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Effect of Custard Apple (*Annona squamosa*) Seed Extract on Quality of Chicken Breast Fillets

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ABSTRACT

Chicken (Broiler) breast fillets were dipped separately for 10 min in distilled water, 100 ppm solution of BHT and 0.1, 0.3 and 0.5% aqueous solutions of custard apple (*Annona squamosa*) seed extract (CSE), stored under refrigeration (4±1°C) and analyzed on 0th, 3rd, 6th and 9th day of storage. Ascorbic acid content, Total Phenolic content, Ferric Reducing Antioxidant Power and DPPH radical scavenging assay of CSE were respectively 645.07±0.32 µgAA/mg, 234.30±0.44 µgGAE/mg, 882.95±0.34 µMFe (II) equi/g and 78.35±0.49%. The fillets treated with 0.5% solution of CSE had significantly ($p<0.01$) lower score than the control on 5 point hedonic scale on zero day of storage. However, they were acceptable till ninth day with significantly ($p<0.01$) higher scores than the controls. The ERV, WHC, pH, TBARS value, Tyrosine value, Total plate count and Psychrophillic count of the ninth day samples treated with 0.5% CSE were respectively 18.06±0.14ml, 63.63±0.46%, 5.72±0.01, 0.490±0.002 mgMDA/Kg, 9.78±0.20 mg/100g, 5.07±0.01 log10CFU/g and 3.83±0.01 log10CFU/g.

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

INTRODUCTION

Oxidative rancidity and microbial growth are the major problems causing quality deterioration of chicken, therefore, to maintain its safety and quality application of preservation technologies is necessary. One of the possibilities to achieve this is use of natural preservatives which prevent the growth of spoilage and pathogenic microorganisms (Chouliara and Kontominas, 2006) and lipid oxidation (Kumar *et al.*, 2015). 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 (Matan *et al.,* 2006, Suppakul *et al.,* 2003).

Chicken meat is more vulnerable to lipid oxidation as it is relatively rich in unsaturated fatty acids (Valsta *et al.,* 2005). Synthetic antioxidants are used in food industries for controlling alterations in sensory parameters. However, use of synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ) in preserving foods is under strict regulation in many countries because of their associated toxic and carcinogenic effects (Jo *et al.,* 2006). Consequently, there is interest from the scientists and manufacturers in using naturally occurring preservatives with antimicrobial and antioxidant properties.

Many workers have documented that custard apple (*Annona squamosa*) pulp, peel, seeds, leaves, bark and roots are good sources of natural antioxidants and antimicrobial compounds (Saha 2011; Abdalbasit *et al.,* 2012; Srivastava *et al.,* 2013; Gowdhami *et al.,* 2014; Roy and Lingampeta, 2014). However, literature is not available on its use in preservation of meat and meat products. Hence, this study was undertaken to assess the effect of CSE on shelf life of the chicken breast fillets as evidenced by the sensory attributes when stored aerobically in LDPE pouches under refrigeration (4±1°C).

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MATERIALS AND METHODS

Boneless and diskinned chicken breast fillets of uniform size, shape and weight (200±10 gm) obtained 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 55µ thickness were purchased from the local market and used for packaging of the breast fillets. Fresh, healthy and ripened custard apple (*Annona squamosa*) fruits of 'Balanagar' variety were procured from orchard of a farmer nearby the Nagpur Veterinary College, Nagpur.

Proximate analysis of custard apple seeds

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

Preparation of custard apple seed extract (CSE)

After washing with drinking water the custard apple fruits were cut manually and seeds were separated from the pulp.

Seeds were dried in hot air oven at 50°C till constant weight was attained. Dried seeds 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. Extract was prepared by the method suggested by Jagtap and Bapat (2012) and Gowdhami *et al.,* (2014) with slight modification. Seed 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 no. 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. Hygienic conditions were maintained during all stages of extract preparation.

Estimation of total phenolic content (TPC)

Total phenolic content of CSE was estimated according to the method of Singleton and Rossi (1965) with slight modification. 200 µl of extract sample (1mg/ml) 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.

Estimation of ascorbic acid content

Ascorbic acid content of the CSE was determined as suggested by Benites *et al.,* (2013) with slight modifications. 4 ml extract sample dissolved in 0.1% DMSO (2.5, 5 and 10 mg/ml) 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 blank. Results were expressed as µgAA/mg of extract by using ascorbic acid standard curve.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay of CSE was performed according to Faria *et al.,* (2005) with slight modifications. FRAP reagent [10 Vol of 300mM acetate buffer pH 3.6 (3.1g sodium acetate + 16ml 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. 3 ml FRAP reagent was mixed with 200 µl of extract (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.

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

DPPH radical scavenging activity of CSE was estimated according to Harbarne (1973) with slight modifications. 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.

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

Where, A = Absorbance of control and
A1= Absorbance of sample

Treatment of meat samples

Breast fillets were trimmed to remove visible connective tissue and subcutaneous fat, washed with chilled drinking water, drained for 10 min and dipped in 0.1 (T1), 0.3 (T2) and 0.5% (T3) chilled aqueous solutions of CSE for 10 min. Meat samples treated with 100 ppm aqueous solution of BHT (T4) and Distilledwater (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 complete processing of the meat samples.

Sensory analysis of meat samples

Meat samples were removed from the LDPE pouches on 0^{th}, 3^{rd}, 6^{th} and 9^{th} day of storage and offered to semi-trained panellists 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) wherein 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) with slight modifications.

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 seed

Proximate analysis revealed that custard apple seeds contained 48.97, 19.68, 16.66, 42.40, 2.00 and 19.26% moisture, crude protein, ether extract, crude fibre, total ash and NFE, respectively.

Antioxidant activity of CSE

Data on DPPH and FRAP assay revealed that CSE possessed good antioxidant activity which could be attributed to its high 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. seeds was 0.091 mg GAE/g and C50 value for DPPH activity was 790 µg/ml.

Table 1: Antioxidant activity of CSE.

TPC (µg GAE/mg)	Ascorbic acid (µg AA/mg ext)	DPPH Radical scavenging assay (% RSA	FRAP (µM Fe (II) equi/g)
234.30 ±0.44	645.07±0.32	78.35±0.49	882.95 ±0.34

Effect of CSE on sensory attributes of chicken breast fillets

The results on effect of varying levels of CSE on sensory attributes of chicken fillets are presented in Table 2.

Colour

Zero day results revealed that both the controls were liked extremely, whereas T1, T2 and T3 were liked moderately, wherein scores of T2 and T3 were significantly ($p<0.01$) lower than both the controls and T1 indicating adverse effect of CSE on colour of meat samples which could be attributed to greenish tinge imparted by CSE to the meat samples, however it was within the acceptable limit. Colour score of both the controls was reduced significantly ($p<0.01$) on third day but was within the acceptable limit; however those were unacceptable from sixth day onwards. There was non-significant reduction in the colour score of T2 samples till sixth day and T3 samples till ninth day of the storage which indicated that CSE maintained natural colour of the samples in acceptable limit for longer period than the controls. Gradual decrease in liking of colour of both the controls and T1 with storage periods 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 have been documented by Khare *et al,.* (2016b) for chicken breast fillets coated with carrageenan, citric acid and cinnamon oil.

Odour

On zero day both the controls were liked extremely, whereas

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

Treatments	Day 0	Day 3	Day 6	Day 9	F value
			Colour		
T1	4.36 ± 0.12^{ABb}	4.19 ± 0.12^{b}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	521.90**
T2	4.22 ± 0.13^{Ab}	3.95 ± 0.11^{b}	4.00 ± 0.10^{Bb}	2.83 ± 0.14^{Ba}	26.37**
T3	4.17 ± 0.10^{A}	4.19±0.10	4.05 ± 0.13^{B}	3.91 ± 0.10^{C}	1.37^{NS}
T4	4.58 ± 0.07^{BCc}	4.36 ± 0.09^{b}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	1201.93**
T5	4.78 ± 0.03^{Cc}	4.16 ± 0.08^{b}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	2438.70**
F Value	7.16**	2.21^{NS}	525.40**	292.55**	
			Odour		
T1	4.17 ± 0.11^{Bc}	2.55 ± 0.10^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	423.90**
T2	3.78 ± 0.07^{Bc}	3.78 ± 0.06^{Cc}	4.00 ± 0.06^{Cb}	2.00 ± 0.06^{Aa}	222.98**
T3	3.64 ± 0.12^{A}	4.03 ± 0.13^{C}	4.30 ± 0.12^{C}	4.05 ± 0.10^{C}	5.46^{NS}
T4	4.58 ± 0.13^{Cc}	3.08 ± 0.09^{Bb}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	489.06**
T5	4.66 ± 0.06^{Cc}	2.50 ± 0.13^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	595.86**
F Value	21.61**	43.12**	844.00**	608.47**	
			General Acceptability		
T1	4.20 ± 0.17^{Cc}	2.94 ± 0.07^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	293.36**
T2	4.08 ± 0.10^{Bc}	4.30 ± 0.15^{Cc}	3.47 ± 0.08^{Bb}	1.61 ± 0.06^{Aa}	137.41**
T3	3.75 ± 0.15^{Aa}	4.50 ± 0.06^{Cc}	4.30 ± 0.08^{Cbc}	4.03 ± 0.17^{Cab}	6.88*
T4	4.63 ± 0.07^{Cc}	4.08 ± 0.07^{Bb}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	1602.31**
T5	4.83 ± 0.06^{Cc}	3.02 ± 0.12^{Ab}	1.00 ± 0.00^{Aa}	1.00 ± 0.00^{Aa}	784.83**
F value	13.33**	52.70**	1022.94**	273.82**	

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. n=6, T1: 0.1% CSE; T2: 0.3% CSE; T3: 0.5% CSE; T4: BHT; T5: Distilled water.

T1, T2 and T3 were liked moderately, wherein T3 was the lowest and significantly ($p<0.01$) lower than T1, T2 and both the controls indicating adverse effect of CSE on odour of meat samples, but within the acceptable threshold. This could be attributed to characteristic odour imparted by CSE to the meat samples. On third day T1 and both the controls were neither liked nor disliked, whereas T2 and T3 were liked moderately with significantly ($p<0.01$) higher score in which T3 was the highest and continued to be the highest till end i.e. ninth day of storage which indicated good effect of CSE on odour of the meat samples. T3 was liked moderately on sixth as well as ninth day, whereas T2 was liked moderately on sixth and disliked moderately on ninth day. The results demonstrated that the CSE maintained odour of the meat samples in acceptable limit for longer period compared to the controls, in which odour of T3 with the highest (0.5%) concentration of CSE was acceptable till ninth day of storage. This could be attributed to the antioxidant and antibacterial activity of CSE demonstrated during the experiment. There was significant ($p<0.01$) and gradual decrease in liking of odour of T1, T2 and both the controls with storage periods 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). However, odour of the T3 meat samples was maintained within the acceptable limit till end of the storage period i.e. ninth day, probably due to its comparatively higher antioxidant and antibacterial activity, considering higher concentration of CSE. Similar findings have been documented by Pavelkova *et al.,* (2013) for chicken breast treated with oregano essential oil and by Khare *et al.,* (2016b) for chicken breast fillets treated with edible coating of carrageenan, citric acid and cinnamon oil where samples had lower scores initially but the scores increased on third and fifth day of storage.

General acceptability

There was highly significant ($p<0.01$) difference between the storage periods for T1, T2 and both the controls, whereas it was significant ($p<0.05$) for T3 indicating gradual decrease in general acceptability of all the treatments with storage periods owing to production of off odours due to lypolysis and proteolysis caused by increased microbial load of the meat samples as well as fading of colour of the chicken with storage duration. On zero day both the controls were liked extremely, whereas T1, T2 and T3 were liked moderately, wherein T3 was the lowest and significantly ($p<0.01$) lower than all other treatments indicating adverse effect of CSE on general acceptability of the meat samples, though it was within the acceptable limit. This could be attributed to characteristic colour and odour imparted by CSE to the meat samples. On third day T1 and negative control were neither liked nor disliked, whereas T2, T3 and positive control were moderately liked, wherein T2 and T3 were significantly ($p<0.01$) higher than both the controls as well as T1 indicating good effect of CSE on general acceptability of the meat samples. T3 was liked moderately on sixth as well as ninth day with significantly ($p<0.01$) higher score than T2 which was neither liked nor disliked on sixth, whereas disliked moderately on ninth day. The results demonstrated that the CSE maintained general acceptability of meat samples in acceptable limit for longer period compared to the controls, in which T3 having the highest (0.5%) concentration of CSE was acceptable till ninth day of storage probably due to the antioxidant and antibacterial activity of CSE demonstrated during the experiment. Similar results have been documented by Khare *et al.,* (2016a) while studying effect of alginate, citric acid, calcium chloride and cinnamon oil edible coating on shelf life of chicken fillets under refrigeration conditions.

Effect of CSE on physicochemical properties of chicken breast fillets

Results of the effect of varying levels of CSE on physicochemical attributes of chicken breast fillets are presented in Table 3. Both the controls and samples treated with 0.1% CSE were evaluated as unacceptable on third day of storage during sensory evaluation; hence their analysis was not conducted further.

pH of all the treatments was significantly ($p<0.01$) lower than the controls on zero and third day, indicating the effect of CSE on meat samples which persisted till end i.e. ninth day of the storage. There was increase in pH of all the treatments on third day, however it was significant ($p<0.01$) for controls and T1 and nonsignificant for T2 and T3. pH of T2 samples initially increased and then decreased from sixth day till end of the storage, while that of T3 samples gradually increased till end of the storage. This change is pH was nonsignificant for T2 and significant ($p<0.01$) for T3 but it was within the acceptable limit. Decrease in pH might be attributed to acid production by lactic acid bacteria during initial phases of storage and increase in pH could be attributed to alkalinizing substances produced by the microbes and ammonia due to amino acid degradation (Jay, 1966).

Effect of the treatments on ERV, WHC, TBARS value and tyrosine value of meat samples was nonsignificant on zero and significant on all other days of the storage. ERV and WHC increased, whereas TBARS and tyrosine values decreased significantly ($p<0.01$) with increase in the concentration of CSE. ERV and WHC decreased, whereas TBARS and tyrosine values of all the treatments increased significantly ($p<0.01$) with advancement of the storage periods. However, those of the meat samples treated with 0.3 and 0.5% CSE were within the acceptable limit of spoilage up to ninth day. This supported the results of sensory evaluation and could be attributed to the antibacterial and antioxidant activity of CSE 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.

Effect of CSE on microbiological parameters of chicken breast fillets

Results of effect of varying levels of CSE on microbiological

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

Treatments	Day 0	Day 3	Day 6	Day 9	F value
			pH		
T1	5.81 ± 0.01^{Ba}	5.97 ± 0.02^{ABb}	Rejected	Rejected	75.08**
T2	5.72 ± 0.01^{A}	6.07 ± 0.32^{AB}	5.75 ± 0.01^{B}	5.65 ± 0.01^{A}	1.28^{NS}
T3	5.67 ± 0.01^{Aa}	5.68 ± 0.01^{Aa}	5.70 ± 0.01^{Aab}	5.72 ± 0.01^{Bb}	4.93**
T4	5.94 ± 0.03^{Ca}	6.17 ± 0.02^{Bb}	Rejected	Rejected	34.59**
T5	5.98 ± 0.05^{Ca}	6.17 ± 0.02^{Bb}	Rejected	Rejected	15.73**
F value	27.05**	1.92^{NS}	18.84**	21.75**	
			ERV ml		
T1	21.07 ± 0.57^{b}	18.35 ± 0.26^{Aa}	Rejected	Rejected	18.86**
T2	21.91 ± 0.79^{d}	20.27 ± 0.67^{Bc}	17.99 ± 0.16^{Ab}	13.04 ± 0.35^{Aa}	48.89**
T3	22.39 ± 0.97^{c}	20.52 ± 0.74^{Bbc}	19.00 ± 0.32^{Bab}	18.06 ± 0.14^{Ba}	8.83**
T4	21.47 ± 0.45^{b}	18.58 ± 0.27^{Aa}	Rejected	Rejected	30.27**
T5	21.64 ± 0.55^{b}	18.49 ± 0.22^{Aa}	Rejected	Rejected	28.46**
F value	0.51^{NS}	4.75**	7.85*	179.43**	
			WHC%		
T1	65.40 ± 0.46^{b}	63.15 ± 0.53^{Aa}	Rejected	Rejected	10.26**
T2	66.64 ± 0.43^{c}	65.54 ± 0.47^{Bc}	62.99 ± 0.45^{Ab}	60.07 ± 0.62^{Aa}	34.71**
T3	67.67 ± 0.45^{c}	67.11 ± 0.44^{Bc}	65.66 ± 0.55^{Bb}	63.63 ± 0.46^{Ba}	14.44**
T4	65.77 ± 0.64^{b}	62.91 ± 0.82^{Aa}	Rejected	Rejected	7.58*
T5	65.63 ± 0.78^{b}	61.23 ± 0.85^{Aa}	Rejected	Rejected	14.62**
F value	2.70^{NS}	12.99**	14.16**	21.39**	
			TBARS value mg MDA/kg		
T1	0.239 ± 0.001^{a}	0.337 ± 0.001^{Cb}	Rejected	Rejected	3951.79**
T2	0.241 ± 0.001^{a}	0.277 ± 0.001^{Bb}	0.425 ± 0.003^{Bc}	0.552 ± 0.002^{Bd}	5248.75**
T3	0.240 ± 0.001^{a}	0.252 ± 0.001^{Ab}	0.391 ± 0.002^{Ac}	0.490 ± 0.002^{Ad}	6359.68**
T4	0.242 ± 0.001^{a}	0.404 ± 0.002^{Db}	Rejected	Rejected	3294.23**
T5	0.239 ± 0.002^{a}	0.510 ± 0.002^{Eb}	Rejected	Rejected	10817.78**
F value	0.50^{NS}	4361.94**	120.56**	396.74**	
			Tyrosine mg/100g		
T1	7.28 ± 0.37^{a}	8.76 ± 0.40^{Ab}	Rejected	Rejected	7.53*
T2	7.74 ± 0.42^{a}	8.85 ± 0.51^{Aab}	9.96 ± 0.23^{Bb}	11.91 ± 0.36^{Bc}	20.22**
T3	7.37 ± 0.37^{a}	8.02 ± 0.30^{Aab}	8.48 ± 0.31^{Ab}	9.78 ± 0.20^{Ac}	11.33**
T4	7.65 ± 0.44^{a}	9.04 ± 0.47^{Ab}	Rejected	Rejected	4.67*
T5	7.46 ± 0.27^{a}	10.33 ± 0.29^{Bb}	Rejected	Rejected	54.07**
F value	0.26^{NS}	4.38**	14.58**	26.26**	

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. n=6, T1: 0.1% CSE; T2: 0.3% CSE; T3: 0.5% CSE; T4: BHT; T5: Distilled water.

quality of chicken breast fillets stored at 4±1°C are presented in Table 4. Chicken breast fillets treated with both the controls and T1 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 psychrophillic count differed significantly ($p<0.01$) between the treatments for all the storage periods as well as between the storage periods for all the treatments. Both the bacterial counts were significantly ($p<0.01$) lower in treatments than the controls and the counts decreased with increasing concentration of CSE, which could be attributed to the antibacterial activity of the CSE. TVC as well as psychrophillic count of the meat samples treated with 0.3 and 0.5% CSE were within the acceptable limit till the end i.e. up to ninth 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 and ascorbic acid content of hydroethanolic (50%) extract of custard apple (*Annona squamosa*) seed were respectively 234.30±0.44 µgGAE/mg and 645.07±0.32 µgAA/mg of the extract as a result of which the extract possessed significant antioxidant as well as antibacterial activity. The extract demonstrated significant increase in

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

Treatments	Day 0	Day 3	Day 6	Day 9	F value
			TVC log10 CFU/g		
T1	4.05 ± 0.013^{Ca}	5.17 ± 0.01^{Bb}	Rejected	Rejected	5569.99**
T2	3.97 ± 0.013^{Ba}	4.62 ± 0.02^{Ab}	4.94 ± 0.01^{Bc}	6.10 ± 0.01^{Bd}	5710.27**
T3	3.84 ± 0.013^{Aa}	4.57 ± 0.02^{Ab}	4.82 ± 0.01^{Ac}	5.07 ± 0.01^{Ad}	1983.20**
T4	4.18 ± 0.010^{Da}	5.84 ± 0.05^{Cb}	Rejected	Rejected	935.71**
T5	4.26 ± 0.008^{Ea}	6.13 ± 0.01^{Db}	Rejected	Rejected	20074.14**
F value	213.82**	720.34**	72.49**	6871.64**	
			Psychrophillic Count log10 CFU/g		
T1	00.00^{a}	3.71 ± 0.02^{Bb}	Rejected	Rejected	24007.15**
T2	00.00^{a}	3.67 ± 0.02^{Bb}	3.77 ± 0.02^{Bc}	4.16 ± 0.00^{Bd}	22279.74**
T3	00.00^{a}	3.55 ± 0.01^{Ab}	3.63 ± 0.01^{Ac}	3.83 ± 0.01^{Ad}	30727.03**
T4	00.00^{a}	3.83 ± 0.01^{Cb}	Rejected	Rejected	164142.89**
T5	00.00^{a}	3.88 ± 0.01^{Cb}	Rejected	Rejected	164947.44**
F value	00.00	69.11**	41.40**	556.75**	

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. n=6, T1: 0.1% CSE; T2: 0.3% CSE; T3: 0.5% CSE; T4: BHT; T5: Distilled water.

shelf life of the chicken breast fillets stored at refrigeration (4±1°C) with acceptable sensory scores supported by the increased/decreased physicochemical attributes and microbiological quality of the fillets. Chicken breast fillets treated with 0.5% aqueous solution of CSE were sensorialy acceptable up to 9th day of storage at 4±1°C without alteration in physicochemical parameters and acceptable microbiological quality.

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