control and T1. It might be attributed to the formation of better protein fat gel, three dimensional solid lattices structure in the broccoli powder incorporated emu meat nuggets. Increase in emulsion stability on incorporation of fiber in meat products was also reported by Mehta et al. (2013). Kumar and Tanwar (2011) also reported an increase in ES on incorporation of ground mustard in chicken nuggets.

The aw of the emu meat nuggets decreased significantly (p<0.05) with an increase in level of incorporation of the broccoli powder. It was significantly lower (p<0.05) in all treated products than control. This might be due to increase in water binding capacity of the products. The pH value of product increased significantly (p<0.05) in T2 and T3 as compared to control and T1. It is attributed to the pH of the broccoli.

The moisture content in emu meat nuggets was in the range of 51.08±0.48 to 67.43±0.32 percent. The moisture content was decreased with the increase in level of incorporation of broccoli powder in the product. This might be due to hygroscopic nature of the broccoli powder. Similar findings were also reported by many researchers on addition of different powders and fat replacers (Mendiratta et al., 2013; Kumar and Sharma, 2004; Mansour and Khalil, 1999). The ash content did not vary significantly (p<0.05) with the different levels of incorporation of broccoli powder. The protein and fat content of all the treatments were comparable. The protein and fat content of the control were significantly higher than treatments. This might be due to the lower protein and fat contents in treated products due to replacement of lean emu meat with broccoli powder.

**Table 2:** Effect of broccoli powder on the aw, pH and moisture of the emu meat nuggets incorporated with broccoli powder (Mean±SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking yield</td>
<td>86.18±0.42</td>
<td>86.42±0.45</td>
<td>86.45±0.38</td>
<td>88.02±0.48</td>
</tr>
<tr>
<td>Emulsion stability</td>
<td>91.21±0.91</td>
<td>91.81±0.30</td>
<td>92.98±0.18</td>
<td>93.50±0.38</td>
</tr>
<tr>
<td>aw</td>
<td>0.85±0.01</td>
<td>0.83±0.01</td>
<td>0.79±0.002</td>
<td>0.71±0.01</td>
</tr>
<tr>
<td>pH</td>
<td>6.21±0.01</td>
<td>6.28±0.01</td>
<td>6.35±0.01</td>
<td>6.39±0.01</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>67.43±0.32</td>
<td>64.87±0.30</td>
<td>63.76±0.41</td>
<td>62.08±0.48</td>
</tr>
<tr>
<td>Protein</td>
<td>19.85±0.37</td>
<td>18.85±0.22</td>
<td>18.40±0.32</td>
<td>17.86±0.72</td>
</tr>
<tr>
<td>Fat</td>
<td>8.52±0.75</td>
<td>7.85±0.52</td>
<td>7.59±0.62</td>
<td>7.38±0.22</td>
</tr>
<tr>
<td>Ash</td>
<td>3.28±0.02</td>
<td>3.78±0.04</td>
<td>3.85±0.07</td>
<td>3.95±0.08</td>
</tr>
</tbody>
</table>

n=6; T1=4% broccoli powder, T2= 6% broccoli powder, T3=8% broccoli powder

Mean±SE with different superscripts row wise differ significantly (p<0.05)

DPPH radical scavenging activity: DPPH free radical scavenging activity of emu meat nuggets with broccoli powder and control are shown in Fig 1. The percent radical scavenging activity of broccoli powder incorporated emu meat nuggets was increased significantly (p<0.05) with the increase in the levels of incorporation. DPPH free radical scavenging by antioxidants is due to their hydrogen donating ability; the more the number of hydroxyl groups, the higher the possibility of free radical scavenging ability (Chen and Ho, 1995). Kim et al. (2013) also reported the radical scavenging activity of broccoli powder. Banerjee et al. (2012) reported the DPPH radical scavenging activity of 2.25 mg and 3 mg broccoli powder was comparable to the activity of 50 and 100 ppm BHT, respectively. A linear correlation between radical scavenging activity and poly-phenolic content has been reported in an extensive range of vegetables and fruits (Robards et al., 1999; Kim et al., 2013).

**ABTS** assay: ABTS** scavenging activity showed increased significantly with the increasing levels of -
broccoli powder incorporation of emu meat nuggets. It has been observed that all the treated samples exhibited significantly (p<0.05) higher ABTS scavenging activity than control Fig 1. Amongst the treatments, sample with 8% broccoli (T3) showed highest ABTS activity followed T2 and T1. Control samples showed comparatively low rate percent inhibition in ABTS activity than treated samples. Several researchers reported ABTS activity of broccoli (Kim et al., 2013; Banerjee et al., 2012; Finley, 2003).

**Total phenolics:** The total phenolics content increased significantly with the increasing levels of incorporation of broccoli powder in emu meat nuggets (Fig 2). This could be due to the presence of natural antioxidants, including carotenoids, tocopherols, ascorbic acid and flavonoids in broccoli powder (Azuma et al., 1999; Kurilich et al., 1999; Banerjee et al., 2012). According to Dominguez et al. (2010) total phenolic contents (mg/g of dry matter) in the broccoli are different in the leaves and stalk and reported to be 99.37-135.64 and 8.13-11.74, respectively.

**Instrumental colour profile:** The level of incorporation of broccoli powder directed the decrease in the L* and a* value (Fig 3). The decrease in redness and yellowness (b* values) could be due to the innate green coloration of broccoli which turned to dark on cooking. Banerjee et al. (2012) also reported significant decrease (p<0.05) in chroma value upon the incorporation of broccoli powder extract at 1.5 and 2% levels and also concluded that the yellowness and chroma values of products with BPE slightly decreased at higher levels. Mitsumoto et al. (2005) have reported the discoloration of chicken meat patties with the addition of natural antioxidants like tea catechins.

![Antioxidant potential of emu meat nuggets incorporated with broccoli powder](image1)

n=6; T1=4% broccoli powder, T2= 6% broccoli powder, T3=8% broccoli powder

![Total phenolics](image2)

n=6; T1=4% broccoli powder, T2= 6% broccoli powder, T3=8% broccoli powder
Fig 2: Total phenolics contents in emu meat nuggets incorporated with broccoli powder

![Graph showing total phenolics contents](image)

n=6; T1=4% broccoli powder, T2= 6% broccoli powder, T3=8% broccoli powder

Fig 3: Instrumental colour profile of emu meat nuggets incorporated with broccoli powder

[Graph showing instrumental colour profile]

Table 3: Sensory attributes of emu meat nuggets incorporated with broccoli powder (Mean±SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance and Colour</td>
<td>7.14±0.08</td>
<td>6.89±0.06</td>
<td>6.32±0.06</td>
<td>6.00±0.08</td>
</tr>
<tr>
<td>Flavour</td>
<td>7.17±0.08</td>
<td>6.31±0.08</td>
<td>6.04±0.07</td>
<td>5.67±0.08</td>
</tr>
<tr>
<td>Tenderness</td>
<td>7.06±0.08</td>
<td>6.52±0.07</td>
<td>6.04±0.08</td>
<td>5.26±0.08</td>
</tr>
<tr>
<td>Juiciness</td>
<td>7.03±0.09</td>
<td>6.78±0.08</td>
<td>6.00±0.06</td>
<td>5.00±0.09</td>
</tr>
<tr>
<td>Overall acceptability</td>
<td>7.11±0.09</td>
<td>6.52±0.07</td>
<td>6.04±0.06</td>
<td>5.11±0.09</td>
</tr>
</tbody>
</table>

n=6; T1=4% broccoli powder, T2= 6% broccoli powder, T3=8% broccoli powder

Mean±SE with different superscripts row wise differ significantly (p<0.05)

**Sensory analysis:** Mean sensory scores for the control and emu meat nuggets with different levels of broccoli powder incorporated are given in Table 3. The colour and appearance scores of T3 were lowest (6.00±0.08). Nuggets with higher level of incorporation of broccoli powder showed a significant (p<0.05) decreasing trend for color and appearance, flavor, tenderness juiciness and overall acceptability. This reflects that the higher level of incorporation of broccoli powder into emu meat nuggets have resulted in negative impact on consumer perception. Banerjee et al. (2012) reported a non-significant difference in the organoleptic characteristics of control and broccoli powder extract and BHT incorporated goat meat nuggets.

**Conclusions**

Results concluded that the oxidative stability of the emu meat nuggets improved with the incorporation of broccoli powder in emu meat nuggets and it improved with the level of incorporation of broccoli powder, however the sensory quality attributes were affected with the higher levels of broccoli powder. The further research is recommended to develop processing strategies for the improvement of the sensory attributes of developed functional emu meat nuggets.
References


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