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## Effect of orange peel and *Moringa oleifera* extracts incorporation on quality of raw and cooked sausages

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**Abstract**

The study was conducted to evaluate the effect of aqueous and ethanolic extracts of orange peel and *Moringa oleifera* leaves on the sensory, proximate and physico-chemical properties of raw meat emulsion and cooked chicken sausages. It was found that treatments incorporated with 3% extracts of orange peel and *Moringa oleifera* leaves were comparable during sensory evaluation to control and BHT (100ppm) sausages. Addition of extracts did not cause any deleterious effect on the physico-chemical quality and proximate composition of various treatments in case of both raw and cooked sausages. It was concluded that 3% extract of natural antioxidants can be incorporated in chicken meat to develop chicken sausages without having compromised any quality parameters.

**Keywords:** Orange peel, *Moringa oleifera*, antioxidants, physico-chemical properties

**Introduction**

With the changing socioeconomic status as well as due to fast and busy life, people are fascinated by convenient food products including the precooked, processed and restructured meat products. Meat is one of the most perishable food items consumed worldwide. Quality deterioration in meat and meat products occurs due to oxidation of lipids and proliferation of spoilage microbes. It is a major concern due to loss of sensory and nutritional quality besides loss of economic value (Estevez, 2017) [5]. Oxidative reactions are enhanced after mincing, cooking, salting and refrigerated storage due to interactions of unsaturated fatty acids with prooxidant substances such as non heme iron (Estevez and Cava, 2004; Goulas and Kontominas, 2007) [6, 9]. Such interactions imparts negative changes on the quality attributes like colour, flavour, texture and also has a negative impact on the nutritional status of the product (Gonzalez *et al.*, 2008) [8].

Further, as awareness is increasing among consumers about the health related issues arising from the consumption of various processed meat products, their interest is growing more towards the consumption of products having natural ingredients than the synthetic, which promote health and lower the risk of diseases. The synthetic compounds with antioxidant properties like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) etc. are not encouraging in meat and allied industry due to their toxic potential and carcinogenic effect (Jayaprakasha *et al.* 2003) [11].

In vogue of present scenario, research has focused on medicinal plants to extract natural and low-cost antioxidants that can replace synthetic additives such as BHA and BHT. Utilization of natural preservatives offer adequate microbial safety replaces the toxic effect of chemical preservatives and has a health promoting effect. Moreover, utilization of natural preservatives has rendered high nutritional value foods microbiologically safe and free from chemical preservatives (Singh *et al.*, 2010) [21]. Processing industries of agricultural products and food generate phenolic rich waste products in substantial quantities which could be used as a valuable source of natural polyphenols.

Mandarin orange (*Citrus reticulata*) is most common among citrus fruits grown and it occupies nearly 40% of the total area under citrus cultivation in India. In citrus fruits, peels are reported to possess highest amounts of polymethoxylated flavones (PMFs) compared to other edible parts of the fruit (Manthey and Grohmann, 2001; Wang *et al.*, 2014) [13, 25]. According to Marin *et al.* (2002) [14] most of citrus by-products could be used as functional ingredients when

designing healthy foods (functional foods), especially non digestible carbohydrates (dietary fiber) and bioactive compounds (ascorbic acid and flavonoids).

*Moringa oleifera* Lam. (Family: Moringaceae) commonly known as horseradish tree, drumstick tree, moringa tree and marum tree is a medium sized, evergreen tree and widely grown in tropical and subtropical regions. It is not only a source of naturally occurring antioxidants (Dillard and German 2000)<sup>[4]</sup> but also used in traditional medicines due to its various pharmacological properties. The major phytochemical constituents in the leaves are phenolic compounds and flavonoids such as cryptochlorogenic acid, isoquercetin and astragalins (Verma *et al.*, 2009; Vongsak *et al.*, 2012)<sup>[23, 24]</sup>. Drumstick leaves have been cited in scientific literature as having antibiotic, antioxidant, antitrypanosomal, antiulcer, hypocholesterolemic and antispasmodic activities (Fahey, 2005)<sup>[7]</sup>. Das *et al.* (2012)<sup>[2]</sup> revealed that incorporation of 0.1 % MLE to goat meat patties did not have any negative effect on sensory quality of patties and they were acceptable even after 15 days of refrigerated storage. Najeeb *et al.* (2014)<sup>[15]</sup> analysed the proximate composition of restructured chicken meat block treated with 1% mint, drumstick and curry leaves powder and concluded that proximate composition (moisture 72.2–72.3 %, protein 19.2–19.4 %, fat 4.2–4.3 % and total ash 2.3–2.4 %) of fresh and refrigerated stored control and treated products was comparable.

## Material and Methods

### Materials Broiler chicken

#### Broiler chicken

Healthy birds of 6 weeks of age were procured from the Livestock farm, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. Birds were slaughtered in the slaughter house of Department of Livestock Products Technology as per scientific standard procedure. The dressed chicken were washed thoroughly, packaged and stored at  $-18\pm 1^{\circ}\text{C}$  till further use. The frozen chunks were drawn as per requirement and thawed overnight in a refrigerator ( $4\pm 2^{\circ}\text{C}$ ) and were used for further study.

### Preparation of orange peel and *Moringa oleifera* leaves extract

Orange peels were collected from local market juice shops of Hisar and *Moringa oleifera* leaves from tree located in university campus. Peels and leaves were washed and dried separately in the tray air drier at temperatures of  $50\pm 5^{\circ}\text{C}$  for 24–48 hours. After drying, both the samples were ground to fine powder. The powder was sieved and used to prepare aqueous and ethanolic extracts. Aqueous and ethanolic (by using 70% ethanol) extracts of orange peel and *Moringa oleifera* leaves were prepared by using 10 g sample in 100 ml of solvent with the incubation time of 6 hours with frequent shaking. Both the mixture of aqueous and ethanolic were filtered through muslin cloth and then filtered through Whatman No. 42 filter paper separately to obtain the respective plant extract. Fresh extract was used during product development and test procedures.

### Preparation of chicken sausages

The frozen deboned chicken meat was minced in an electrical meat mincer (3mm plate). Sausages were prepared by making meat emulsion using minced meat, common salt (2 %), sodium nitrite (0.02%), spice mix (2 %), condiments paste (3 %), vegetable oil (15%), semolina (5 %) and ice flakes/chilled

water (10 %). All ingredients were properly mixed in the electric meat mixer for sufficient time to make a fine emulsion. Treatments consisted of 3% of different extracts (orange aqueous, orange ethanolic, moringa aqueous, moringa ethanolic, combination of aqueous extracts and combination of ethanolic extracts) along with other ingredients. Emulsion thus formed was filled in cellulose casings by hand operated sausage filling machine. The sausages were steam cooked for 35 min. The cooked sausages were taken out, cooled in chilled water for 5 min and casings were peeled off. They were packaged in LDPE bags and stored at refrigerated temperature ( $4\pm 2^{\circ}\text{C}$ ) for further analysis.

### Analysis of samples

#### pH

The pH of cooked sausages was determined (Trout *et al.*, 1992)<sup>[22]</sup> with pH meter. Ten grams of sample was homogenized with 50 ml of distilled water for 1 minute using pestle and mortar. The electrode was dipped into the suspension and the pH value of the sample was recorded.

#### Cooking yield

The weight of raw sausages and cooked sausages was recorded and yield was expressed as percentage by using the following formula:

$$\text{Cooking yield} = \frac{\text{Weight of the cooked sausages}}{\text{Weight of raw sausages}} \times 100$$

#### Water holding capacity (WHC)

WHC was estimated according to Wardlaw *et al.* (1973)<sup>[26]</sup> with slight modification. In a 100 ml polycarbonate centrifuge bottle, finely minced meat sample (20 g) was taken and then 30 ml of 0.6 M NaCl solution was added to it, mixed with glass rod and stirred for 2 minutes on a mechanical shaker. After holding for 15 minutes at  $4^{\circ}\text{C}$  in order to allow the effect of salt to reach equilibrium, the meat slurry was again stirred for 1 minute on a shaker and immediately centrifuged at 5000 rpm for 10 minutes at  $-9^{\circ}\text{C}$  in a refrigerated centrifuge. The supernatant volume was measured and difference between the added and decanted solution was expressed as percentage of the initial weight of meat.

#### Proximate composition

The proximate composition was estimated using standard procedures as per AOAC (2005)<sup>[11]</sup>.

#### Moisture

Finally chopped sample (30 g) was weighed in dried aluminium dish and kept in hot air oven with lid opened at  $105\pm 5^{\circ}\text{C}$  for 16–18 h. After cooling in dessicator, loss in weight was calculated as moisture of the sample.

#### Protein

Protein estimation was done by using semiautomatic instrument by Pelican Equipments, Chennai. 0.25 g of moisture free sample and 10 ml of conc.  $\text{H}_2\text{SO}_4$  were transferred to a Kjeldahl digestion tube. A pinch of catalytic mixture was added and digestion was carried out till the appearance of blue green clear solution. After cooling, aliquot was diluted with distilled water. The diluted aliquot was made alkaline by mixing with 40% NaOH solution and was distilled. Liberated ammonia was collected in a conical flask containing 25 ml of boric acid solution and 2–3 drops of

mixed indicator. Contents of flask containing boric acid were titrated against 0.1 N H<sub>2</sub>SO<sub>4</sub>.

$$\text{Protein (\%)} = \frac{14 \times \text{Normality of acid used} \times \text{volume of acid used} \times 6.25 \times 100}{\text{Weight of sample (g)} \times 1000}$$

### Fat

The fat content in cooked product was estimated by solvent extraction method as per AOAC (2005) [1] using Socs Plus (SCS-6-AS, Pelican Industries, Chennai). Two grams of dried, ground sample was taken in an extraction thimble (Whatman No. 1 filter paper) fitted in a specially designed beaker. The initial weight of the empty beakers was noted (W<sub>1</sub>). The thimbles with the samples were placed in the beakers containing around 80 ml of solvent (petroleum ether). The extraction was carried out automatically using 3 segments programme. After the process was over, the beakers containing the fat residue were placed in hot air oven (100°C) for 20-30 minutes. The beakers were removed and cooled in desiccators. The final weight of the beakers was noted as W<sub>2</sub>. The fat percentage in the sample was calculated using the following formula:

$$\text{Fat \%} = \frac{\text{Final weight of beaker (W}_2\text{)} - \text{Initial weight of beaker (W}_1\text{)}}{\text{Weight of sample}} \times 100$$

### Ash

The ash content was estimated as per AOAC (2005) [1]. Moisture free sample (around 2 g) was taken in pre-weighed moisture free crucible. The crucibles were then placed on a hot plate for charring. After charring, the crucibles were transferred to muffle furnace set at 550°C for around 2 h. After cooling of the furnace, the crucibles were taken out in desiccators and final weight was recorded. Ash was calculated as the difference between weight of empty crucible and weight after ashing.

### Sensory evaluation

A four member experienced panel of judges consisting of teachers of the Department evaluated the samples for the sensory attributes of colour and appearance, flavour, texture, tenderness, juiciness and overall acceptability using 8-point descriptive scale (Keeton, 1983) [12], where 8= excellent and 1= extremely poor. The test samples were presented to the panelists after assigning the suitable codes.

### Statistical analysis

Each experiment was replicated thrice and each parameter was analyzed in duplicate. The data observed was analyzed using SPSS version 16.0 (SPSS, Chicago, Ill). One-way analysis of variance (ANOVA) was used for various parameters followed by tabulation of data. The level of significance were tested by comparing mean values using the least significant difference (LSD) test at 5 % levels (Snedecor and Cochran 1967) [19].

## Results and Discussion

### Sensory evaluation of chicken sausages

Colour and appearance scores were comparable between control and extract treated sausages. Similar findings were observed by Nowak *et al.* (2016) [17] in pork sausages supplemented with extracts of cherry and blackcurrant leaves. Narkhede (2012) [16] also observed non significant variations in appearance scores of chicken nuggets containing natural antioxidants as compared to control, supporting the results of

present study. Addition of extracts did not influence the flavour scores of fresh sausages as evident from comparable values. No significant difference was noticed in texture, juiciness and tenderness scores between control, BHT and extract treated sausages. The scores for OAA were similar for all the treatments and values ranged from 7.50 to 7.75. These observations were consistent with the study of Das *et al.* (2012) [2] who reported that addition of MLE had no effect on the sensory attributes. Devatkal *et al.* (2010) [3] also observed that sensory evaluation of goat meat patties treated with kinnow rind, pomegranate and seed powder indicated no significant differences.

### Proximate composition of raw meat emulsion and chicken sausages

In present study the moisture contents of the control and treated chicken meat emulsion were comparable, ranged from 65.06 to 66.53%. The moisture content was similar due to similar composition of emulsion and addition of different extracts had no effect. Similarly the protein and fat content of the control and treated emulsion did not show any distinguished variation. Protein was found to be varying from 15.84 to 16.62%. Hazra *et al.* (2012) [10] depicted that moisture, protein and ether extract did not differ significantly in control and 2% moringa leaves extract treated cooked buffalo meat. The ash content was alike in control and various treatments of the emulsion. The cooked sausages also followed similar trend as observed in raw emulsion. The results were also in conformance with Najeeb *et al.* (2014) [15] who revealed that proximate composition of restructured chicken meat blocks treated with 1% mint, drumstick and curry leaves powder separately was comparable with control and reference products (200 ppm BHT). The addition of extracts did not alter the proximate composition of chicken sausages. There was slight decrease in moisture and increase in protein of the cooked sausages as compared to emulsion which was expected as there is moisture loss during cooking.

### Physico-chemical composition of raw meat emulsion and chicken sausages

Addition of extracts did not affect the physico-chemical characteristics of the chicken sausages. The pH of the raw emulsion was comparable among different treatments. The pH values of cooked sausages also demonstrated similar trend. The recorded pH values ranged from 5.99 in OPAE 3 to 6.18 in OMAE 1 and MLEE 3. The results for pH values were in accordance with the findings of Naveena *et al.* (2008) who reported no significant difference in the pH of pomegranate rind powder (PRP) incorporated patties in comparison to control. Rojas and Brewer (2007) [18] also revealed that incorporation of grape seed extract, oleoresin rosemary and water-soluble oregano extract in beef and pork patties did not affect the pH values of patties during refrigerated storage.

Water holding capacity ranged from 43.70 to 45.95 in raw emulsion of chicken sausages treated with natural antioxidants. No significant difference was observed between control and treatments in water holding capacity. The results were similar to Shah *et al.* (2015) [20] who did not notice any significant variation in WHC of Moringa leaves extract treated and control beef samples.

Incorporation of extracts did not affect the cooking yield of the sausages as control, BHT and extract treated sausages had statistically comparable cooking yields. The recorded cooking yield varied from 92.94% to 95.57%. Comparable results were observed by Hazra *et al.* (2012) [10] who reported that

cooked ground buffalo meat treated with 1, 1.5 and 2% of drumstick leaves did not show any significant difference in the cooking yield compared to the control. Das *et al.* (2012)<sup>[2]</sup> also reported that there was no variation in cooking yield of control and raw ground goat meat patties treated with 0.1% MOL extract. Najeib *et al.* (2014)<sup>[15]</sup> demonstrated that the cooking yield of the restructured chicken slices treated with 1% powders of moringa, curry, mint and BHT (200 ppm) had no significant difference.

It can be concluded that 3% level of incorporation of various extracts of orange peel and *Moringa oleifera* leaves in raw chicken meat emulsion and cooked sausages had no significant effect on the sensory, proximate composition and physico chemical properties. These results indicate that orange and *Moringa oleifera* can be used as antioxidants in meat without any detrimental effect on the various quality parameters.

**Table 1:** Effect of different extracts of orange peel and *Moringa oleifera* leaves on proximate composition of raw chicken meat emulsion (Mean±SD) (n=6)

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	65.90±0.85	16.32±0.50	13.16±0.51	2.15±0.08
BHT	66.53±0.61	16.31±0.40	12.63±0.41	2.13±0.05
<b>Aqueous Extracts</b>				
OPAE 3	66.12±0.92	16.43±0.83	12.70±0.80	2.15±0.15
MLAE 3	65.17±0.76	16.62±0.65	12.29±0.34	2.25±0.06
OMAE 1	65.58±1.02	15.90±0.45	13.19±0.50	2.16±0.09
<b>Ethanollic Extracts</b>				
OPEE 3	66.44±0.50	15.84±0.31	12.44±0.91	2.17±0.11
MLEE 3	66.18±0.83	15.92±0.43	12.08±1.07	2.22±0.13
OMEE 1	65.06±1.06	16.52±0.49	13.05±0.57	2.20±0.08

BHT-100 ppm BHT, OPAE 3-3 ml of orange peel aqueous extract, MLAE 3-3 ml of moringa leaves aqueous extract, OPEE 3-3 ml of orange peel ethanolic extract, MLEE 3-3 ml of moringa ethanolic extract, OMAE 1-1.5 ml of orange peel aqueous extract +1.5 ml of moringa leaves aqueous extract, OMEE 1-1.5 ml of orange peel ethanolic extract +1.5 ml of moringa leaves ethanolic extract

**Table 2:** Effect of different extracts of orange peel and *Moringa oleifera* leaves on proximate composition of cooked chicken meat sausages (Mean±SD) (n=6)

Treatments	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	64.10±1.37	17.23±0.57	13.14±0.94	2.24±0.30
BHT	63.63±1.41	17.51±0.94	12.94±0.71	2.29±0.38
<b>Aqueous Extracts</b>				
OPAE 3	63.17±1.65	18.16±0.84	13.17±0.79	2.40±0.46
MLAE 3	64.32±1.33	17.14±1.32	13.02±0.60	2.60±0.33
OMAE 1	64.36±1.96	17.13±0.64	13.25±0.59	2.49±0.27
<b>Ethanollic Extracts</b>				
OPEE 3	63.64±1.59	17.52±0.85	13.05±0.94	2.72±0.31
MLEE 3	63.76±1.75	17.42±0.86	12.32±0.63	2.33±0.34
OMEE 1	63.45±1.32	17.67±0.72	12.42±0.98	2.67±0.28

BHT-100 ppm BHT, OPAE 3-3 ml of orange peel aqueous extract, MLAE 3-3 ml of moringa leaves aqueous extract, OPEE 3-3 ml of orange peel ethanolic extract, MLEE 3-3 ml of moringa ethanolic extract, OMAE 1-1.5 ml of orange peel aqueous extract +1.5 ml of moringa leaves aqueous extract, OMEE 1-1.5 ml of orange peel ethanolic extract +1.5 ml of moringa leaves ethanolic extract

**Table 3:** Effect of different extracts of orange peel and *Moringa oleifera* leaves on physico-chemical properties of chicken meat (Mean±SD) (n=6)

Treatments	pH (Raw emulsion)	Water holding capacity (%)	Cooking yield (%)	pH (Cooked)
Control	6.11±0.25	45.82±1.15	92.94±2.63	6.26±0.15
BHT	6.06±0.39	45.86±1.26	94.32±2.17	6.12±0.25
<b>Aqueous Extracts</b>				
OPAE 3	5.99±0.36	45.18±2.40	93.88±2.60	6.21±0.10
MLAE 3	6.15±0.33	45.96±1.57	94.43±2.86	6.23±0.11
OMAE 1	6.18±0.37	43.97±1.73	93.54±1.93	6.23±0.08
<b>Ethanollic Extracts</b>				
OPEE 3	6.05±0.29	43.70±2.59	95.57±2.26	6.25±0.07
MLEE 3	6.18±0.31	43.70±2.29	92.79±1.29	6.22±0.12
OMEE 1	6.13±0.22	43.81±2.00	93.92±1.07	6.24±0.11

BHT-100 ppm BHT, OPAE 3-3 ml of orange peel aqueous extract, MLAE 3-3 ml of moringa leaves aqueous extract, OPEE 3-3 ml of orange peel ethanolic extract, MLEE 3-3 ml of moringa ethanolic extract, OMAE 1-1.5 ml of orange peel aqueous extract +1.5 ml of moringa leaves aqueous extract, OMEE 1-1.5 ml of orange peel ethanolic extract +1.5 ml of moringa leaves ethanolic extract

**Table 4:** Effect of different extracts of orange peel and *Moringa oleifera* leaves on sensory scores of chicken meat sausages packaged in aerobic conditions and stored at 4±2°C (Mean±SD) (n=12)

Treatments	Colour	Flavour	Texture	Juiciness	Tenderness	OAA
Control	7.75±0.40	7.75±0.45	7.83±0.39	7.83±0.39	7.83±0.39	7.75±0.45
BHT	7.67±0.49	7.70±0.45	7.75±0.49	7.67±0.49	7.79±0.33	7.70±0.45
<b>Aqueous Extracts</b>						
OPAE 3	7.67±0.52	7.70±0.54	7.75±0.45	7.71±0.45	7.67±0.49	7.75±0.62
MLAE 3	7.67±0.49	7.67±0.65	7.67±0.49	7.58±0.51	7.67±0.39	7.75±0.45
OMAE 1	7.71±0.39	7.67±0.49	7.42±0.49	7.67±0.49	7.63±0.51	7.67±0.49
<b>Ethanollic Extracts</b>						
OPEE 3	7.67±0.78	7.54±0.67	7.67±0.49	7.71±0.45	7.75±0.45	7.67±0.49
MLEE 3	7.67±0.78	7.50±0.67	7.46±0.51	7.50±0.52	7.67±0.49	7.58±0.51
OMEE 1	7.67±0.49	7.46±0.50	7.52±0.51	7.58±0.51	7.50±0.52	7.50±0.52

Means with different superscripts in a row differ significantly ( $P \leq 0.05$ )

BHT-100 ppm BHT, OPAE 3-3 ml of orange peel aqueous extract, MLAE 3-3 ml of moringa leaves aqueous extract, OPEE 3-3 ml of orange peel ethanolic extract, MLEE 3-3 ml of moringa ethanolic extract, OMAE 1-1.5 ml of orange peel aqueous extract +1.5 ml of moringa leaves aqueous extract, OMEE 1-1.5 ml of orange peel ethanolic extract +1.5 ml of moringa leaves ethanolic extract

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