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Quality Assessment of Chicken Breast Fillets Treated with Custard Apple (*Annona squamosa*) Leaves Extract

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ABSTRACT

Background: Meat and meat products are perishable due to high content of moisture and easy availability of other nutrients. Oxidative rancidity and microbial growth are the major causes of their spoilage. Synthetic antioxidants *viz*. BHA, BHT, TBHQ *etc.* are used in the chicken processing industry for maintaining quality of chicken products. However, due to evidence of their toxic effects on human health there is interest in the scientists and manufacturers to use natural preservatives with antioxidants and antimicrobials properties. Hence, the study was undertaken to assess the effect of custard apple (*Annona squamosa*) leaves extract (CLE), on the quality of chicken breast fillets.

Methods: Chicken breast fillets were treated separately by dipping in 0.1, 0.3 and 0.5% aqueous solutions of CLE, distilled water and 100 ppm solution of BHT. Samples were stored under refrigeration (4±1°C) conditions and analyzed on 0th, 3rd, 6th and 9th day of storage.

Result: Total phenolic content, ascorbic acid content, DPPH radical scavenging assay and ferric reducing antioxidant power of CLE were 57.13±0.22 µgGAE/mg, 163.23±0.40 µgAA/mg, 32.20±0.42% and 132.43±0.50 µMFe (II) equi/g respectively. The fillets treated with 0.5% solution of CLE were acceptable till sixth day with significantly ($p<0.01$) higher sensory scores than the controls. The pH, WHC, ERV, tyrosine value, TBARS value, total plate count and psychrophilic count of the sixth day samples treated with 0.5% CLE were 5.72±0.01, 65.32±0.31%, 19.40±0.59 ml, 9.50±0.37 mg/100 g, 0.316±0.002 mgMDA/kg, 4.90±0.01 log10CFU/g and 3.68±0.02 log10CFU/g respectively. It can be concluded that 0.5% aqueous solution of CLE can be used for extension of shelf life of chicken breast fillets up to 6 days at refrigeration (4±1°C).

Key words: Custard apple extract, Chicken fillets, DPPH, TPC, TBARS, Tyrosine.

INTRODUCTION

Fresh chicken meat is highly perishable. Microbial growth and oxidative rancidity are the major problems causing quality deterioration and shelf life reduction of chicken. Therefore, use of preservation technologies is necessary to maintain its safety and quality. Chicken meat is more vulnerable to lipid oxidation as it is relatively rich in unsaturated fatty acids (Valsta *et al.,* 2005). Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) are worldwide used for controlling alterations in sensory parameters of chicken and its products. However, their use is under strict regulation in many countries because of their associated toxic and carcinogenic effects (Jo *et al.,* 2006). Therefore, there is rising interest from the manufacturers and scientists in using naturally occurring preservatives with antimicrobial and antioxidant properties. Natural preservatives include extracts of herbs and spices (Botsoglou *et al.,* 2003) rich in phenolic compounds such as flavonoids and phenolic acids, which exhibit a wide range of biological effects, including antioxidant and anti-microbial (Suppakul *et al.,* 2003). Many workers have documented that custard apple (*Annona squamosa*) leaves are good sources of natural antioxidants and antimicrobial compounds (Saha 2011; 2012; Gowdhami *et al.,* 2014; Roy and Lingampeta, 2014). However, literature is not available on its use in the preservation of meat and meat products. Hence, this study was undertaken to assess the effect of custard apple (*Annona squamosa*) leaves extract (CLE) on the quality of the chicken breast fillets stored under refrigeration (4±1°C).

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MATERIALS AND METHODS

Deskinned boneless breast fillets of uniform weight (200±10 gm), shape and size from healthy chicken (broiler) birds with average age of 35 days and 2.0 kg live weight were procured hygienically from the local market. Low density polyethylene (LDPE) pouches of 55 µ thickness were used for packaging of the fillets. Mature leaves of custard apple (*Annona squamosa*) trees of 'Balanagar' variety were procured from orchard of a farmer near by Nagpur city.

Proximate analysis of custard apple leaves

Proximate analysis of custard apple leaves was conducted by following standard procedures of AOAC (1995).

Preparation of custard apple leaves extract (CLE)

Custard apple leaves were first washed with drinking water and then with distilled water and air dried (27±2°C) by spreading on the uniform platform for 7 days *i.e.* till constant weight was attained. Dried leaves were powdered using mixer grinder, sieved through laboratory sieve of 30 mesh size to maintain the particle size of 0.49 mm, packaged in LDPE container and stored at -20°C till further use for 5 days. Extract was prepared by the method suggested by Gowdhami *et al.,* (2014) with slight modification. Leaves powder was mixed with 50% aqueous ethanol solution in 1:5 ratio and stored at room temperature (27±1°C) for 24 hrs with occasional stirring. The mixture was strained through four layered muslin cloth and then filtered through Whatman filter paper number 1. The filtrate was concentrated in stainless steel plates kept at 50°C in hot air oven till constant weight was attained. Dried extract was collected and stored at -20°C in airtight LDPE container till further use for 6 weeks. Hygienic conditions were maintained during all stages of extract preparation.

Estimation of ascorbic acid content

Ascorbic acid content of the CLE was determined as suggested by Benites *et al.,* (2013) with slight modifications. Extract sample was dissolved in 0.1% aqueous solution of dimethylsulfoxide (DMSO) @ 2.5, 5 and 10 mg/ml. Four ml of this sample was added to 1 ml of 2,4 dinitrophenylhydrazine reagent (2,4-DNHP), mixed thoroughly and allowed to stand undisturbed for 30 min. Absorbance was read in triplicate at 515 nm on UV-VIS spectrophotometer (Schemadzu - Model No. UV-1800) using 0.1% DMSO as a blank. Results were expressed as µgAA/mg of extract by using ascorbic acid standard curve.

Estimation of total phenolic content (TPC)

Total phenolic content (TPC) of CLE was estimated according to the method of Singleton and Rossi (1965) with slight modification. CLE (1 mg/ml) 200 µl was taken in a test tube and volume was made up to 2 ml by adding distilled water. Folin-Ciocalteu reagent 300 µl was added and mixture was kept undisturbed for 5 min. Then 800 µl of Na_2CO_3 (20%) was added and volume was made up to 5 ml by adding distilled water. The mixture was incubated at room temperature for 30 min and absorbance was taken at 765 nm on UV-VIS spectrophotometer (Schemadzu - Model No. UV-1800). TPC was calculated from the standard curve of gallic acid and expressed as µgGAE/mg of extract.

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) radical scavenging assay

DPPH radical scavenging activity of CLE was estimated according to Harbarne (1973). 100, 200, 400, 600, 800 and 1000 µg of sample and BHT (dissolved in 50% ethanol) were taken in different test tubes. 1 ml of 0.1 mM DPPH methanolic solution was added and volume was made up to 5 ml by adding methanol. The mixture was shaken vigorously and incubated at room temperature in dark place for 30 min. Control was prepared with methanol and DPPH without adding sample. Absorbance was read at 517 nm on UV-VIS spectrophotometer (Schemadzu - Model No. UV-1800). Radical scavenging activity was calculated using following formula.

$$\text{Radical scavenging activity (\%)} = \frac{A1}{A\text{-}A1} \times 100$$

Where
A= Absorbance of control.
A1= Absorbance of sample.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay of CLE was performed according to Faria *et al.,* (2005). FRAP reagent [10 Vol of 300 mM acetate buffer pH 3.6 (3.1 g sodium acetate + 16 ml glacial acetic acid) + 1 Vol of 10 mM TPTZ in 40 mM HCl +1 vol of 20 mM $FeCl_3$] was diluted to 1/3 with methanol and pre warmed to 37°C. FRAP reagent 3 ml was mixed with 200 µl of CLE (1 mg/ml). The mixture was shaken well and incubated at 37°C in water bath for 30 min. Blank samples were also incubated at same temperature and time. The absorbance of samples was read at 593 nm against blank using UV-VIS spectrophotometer (Schemadzu - Model No. UV-1800). Standard curve of $FeSO_4.7H_2O$ was used and results were recorded as µM Fe (II) equivalent/g dry weight of sample.

Treatment of meat samples

Samples of breast fillets were cleaned by removing visible connective tissue and subcutaneous fat by trimming and washed with chilled distilled water, drained for 10 min and dipped in 0.1 (T1), 0.3 (T2) and 0.5% (T3) chilled aqueous solutions of CLE for 10 min. Meat samples treated with 100 ppm aqueous solution of BHT (T4) and distilled water (T5) were used as negative and positive control, respectively. The fillets were then drained for 10 min, packaged in LDPE pouches of 55 µ thickness and stored under refrigeration (4±1°C) till evidence of spoilage was detected during sensory evaluation. Hygienic conditions were maintained during processing of the fillet samples.

Sensory analysis of meat samples

Fillet samples were removed from the LDPE pouches on 0th, 3rd, 6th and 9th day of storage and offered to semi-trained panelists which included six judges to assess the sensory attributes *viz.* colour, odour and general acceptability by using 5-point hedonic scale (Dzomba *et al.,* 2014) where in 5 denoted liked extremely, 4 liked moderately, 3 neither liked nor disliked, 2 disliked moderately and 1 disliked extremely.

Physico-chemical analysis

pH, extract release volume (ERV), water holding capacity (WHC), thiobarbituric acid reactive substances (TBARS) value

and tyrosine value of the meat samples were determined according to the procedure described respectively by AOAC (2012), Strange *et al*., (1977), Wardlaw *et al.,* (1973), Witte *et al.,* (1970) and Strange *et al.,* (1977).

Microbiological analysis

Total viable count (TVC) and psychrophillic count of the meat samples were determined by following the standard methods of APHA (1984).

Statistical analysis

The experiment was repeated three times with two replicates and the data generated during the study were analyzed by Dunken test through "SPSS-20.0" software package as per standard methods (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Composition of custard apple leaves

The fresh custard apple (*Annona squamosa*) leaves contained 37.89, 20.99, 3.20, 13.62, 11.16 and 51.03% of moisture, crude protein, ether extract, crude fibre, total ash and NFE respectively.

Antioxidant activity of CLE

CLE possessed the antioxidant activity which could be attributed to its content of phenolic compounds and ascorbic acid (Table 1). Chandrashekhar and Kulkarni (2015) reported that, total phenolic content of ethanol extract of *Annona squamosa* L. leaf was 0.114 mg GAE/g and IC50 value for DPPH activity was 80 µg/ml.

Effect of CLE on sensory attributes of chicken breast fillets

The results on effect of varying levels of CLE on sensory attributes of chicken fillets stored at refrigeration (4±1°C) are presented in Table 2.

Colour

Colour scores of T2 and T3 on zero day were significantly ($p<0.01$) lower than controls and T1 indicating the adverse effect of CLE on colour of meat samples which could be attributed to greenish tinge imparted by CLE to the meat samples, however, it was within the acceptable limit. The colour score of both the controls and T1 was reduced significantly ($p<0.01$) on third day but was within the acceptable limit; however, it was unacceptable from sixth

Table 1: Antioxidant activity of CLE.

TPC (µg GAE/mg)	Ascorbic acid (µg AA/mg)	DPPH RSA (% RSA)	FRAP (µM Fe (II) equi/g)
57.13±0.22	163.23±0.40	32.20±0.42	132.43±0.50

Table 2: Effect of varying levels of CLE on the sensory attributes of chicken breast fillets stored at 4±1°C.

Treatment	Day 0	Day 3	Day 6	Day 9	F value
Colour					
T1	4.53 ± 0.08^{Bc}	3.49 ± 0.12^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	650.14**
T2	3.86 ± 0.10^{Ab}	4.04 ± 0.09^{BCb}	4.01 ± 0.14^{Cb}	3.15 ± 0.15^{Ba}	11.74**
T3	3.67 ± 0.14^{A}	3.82 ± 0.10^{B}	3.78 ± 0.11^{B}	3.51 ± 0.16^{C}	1.08^{NS}
T4	4.78 ± 0.04^{BCc}	4.11 ± 0.13^{Cb}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	925.95**
T5	4.81 ± 0.04^{Cc}	4.33 ± 0.08^{Cb}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	2357.94**
F value	35.80**	9.74**	46.87**	164.50**	
Odour					
T1	4.36 ± 0.12^{Bc}	3.07 ± 0.10^{Bb}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	464.70**
T2	3.76 ± 0.09^{Ab}	4.18 ± 0.11^{Cc}	4.15 ± 0.09^{Bc}	2.28 ± 0.08^{Ba}	92.32**
T3	3.53 ± 0.12^{Ab}	4.35 ± 0.14^{Cc}	4.19 ± 0.09^{Bc}	2.54 ± 0.06^{Ca}	58.42**
T4	4.65 ± 0.05^{Cc}	2.50 ± 0.14^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	516.49**
T5	4.74 ± 0.06^{Cc}	2.17 ± 0.08^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	1380.63**
F value	34.01**	71.39**	912.45**	291.13**	
General acceptability					
T1	4.15 ± 0.12^{aBc}	2.93 ± 0.09^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	441.54**
T2	4.02 ± 0.06^{Bb}	4.40 ± 0.12^{Bc}	3.90 ± 0.15^{Bb}	2.45 ± 0.07^{Ba}	64.58**
T3	3.50 ± 0.16^{Ab}	4.46 ± 0.13^{Bd}	4.02 ± 0.06^{Bc}	2.92 ± 0.09^{Ca}	34.09**
T4	4.50 ± 0.11^{Cc}	3.06 ± 0.15^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	342.28**
T5	4.92 ± 0.03^{Dc}	2.88 ± 0.09^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	1504.95**
F value	25.86**	48.67**	494.54**	316.64**	

Means±(S.E.) bearing different superscripts (between column small letters and between rows capital letters) differ significantly. *Significant value ($p<0.05$); **Highly significant value ($p<0.01$); NS: Non-significant value. n=6, T1: 0.1% CLE; T2: 0.3% CLE; T3: 0.5% CLE; T4: BHT; T5: Distilled water.

day onwards. Colour score of T2 samples decreased significantly on ninth day, whereas, that of T3 samples was maintained in acceptable limit for longer period than the controls. Gradual decrease in liking of colour of both the controls and T1 could be attributed to increase in microbial load of those meat samples with storage periods leading to fading of natural colour of the chicken. Similar trend has been documented by Khare *et al.,* (2016b) for chicken breast fillets coated with carrageenan, citric acid and cinnamon oil. Simitzia *et al*., 2008 and Badee *et al*., 2013 reported that natural antioxidants may retard color loss in meat by delaying formation of metmyoglobin.

Odour

Odour scores of T2 and T3 on zero day were significantly ($p<0.01$) lower than T1 and both the controls indicating adverse effect of CLE on odour of meat samples, but within the acceptable limit. This could be attributed to characteristic odour imparted by CLE to the meat samples. On third day the scores of T1 and both the controls were significantly ($p<0.01$) lowered and samples were unacceptable, whereas those of T2 and T3 were significantly ($p<0.01$) increased. However, the scores of T2 and T3 samples were decreased significantly ($p<0.01$) on the ninth day. The results demonstrated that the CLE maintained odour of the meat samples in acceptable limit for longer period as compared to the controls. Odour of the T3 meat sample was acceptable till sixth day, probably due to its comparatively higher antioxidant and antibacterial activity due to higher concentration of CLE. Production of off odours could be attributed to the lipid peroxidation and proteolysis owing to increased microbial load of the meat samples with storage periods (Jay, 1966). Similar findings have been recorded by Badee *et al*., (2013) for chicken drumstick treated with marjoram essential oil, Pavelkova *et al.,* (2013) for chicken breast treated with oregano essential oil and by Khare *et al.,* (2016b) for chicken breast fillets treated with an edible coating of carrageenan, citric acid and cinnamon oil.

General acceptability

General acceptability of T3 samples was significantly ($p<0.01$) lower than all other treatments on zero day indicating the adverse effect of CLE on the acceptability of the meat samples, though it was within the acceptable limit. This could be attributed to the characteristic colour and odour imparted by CLE to the meat samples. On third day both the controls and T1 were unacceptable with significantly ($p<0.01$) lower scores, whereas scores of T2 and T3 were significantly ($p<0.01$) increased indicating good effect of CLE on general acceptability of the meat samples. However, acceptability score of T2 and T3 significantly ($p<0.01$) decreased on ninth day. The results demonstrated that, the CLE maintained general acceptability of the meat samples in acceptable limit for longer period compared to the controls, in which T2 and T3 were acceptable till sixth day of storage probably due to the antioxidant and antibacterial activity of CLE demonstrated during the experiment. A gradual decrease in general acceptability of all the treatments with storage periods could be attributed to production of off odours due to lipolysis and proteolysis caused by increased microbial load of the meat samples as well as fading of colour of the chicken with storage duration. Similar results have been documented by Badee *et al*., (2013) who reported the extension of shelf life of chicken drumsticks treated with 0.1% and 0.2% marjoram essential oil up to 9 days and 12 days respectively compared to 7 days with untreated samples. Results were also in agreement with those reported by Khare *et al.,* (2016a) while studying effect of alginate, citric acid, calcium chloride and cinnamon oil edible coating on the shelf life of chicken fillets under refrigeration conditions.

Effect of CLE on physicochemical properties of chicken breast fillets

Results of the effect of varying levels of CLE on physicochemical attributes of chicken breast fillets stored at refrigeration (4±1°C) are presented in Table 3. Both the controls and T1 samples were evaluated as unacceptable by the judges on third day of storage, hence they were discarded and their evaluation was not conducted.

The pH of all treatments was significantly ($p<0.01$) lower than the controls on zero and third day. There was significant ($p<0.01$) increase in pH of all the samples on third day after which pH of T2 samples significantly ($p<0.01$) decreased from sixth day till end of the storage, while that of T3 samples decreased significantly ($p<0.01$) on ninth day. A decrease in pH might be attributed to acid production by lactic acid bacteria while increase in pH could be attributed to alkalinizing substances produced by the microbes and ammonia due to amino acid degradation (Jay, 1966).

ERV and WHC of T3 samples was significantly ($p<0.01$) higher than all other treatments and controls on zero and third day. Whereas, there was a significant ($p<0.01$) decrease in ERV and WHC of all the treatments with storage periods. Kandeepan and Biswas (2007) and Jayanthi *et al.,* (2017) have documented similar results for buffalo and goat meat respectively.

Difference in TBARS and tyrosine values of meat samples was significant on all days except zero day of the storage. The TBARS values off all the treatments were significantly ($p<0.01$) lower than the controls on zero and third day, while that of T3 was significantly ($p<0.01$) lower than T2 on sixth and ninth day. The results were in agreement with those recorded by Sheikh Dalia (2014) who revealed lower TBARS values in chicken breast meat coated with gum arabic and plantago during entire storage period of 21 days under refrigeration. Tyrosine value of T2 and T3 samples was significantly ($p<0.01$) lower than T1 and controls on third day and that of T3 was significantly ($p<0.01$) lower than T2 on sixth and ninth day. The TBARS and tyrosine values of all the samples increased significantly ($p<0.01$) with advancement of the storage periods. However, those of the meat samples treated with 0.3 and 0.5% CLE were within the acceptable limit of spoilage up to sixth day.

This supported the results of sensory evaluation and could be attributed to the antibacterial and antioxidant activity of CLE demonstrated during the experiment. Similar results have been documented by Khare *et al.,* (2016b) in chicken breast fillets with edible coating of carrageenan, citric acid and cinnamon oil. The results were also in agreement with Santosh Kumar *et al.,* (2014) who revealed that tyrosine value of chicken increased significantly (P<0.05) during chilled storage.

Effect of CLE on microbiological parameters of chicken breast fillets

Results of effect of varying levels of CLE on microbiological parameters of chicken breast fillets stored at 4±1°C are presented in Table 4. Both the controls and T1 samples were evaluated as unacceptable by the judges on third day of storage, hence they were discarded and their evaluation was not conducted further. TVC and psychrophilic counts significantly (p<0.01) differed between the storage periods for all the treatments as well as between the treatments for all the storage periods. Both the bacterial counts were significantly (p<0.01) lower in treatments than the controls and the counts decreased with increasing concentration of CLE, which could be attributed to the antibacterial activity of the CLE demonstrated during the experiment. TVC as well as psychrophilic count of the meat samples treated with 0.3 and 0.5% CLE were within the acceptable limit up to

Table 3: Effect of CLE on physicochemical properties of chicken breast fillets stored at refrigeration (4±1°C).

Treatment	Day 0	Day 3	Day 6	Day 9	F value
pH					
T1	5.82±0.01^{Ba}	5.97±0.01^{Bb}	Rejected	Rejected	139.28**
T2	5.73±0.01^{Aa}	6.11±0.04^{CDc}	5.90±0.02^{Bb}	5.68±0.02^{a}	71.32**
T3	5.67±0.01^{Aa}	5.71±0.01^{Abc}	5.72±0.01^{Ac}	5.68±0.01^{ab}	6.46**
T4	5.88±0.03^{Ca}	6.05±0.05^{Cb}	Rejected	Rejected	10.47**
T5	5.93±0.04^{Ca}	6.18±0.01^{Db}	Rejected	Rejected	33.01**
F value	21.96**	42.93**	114.72**	00.00^{NS}	
ERV (ml)					
T1	21.60±0.23^{Ab}	19.88±0.24^{Ba}	Rejected	Rejected	27.43**
T2	22.03±0.73^{Ac}	20.80±0.66^{Bc}	18.70±0.20^{b}	13.77±0.20^{Aa}	50.60**
T3	23.81±0.29^{Bd}	22.27±0.17^{Cc}	19.40±0.59^{b}	18.26±0.21^{Ba}	52.18**
T4	22.05±0.34^{Ab}	18.41±0.34^{Aa}	Rejected	Rejected	57.01**
T5	20.97±0.33^{Ab}	17.95±0.25^{Aa}	Rejected	Rejected	52.46**
F value	6.25**	22.28**	1.28^{NS}	236.23**	
WHC (%)					
T1	65.30±0.50^{Ab}	63.63±0.43^{Aa}	Rejected	Rejected	6.45**
T2	66.12±0.35^{Ac}	65.57±0.38^{Cc}	63.44±0.40^{Ab}	60.07±0.62^{Aa}	37.07**
T3	67.75±0.27^{Bc}	67.19±0.15^{Dc}	65.32±0.31^{Bb}	62.80±0.37^{Ba}	60.62**
T4	65.19±0.63^{Ab}	62.77±0.44^{Aa}	Rejected	Rejected	9.96**
T5	65.87±0.57^{Ab}	62.04± 0.38^{Aa}	Rejected	Rejected	31.11**
F value	4.56**	32.16**	13.89**	14.22**	
TBARS value (mg MDA/kg)					
T1	0.226±0.003^{ABa}	0.236±0.001^{Bb}	Rejected	Rejected	12.54**
T2	0.221±0.002^{Aa}	0.228±0.001^{Ab}	0.334±0.002^{Bc}	0.486±0.001^{Bd}	4875.16**
T3	0.222±0.002^{Aa}	0.225±0.001^{Aa}	0.316±0.002^{Ab}	0.477±0.001^{Ac}	5377.95**
T4	0.225±0.003^{ABa}	0.416±0.004^{Cb}	Rejected	Rejected	1734.26**
T5	0.231±0.001^{Ba}	0.518±0.001^{Db}	Rejected	Rejected	37857.82**
F value	2.69**	4317.43**	42.50**	21.51**	
Tyrosine (mg/100 g)					
T1	8.30±0.49^{a}	10.61±0.73^{Bb}	Rejected	Rejected	6.97*
T2	7.74±0.37^{a}	8.76±0.39^{Ab}	10.80±0.26^{Bc}	12.84±0.24^{Bd}	49.03**
T3	7.93±0.31^{a}	8.48±0.45^{Aab}	9.50±0.37^{Ab}	10.98±0.44^{Ac}	11.47**
T4	7.46±0.27^{a}	10.33±0.20^{Bb}	Rejected	Rejected	73.78**
T5	7.93±0.23^{a}	12.09±0.33^{Cb}	Rejected	Rejected	104.18**
F value	0.78^{NS}	10.52**	8.05**	13.69**	

Means±(S.E.) bearing different superscripts (between column small letters and between rows capital letters) differ significantly. *Significant value (p<0.05); **Highly significant value (p<0.01); NS: Non-significant value. n=6, T1: 0.1% CLE; T2: 0.3% CLE; T3: 0.5% CLE; T4: BHT; T5: Distilled water.

Table 4: Effect of CLE on microbiological parameters of chicken breast fillets stored at refrigeration (4±1°C).

Treatment	Day 0	Day 3	Day 6	Day 9	F value
TVC (log10 CFU/g)					
T1	4.07 ± 0.01^{Ca}	$5.23.01^{Cb}$	Rejected	Rejected	7081.45**
T2	3.90 ± 0.02^{Ba}	4.72 ± 0.03^{Bb}	5.02 ± 0.01^{Bc}	7.06 ± 0.01^{Bd}	4343.56**
T3	3.67 ± 0.02^{Aa}	4.59 ± 0.02^{Ab}	4.90 ± 0.01^{Ac}	6.45 ± 0.01^{Ad}	4807.41**
T4	4.24 ± 0.01^{Da}	6.06 ± 0.01^{Db}	Rejected	Rejected	17575.44**
T5	4.31 ± 0.01^{Ea}	6.17 ± 0.01^{Eb}	Rejected	Rejected	24037.00**
F value	293.12**	1603.09**	46.58**	2203.25**	
Psychrophilic count (log10 CFU/g)					
T1	00.00^{a}	3.87 ± 0.02^{Cb}	Rejected	Rejected	68890.00**
T2	00.00^{a}	3.81 ± 0.02^{Bb}	3.89 ± 0.01^{Bc}	4.13 ± 0.01^{Bd}	21096.38**
T3	00.00^{a}	3.61 ± 0.02^{Ab}	3.68 ± 0.02^{Ac}	3.83 ± 0.02^{Ad}	10987.73**
T4	00.00^{a}	3.95 ± 0.01^{Db}	Rejected	Rejected	142440.64**
T5	00.00^{a}	4.03 ± 0.01^{Eb}	Rejected	Rejected	166375.00**
F value		90.92**	63.27**	247.46**	

Means±(S.E.) bearing different superscripts (between column small letters and between rows capital letters) differ significantly. *Significant value ($p<0.05$); **Highly significant value ($p<0.01$); NS: Non-significant value. n=6, T1: 0.1% CLE; T2: 0.3% CLE; T3: 0.5% CLE; T4: BHT; T5: Distilled water.

sixth day of storage, supporting the results of sensory evaluation. Similar results have been documented by Khare *et al.,* (2016a) for total viable count of chicken breast fillets stored at 4±1°C.

CONCLUSION

Total phenolic content and ascorbic acid content of hydro-ethanolic (50%) extract of custard apple (*Annona squamosa*) leaves were 57.13±0.22 µgGAE/mg and 163.23±0.40 µgAA/mg of the extract respectively, as a result of which the extract exhibited significant antioxidant activity. The extract improved shelf life of the chicken breast fillets stored at refrigeration (4±1°C) with acceptable sensory scores, physicochemical attributes and microbiological quality of the fillets. Chicken breast fillets treated with 0.3% and 0.5% aqueous solution of CLE were organoleptically acceptable up to 6th day of storage at 4±1°C without alteration in physicochemical parameters and acceptable microbiological quality. From the present investigation it can be concluded that 0.3% and 0.5% aqueous solution of CLE can be used for extension of shelf life of chicken breast fillets up to 6 days at refrigeration (4±1°C). However, further research is necessary for development of technology for production of natural antioxidant of commercial value by using custard apple (*Annona squamosa*) leaves.

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