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COMPARATIVE STUDY OF EFFICACY OF CLOVE, CINNAMON, BLACK PEPPER AND ROSEMARY ESSENTIAL OILS AS ANTIOXIDANTS IN EMU (Dromaius Novaehollandiae) MEAT BALLS

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The present study was conducted to develop emu meat balls with better sensory attributes by incorporating various essential oils such as clove, rosemary, black pepper and cinnamon. Three different levels (0.02%, 0.04% and 0.06%) of each essential oil were incorporated into emu meatballs and the optimum concentration was selected based on various evaluation criteria such as sensory parameters, physicochemical, oxidative stability and proximate analysis using standard procedures. The essential oils and their concentrations had no significant effect on the cooking yield and cooking loss between treated samples (essential oil incorporated meat balls). The pH values were significantly higher ($P<0.05$) in control when compared to the treated samples. The moisture content and protein showed significant difference ($P<0.05$) between control and treated samples. The radical scavenging activity of treated samples had higher significant differences ($P<0.001$) based on the essential oils. The sensory attributes of meatballs incorporated with 0.02% of black pepper and clove essential oil scored high. Based on this finding, it may be suggested that 0.2% of black pepper and clove essential oil may be used to enhance the sensory attributes and nutritional properties of emu meatballs with minimal changes compared to control sample (without essential oil).

Keywords: Emu meatball, Essential oils, Physiochemical analysis, Sensory analysis

INTRODUCTION

Emus (*Dromaius novaehollandiae*) are the second largest member of the group of flightless birds, which include the emu, ostrich, rhea, cassowary and kiwi. The emu is totally marketable bird as it is reared for meat, oil, skin, feathers, eggs and toes. The emu oil is rendered from the fat of the emu and is extensively used for its antibacterial, anti allergic, anti-inflammatory and non toxic. The American Heart Association believes emu meat as a healthy alternative to beef. Emu meat has dark red appearance and resembles more with the red meat. Thus it has low-fat, low-cholesterol and high in iron, zinc, phosphorus, magnesium, vitamin B_2, B_6 and B_{12}. 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). Emu 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. Lipid oxidation and bacterial contamination are the main factors that determine food quality loss and shelf life reduction of meat and meat products and hence incorporating natural antioxidants is the best way to reduce the losses. Oxidative

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processes in meat lead to the degradation of lipids and proteins which, in turn, contribute to the deterioration in flavor, texture and color of displayed meat products (Decker *et al.*, 1995). As the food industry is facing great challenges to produce safe, and at the same time food without synthetic chemical preservatives, essential oils make their way into the scientific focus. Herbs of the Lamiaceae family, mainly oregano (*Origanumvulgare* L.), rosemary (*Rosmarinusofficinalis* L.), and sage (*Salvia officinalis* L.), have been reported as having significant antioxidant capacity (Fernandez *et al.*, 2005). Individual components of Essential Oils are also used as food flavorings, either extracted from plant material or synthetically manufactured (Oosterhaven *et al.*, 1995). This synergistic effect of components of essential oils was shown by evaluating two natural extracts of rosemary and lemon balm as antioxidants in cooked pork patties (Lara *et al.*, 2011). A number of essential oil components such as carvacrol, carvone, cinnamaldehyde, citral, p-cymene, eugenol, limonene, menthol and thymol have been registered by the European Commission and considered to present no risk to the health of the consumer. Newly, various studies reported the incorporation of the essential oils in comminuted meat products with improved quality and health benefits (Ahn *et al.*, 2002). United States Food and Drug Administration (FDA) has classified the substances as Generally Recognized As Safe (GRAS) or as approved food additives. Also there is rising concern of safety in regards of synthetic food additives which again hiked the interest of using essential oils as natural food additives and preservatives. Therefore there is much more attention which has been focused on the use of essential oils and spices to improve sensory characteristics, retard lipid oxidation and extend the shelf life of meat and meat products. Antioxidants are added to fresh and processed meat and meat products to prevent lipid oxidation, retard development of off-flavors, and improve color stability. In the present work, the efficacy of four different essential oils was studied in improving the sensory parameters, physiochemical analysis and antioxidant activity in developed emu meatballs.

MATERIALS AND METHODS

Fresh boneless and skinless emu (*Dromaius novaehollandiae*) leg muscle samples were collected from a leading emu slaughtered firm in Chennai, Tamilnadu, India. The birds were 15-24 months old and weighing 45-50 kg. The emu meat samples for the experiments were collected within 6 h of post-mortem from the farm. The samples were wrapped in sterile polyethylene bags in a thermocole container filled with ice and transported to the laboratory for further processing. The meat was chilled overnight at 4±1 °C in refrigerator till further use. Meat was washed thoroughly in good quality water to remove any unwanted particle if present on meat surface. Four essential oils namely blackpepper, clove, cinnamon and rosemary oils were obtained from local shop (Aromax Trading Pvt Ltd., Chennai). The ingredients of spice mix included green condiments (onion, garlic, and ginger at the ratio of 3:1:1), coriander powder, toast powder and salt were procured from local market.

Preparation of Spice Mix

The spice mix was prepared using onion (15%), garlic (3%), ginger (3%), green chillies (1.5%), coriander powder (1.5%) and salt (1.5%). The onions were peeled, washed and sliced into small pieces. After removing the extraneous matter the condiments were chopped into very smaller sizes to get a perfect paste. All the above ingredients were ground in grinder (Philips grinder, India) to form a fine paste.

Preparation of Emu Meat Balls

Meat was cut into small pieces and ground in a meat mincer (Philips grinder,India) to get a perfect minced emu meat. Five different ratios of meat spice emulsions (meat: spice mix) was prepared like 50:50, 60:40, 70:30, 80:20 and 90:10. Small balls out of each emulsion were made by hand (15 g). The meatballs were added to the hot oil (175 °C) and were fried for 2 to 5 minutes, stirring frequently, until well browned on all sides. The preliminary trials were conducted to access the best ratio of emulsions on the basis of sensory evaluation by trained sensory panel of 11 panelists. On the basis of sensory evaluation, essential oils (clove, rosemary, black pepper and cinnamon) were added to the optimized developed meat ball. The control and treated samples were prepared with varying levels of essential oil concentrations such as 0, 0.2, 0.4 and 0.6% for each essential oil (Table 1).

Proximate Analysis

The proximate analysis of the meat ball was carried out according to Association of Official Analytical Chemists (AOAC, 2002). All the analysis were done in triplicates.

Physiochemical Analysis

The pH was determined using a standardized electrode attached to a digital pH meter for control and treated samples. Cooking yield was determined by measuring the difference of the sample weight before and after cooking (Kumar *et al.*, 2013).

Table 1: Formulation of Control and Treated Emu Meat Balls (% w/w)

Essential Oils	Concentration (%)	Emu Meat	Spice Mix	Total
Control	-	70	30	100
Clove	0.2	69.8	30	100
	0.4	69.6	30	100
	0.6	69.4	30	100
Cinnamon	0.2	69.8	30	100
	0.4	69.6	30	100
	0.6	69.4	30	100
Black pepper	0.2	69.8	30	100
	0.4	69.6	30	100
	0.6	69.4	30	100
Rosemary	0.2	69.8	30	100
	0.4	69.6	30	100
	0.6	69.4	30	100

The cooking loss of emu meat balls was expressed as average proportion of the samples from each treatment.

Sensory Evaluation

The sensory evaluation of meatballs was conducted by eleven trained panel members based on the sensory quality attributes such as appearance, color, flavor, tenderness, juiciness and overall acceptability using 9 point hedonic scale (Keeton, 1983).

DPPH Radical Scavenging Activity

The antioxidant activity of essential oils is related to the presence of high phenol compounds. Antioxidants are compounds that eliminate undesirable effects of reactive oxygen in foods and neutralize free radicals, which prevent the breakdown of nutrients in perishable foods (Velasco and Williams, 2011). The ability to scavenge 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radical by added antioxidants as essential oils in the product was estimated following the method described by Kumar *et al.* (2013). Fresh DPPH solution was prepared in ethanol before every measurement. In this method, about 5 g of sample was mixed with 20 ml of ethanol for 2 min. From this solution 1 ml of the filtrate was mixed with 1ml of 0.1 M 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 test tubes with samples were incubated at room temperature (27±1 °C) under dark for measurement of absorbance in time t = 20 min (t_{20}). Ethanol was used as blank sample. Gallic acid (200-600 μM/m1) was used as a standard. The free radical scavenging activity was calculated as a decrease of absorbance from the equation:

Statistical Analysis

The results were expressed as mean ± standard deviation of the batches under study (n = 3). The data obtained from various trials under each experiment was subjected to two way ANOVA statistical methods for analysis of variance and Duncan's Multiple Range Test using SPSS version 19 statistical software package in order to study the effect of essential oils on developed Emu meatballs.

RESULTS AND DISCUSSION

Optimization of Spice mix

On the basis of sensory evaluation, the emu meatballs prepared from 70:30 emulsion were selected for further studies such as optimization of essential oil and concentration

Proximate Composition and Physiochemical Analysis

The effect of essential oil and its concentration on proximate composition and physiochemical parameters of Emu meat balls are given in Table 2. Data indicated that there was significant decrease (P<0.05) in the cooking loss in the treated samples compared to control meatballs. There was no significant differences observed between the treated samples based on essential oils as well as their concentrations (P>0.05). The cooking loss was occurred by loss of fat and evaporation of moisture during frying. Various researchers also reported that nonmeat ingredients such as flaxseed flour (Bilek and Turhan, 2009), ricebran (Huang *et al.*, 2005), okara (Turhan *et al.*, 2009) and lemon albedo (Aleson-Carbonell *et al.*, 2005) reduced the cooking loss of meatballs. The cooking yield in control meatball sample was significantly lower (P<0.05) than samples treated with essential oils. There was no significant differences observed between the treated samples based on essential oils and their concentrations (P>0.05). Garcia (2002) also reported an increase in the cooking yield and moisture retention in dry fermented sausage after the incorporation of the test ingredients like cereals and fiber.

Table 2: Effect of Essential Oils and their Concentrations on Proximate Composition (%) of Treated Emu Meatballs

Samples	Concentration	Cooking Yield (%)	Cooking Loss (%)	pH	Moisture Content (% w.b)	Protein	Ash	Fat
Control	-	86.17^{a}±0.08	1.51^{a}±0.02	6.54^{a}±0.01	67.68^{a}±0.56	19.69^{a}±0.07	3.30^{a}±0.07	3.35^{a}±0.02
Clove	0.2	88.93^{b}±0.96	1.13^{b}±0.01	6.19^{b}±0.01	66.77^{b}±0.57	19.81^{b}±0.01	3.76^{b}±0.01	3.74^{b}±0.09
	0.4	88.33^{b}±0.17	1.15^{b}±0.02	6.07^{c}±0.01	66.77^{b}±0.57	19.81^{b}±0.01	3.82^{c}±0.05	3.79^{b}±0.05
	0.6	88.76^{b}±0.13	1.15^{b}±0.04	5.89^{d}±0.09	67.83^{c}±0.02	19.87^{c}±0.03	3.86^{c}±0.05	3.79^{b}±0.10
Cinnamon	0.2	88.33^{b}±0.17	1.12^{b}±0.01	6.19^{b}±0.01	66.77^{b}±0.57	19.81^{b}±0.01	3.82^{c}±0.01	3.74^{b}±0.09
	0.4	88.33^{b}±0.17	1.13^{b}±0.02	6.07^{c}±0.01	67.83^{c}±0.02	19.81^{b}±0.02	3.86^{c}±0.05	3.74^{b}±0.08
	0.6	88.76^{b}±0.13	1.13^{b}±0.01	5.99^{d}±0.01	67.83^{c}±0.57	19.84^{b}±0.01	3.88^{c}±0.10	3.79^{b}±0.09
Black pepper	0.2	88.33^{b}±0.17	1.11^{b}±0.02	6.17^{b}±0.01	66.77^{b}±0.57	19.85^{c}±0.03	3.80^{b}±0.01	3.74^{b}±0.09
	0.4	88.33^{b}±0.16	1.13^{b}±0.17	6.09^{c}±0.01	66.77^{b}±0.57	19.85^{c}±0.01	3.81^{c}±0.01	3.76^{b}±0.09
	0.6	88.77^{b}±0.13	1.13^{b}±0.02	6.05^{c}±0.03	67.83^{c}±0.02	19.88^{c}±0.06	3.86^{c} ±0.05	3.77^{b}±0.08
Rosemary	0.2	88.33^{b}±0.16	1.12^{b}±0.02	6.19^{b}±0.11	66.77^{b}±0.57	19.84^{b}±0.03	3.70^{b}±0.10	3.74^{b}±0.08
	0.4	88.33^{b}±0.16	1.13^{b}±0.17	6.06^{c}±0.03	67.77^{b}±0.73	19.84^{b}±0.03	3.77^{b}±0.11	3.77^{b}±0.08
	0.6	88.76^{b}±0.13	1.15^{b}±0.04	5.99^{d}±0.01	67.83^{c}±0.02	19.85^{c}±0.01	3.80^{b}±0.10	3.79^{b}±0.10

Note: Datas are expressed as mean ± standard deviation. Data in the same row bearing different superscripts are statistically different at 5% level of significance (P<0.05).

The pH value of control meat ball is significantly higher (P<0.05) than the treated meat balls. There was high significant differences (P<0.001) between the concentrations of essential oils. The pH decreased with increasing concentration of essential oils. The essential oils, which are natural antioxidants, have acidic compounds, which decrease the pH on incorporation (Burt, 2004). Lara *et al.* (2011) also observed the decrease in pH during incorporation of natural antioxidants in pork patties. The moisture content showed significant difference (P<0.05) between control and treated samples. There was no significant differences between essential oils and high significant differences (P<0.001) between their concentration were observed for moisture content. The protein content showed significant differences (P<0.05) between the treated samples based on the essential oils and their concentration variations. There was no significant differences in fat content in treated samples (P>0.05). The fat content in treated samples showed significant differences (P<0.05) based on the concentrations of essential oils.

Sensory Analysis

Mean sensory scores for control and treated samples are shown in Table 3. The appearance and color showed significant difference (p<0.05) between the concentrations of essential oils in treated samples. The flavor was the major parameter in sensory attributes and showed high significant difference (P<0.001) between concentrations of essential oils. The meatballs treated with 0.2% black pepper essential oil maintained the flavor as control samples. The treated samples with higher concentration of essential oils showed a lower impact on the flavor and overall acceptability. Juiciness and tenderness had significant differences (P<0.05) between treated samples.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of control and treated Emu meatballs are presented in Table 4. The percentage of radical scavenging activity of treated samples had higher significant differences (P<0.001) based on the essential oils. There was no significance difference observed in concentration of within each essential oil, but significant

Table 3: Effect of Essential Oils and their Concentrations on Sensory Attributes of Treated Emu Meatballs

Sample	Concentration	Appearance and Colour	Flavour	Juiciness	Tenderness	Overall Acceptability
Control	-	7.66^{a}±0.33	8.00^{a}±0.33	7.67^{a}±0.33	7.66^{a}±0.33	7.56^{a}±0.23
Clove oil	0.2	7.66^{a}±0.33	7.33^{b}±0.33	7.66^{a}±0.33	7.66^{a}±0.33	7.56^{a}±0.23
	0.4	7.33^{b}±0.33	6.66^{c}±0.33	7.33^{b}±0.33	7.66^{a}±0.33	7.13^{c}±0.29
	0.6	7.33^{b}±0.33	5.66^{d}±0.33	7.33^{b}±0.33	7.33^{b}±0.33	6.90^{d}±0.20
Cinnamon oil	0.2	7.66^{a}±0.33	6.66^{c}±0.33	7.66^{a}±0.33	7.66^{a}±0.33	7.36^{b}±0.33
	0.4	7.33^{b}±0.33	7.00^{b}±0.57	7.33^{b}±0.33	7.66^{a}±0.33	7.30^{b}±0.20
	0.6	7.33^{b}±0.33	6.33^{c}±0.33	7.33^{b}±0.33	7.33^{b}±0.33	7.01^{c}±0.16
Black pepper oil	0.2	7.66^{a}±0.33	8.00^{a}±0.53	7.66^{a}±0.33	7.66^{a}±0.33	7.56^{a}±0.21
	0.4	7.66^{a}±0.33	7.33^{b}±0.33	7.33^{b}±0.33	7.67^{a}±0.33	7.40^{b}±0.11
	0.6	7.33^{b}±0.33	7.00^{b}±0.00	7.33^{b}±0.33	7.33^{b}±0.33	7.33^{b}±0.08
Rosemary oil	0.2	7.65^{a}±0.33	5.66^{d}±0.33	7.66^{a}±0.33	7.66^{a}±0.33	7.13^{c}±0.23
	0.4	7.33^{b}±0.33	5.66^{d}±0.33	7.33^{b}±0.33	7.33^{b}±0.33	6.91^{d}±0.06
	0.6	7.33^{b}±0.33	5.33^{d}±0.33	7.00^{c}±0.00	7.00^{c}±0.00	6.63^{d}±0.06

Note: Datas are expressed as mean ± standard deviation. Data in the same row bearing different superscripts are statistically different at 5% level of significance (P <0.05).

Table 4: Effect of Essential Oils and their Concentrations on the Oxidative Stability of Emu Meatballs

Sample	Concentration	DPPH Radical Scavenging Activity (%)
Control	-	19.83^{a}±0.94
Clove oil	0.2	65.52^{b}±0.60
	0.4	66.11^{b}±0.09
	0.6	67.65^{b}±0.06
Cinnamon oil	0.2	71.19^{c}±0.49
	0.4	71.38^{c}±0.45
	0.6	72.36^{c}±0.47
Black pepper oil	0.2	66.36^{b}±0.04
	0.4	66.29^{b}±0.56
	0.6	69.09^{c}±0.26
Rosemary oil	0.2	4.51^{d}±0.32
	0.4	4.53^{d}±0.31
	0.6	4.64^{d}±0.34

Note: Datas are expressed as mean ± standard deviation. Data in the same row bearing different superscripts are statistically different at 5% level of significance (P <0.05).

different was observed between essential oils. The DPPH free radical scavenging ability of antioxidants is due to their hydrogen donating ability which means that more the number of hydroxyl groups, the higher the possibility of free radical scavenging ability (Cwen and Chit, 1995).

CONCLUSION

The results from this research work concluded that the concentrations of essential oils had significant effect on the protein, moisture content, pH and ash percentage of Emu meatballs. There was improvement in the oxidative stability with the incorporation of essential oils in the Emu meatballs. The clove, cinnamon and black pepper was significantly higher in maintaining a low level of oxidation in samples than rosemary essential oil. The clove, cinnamon and black pepper was significantly higher in maintaining a low level of oxidation in samples than rosemary essential oil. However, the sensory attributes showed the maximum acceptance for meatballs treated with 0.2% of black pepper and clove essential oils. Further research is recommended for extending the shelf life of emu meatballs by incorporating 0.2% of clove and black pepper essential oils.

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