Defence Life Science Journal, Vol. 3, No. 1, January 2018, pp. 51-58, DOI : 10.14429/dlsj.3.12016

Evaluation of Antioxidant Activity, Radical Scavenging, and Reducing Power of Clove Oil and Clove Oleoresin in Comparison with Natural and Synthetic Antioxidants in Chevon *(Capra aegagrus hircus)* and Chicken Meat

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ABSTRACT

The antioxidant effects of clove oil (CO) and clove oleoresin (COR) in two species of meat i.e. chicken and chevon during refrigerated storage (4±1°C) were investigated. The antioxidant potential (AOP) and radical scavenging activities were compared with natural (α-Tocopherol and *L*-ascorbic acid) and synthetic antioxidants (BHA and TBHQ). CO & TBHQ, BHA and COR and L-ascorbic acid and α-Tocopherol produced 84-79 per cent, 68-77 per cent, and 68-59 per cent AOP respectively in both species. DPPH and ABTS methods of scavenging assay established better scavenging capability of CO and TBHQ in comparison with other treatments. Significant reduction ($p<0.05$) in FRAP values was observed in CO & TBHQ in both species of meat. The order of antioxidant potential, scavenging activity and reducing power was in the order of CO>TBHQ>COR>BHA>Tocopherol>L-ascorbic acid. All the antioxidant assays analysed demonstrated a very significant correlation ($p<0.05$) between each other. The results suggest that CO and COR through their antioxidant effects are potentially useful in preserving meat products.

Keywords: Clove oil; Clove oleoresin; Antioxidant potential; Reducing power; Meat

NOMENCLATURE

CO	Clove oil
COR	Clove Oleoresin
AOP	Antioxidant potential
BHA	Butylated hydroxyanisole
TBHQ	Tertiary Butyl Hydro Quinone
DPPH	2, 2- diphenyl-1-picrylhydrazyl
ABTS	2, 2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid)
FRAP	Ferric reducing antioxidant power
Fe^{3+}	Ferric Iron
β	Beta
TBARS	Thiobarbituric acid reactive substances
TBA	Thiobarbituric acid
ml	Millilitre
mM	MilliMolar
min	minute
TPTZ	Tripyridyl triazine
pH	Potential Hydrogen
a*	Redness
v:v:v	Volume:volume:volume
Fe^{2+}	Ferrous Iron
nm	Nanometer
v/v	Volume/volume
ANOVA	Analysis of Variance
OH^-	hydroxyl radical
$O2^-$	Oxygen ion
ppm	Parts per million
Eq./kg	Equivalent per kilogram

Received : 03 October 2017, Revised : 05 December 2017
Accepted : 06 December 2017, Online published : 15 December 2017

1. INTRODUCTION

Autoxidation of lipids is a natural process which affects fatty acids and leads to oxidative deterioration of meat and off-flavours development[1]. Oxidation of lipids in meat depends on several factors including fatty acid composition, the level of the antioxidant vitamin E and pro-oxidants such as the free iron present in muscles. Poly unsaturated fatty acids are more susceptible to lipid oxidation. Lipid oxidation is one of the primary causes of deterioration in food system during cooking and storage leading to the development of off-flavour, loss of colour and texture, decrease in nutritive value and production of potentially toxic compounds[2,3]. To avoid or delay the autoxidation process, antioxidants have been utilised with the practice being carried out successfully for over 50 years. Because of the growing concern for the potential health hazard of synthetic antioxidants, there is a renewed interest in the use of naturally occurring substances[4].

Antioxidants reduce free radicals mostly by single electron transfer and hydrogen atom transfer. Numerous assays have been often used to determine antioxidant capacities in meat and their products including 2, 2- azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS)[5], ferric reducing antioxidant power (FRAP)[6] and 2, 2- diphenyl-1-picrylhydrazyl (DPPH)[7]. ABTS, FRAP and DPPH are methods that measure the single electron transfer. Natural antioxidants are multifunctional during processing; their activity cannot be evaluated by a single method in heterogeneous foods like meat and meat products[8]. Therefore, two or more radical scavenging capacity assays are essential to investigate heterogeneous

samples in view of the fact that each assay involves in different chemical mechanisms. ABTS, DPPH, and Fe^{+3} (FRAP) are the three scavenging assays to assess in vitro antioxidant activity of meat. ABTS radical scavenging estimates single electron-transfer capabilities[9] whereas DPPH radical scavenging allows evaluation of the hydrogen-donating potency compounds[10] and Fe^{+3} probes in FRAP assay reflects the reductive antioxidant power[11]. FRAP is sensitive to single electron transfer where as ABTS for single electron and hydrogen atom transfer.

Muscle foods have low oxidative stability and are very susceptible to rancidity during processing and storage. Various studies have revealed that lipid and protein oxidation in meat and meat products can be minimised through the application of antioxidants[12]. Spices have long been known for their preservative and health properties as antimicrobials and antioxidants[13-15]. Clove is one of the important spices that have long been recognised to possess essential oils exhibiting preservative properties. It has also been used in the traditional Indian cuisines either whole or in ground form as flavourings. Clove Oils and clove oleoresins have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties[2,16-18]. This may be due to the presence of major compounds present in them abundantly viz., Eugenol, β-caryophyllene, eugenyl acetate and other minor components[19].

Clove has been shown to avert discoloration of raw pork during storage at room temperature and was stated to be the strongest antioxidant amongst spice and herb extracts including cinnamon, oregano, pomegranate peel and grape seed in retarding lipid oxidation[20]. The ethanolic extract of clove has been employed efficiently in enhancing the shelf life of fresh mutton up to 4 days at 25±2°C[21]. Incorporation of clove oil in combination with lactic acid or vitamin C showed a reduction in lipid oxidation and preservation of high colour a* value with improvement in sensory colour in buffalo meat during retail display[22]. However, no information relating the potential of clove oil and clove oleoresin in stabilising meat products has been reported so far. Thus, the current study aimed to investigate the effects of adding clove oil and clove oleoresin on the antioxidant activity, radical scavenging and reducing power of chevon and chicken meat in comparison with synthetic antioxidants (BHA and TBHQ) and natural antioxidants (L-Ascorbic acid and α-Tocopherol).

2. MATERIALS AND METHODS

2.1 Raw Materials and Chemicals

All the reagents and chemicals utilised as part of the investigation were of Analar grade and obtained from M/s. BDH Company, Sigma Chemical Co. Ltd, U.K and Rankem Chemicals, India. Clove oil (CO) and Clove oleoresin (COR) was obtained from M/s Synthite Industries Limited, Kolencherry, Kerala.

2.2 Meat samples

Fresh chicken (leg portion) and chevon (goat meat) were purchased from local market, Mysuru, India within 1 h - 2 h after slaughter and allowed rigor mortis to set in. It was then washed thoroughly under running water to remove foreign particles adhered to the surface of pieces and the fat content of chicken and chevon mince used in the study is 4-5 % and 5-6 % respectively. The deboned meat was then minced in a mincer (Hobart, Model 4812-CE, Offenburg, Germany) and used for further analysis.

2.3 Sample Preparation

Following mincing, the samples were divided into 50 g each portions. Synthetic antioxidants BHA and TBHQ were incorporated at the permitted level (200 ppm). Natural antioxidants L - Ascorbic acid (200 ppm), α-Tocopherol (200 ppm), Clove oil (1000 ppm) and Clove oleoresin (1000 ppm) were separately added to minced meat samples and mixed thoroughly. Treated samples were packed in polypropylene pouches and cooked till an internal temperature of 80 °C. The samples were cooled to room temperature and comparative evaluation of the antioxidant potential was established during refrigerated storage for a period of 21 days.

2.4 Antioxidant Assays

2.4.1 Antioxidant Potential by TBARS Method

The antioxidant potential expressed in terms of percentage of antioxidant activity was calculated by the Eqn (1)[23].

$$\%\text{AOP} \frac{\left[\begin{array}{l}\text{TBARS value of the control} \\ -\text{ TBARS of the test sample}\end{array}\right] \times 100}{\left[\text{TBARS value of the control}\right]} \quad (1)$$

TBARS values were expressed as mg malonaldehyde/kg sample and estimated colorimetrically using TBA[24]. This method was employed to evaluate the antioxidant activity of natural and synthetic antioxidants in different species of meat.

2.4.2 Antioxidant Assay by DPPH Method

To estimate and to compare the antioxidant activity of clove oil and clove oleoresin with natural and synthetic antioxidants, radical scavenging activity method was employed. The scavenging effects of samples of DPPH radical were monitored according to the method of Yen and Chen[25]. Briefly a 2.0 ml aliquot of test sample (in methanol) was added to 2 ml of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min and then left to stand at room temp for 30 min in the dark and its absorbance was read at 517 nm. The ability to scavenge the DPPH radical was calculated using equation

$$\text{Scavenging effect } (\%) \left[\frac{1-(\text{A sample} - \text{A sample blank})}{\text{A control}}\right] \times 100 \quad (2)$$

where the A control is the absorbance of the control (DPPH solution without sample), the A sample is the absorbance of the test sample (DPPH solution + test sample) and the A sample blank is the absorbance of the sample only (without DPPH solution).

2.4.3 FRAP Assay

The ferric reducing antioxidant power method[26] was used for determination of reducing power. FRAP solution was prepared by diluting an aqueous solution of 10 mM TPTZ and 20 mM ferric chloride in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v) as described by Benzie

and strain[11]. Increases in absorbance due to the formation of a coloured TPTZ–Fe^{2+} complex were monitored at 595 nm in a UV/Vis Perkin–Elmer Lambda EZ210 spectrophotometer. A Trolox reference curve was used.

2.4.4 ABTS Assay

ABTS assay was carried out as per the method of Cai[27], *et al.* The ABTS radical cation (ABTS*) solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate and incubated in the dark at room temperature for 16 h. The ABTS* solution was then diluted with 80 % (v/v) ethanol to obtain an absorbance of 0.700±0.005 at 734 nm. ABTS* solution (3.9 ml) was added to 0.1ml of the test sample (pre-diluted at a ratio of 1:50) and mixed vigorously. The reaction mixture was allowed to stand at 23 ºC for 6 min and the absorbance was recorded at 734 nm immediately. A standard curve was obtained by using ascorbic acid in 80 % ethanol. The % ABTS which was scavenged ($\%ABTS_{sc}$) was calculated using the formula:

$$\%ABTS_{sc} = (A_{con} - A_{sample}) \times 100 / A_{con}$$

where A_{con} is the absorbance of the control and A_{sample} is the absorbance of the sample read at 734 nm.

2.4.5 Statistical Analysis

Two-way analysis of variance (ANOVA) of the data was carried out using the SPSS 17 for Windows (SPSS Statistical Software, Inc., Chicago, IL, USA) software package. One way ANOVA was found significant ($p<0.05$), Regression analysis for the correlation was performed and correlation coefficient was established using the software Curve Expert 1.3[28].

3. RESULTS AND DISCUSSION

3.1 Antioxidant Potential of Clove Oil and Clove Oleoresin

Comparative evaluation of antioxidant potential of clove oil and clove oleoresin with synthetic (TBHQ and BHA) and natural antioxidants (Tocopherol and Ascorbic acid) in two species of meat i.e. chicken and Chevon meat have been depicted in Tables 1 and 2, respectively. The percentage of AOP calculated by estimating the TBARS of control and treated samples during refrigerated storage for a period of 21 days have been given in Tables 1 and 2. From the data on AOP for chicken and Chevon meat, it could be seen that no significant difference ($p<0.05$) exist between the species initially as well as during storage. But significant difference ($p<0.05$) in AOP have been noticed with clove oil when compared to other treatments except in the case of TBHQ. The higher percentage of AOP (79-84 %) recorded in the case of clove oil which is similar to TBHQ in both chicken and chevon meat is a clear indicator of the ability of clove oil at 1000 ppm level to inhibit the lipid oxidative changes taking place during storage. Clove oil is a constituent of many powerful antioxidant components like eugenol, β-caryophyllene and eugenyl acetate which are known to inhibit oxidation of lipids by scavenging free radicals[29].

Out of the treatments, BHA produced antioxidant activity similar to that of COR (68-77 %) initially as well as during storage as seen from the Tables 1 and 2. Both natural antioxidants did not differ significantly ($p>0.05$) in their antioxidant potential initially and during storage in the case of both species of meat but recorded a significant difference ($p<0.05$) with other treatments. The positive effect of natural antioxidants in improving the oxidative stability of lipids in cooked chicken meat has been reported[30] and the effect of ascorbic acid in reducing the oxidative changes in ground beef and turkey was reported by Craig[31], *et al*. Looking at the variation in the percentage of antioxidant potential illustrated by the treatments, it can be interpreted that clove oil and TBHQ produced the maximum effectiveness with significant difference ($p<0.05$) in comparison with other treatments. From the table, after examining the data pertaining to the antioxidant activity of treatments with reference to chicken and mutton it can be emphasised that the effectiveness of all this treatments were more in mutton in comparison with chicken. The reason for this may be due the higher unsaturation of chicken meat which may lead to the higher rate of oxidation in comparison with mutton[32]. The order of antioxidant potential as obtained from the Tables 1 and 2 can be summarised as follows CO> TBHQ>COR>BHA>Tocopherol>Ascorbic acid.

Table 1. Antioxidant potential of clove oil and clove oleoresin in comparison to natural and synthetic antioxidants in chicken during refrigerated storage (4±1°C)

Samples	Storage period (Days)			
	0	7	14	21
BHA	75.11±1.33^a	73.16±1.14^a	72.05±1.07^a	68.13±0.91^a
TBHQ	82.93±0.98^b	80.88±0.88^b	78.46±1.33^b	76.11±0.98^b
Ascorbic acid	65.16±0.44^c	63.09±1.04^c	61.94±1.09^c	59.04±0.87^c
Tocopherol	66.16±1.77^c	65.01±1.34^c	60.98±1.22^c	58.11±1.04^c
Clove oil	83.15±1.26^b	81.11±1.15^b	80.64±1.78^b	79.19±1.90^b
Clove oleoresin	76.14±1.43^a	74.55±1.44^a	72.93±1.15^a	67.93±1.06^a

All values are mean ± standard deviation of data from three independent experiments. Different lowercase letters (a-c) in the same column indicate significant difference ($p<0.05$).

Table 2. Antioxidant potential of clove oil and clove oleoresin in comparison to natural and synthetic antioxidants in Chevon during refrigerated storage (4±1°C)

Samples	Storage period (Days)			
	0	7	14	21
BHA	77.09±1.14^a	75.09±0.46^a	73.17±0.91^a	70.99±2.05^a
TBHQ	83.94±0.91^b	81.46±2.04^b	80.11±0.91^b	77.46±1.16^b
Ascorbic acid	66.39±0.98^c	64.09±1.11^c	63.26±0.91^c	60.09±0.78^c
Tocopherol	68.41±1.16^c	66.93±1.39^c	65.29±1.09^c	60.19±0.45^c
Clove oil	84.82±1.33^b	82.44±1.24^b	81.81±1.55^b	80.45±1.16^b
Clove oleoresin	77.71±1.75^a	75.16±1.25^a	73.18±1.29^a	68.03±1.18^a

All values are mean ± standard deviation of data from three independent experiments. Different lowercase letters (a-c) in the same column indicate significant difference ($p<0.05$).

3.2 DPPH and ABTS Radical Scavenging Activity

Evaluation of the free radical scavenging activity is very important to establish and assess the antioxidant potential. Since the lipid peroxidation process involves or/and is triggered by the formation of free radicals and scavenging capacity of these radicals by antioxidants will determine the potential of antioxidants. DPPH is a useful reagent for investigating the free radical scavenging activities of compounds. The principle of this method is the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidants due to the formation of non-radical form DPPH-H by the reaction[33]. DPPH radical scavenging activity of clove oil and clove oleoresin along with natural and synthetic antioxidants in chicken and chevon meat have been carried out and the percentage of activity has been depicted in Fig. 1(a) and 1(b), respectively. Among the seven treatments in each species CO and TBHQ produced the best radical scavenging activity initially and during refrigerated storage. The values were significantly different ($p<0.05$) from all other treatments. This may be due to the capacity of eugenol and TBHQ in scavenging several free radicals such as OH^-, O^{2-}, etc which are normally formed in lipid oxidation process[34]. From the figure it can be seen that even though all the treatments in both species were effective in scavenging free radicals with varied potential, CO at 1000 ppm produced better scavenging capacity in comparison with others. The order of DPPH scavenging activity was found to be CO>TBHQ>OR>BHA>Tocopherol>Ascorbic acid.

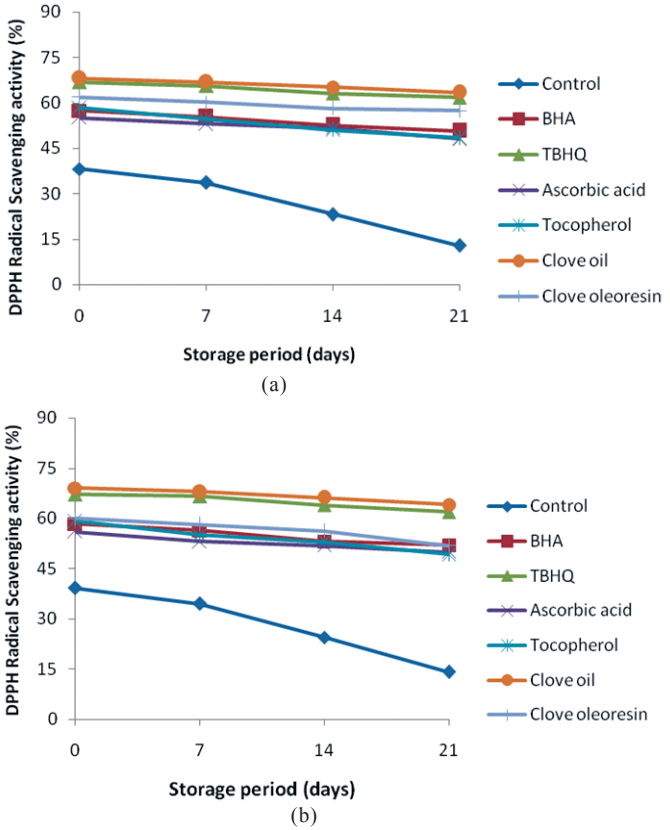

Figure 1. DPPH radical scavenging activity clove oil and clove oleoresin in comparison to natural and synthetic antioxidants in : (a) Chicken during refrigerated storage (4±1 °C) and (b) Chevon meat during refrigerated storage (4±1 °C).

The other procedure to establish the antioxidant and radical scavenging activity which is normally employed with DPPH is ABTS method. Several researchers have reported the efficacy of employing ABTS method to evaluate the AOPs of treatments by estimating the radical scavenging activity[29,35,36]. The data on ABTS for the six treatments along with control for both the species have been given in Fig. 2(a) and 2(b). From the data it can be elucidated that as in the case of DPPH here also CO and TBHQ exhibited superior potential in radical scavenging capacity in comparison with other treatments[37] and control. The trend obtained is nearly similar to the DPPH activity as described earlier. The order of % of ABTS activity was found to be CO>TBHQ>COR>BHA >Tocopherol > Ascorbic acid. ABTS scavenging antioxidant capacities of lipophilic and hydrophilic extracts of chicken meat was reported by Sacchetti[38], *et al*.

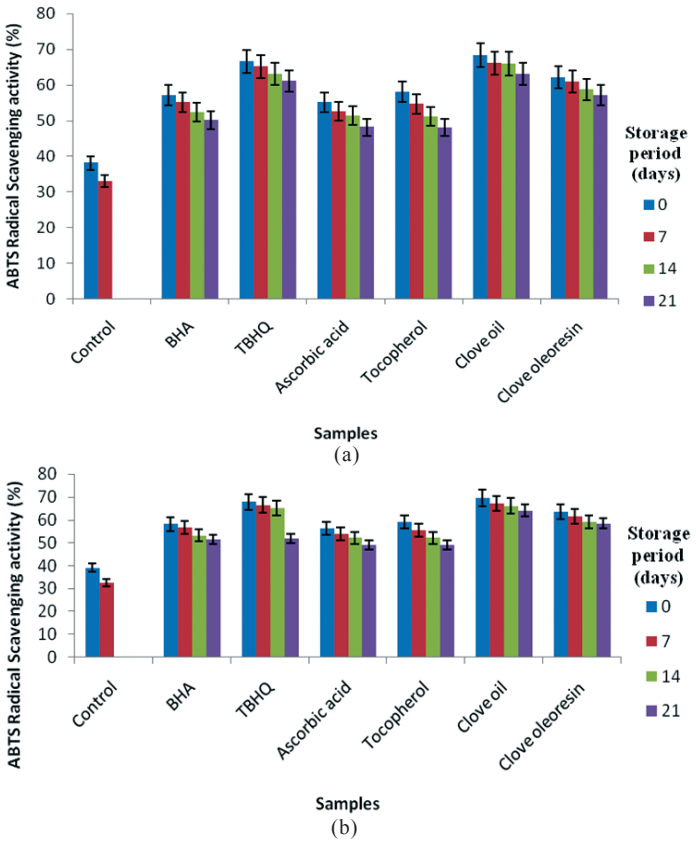

Figure 2. ABTS radical scavenging activity clove oil and clove oleoresin in comparison to natural and synthetic antioxidants in : (a) Chicken meat during refrigerated storage (4±1 °C) and (b) Chevon meat during refrigerated storage (4±1 °C).

3.3 FRAP Assay

Studies were carried out further to establish and confirm the ability of these treatments in comparison with natural and synthetic antioxidants to evaluate the reducing power, as this is correlated to its electron transfer ability. This can be taken as an indicator of antioxidant potential. Plant extracts are reported to have reducing power and this can be directly correlated with the antioxidant activity[4].

Both species of meat after treatments were subjected for FRAP assay to assess the reducing power, because in this assay the reductants existing in the extract cause reduction of Fe^{3+} to Fe^{2+} form which can be monitored spectrophotometrically. The data obtained for clove oil and clove oleoresin in comparison with natural and synthetic antioxidants have been reflected in Fig. 3(a) and 3(b), respectively for chicken and chevon. From the data expressed, as mmol Trolox Eq./kg of meat, the CO and TBHQ samples produced higher values of FRAP (3.7 to 5.9) in both chicken and chevon meat initially and during storage. No significant difference ($p>0.05$) in FRAP values were observed in CO and TBHQ treatment indicating similar characteristics of reducing capacity. Significant reduction ($p<0.05$) in FRAP values was observed between CO and TBHQ with other treatments initially and during storage in both species of meat. The phenolic compounds present in clove oil produces redox properties which can play an important role in the absorption and neutralisation of free radicals and quenching singlet oxygen[39]. From the Figures, it could be seen that during storage period, significant reduction ($p<0.05$) in FRAP values were observed in the case of ascorbic and tocopherol as well as BHA. This can be attributed to the poor ability of these antioxidants to reduce Fe^{3+} to Fe^{2+} form[35]. The findings reported in the study are in accordance with the earlier studies on the antioxidant potential and radical scavenging capability reported in the Tables 1 and 2 and Figs. 1, 2, 3, and 4.

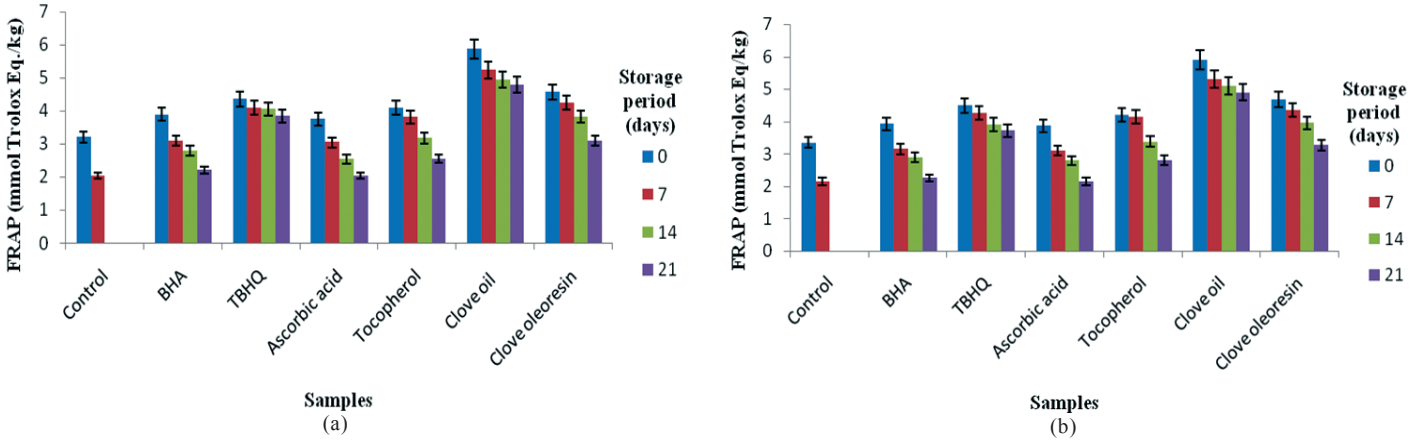

Figure 3. Reducing power of clove oil and clove oleoresin in comparison to natural and synthetic antioxidants in : (a) Chicken meat during refrigerated storage (4±1°C) and (b) Chevon meat during refrigerated storage (4±1°C).

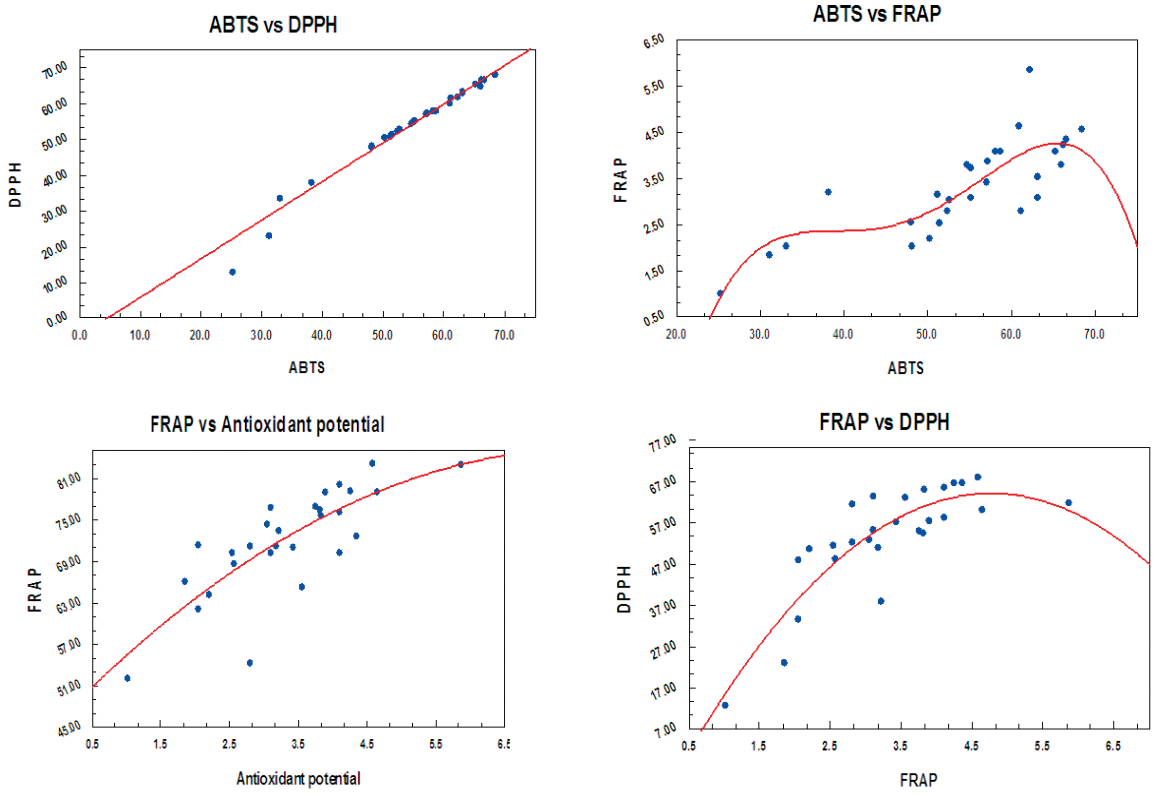

Figure 4. Correlation characteristics of radical scavenging activity (DPPH and ABTS), Reducing power (FRAP) and antioxidant potential.

3.4 Correlation between Radical Scavenging Capacity Assays (DPPH, ABTS and FRAP)

The radical scavenging capacity assays for lipid oxidation were subjected for linear correlation analysis to establish the best fit equations. Since the trend obtained for both the species were of the same pattern as per the earlier discussion, for establishing the correlation parameters amongst the DPPH, ABTS and FRAP values for both chicken and mutton meat for one parameter were correlated with other (Figs. 1-3 and Tables 1 and 2). The regression analysis for the data were established using curve expert 1.4 software and the best fit equations for the correlation studies have been represented in Fig. 4. The equations for the ABTS *vs* DPPH, ABTS *vs* FRAP, FRAP *vs* DPPH and FRAP *vs* antioxidant potential were found to be $y = -4.97+1.08x$; $y = -4.62+4.40x+1.14x^2$, $y= -1.40+3.27x+-3.44x^2$ and $y = 4.59+1.10x+-6.56x^2$ with correlation coefficient of 0.98, 0.81, 0.86 and 0.79 respectively.

From the correlation coefficients it can be seen that DPPH exhibited a better coefficients index in comparison with ABTS and FRAP and it is significantly ($p<0.05$) different. Linear fit model was found to be the best fit model for ABTS *vs* DPPH data, quadratic fit model fitted best for FRAP *vs* DPPH and FRAP *vs* antioxidant potential, Polynomial model for ABTS *vs* FRAP analysis respectively. The above regression analysis reveals that there is a positive correlation between all the three free radical scavenging assays. Establishing the correlation pattern for these assays clearly indicate the efficacy of employing these parameters to determine and estimate the antioxidant potential of clove oil and clove oleoresin in comparison with natural and synthetic antioxidants.

4. CONCLUSIONS

The results clearly demonstrate the antioxidant potential of clove oil and clove oleoresin in comparison with synthetic (TBHQ and BHA) and natural (Tocopherol and Ascorbic acid) antioxidants in inhibiting lipid oxidation and extension of shelf life of cooked chicken and chevon meat during storage at 4±1° C for 21 days. The antioxidant properties of clove oil and clove oleoresin showed that cloves had good antioxidant activity with higher polyphenol and flavonoid contents. Clove oil and clove oleoresin exhibited potent antioxidant activity in both chicken and chevon meat. The results clearly demonstrate the effectiveness of clove oil and clove oleoresin as potential natural antioxidants in replacing the synthetic antioxidants. Establishing AOP, scavenging activity and reducing power and comparing with the usual natural and synthetic antioxidants will throw more light on the antioxidant capability of clove oil in the application of meat and poultry products development.

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