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Effect of Black Cumin and Arjuna Fruit Extract on Lipid Oxidation in Pork Nuggets during Refrigerated Storage

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ABSTRACT

In this study, the efficacy of arjuna fruit extract (AFE, 1.0 %) and black cumin extract (BCE, 1.5%) in retarding lipid oxidation of pork nuggets was investigated and compared with a synthetic antioxidant, butylated hydroxytoluene (BHT, 100 ppm) and control sample during refrigerated storage at 4±1oC for 20 days under aerobic packaging. The total phenolics content of AFE treated cooked pork nuggets was significantly ($p < 0.05$) higher compared to control but nuggets with BCE extract had comparable total phenolics with BHT treated nuggets. However, incorporation of AFE, BCE or BHT did not have any effect on the cooking yield of pork meat nuggets. The increase in pH during storage was significantly ($p>0.05$) less in all treated samples as compared to control. The pork nuggets with AFE had significantly ($p<0.05$) lower mean total aerobic plate count than control. In samples incorporated with AFE and BCE, thiobarbituric acid reactive substances (TBARS) value, peroxide formation, free fatty acids were significantly ($p<0.05$) lower than control even on 20th day of storage. Sensory evaluation revealed that incorporation of AFE and BCE improved the flavour and overall acceptability scores of pork nuggets than control and prolonged the shelf-life of the product up to 20 days by inhibiting lipid oxidation and microbial growth. From the above findings, it is concluded that AFE at 1 % and BCE at 1.5 % were effective and could be used as natural antioxidant in retarding lipid oxidation of processed muscle foods.

Keywords: *Black cumin, Arujna fruit, Meat, Lipid oxidation*

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INTRODUCTION

Meat and meat products are one of the major sources of essential nutrients such as protein, fat, and minerals in our daily diet. However, due to the fat content, mainly unsaturated fatty acids, these products are easily oxidized during storage. Off flavours due to lipid oxidation not only deteriorates the quality and shelf life of muscle foods but also reduces its sensory quality and consumer acceptability (Georgantelis *et al.* 2007; Das *et al.* 2016). Besides, protein oxidation negatively affects the physico-chemical properties and nutritional value of muscle foods (Lund *et al.* 2007; Estevez 2011) and could also affect the tenderness and water holding capacity of meat during storage due to structural changes/ modification of amino acids leading to carbonyl formation and decreased sulfhydryl content (Bekhit *et al.* 2013; Turgut *et al.* 2016). Moreover, primary and secondary oxidation products of the lipid oxidation react with protein and amino acids and form transverse cross-links resulting in a reduction of protein digestibility (Morzel *et al.* 2006). Therefore, delaying two factors i.e. lipid and protein oxidation is important to maintain the quality of meat products for longer period.

In processed food industry and in particular, meat industry, antioxidants are often used to prevent lipid oxidation and may be for controlling or minimizing off-odor development in meat products. Therefore, additives of natural origin over synthetic ones have been the subject of interest for many investigators in recent years. Studies using plant extracts of rosemary (Lund *et al.* 2007), potato peel (Kanatt *et al.* 2005), phenolics from pomegranate peel (Naveena *et al.* 2008), catechins from tea (Mitsumoto *et al.* 2005) have shown positive effect on the inhibition of lipid and/or protein oxidation in food products. However, studies involving the efficacy of extracts from black cumin (*Nigella sativa L.*) seed and Arjuna (*Terminalia arjuna*) fruit in muscle food system are scanty, although both are extensively used in preparation of traditional herbal medicine for treatment of various ailments. Black cumin is well known for its nutritional and pharmaceutical uses. Various scientific investigations on the seed and its oil indicate a number of bioactivities for the plant, which include antibacterial, antifungal and antioxidant activities (Toma *et al.* 2015; Chauhan *et al.* 2018a). Likewise, plant components of Arjuna, contain different active components and possesses antimicrobial, cytotoxic, antidiabetic (Ragavan and Krishnakumari 2006), antidiarrheal, antidysentric (Alawa *et al.* 2002) and hepatoprotective (Doorika and Ananthi 2012) and antioxidant (Chauhan *et al.* 2018b) activities.

In spite of having enormous medicinal importance and antioxidant activity, hardly any study has been conducted to investigate the efficacy of extracts from black cumin seed and arjuna fruit in muscle food system. Keeping this in view, the present study was carried out to explore the antioxidant efficacy of black cumin seed and Arjuna fruit extract in retarding lipid oxidation of cooked pork nuggets during refrigerated storage.

MATERIALS AND METHODS

Preparation of black cumin seed and arjuna fruit extracts: Black cumin (*N. sativa*) seeds and T. arjuna fruits were obtained from the local supermarket (Kolkata, India), cleaned to remove extraneous dirt (in case of T. arjuna, outer part of these fruits was scrapped), dried in a hot air oven at 45±2°C for 8-10 hours and ground in a grinder (Kenstar, Mumbai, India) to obtain fine powder. A 100 g

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of powder each of black cumin seed and Arjuna fruit was refluxed with distilled water (1000 mL) for 2 h. Each extract was filtered through cheese cloth after cooling. The residue was refluxed again for an hour. All the filtrates were collected and centrifuged (Hermle Z326K, Germany) at 10,000 X g for 15 min. The filtrate was again passed through filter paper to get Arjuna fruit extract (AFE) and black cumin extract (BCE).

Analysis of total phenolics content: The total phenolics content (TPC) of AFE and BCE was estimated by the Folin-Ciocalteu method following the procedure laid by Singleton and Rossi (1965). The results were expressed as mg of gallic acid equivalents (GAE) per 100 g of powder extract.

Antioxidant activity of extracts: The antioxidant activity of both the extracts was evaluated with DPPH scavenging assay (Fargere *et al*. 1995). The per-cent Free radical scavenging activity (FRSA) was calculated using the following formula:
FRSA (%) = (Absorbance-Control- Absorbance-Sample/ Absorbance-Control) x 100. The ferric reducing antioxidant power assay of the AFE and BCE was determined according to the method of Oyaizu (1986). An increase in the absorbance of the reaction mixture indicated the reducing power of the sample.

Preparation of pork nugget: Minced pork meat was purchased from a commercial producer (West Bengal Livestock Development Corporation, Kolkata) and meat nuggets were prepared following a standardized method (Das *et al.* 2016) using ingredients: minced pork (73%), ice flakes (10%), refined vegetable oil (7%), garlic-onion paste (3%) and wheat flour (3 %), dry spice mix (1.8%), salt (1.5%), sodium tripolyphosphate (0.3%) and sodium nitrite (150 ppm). With all these ingredients, 4 batches were formed for emulsion preparation. Salt, sugar, phosphate and nitrite were thoroughly mixed to minced pork meat in a bowl chopper for emulsion preparation and ice flakes were added during chopping to maintain a lower temperature. Garlic-onion paste, dry spice mix, fine wheat flour and chopping were continued until uniform mixing of all the ingredients. BCE (1.5%) and AFE (1%) were added during emulsion preparation adjusting ice flakes to two batches of emulsions (T_1 and T_2). Control group (C) had no antioxidant source but another group (T_3) included BHT (100 ppm) as a reference. Nuggets were prepared from cooked blocks and aerobically packed in low-density polyethylene (LDPE) pouches and kept at refrigerated temperature (4±1°C) for analysis at 0, 5, 10, 15 and 20 days of storage.

pH and cooking yield of pork nuggets: The pH was determined by blending 10 g of nugget sample with 50 mL of distilled water for a minute in a tissue homogenizer (Omni, Germany). Homogenized sample was kept for 5 minutes and then mixed again by shaking. The pH value was recorded with the help of digital pH meter (Model CP 901, Century Instrument Ltd. India) by immersing the electrode directly into the meat suspension. The cooking yield of nuggets was determined by recording the weight of each meat block before and after cooking and expressed in percentage as: weight of cooked meat block/weight of raw meat block × 100.

Determination of Lipid oxidation

Peroxide value: Peroxide value (PV) of nugget sample was determined as per the procedure described by Koniecko (1979) with slight modifications. Briefly, nugget sample (5g) of each group (C, T_1, T_2 and T_3) was blended with anhydrous sodium sulphate and 30 ml chloroform. The mixture was filtered through Whatman filter paper No. 1. To 25 ml aliquot of the filtered chloroform extract; 30 ml of glacial acetic acid and 2 ml of saturated potassium iodide solution were mixed. After 2 min, 100 ml of DW (distilled water) and 2 ml of fresh 1% starch solution were added to the content and titrated immediately against 0.1 N sodium thiosulphate till the end point was reached (non-aqueous layer turned to colourless). The PV was expressed in mEq/kg of the sample.

Thiobarbituric acid reacting substances (TBRAS) values: Lipid oxidation was performed by measuring thiobarbituric acid reacting substances (TBARS) values following the method of Witte *et al.* (1970) with slight modifications. Briefly, 10 g of sample from each treatment group was triturated with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) for 2 min. The content was then quantitatively transferred into a beaker by rinsing with 25 ml of chilled DW, mixed and filtered. Three millilitres of TCA extract (filtrate) was mixed with 3 ml of thiobarbituric acid (TBA) reagent (5mM) in test tube and cooled in a running tap water after boiling in a water bath at 70°C for 35 minutes. A blank sample was made by mixing 3 mL each of 10% TCA and 5 mM TBA reagent. The absorbance was measured at a fixed wavelength of 532 using a UV-VIS spectrophotometer. The TBA value was calculated as mg malonaldehyde (MDA) per kg of the sample by multiplying the absorbance value with a factor of 5.2.

Free fatty acids: For FFA, 5 g of sample with 30 ml chloroform was blended for 2 min in the presence of anhydrous sodium sulphate and was filtered through filter paper (Koniecko 1979). The filtrate was titrated against 0.1 N alcoholic potassium hydroxide, after adding 2 to 3 drops of 0.2 g/100 ml phenolphthalein indicator solution, to get the pink colour end point. The quantity of potassium hydroxide required for titration was recorded as percentage of FFA.

Total plate count: The total plate count of samples from different treatment groups was determined at different storage intervals (0, 5, 15 and 20th day) by using pour plate method (Das *et al*. 2011). A 10 g of meat sample was homogenised in 90 ml of sterile peptone water (0.1%). Appropriate serial dilutions were prepared in 0.1% sterile peptone water and duplicate plated with plate count agar, incubated at 37°C for 48 h. Microbial colonies from the plates were counted and expressed as $\log_{10}$ CFU/g.

Sensory evaluation: A ten-member trained panel evaluated the pork meat nuggets during storage. Prior to analysis, the panellists were trained using 5 extra samples for 3 sessions before they started on real samples on the days of analysis. Samples were placed in covered cups coded with random 3-digit numbers and allowed to stand at room temperature prior to evaluation. The panellists were instructed to evaluate all samples using a seven-point hedonic

scale where 1=unacceptable, 2=very strong, 3=moderately strong, 4=slightly strong, 5=perceptible, 6=barely perceptible, 7=none for rancid odour whereas in case of color, 1=extremely dark brown, 2=very dark brown, 3=dark brown,4=dark red,5=slightly dark red,6=cherry red,7=light cherry. The panellists rated the samples for rancid odour three times repeatedly for 10s with interval of 20s in the analysis.

Statistical analysis: The present study was repeated three times and every time, measurements of all the parameters were done in duplicate. The data analysis was carried out using SPSS software (version 20.0). In case of storage study, data were analysed using two-way ANOVA with interaction taking treatment and storage time as main effects. The values were presented as mean along with standard error (Mean ± SE) and significance level was identified at the 95% confidence level (p<0.05).

RESULTS AND DISCUSSION

Total phenolics content and antioxidant potential of extracts: The total phenolics content and antioxidant potential of both the extracts are presented in Table 1. The results show that AFE contained significantly higher total phenolics content (13.42mg GAE/g) than BCE (4.43mg GAE/g). Chauhan *et al.* (2018b) reported similar range of phenolic content in AFE. As reports on TPC and antioxidant activity of AFE are very scanty, we could correlate our findings with *T. arjuna* bark or leaves extract. Sultana (2009) reported similar range of TPC in aqueous ethanolic extract using *T. arjuna* bark (12.85mg GAE/g dry weight). As far as TPC of BCE is concerned, our results are in close agreement with the reports (4.1 to 8.6 mg GAE/g) of earlier researchers (Thippeswamy and Naidu 2005; Toma *et al.* 2015, Chauhan *et al.* 2018a).

Table 1. Total phenolic contents (TPC) and DPPH radical scavenging activity of black cumin seed and arjuna fruit extract.

Extract	TPC (mg GAE/g)	DPPH IC_{50} (µg/ml)
Black cumin	4.43±0.48b	463±12.22a
Arjuna fruit	13.42±0.77a	11.42±0.45b

Mean ± SE bearing different superscript in a column differ significantly (p< 0.05); n=6

In the present study, significantly higher (p<0.05) DPPH radical scavenging activity with IC_{50} = 11.42 µg/ml was shown by AFE whereas BCE exhibited the lowest antioxidant activity (IC_{50} = 463µg/ml). Khattak and Simpson (2008) in their study found similar results by extracting N. sativa seeds in different solvents. Ratz-Łyko *et al.* (2013) reported an IC_{50} of 500 µg/ml and 350 µg/mL for N. sativa seeds, respectively before and after hydrolysis in a 50% ethanolic extract. Higher results were also reported (IC_{50}=1.24 mg/dry weight) in an 80% methanolic (Thippeswamy and Naidu 2005) and ethanolic extracts (IC_{50} of 624.7 µg/ml) of *N. sativa* seed (Toma *et al.* 2015). Our findings on IC_{50} of AFE can be correlated with IC values ranging from 49.0% to 87.0 % using extracts from barks of medicinal plants like Azadirachta indica, *T. arjuna*, *Acacia nilotica and Eugenia jambolana Lam*. The potential of antioxidant properties of Arjuna leaves and bark extracts in terms of IC_{50} (2.71-7.68 µg/ml) has also been reported (Chatha *et al.* 2014).

Cooking yield and total phenolics content of pork meat nuggets: The total phenolics content and cooking yield of nuggets with AFE (1 %), BCE (1.5%) and 100 ppm BHT is presented in Table 2. The TPC of cooked pork nuggets prepared with 1% AFE extract was significantly (P < 0.05) higher compared to control nuggets but nuggets with 1.5% BCE extract had comparable total phenolics than samples with 100 ppm BHT. In a study, Banerjee *et al.* (2012) reported significantly higher total phenolics content in goat meat nuggets incorporated with BHT than control nuggets. Verma *et al.* (2013) also reported that incorporation of guava powder in meat products formulation significantly increased the phenolics content of final products than control. Similarly, sheep meat nuggets incorporated with 1% and 1.5% litichi fruit pericarp extract had significantly higher total phenolics than nuggets with 100 ppm BHT (Das *et al.* 2016). However, addition of BCE, AFE or BHT did not affect the cooking yield of pork nuggets.

Table 2. Total phenolics content (TPC) and cooking yield of pork meat nuggets

Pork nuggets	TPC (mg GAE/g)	Cooking yield (%)
Control (C)	0.07±0.01c	93.65±0.35
1% AFE (T1)	0.23±0.02a	92.89±0.49
1.5% BCE (T2)	0.19±0.03ab	93.21±0.91
100 ppm BHT (T3)	0.15±0.01b	93.82±0.45

Data (Mean ± SE) bearing different superscript in a column differ significantly (p< 0.05)

Control= no additive; AFE= Arjuna fruit extract; BCE=black cumin extract; BHT= Butylated hydroxytoluene; n=6.

Effect of AFE and BCE on pH of cooked pork meat nuggets: Initially, the mean pH values were similar between control and treatment groups (Table 3). But as storage days progressed, a significant (p>0.05) increase in the pH value of control sample. On the other hand, the increase in pH during storage was significantly (p>0.05) less in treated samples (1% AFE, 1.5% BCE and 100 ppm BHT), which might be due to the inhibitory effects of AFE and BCE on oxidation of protein and lipid and some antimicrobial effects of these additives. This is in agreement with the reports of McCarthy *et al.* (2001) and Carpenter *et al.* (2007) who found no difference in the pH of control and test antioxidants like grape seed, bearberry and rosemary extracts incorporated raw and cooked pork meat products.

Effect of AFE and BCE on total aerobic plate count of cooked pork meat nuggets: The microbial quality of pork nuggets during the storage (0, 5th, 10th and 20th) period is presented in Table 3. Initially, aerobic plate counts of control (C) and treated pork nuggets (T_1, T_2 and T_3) ranged from 2.6 to 4.86, 2.62 to 4.92, 2.7 to 5.32 and 2.64 to 5.62 $\log_{10}$CFU/g, respectively. As the storage period progressed, it was found that pork nuggets

incorporated with 1% AFE was quite effective in lowering microbial load followed by 1.5% BCE over BHT and control, which might be due to its richness in polyphenolic compounds having antimicrobial effect along with antioxidant property. Polyphenols are well documented to have microbiocidal activities against a huge number of pathogenic bacteria and even oxidized polyphenols also have inhibitory activity against bacterial growth (Field and Lettinga 1992). The possible mechanism of polyphenol as antimicrobial may be related to inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, cell envelope transport proteins, non-specific interactions with carbohydrates, etc. (Cowan 1999). In a study, Ramya *et al*. (2008) reported that aqueous extracts of *T. arjuna* leaves and fruits are active towards the Gram negative strains. On the other hand, *N. sativa* seed extract has been shown to possess antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, and Candida albicans* and its essential oils act more against Gram-positive bacteria than the Gram-negative ones. (Haloci *et al*. 2012).

Lipid oxidation of cooked pork nuggets

Effect of AFE and BCE on thiobarbituric acid reactive substances: The TBARS values of control and treated nugget samples during 20 days of refrigerated storage are presented in Table 3. There was an increase ($p<0.05$) in TBARS values in all the groups during storage indicating persistent formation of aldehydes in products. However, the treated pork nuggets (T_1, T_2 and T_3) had significantly lower (37%) TBARS value than the control samples.

TBARS value in nuggets with 1% AFE increased from 0.37–0.94 mg MDA/kg during storage but at a slower rate compared to control, whereas nuggets with BCE had 0.36 - 0.96 mg MDA/kg. The control sample had an initial TBARS value of 0.39 and it reached to 1.29 mg MDA/kg on 20th day of storage study.

Table 3. pH, thiobarbituric acid reactive substances, free fatty acids and peroxide values of control and treated cooked pork meat nuggets during refrigerated storage

	Storage days				
Pork meat nuggets	**0**	**5**	**10**	**15**	**20**
			pH		
C	6.39±0.02^{cA}	6.43±0.03^{cA}	6.55±0.04^{bA}	6.76±0.05^{aA}	6.85±0.02^{aA}
T1	6.37±0.02^{bA}	6.41±0.02^{bA}	6.46±0.05^{abA}	6.55±0.03^{aB}	6.65±0.06^{aB}
T2	6.40±0.01^{aA}	6.43±0.03^{abA}	6.47±0.06^{abA}	6.53±0.05^{bB}	6.69±0.03^{cB}
T3	6.38±0.05^{aA}	6.42±0.04^{aA}	6.44±0.05^{abA}	6.54±0.05^{bB}	6.66±0.06^{cB}
			Aerobic plate count (log10 CFU/g)		
C	2.64±0.08^{cA}	3.42±0.12^{dA}	4.28±0.14^{cA}	4.82±0.20^{bA}	5.62±0.20^{aA}
T1	2.6±0.07^{eA}	3.24±0.14^{dA}	3.66±0.12^{cB}	4.12±0.18^{bB}	4.86±0.16^{aB}
T2	2.62±0.09^{eA}	3.3±0.11^{dA}	3.74±0.1^{cAB}	4.28±0.16^{bB}	4.92±0.14^{aB}
T3	2.7±0.06^{eA}	3.48±0.16^{dA}	3.98±0.14^{cA}	4.68±0.14^{bA}	5.32±0.12^{aA}
			Thiobarbituric acid reactive substances (mg Malonaldehyde/kg sample)		
C	0.39±0.02^{eA}	0.53±.02^{dA}	0.78±0.04^{cA}	1.06±0.02^{bA}	1.29±0.03^{aA}
T1	0.37±0.02^{dA}	0.40±0.02^{dB}	0.58±0.04^{cB}	0.72±0.02^{bB}	0.94±0.03^{aB}
T2	0.36±0.03^{dA}	0.41±0.02^{dB}	0.60±0.02^{cB}	0.75±0.02^{bB}	0.96±0.04^{aB}
T3	0.45±0.08^{dA}	0.39±0.02^{dB}	0.60±0.04^{cB}	0.71±0.04^{bB}	0.92±0.04^{aB}
			Free fatty acid (% oleic acid)		
C	0.12±0.00^{eA}	0.17±0.00^{dA}	0.21±0.01^{cA}	0.32±0.01^{bA}	0.41±0.01^{aA}
T1	0.10±0.01^{eA}	0.12±0.00^{dB}	0.14±0.01^{cB}	0.18±0.01^{bB}	0.24±0.01^{aC}
T2	0.11±0.00^{dA}	0.13±0.00^{dB}	0.15±0.01^{cB}	0.20±0.01^{bB}	0.27±0.01^{aBC}
T3	0.12±0.03^{dA}	0.10±0.01^{dC}	0.15±0.01^{cB}	0.20±0.01^{bB}	0.28±0.01^{aB}
			Peroxide value (mEq/kg sample)		
C	0.65±0.02^{eA}	0.80±0.04^{dA}	1.42±0.04^{aA}	1.24±0.04^{bA}	1.12±0.03^{cA}
T1	0.61±0.01^{cA}	0.68±0.02^{cA}	0.88±0.02^{bB}	1.20±0.02^{aA}	0.94±0.06^{bB}
T2	0.63±0.03^{cA}	0.70±0.06^{cA}	0.90±0.05^{bB}	1.24±0.04^{aA}	1.01±0.04b^{AB}
T3	0.70±0.08^{cA}	0.71±0.06^{cA}	0.88±0.08^{bB}	1.14±0.09^{aA}	1.17±0.08^{bA}

C: Control (no additive); T1= 1% Arjuna fruit extract; T2= 1.5 % black cumin extract; T3= 100 ppm butylated hydroxytoluene; n=6 Data (mean ± SE) with different small letter superscripts in the same row differ significantly ($p<0.05$) Data (mean ± SE) with different capital letter superscripts in the same column differ significantly ($p<0.05$)

Table 4: Effect of Arjuna fruit and black cumin seed extract on sensory attributes of cooked pork meat nuggets during refrigerated storage

	Storage days				
Pork meat nuggets	**0**	**5**	**10**	**15**	**20**
			Appearance		
C	7.10±0.08^{aA}	7.10±0.08^{aA}	6.67±0.08^{bA}	6.27±0.07^{cA}	6.03±0.19^{cC}
T1	7.20±0.10^{aA}	7.12±0.07^{abA}	6.80±0.11b^{cA}	6.53±0.10^{cA}	6.55±0.16^{cAB}
T2	7.28±0.13^{aA}	7.08±0.04^{abA}	6.92±0.17^{bA}	6.87±0.12^{bcA}	6.55±0.10^{cA}
T3	7.05±0.03^{bA}	6.93±0.10^{aA}	6.87±0.10^{c}	6.47±0.12^{bA}	6.22±0.10^{bBC}
			Flavour		
C	6.95±0.21^{aA}	6.68±0.05^{aA}	6.70±0.12^{aA}	6.02±0.12^{bA}	5.93±0.15^{bA}
T1	7.28±0.11^{aA}	7.00±0.14^{aA}	6.92±0.17^{abA}	6.53±0.10^{bcA}	6.45±0.17^{cA}
T2	7.32±0.10^{aA}	6.96±0.21^{abA}	6.73±0.15^{bA}	6.58±0.11^{bcA}	6.50±0.12^{bA}
T3	7.10±0.15^{aA}	7.02±0.08^{aA}	6.67±0.08^{bA}	6.62±0.09^{bA}	6.17±0.13^{cAB}
			Juiciness		
C	6.90±0.19^{aA}	7.12±0.07^{aA}	6.78±0.10^{aA}	6.25±0.17^{bB}	6.13±0.10^{bA}
T1	7.28±0.10^{aA}	7.16±0.03^{abA}	6.82±0.18^{cA}	6.92±0.08^{bcA}	6.32±0.08^{dA}
T2	7.40±0.14^{aA}	7.34±0.11^{aA}	6.73±0.13^{bA}	6.62±0.13b^{AB}	6.45±0.15^{bA}
T3	7.13±0.19^{aA}	7.17±0.06^{aA}	6.64±0.17^{bA}	6.45±0.14^{bB}	6.35±0.13^{bA}
			Texture		
C	6.87±0.10^{aA}	7.12±0.09^{aA}	7.10±0.04^{aA}	6.13±0.07^{bA}	6.37±0.16^{bA}
T1	7.10±0.19^{aA}	7.22±0.10^{aA}	7.07±0.07^{aA}	6.60±0.18^{bA}	6.28±0.05^{bA}
T2	7.20±0.20^{aA}	7.12±0.14^{aA}	7.02±0.07^{aA}	6.48±0.18^{bA}	6.25±0.07^{bA}
T3	7.12±0.19^{aA}	7.2±0.13^{aA}	7.09±0.07^{aA}	6.45±0.19^{bA}	6.42±0.13^{bA}
			Overall acceptability		
C	6.90±0.13^{aA}	6.86±0.08^{aA}	6.75±0.08^{aA}	6.15±0.08^{bB}	5.98±0.10^{bB}
T1	7.17±0.12^{aA}	7.22±0.13^{aA}	6.90±0.12^{aA}	6.48±0.10^{bA}	6.40±0.14^{bA}
T2	7.28±0.15^{aA}	7.18±0.11^{abA}	6.90±0.11^{bA}	6.50±0.07^{cA}	6.50±0.13^{cA}
T3	7.03±0.24^{aA}	7.05±0.11^{aA}	6.85±0.12^{bA}	6.43±0.14^{cAB}	6.25±0.09^{cAB}

C: Control (no additive); T1= 1% Arjuna fruit extract; T2= 1.5 % black cumin extract; T3= 100 ppm butylated hydroxytoluene ; n=18.

Data (mean ± SE) with different small letter superscripts in the same row differ significantly ($p<0.05$).

Data (mean ± SE) with different capital letter superscripts in the same column differ significantly ($p<0.05$).

The TBARS values of cooked nuggets (1% AFE and 1.5% BCE) were comparable and at times better than synthetic antioxidant indicating their effectiveness in inhibiting lipid oxidation in pork product during the storage period. In a study, extracts of grape (*Vitisvini fera L.*) seed and bear berry (*Arctostaphylosuva-ursi L.*) applied on outer surfaces were found effective in retarding lipid oxidation in raw and cooked pork patties (Carpenter *et al.* 2007). Chen *et al.* (1999) noticed a reduction in TBARS in irradiated raw pork patties treated with oleoresin rosemary extract after 3 days of refrigerated storage (4°C). Likewise, guava powder besides supplementing the dietary fiber to sheep meat nuggets was found to retard lipid peroxidation in the product during refrigerated storage (Verma *et al.* 2013).

Effect of AFE and BCE on free fatty acids (FFA) of cooked pork meat nuggets: The FFA values reported in this study ranged from 0.12 to 0.41% in control, T_1 (0.10 to 0.24%), T_2 (0.11 to 0.27%) and T_3 (0.12 to 0.28%) samples, respectively (Table 3). FFA contents increased significantly ($p<0.05$) in control as well as treated groups with the progress in storage period, however, the increase was more in control than the treated samples. The pork meat nuggets incorporated with AFE and BCE had activity comparable with BHT in inhibiting FFA values during storage study. This might be due to the presence of free radical scavenging compounds which prevent the lipolysis and lipid oxidation possibly due to low lipolysis and lipolytic enzyme activity in antioxidant-treated products, leading to low production of free fatty acids (Aguirrezábal *et al.* 2000). In a similar study, Das *et al.* (2011) reported increasing trend of FFA during refrigeration storage of raw ground meat for 9 days. Other workers also suggested similar trends in FFA of chicken (Biswas *et al.* 2012) and goat meat products (Verma and Sahoo 2000).

Effect of AFE and BCE on peroxide value of cooked pork meat nuggets: The peroxide values of the control and treated pork nugget samples were studied over a period of 20 days and are presented in Table 3. The PV (mEq/kg) of control sample remained significantly ($p<0.05$) higher on day 0, 5th and 10th as compared to AFE, BCE and BHT treated samples. The initial PV of the control was 0. 65 mEq/kg and increased to 1.12 mEq/kg after 20 days of storage. Whereas, the initial PVs of pork nuggets were significantly ($P<0.05$) lower than control with AFE (0.61 mEq/kg), BCE (0.63 mEq/kg) and BHT (0.70 mEq/kg) increased to 0.94, 1.01 and 1.17 mEq/kg, respectively during the same period. The PV of the control sample increased until 10th day (1.42±0.04 meq/kg) and decreased thereafter, indicating that after the induction period, the decomposition rate of the hydroperoxides was faster than the production rate (Ladikos and Lougovois 1990). The findings of researchers on grape seed extract in pork frankfurters (Wagh *et al.* 2015) and fresh pork sausage with rosemary extract (Georgantelis *et al.* 2007) also report a maximum PV after 10 and 14 days storage, respectively at 4°C, followed by a decline. In partial agreement, Østerlie and Lerfall (2005) argued that low pH could minimize hydrolytic activity of microorganisms and reduce oxidative rancidity in meat products. The effect of 1% AFE and 1.5% BCE significantly ($p<0.05$) retarding the lipid oxidation of pork nuggets during storage could be due to higher polyphenolic content present in the extracts.

Sensory attributes of pork nuggets: Sensory evaluation is one of the first and most important parameter to judge the overall acceptability by correlating various attributes like odors, flavors, colouration etc. of the product into consideration. Initially, there was no difference ($p < 0.05$) in sensory attributes between treated and control samples. But as the storage period progressed, it was found that control sample had significantly ($p<0.05$) lower sensory scores than that of treated sample (Table 4). Sensory scores for flavour showed significant difference ($p<0.05$) between control and treatment groups, which might be due to pholyphenolic components present in extract preventing lipid oxidation and off-flavour developments. Among the treated products, 1.5% BCE scored higher flavour over other groups which may be due to its flavour enhancing property. Black cumin is well known as a flavouring agent and most commonly added to some food products such as paste, pastry, cheese, pickles and bakery products for flavouring (Cheikh-Rouhou *et al.* 2007). The incorporation of 1% AFE in pork nugg*et al*so showed significantly ($p<0.05$) higher flavour score than control and was comparable with 1.5% BCE treated group. There was no significant difference ($P>0.05$) in juiciness and texture score between control and treatment groups on day 0. But with the progress in storage period, both the parameters in all the tested samples (C, T_1, T_2 and T_3) received lower scores. As far as the overall acceptability of the nuggets with 1 % AFE (T1) and 1.5 % BCE (T2) is concerned, both were significantly ($p<0.05$) better with improved flavour scores in comparison to control nuggets (Table 4). Aroma and flavour are the most important attributes that influence the sensory properties of comminuted meat products extended with non-meat protein additives (Das *et al.* 2008). Although all the samples (C, T_1, T_2 and T_3) were equally acceptable to the panel members up to day 10 but after that control sample received lower overall acceptability scores. As per Cakir *et al.* (2016), addition of black cumin to the cheese samples improve the levels of colour and appearance, odour, flavour, texture and overall acceptability scores than control cheese samples. In this context, Soltanizadeh and Ghiasi-Esfahani (2015) reported that the overall acceptability scores of the beef burgers followed the trends of flavour acceptability scores, reflecting the major influence of flavour on overall acceptance.

CONCLUSIONS

From the above findings and based on the physico-chemical, microbiological and sensory attributes, it is concluded that both the natural antioxidants i.e. AFE at 1 % or BCE at 1.5 % could be used in retarding lipid and protein oxidation and extending shelf-life and overall acceptability of pork meat nuggets during refrigerated storage study.

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