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Research Article

Effect of calcium chloride and gallic acid combination on the extension of postharvest life of *Lagenaria siceraria*, a vegetable with medicinal importance

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ABSTRACT

Lagenaria siceraria (Mol.) Standl. consists of all essential elements that are required for good and normal human health. Post harvest losses occur due to lack of proper packaging material, microbial spoilage, and improper handling during transport. Thus, this study deals with viability, sensory evaluation, antioxidant enzymatic activities of *L. siceraria* fruits by the treatment of a combination of calcium chloride and gallic acid (abbreviated as CG) for 21 days storage at room temperature. The solutions were prepared by the addition of CG in equal amount with different concentrations: 1:1 µM, 2:2 µM, 3:3 µM, and 4:4 µM. The untreated *L. siceraria* fruits survived for 14 days. However, all the coated *L. siceraria* fruits could sustain for 21 days, and the combination of 2:2 µM CG was superior in all aspects. The combination of calcium chloride and gallic acid showed beneficial effects by delaying the ripening process.

Keywords: *L. siceraria*, $CaCl_2$, gallic acid, postharvest

Abbreviations: CAT- Catalase activity; CG- $CaCl_2$ plus Gallic acid; POD- Peroxidase activity; TTA- Titratable acidity; FCR- Folin and Ciocalteu's phenol reagent

INTRODUCTION

Vegetables can be consumed either raw or in cooked form and considered an imperative part of the human diet. They are low in fats and starch, whereas high in vitamins, minerals, and dietary fiber. Numerous nutritionists urge individuals to devour a lot of foods grown from the ground, at least five plants a day regularly being suggested. *Lagenaria siceraria* (Mol.) Standl. i.e. bottle gourd is a popular culinary vegetables in many tropical regions around the world (India, Japan, Thailand, etc.) and grown year-round in tropical climates. It is used in the field of pharmaceutical and dietary formulation (Decker-Walter *et al.,* 2001). It belongs to the Cucurbitaceae family, which is probably one of the earliest vegetables cultivated by man. *L. siceraria* plant is fast-growing, annual yellowish-green in colour containing white pulp with white seeds embedded in spongy flesh. The plant is a climber with various synonyms, like calabash gourd, lauki, long-squash, etc. (Pradhan *et al.,* 2013).

L. siceraria fruits generally has all the essential elements that are needed for good and normal human health (Kubde *et al.,* 2010). The juice of the fruit is used to regulate the blood pressure of hypertensive patients, due to its high

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potassium content (Parle *et al.,* 2011). The cooling property of it aids to prevent constipation and also has cardio-tonic and diuretic properties (Harika *et al.,* 2012). *L. siceraria* fruit juice is a good herbal cure for a nervous disorder like epilepsy and also for fatigue. *L. siceraria* fruit has low fat as well as low plant sterol content and possesses high dietary fiber which helps to lose weight quickly (Parle, 2011). *L. siceraria* fruits cure pain, fever, ulcers, asthma and other bronchial disorders (Kumar *et al.,* 2012). It has the highest choline content, and it acts as a mental healer and a precursor of acetylcholine that is essential for good memory (Parle *et al.,* 2011). The post-harvest losses in *L. siceraria* fruits occur due to lack of proper packaging material, microbial spoilage, and improper handling during transport (Habib-ur-Rahaman, 2003).

An important role of Ca^{2+} is to provide stability and mechanical strength to the cell structure of the fruit. Ca^{2+}, an essential element has been known to play a significant role in conserving the post-harvest quality of vegetables and fruits (Kirkby and Pilbeam 1984; Bangarth, 1979). Calcium shows many effects: it provides strength to the cell wall, hence delaying the senescence as well as fruit ripening. By controlling the transpiration rate, it decreases weight loss (%). Calcium also maintains the membrane functions like phase transition and membrane fluidity (Ullah *et al.,* 2007). Gallic acid is a normally available phenolic acid that can be extracted from gallnuts, oak bark, tea leaves, and other plants (Alkan *et al.,* 2011). Additionally, it is usually regarded as an antioxidant as well as an antimicrobial agent (Hager *et al.,* 2012). Besides, gallic acid is known to be a natural phenolic crosslinker or plasticizer that can improve the mechanical performance of natural polymer materials (Sun *et al.,* 2014). The major objective of this research work is to treat different concentrations of gallic acid and calcium chloride on the *L. siceraria* fruits and examine the effects on colour, sensory evaluation, and biochemical parameters during storage life and the quality of *L. siceraria* fruits.

MATERIALS AND METHODS

Choice of material

Bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) variety 'Pusa Summer Prolific long' with uniform size was chosen as the experimental material. These cultivars are pale green with a prominent bottleneck at the top. *L. siceraria* fruits were purchased from the local market of Anand (22°33'24.1"N 72°58'04.1"E), Gujarat, India. All the fruits used in the experiment were from the same field and same time of harvest (65 days after sowing).

Sample preparation

The chosen fruits were cleaned with a cotton cloth for the expulsion of shallow dirt. The fruits were further cleaned with distilled water and used later for dipping application. The cleaned *L. siceraria* fruits were sunk for 10 sec in the freshly prepared combinations of 1:1 µM, 2:2 µM, 3:3 µM, and 4:4 µM calcium chloride (Mol. wt. 110.98 g/mol) and gallic acid (Mol. wt. 170. 12 g/mol) solution (volume of 300 ml/each *L. siceraria* fruits sunk) prepared with distilled water. The *L. siceraria* fruits without application of gallic acid were considered as control. All the treated and untreated fruits were kept at room temperature (25 ± 2°C) with 55% relative humidity in the sterile aluminum container. The container itself pre-sterilized by washing with acid followed by cleaning with distilled water and dried overnight (precautions of not having a trace of water). Thirty fruits were treated per treatment for each replication. The chemicals were purchased from Sigma Aldrich, India.

Sensory evaluation

The test was assessed on a 5 point scale for quality characteristics; in particular, colour, odour, taste, and overall acceptance. The panel was composed of 5 trained persons. Each time they judged the treated and untreated fruits for colour and odour, based on extremely noteworthy to extremely unlike. Each treated and untreated fruits were cooked in the same fashion each time and tasted and scored the quality.

Weight loss

The *L. siceraria* fruits once washed and utilized for the experiment were considered as a zero-day sample. The weight loss percentage was calculated by the following formula

Weight loss percentage = {(Fresh weight on day zero-Final weight on the day of the experiment)/(Fresh weight on day zero)} x 100%

This formula was used both in the treated and untreated samples after an interval of every seven days for 21 days.

Titratable acidity (TTA)

Five grams of *L. siceraria* fruit slice was weighed and homogenized with 50 ml of distilled water and centrifuged at 8000g for 20 min. For this 20 ml of supernatant was taken and titrated against 0.1 N NaOH with the addition of 1-2 drops of phenolphthalein indicator. The ml of NaOH used was recorded. Sample containing acid (%) was calculated using the following formula

% Acid = (ml of NaOH × mili equivalent factor × 100) per g of fruit extract sample

Here the acid in the *L. siceraria* fruits was citric acid, hence the equivalent factor used was 0.064.

Estimation of protein

Five grams of *L. siceraria* fruit slice was homogenized in 10 ml 80% acetone in mortar and pestle and then centrifuged at 8000 g for 20 min. at 4°C. The supernatant was collected and used for protein estimation. For estimation of protein, sample extract was pipetted out of different aliquots (0.5, 1.0, and 1.5) and volume made up with water up to 2 ml. Then 4.5 ml of reagent I (2% Na_2CO_3 in 0.1 N NaOH) was added. All test tubes were shaken well and incubated at room temperature for 10 min. Then, 5 ml of a solution of sodium tartrate (reagent II) was added followed by 0.5 ml of FCR, and thoroughly mixed and incubated in dark conditions for 30 min. A tube with 2 ml of distilled water along with the reagents I and II with FCR except for standard (Bovine serum albumin and the fruit extract sample) served as blank. The absorbance was read against blank at 660 nm in UV visible spectrophotometer (model number 169; Systronics India). Further, the amount of protein was deduced after plotting the standard graph (Lowry *et al.,* 1951).

Estimation of proline

Two grams of *L. siceraria* fruit slice was extracted. This fruit sample was homogenized in mortar and pestle with 10 ml of 80% acetone and filtered through Whatman No. 2 filter paper. The extraction was repeated and pooled the filtrates. 2 ml of filtrate was taken and 2 ml each of glacial acetic acid and ninhydrin were added and mixed well. The reaction was ended by putting on an ice bath after incubation in a boiling water bath for 20 min. 4 ml of toluene was added, mixed sturdily for 20-30 sec. The toluene layer was aspirated and warm to room temperature. The absorbance was measured for red color at 520 nm against a blank. The amount of proline in the sample can be calculated using the formula [µmoles of proline /g tissue = (µg proline per ml × ml toluene)/115.5 × 5 g of sample] (Bates *et al.,* 1973).

Catalase (CAT) activity

One gram of *L. siceraria* fruit pulp was macerated with 0.1M phosphate buffer (pH 7.0) in pre-chilled mortar and pestle and centrifuged at 8000g for 20 min at 4ºC. The supernatant was used for enzyme activity. 3 ml of phosphate buffer, 2 ml of H_2O_2 and 1 ml of enzyme extract was taken into a test tube. Incubated for 1 min. at 20ºC and then the reaction was stopped by adding 10 ml of 0.7N H_2SO_4. This mixture was titrated against 0.01 N $KMnO_4$ until a faint purple color persists for at least 15 sec. The blank was prepared by adding the enzyme extract to an acidified solution of the reaction mixture at zero time (Sinha, 1972).

Peroxidase (POD) activity

Three grams of *L. siceraria* fruit pulp was homogenized in 10 ml ice-cold phosphate buffer (0.1 mM), pH 6.0 in pre-chilled mortar and pestle. The homogenate was sieved through two folds of muslin cloth. Then centrifuged the homogenate at 8000 g for 20 min. at 4ºC. The supernatant was collected and used as an enzyme source. 1 ml of O-dianisidine, 0.5 ml H_2O_2, 1 ml of phosphate buffer, and 2.4 ml of D/W were added into the test tube. For blank H_2O_2 was barred, however, the extra volume of water was added. The reaction was begun by adding 0.2 ml of an enzyme and incubated at 30ºC for 5 min. Then the reaction was stopped by adding 1 ml of 2 N H_2SO_4. The absorbance was measured at 460 nm (Wang, 2005).

DPPH radical scavenging activity

Five grams of *L. siceraria* fruit pulp was ground with 50 ml of distilled water and centrifuged at 8000 g for 20 min at 4ºC and the supernatant collected was used as enzyme extract. 10 µl of an enzyme extract was made it up to 40

µL with DMSO. Later, 2.96 ml of 0.1 mM DPPH solution was added. This mixture was then incubated in dark conditions for 20 min at room temperature. Then, the absorbance of the mixture was measured at 517 nm. As a control, 3 ml of DPPH was taken. The % radical scavenging activity of the sample extracts was determined by using the following formula: [% Radical Scavenging Activity = (Abs. of control – Abs. of sample × 100)/Abs. of control] (Molyneux, 2004).

Statistical analysis

The tests were led by seeking Completely Randomized Design (CRD). Every parameter analyzed was led three times with 30 tests for each replication. Later the acquired information was statistically scrutinized utilizing two-way analysis of variance (ANOVA). To examine the least significant difference among the treatments, Duncan's multiple range test (Duncan, 1955) was followed for the data (means ± SD) and ascertained at $P < 0.05$ with the aid of SPSS (Version 20, SPSS Inc. Chicago, USA) software.

RESULTS AND DISCUSSION

Sensory evaluation

The combination of calcium chloride plus gallic acid (abbreviated as CG) treated *L. siceraria* fruits extended their shelf life to a hefty 21 days, whilst, to control where the same was 14 days both in the room temperature condition. The detailed sensory evaluation parameter gave an immense overview of the treated material on expensed shelf life in *L. siceraria* fruits. The sensory characteristics of *L. siceraria*-fruits stored at room temperature were presented in Table 1. The analogy featured in color scores of the different combinations after 21 days at room temperature pointed out the change in the color of the samples. At the finishing point of the storage period, the 2:2 µM CG treated *L. siceraria* fruits was sensory acceptable and it proved to retain the quality. The colour changes for the CG treated *L. siceraria* fruits compared to untreated were insignificant (Figure 1). These sensory tests were also proved to be reasonable during the assessed parameters. No such previous study on the color of *L. siceraria* fruits were measured albeit, only 14 days of extended shelf life were observed (Patil, 2008).

Table 1: Sensory evaluation of treated and untreated bottle gourd stored at room temperature for 21 days. The values are mean±standard deviation

Days	Coating Amount ($CaCl_2$:Gallic acid coating)	Colour	Odour	Overall
0	Control	5.0±0.00	5.0±0.00	5.0±0.00
	1:1 µM	5.0±0.00	5.0±0.00	5.0±0.00
	2:2 µM	5.0±0.00	5.0±0.00	5.0±0.00
	3:3 µM	5.0±0.00	5.0±0.00	5.0±0.00
	4:4 µM	5.0±0.00	5.0±0.00	5.0±0.00
7	Control	4.0±0.00	4.0±0.00	4.0±0.00
	1:1 µM	3.0±0.00	3.4±0.02*	3.2±0.01*
	2:2 µM	5.0±0.00	4.6±0.03*	4.8±0.02*
	3:3 µM	3.0±0.00	3.3±0.04*	3.1±0.01*
	4:4 µM	3.0±0.00	3.4±0.05*	3.2±0.06*
14	Control	3.0±0.00	3.2±0.03*	3.1±0.03*
	1:1 µM	2.8±0.02*	2.6±0.04**	2.7±0.05*
	2:2 µM	4.8±0.06*	4.4±0.01*	4.6±0.05*
	3:3 µM	2.7±0.03*	3.1±0.05*	2.9±0.03*
	4:4 µM	2.4±0.06*	3.0±0.00	2.7±0.03*
21	Control	-	-	-
	1:1 µM	2.5±0.02*	2.1±0.03*	2.3±0.04*
	2:2 µM	4.5±0.02*	4.0±0.00	4.2±0.03*
	3:3 µM	2.3±0.03*	2.6±0.04*	2.4±0.04*
	4:4 µM	2.0±0.00	2.1±0.03*	2.0±0.04*

*Significant at P<0.05

Weight loss

The CG application influenced the weight loss of *L. siceraria* fruits during the entire post-harvest period i.e. 21 days. The 2:2 µM CG treated *L. siceraria* was found noteworthy against loss of weight in contrast to other concentrations and control. The highest weight loss i.e. 18.8% was shown by the 1:1 µM CG treated *L. siceraria* fruits after 21 days in contrast to the controlled *L. siceraria* fruits which were deteriorated after 14 days. The lowest 15.3 % weight loss was observed with 2:2 µM CG treated *L. siceraria* fruits for a storage period of 21 days (Table 2). The values of weight loss were significant at P<0.05 (Table 3). The fresh fruits during the storage period lost the shine concomitant with a 3-10 % loss of weight (Ben-Yehoshua and Rodov, 2002). This loss of weight in the *L. siceraria* fruits might be due to the reduction in moisture level, rapid respiration leads to shrinkage in the fruit size. This

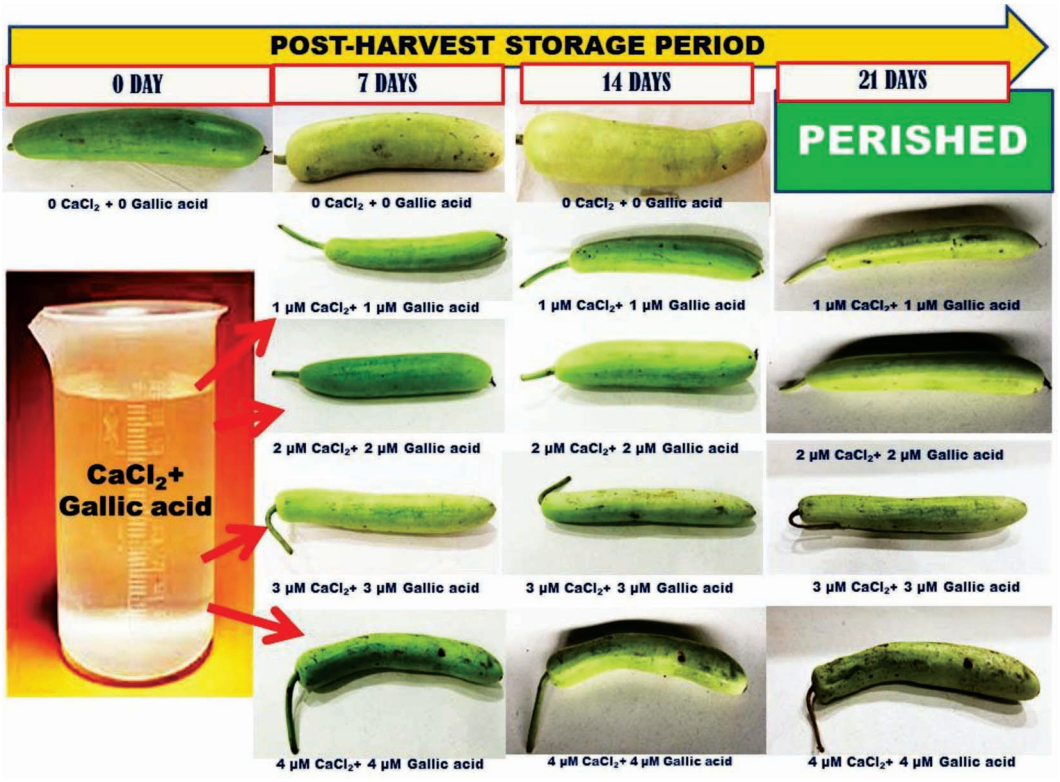

Figure 1: Effect of $CaCl_2$+ Gallic acid application and storage period on the skin colour of bottle gourd (*Lagenaria siceraria*)

loss occurs importantly through the physical characteristics and cuticle of *L. siceraria* fruit, or both (Lownds *et al.,* 1993). Moreover, this loss of weight is due to the release of a carbon atom during every cycle of respiration (Kablan *et al.,* 2008). Similar reports were displayed in bell peppers, green chilies, and capsicum (Xing *et al.,* 2011; Panigrahi *et al.,* 2017; 2018; Patel *et al.,* 2018). Therefore, the present combination(s) of exogenous treatment could manage the loss of weight.

Titratable acidity

The 7 days interval of titratable acidity measurement provided a reason that a depletion of the values in *L. siceraria* fruits for a storage period of 21 days. The highest reduction in TTA percentage values in control was from 10.1 ± 0.002 to 3.5 ± 0.46 and this was observed within 14 days post-harvest of *L. siceraria* fruits. Among all the treatments applied, the least TTA values were observed in 2:2 µM CG treated *L. siceraria* fruits. However, the other two CG treated *L. siceraria* fruits were observed which showed the reduction in TTA value but more than 2:2 µM CG treated *L. siceraria* fruits (Table 2). These results were well supported and significant at $P<0.05$ (Table 3). This characteristic of TTA reduction during the postharvest period in fruits is a significant feature (Panigrahi *et al.,* 2018; Patel *et al.,* 2018).

Total protein content

The principle in support of this method is the reaction between peptide nitrogen with copper ions under alkaline conditions and later the Folin-Ciocalteay phosphomolybdic phosphotungstic acid changes to heteropolymolybdenum blue following copper-catalyzed oxidation of aromatic acids (Everette, 2010). The fluctuation in pH can influence the test. Gradually the value of protein contents decreases as proteins were converted into amino acids. Due to CG application, this decrease in protein content can be checked but depends on the combinations and concentration of these applications. In this study, 2:2 µM CG treated *L. siceraria* fruits showed a higher amount of protein (4.788 ± 0.104 µg/g) during the storage period on 21 d whereas the control possessed 3.54 µg/g protein after 14 days (Table 2). The other combinations of CG also showed better protein content as compared to the control of *L. siceraria* fruits.

Table 2: Effect of $CaCl_2$ + Gallic acid application and storage period on weight loss, titratable acidity (TTA), protein content, proline content, catalase activity, peroxidase activity, and DPPH radical scavenging activity (DPPH RSA) in bottle gourd (*Lagenaria siceraria*)

$CaCl_2$Gallic acid (µM)	Storage (d)	Weight loss (%)	TTA (% acidity)	Protein content (µg g^{-1} extract)	Proline content (µmol g^{-1} extract)	Catalase activity (nmol H_2O_2 min^{-1} g^{-1} extract)	Peroxidase activity (U g^{-1} extract)	DPPH RSA (%)
0	0	0.0±0.00	10.1±0.002b	5.812±0.314a	0.649±0.016h	3.163±0.075a	0.047±0.034a	5.241±0.581a
(Control)	7	17.9±1.9abcd	9.6±0.019b	4.261±0.293b	0.729±0.035g	2.253±0.150f	0.029±0.007ab	7.245±1.294b
	14	16.9±1.9abcd	3.5±0.46a	4.091±1.249b	0.887±0.020f	1.906±0.075gh	0.014±0.011ab	5.953±0.539ab
	21	-	-	-	-	-	-	-
1:1	7	20.0±1.7abc	4.2±0.02bc	4.534±0.792bc	0.708±0.039f	2.816±0.150bcd	0.024±0.025abc	5.895±2.870abc
	14	19.4±2.7abcd	2.1±0.014cd	4.366±0.276cd	0.836±0.031de	2.643±0.075de	0.016±0.011bcd	7.270±1.656abcd
	21	18.8± 2.7cd	1.2±0.001ef	3.220±0.626fg	1.002±0.012a	2.080±0.130fgh	0.014±0.086bcd	4.470±3.148cd
2:2	7	19.4±8.4ab	9.3±0.007ab	4.820±0.092ef	0.839±0.035gh	2.990±0.130ab	0.041±0.019ef	6.672±2.33ab
	14	16.7±9.02ab	6.8±0.014bc	4.803±0.285bcd	0.958±0.046f	2.860±0.130bcd	0.045±0.011ef	7.321±1.561ab
	21	15.3±7.88abcd	3.8±0.010bc	4.788±0.104bc	1.365±0.043d	2.513±0.300e	0.036±0.037efg	5.599±8.613abcd
3:3	7	19.3±2.1abcd	5.5±0.007ab	4.774±0.471bcd	0.752±0.041g	2.903±0.270bc	0.038±0.033fgh	4.657±1.276ef
	14	16.8.± 2.8abcd	1.7±0.007bcd	4.633±0.259bcd	0.900±0.056ef	2.686±0.075cde	0.026±0.019fg	7.043±1.235efg
	21	15.4±1.9d	1.7±0.007abcd	4.291±0.175abcd	1.204±0.070b	2.123±0.150fg	0.016±0.011abfgh	4.622±1.160ef
4:4	7	19.5±8.8abc	8.5±0.007efg	4.713±0.051efg	0.651±0.044h	2.253±0.150f	0.035±0.034efg	4.771±2.365fgh
	14	18.1±1.8abcd	5.9±0.014fgh	4.624±0.773fgh	0.874±0.069f	2.166±0.075f	0.025±0.008abc	6.342±3.551fgh
	21	16.5±1.7bcd	2.5±0.001fgh	4.481±0.234ef	1.087±0.068c	1.863±0.075i	0.016±0.005abcd	4.738±0.516gh

The data represents mean±standard deviation. Data for each column followed by the different alphabets are significantly different according to Duncan's multiple range test (Duncan, 1955) at $P<0.05$. The absence of data ('-') represent the unavailability of information due to postharvest deterioration of fruit.

The results obtained are similar to the previous study (Patil *et al.,* 2008). The two-way ANOVA value was also in favour of the CG combination and significant with the storage condition at P<0.05 (Table 3).

Proline estimation

The proline content enhances during the advent conditions of the biotic and abiotic stress introduced into the plants and helps in the tolerance level of the plant to drought stress (Verbruggen and Hermans, 2008; Hare *et al.,* 1998). The closed imino ring of proline influences the secondary structure of proteins and also reduces the rate of peptide formation between proline and the other amino acids. Once detached from the plants the fruits start feeling the stress and as a result, the levels of proline increased as in the case of *L. siceraria* fruits with increasing storage period. Like the previous parameter, the highest amount of proline was obtained in 2:2 µM CG treated *L. siceraria* fruits followed by 3:3 µM CG treated *L. siceraria* fruits. The least level of proline was obtained in control with a value of 0.887 ± 0.020 µmol/g extract after 14 days as it was shriveled when measured at regular intervals of 7 days (Table 2). The significance of this proline accumulation was justified at P<0.05 in two-way ANOVA assessment (Table 3). Thus, the combination of gallic acid and $CaCl_2$ could be a significant application that is effective in extending the shelf life of *L. siceraria* fruits up to 21 days.

Catalase activity

Catalase (abbreviated as CAT) activity of different enzyme samples was analysed by using the titration method. Dichromate in acetic acid is reduced by various types of oxidizable compounds including free sugars, and basic amino acids. The concentration of these compounds in the assay system is usually too low to produce an appreciable interference in the determination of hydrogen peroxide (Sinha, 1972). The senescence of plant parts may be associated with the defensive system including catalase, peroxidase, and antioxidant activities (Sigaud-Kutner *et al.,* 2005; Xu *et al.,* 2009). CAT activity declined during the increasing storage period due to the reduction in enzymatic potential. Results of the present study showed that catalase activity was significantly decreased. Moreover, 2:2 µM CG treated *L. siceraria* fruits showed better and

Table 3: Two-way ANOVA showing individual and interaction effect of the $CaCl_2$ + Gallic acid application and storage period on weight loss, titratable acidity (TTA), protein content, proline content, catalase activity, peroxidase activity, and DPPH radical scavenging activity (DPPH RSA), in bottle gourd (*Laegeneria siceraria*). *Significant at P<0.05

Source	df	Weight loss		TTA		Protein content		Proline content		Catalase activity		Peroxidase activity		DPPH RSA	
		Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F	Mean square	F
Corrected model	19	6029.559	14.262	0.017	1.524	4.913	22.748	0.229	141.226	1.649	91.496	0.001	1.158	778.052	9.856
$CaCl_2$ Gallic acid conc.	4	6452.127	15.262	0.018	1.622	4.533*	20.991*	0.252	155.428	2.074	115.055	0.001	1.296	237.778*	3.012*
Storage duration	3	14333.150	33.904	0.023	2.107	15.118	70.005	0.263	162.324	5.386	298.807	0.003	4.594	2775.181	35.156
$CaCl_2$ conc. Gallic acid × Storage duration	12	3812.802*	9.019*	0.015*	1.345	2.488*	11.519*	0.213*	131.217*	0.574*	31.815*	0.000*	0.253*	458.860*	5.813*
Error	40	422.759		0.011		0.216		0.002		0.018		0.001		78.940	
Total	60														

higher CAT activity over control and other combinations. The CAT activity rose to 1.606 nmol H_2O_2 min^{-1} g^{-1} extract which was 5 times better as compared to control after 21 days of storage where the control *L. siceraria* fruits in its last stage of storage. This indicated the CG application could effectively reduce the catalase activity during the storage intervals of 7 days. The active oxygen species need the implication of several antioxidant enzymes alongside the non-enzymatic antioxidants (Xu *et al.,* 2009). The previous study by Xing *et al.,* (2011) showed the extension of peppers up to 35 days with similar types of CAT activity.

Peroxidase activity

Peroxidase is an important oxy-radical detoxification enzyme in plant tissue. This enzyme catalyzes more than one reaction and acts on several substrates, not only causing browning of fruits but also leading to discoloration, off-flavors, and nutritional damage (Alikhani, 2014). The present work revealed a decrease in peroxidase activity in treated as well as control fruits. However, a better result was observed in 2:2 µM CG treated *L. siceraria* fruits concentration than other fruits treated with CG. The maximum period the untreated *L. siceraria* fruits could survive was 14 days at room temperature with a value of POD 0.014 ± 0.011 Ug^{-1} whilst 0.036 ± 0.037 U^{-1} POD value for 2:2 µM CG treated *L. siceraria* fruits after 21 days of postharvest (Table 2). This suggests that the contribution of CG applications for these many storage days maintained the quality of *L. siceraria* fruits. The combination of CG was significant at $P<0.05$ (Table 3). Similar POD results were obtained in pepper albeit for 35 days (Xing *et al.,* 2011). In response to stress, plants induce the activity of POD and CAT enzymes (Jahnke *et al.,* 1991).

DPPH radical scavenging activity

The radicals created during the postharvest can be scavenged by DPPH (2,2-diphenyl-1-picrylhydrazyl), a consistent free radical, which nullifies the effect of antioxidants. The CG applications on *L. siceraria* fruits scavenged the DPPH radicals by maximum percentages 5.599 ± 8.613, 4.738 ± 0.516, 4.622 ± 1.16 and 4.470 ± 3.148 respectively in 2:2 µM, 4:4 µM, 3:3 µM, and 1:1 µM and also improved the postharvest life extension of 21 days (Table 2). In contrast, the untreated *L. siceraria* fruits could survive for 14 days with a DPPH scavenging value of 5.953 ± 0.539 percentage. These values are well significant at $P<0.05$ (Table 3). An initial increase followed by a decrease in the value of DPPH radical was reported in tomato and fresh-cut pears (Oms-Oliu *et al.,* 2008) as also observed in our present study. It is important to discuss here that the DPPH radical activities are also influenced by several other factors such as environmental and genetic makeup, protocol handled during and after postharvest of fruits (Dumas *et al.,* 2003).

CONCLUSION

The results of this investigation imply that the application of gallic acid in combination with calcium chloride on *L. siceraria* fruits showed a considerable change of sensory characteristics including colour, and odour, as well as weight loss. The level of DPPH radical scavenging activity and proline concentration increased under stress conditions as compared to control. The other activities such as catalase, peroxidase, protein, and titratable acidity decreased at room temperature conditions. Thus, the combination of gallic acid and calcium chloride showed beneficial effects by delaying the ripening process. The treatment of gallic acid and calcium chloride (1:1 µM, 2:2 µM, 3:3 µM, and 4:4 µM), extended the shelf-life of *L. siceraria* fruits up to 21 days. But, a 2:2 µM concentration of gallic acid and $CaCl_2$ showed a significantly better result in comparison with controlled samples. Hence, the 2:2 µM of CG application is considered better to extend the shelf-life of *L. siceraria* fruits. It can be said that calcium chloride and gallic acid (CG) solution can be tested for expansion of the shelf life of other fruits and vegetables.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Alkan D, Aydemir LY, Arcan I, Yavuzdurmaz H, Atabay HI, Ceylan C and Yemenicioglu A (2011). Development of flexible antimicrobial packaging materials against *Campylobacter jejuni* by incorporation of gallic acid into zein-based films. *J. Agric. Food Chem.*, 59(20): 1003–1010.

Alikhani M (2014). Enhancing safety and shelf life of fresh-cut mango by application of edible coatings and microencapsulation technique. *Food Sci. Nutr.*, 2(3): 210-217.

Bangarth F (1979). Calcium-related physiological disorders of plants. *Ann. Rev. Phytopathol*. 17: 97-122.

Bates LS, Waldren RP and Teare ID (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil,* 39: 205-207.

Ben-Yehoshua S and Rodov V (2002). Transpiration and water stress. In: Barz J, Brecht JK (eds) *Postharvest physiology and pathology of vegetables*. Marcel Dekker, Inc., New York, USA, pp 111–159.

Bernal MA, Calderon AA, Pedreno MA, Munoz R, Barcelo RA and Caceres FM (1993). Dihydrocapsaicin oxidation by *Capsicum annuum* (var. annuum) peroxidase. *J. Food Sci.,* 58(3): 611–613.

Decker-Walters D, Staub J, Lopez-Sese A and Nakata E (2000). Diversity in landraces and cultivars of bottle gourd (*Lagenaria siceraria*; Cucurbitaceae) as assessed by random amplified polymorphic DNA. *Genet. Resour. Crop Evol.,* 48: 369-380.

Dumas Y, Dadomo M, Di Lucca G and Grolier P (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric*., 83: 369–382.

Duncan DB (1955). Multiple range and multiple F test. *Biometrics.,* 11: 1-42.

Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW and Walker RB (2010). A thorough study of reactivity of various compound classes toward the Folin-Ciocalteu Reagent. *J. Agric. Food Chem.,* 58 (14): 8139–8144.

Habib-ur-Rahaman AS (2003). Bottle gourd (*Lagenaria siceraria*) a vegetable for good health. *Nat. Prod. Rad.,* 2: 249-256.

Hager AS, Vallons K J and Arendt EK (2012). Influence of gallic acid and tannic acid on the mechanical and barrier properties of wheat gluten films. *J. Sci. Food Agric.,* 60(24): 6157–6163.

Hare PD, Cress WA and Van Staden J (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.,* 21: 535–553.

Harika M, Gasti VD, Shantappa T, Mulge R, Shirol AM. Mastiholi AB and Kulkarni MS (2012). Evaluation of bottle gourd genotypes [*Lagenaria siceraria* (Mol.) Standl.] for various horticultural characters. *Karnataka J. Agric. Sci.,* 25(2): 241-244.

Jahnke LS, Hull MR and Long SP (1991). Chilling stress and oxygen metabolizing enzyme in *Zea mays* and *Zea diploperennis*. *Plant Cell Environ.,* 14: 97–104.

Kablan T, Koffi NR, Marina K and Oule MK (2008). The effects of different storage temperatures on the quality of bell pepper (*Capsicum annuum* L.). *Agricultural J.,* 3(2): 157–162.

Kirkby EA and Pilbeaam DJ (1984). Calcium as a plant nutrient. *Plant cell Environ.,*7: 397-405.

Kubde MS, Khadabadi SS, Farooqui IA and Deore SL (2010). *Lagenaria siceraria*: Phytochemistry, pharmacognosy and pharmacological studies. *Report Opin.*, 2: 91-98.

Kumar A, Partap S, Sharma NK and Jha KK (2012). Phytochemical ethnobotanical and pharmacological profile of *Lagenaria siceraria*-A review. *J. Pharmacogn. Phytochem.,* 1(3): 24-31.

Lin WC (2005). Quality of stored greenhouse sweet peppers influenced by storage temperatures and pre-harvest factors. *Information and Technology for Sustainable Fruit and Vegetable Production* (pp. 101–110). *Frutic*., 05, 12–16, Montpellier France.

Lownds NK, Banaras M and Bosland PW (1993). Relationships between postharvest water loss and physical properties of pepper fruit (*Capsicum annuum* L.). *Hort. Sci.,* 28(12): 1182-1184.

Lowry OH, Rosebrough NJ, Farr AL and Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* 193(1): 265-275.

Molyneux P (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin*. *J. Sci. Tech.,* 26(2): 211-219.

Oms-Oliu G, Soliva-Fortuny R and Martín-Belloso O (2008). Edible coatings with antibrowning agents to maintain sensory quality and antioxidant properties of fresh-cut pears. *Postharvest Biol. Technol.,* 50: 87–94.

Panigrahi J, Gheewala B, Patel M, Patel N and Gantait S (2017). Gibberellic acid coating: A novel approach to expand the shelf-life in green chili (*Capsicum annuum* L.). *Sci. Hort.,* 225: 581-588.

Panigrahi J, Patel M, Patel N, Gheewala B and Gantait S (2018). Changes in antioxidant and biochemical activities in castor oil-coated *Capsicum annuum* L. during postharvest storage. *3 Biotec.,* 8: 280.

Patel N, Gantait S and Panigrahi J (2018). Extension of postharvest shelf-life in green bell pepper (*Capsicum annuum* L.) using exogenous application of polyamines (spermidine and putrescine). *Food Chem.,* 275: 681-687.

Parle M and Kaur S (2011). Is bottle gourd a natural guard? *Int. Res. J. Pharm.,* 2(6): 13-17.

Patil PD, Parmar BR, Bhalerao PP and Bhalerao RR (2010). Effect of storage and packing materials on shelf life of bottle gourd (*Lagenaria siceraria* L.) cv. Pusa Naveen. *The Asi. J. Hort.,* 5(1): 214-217.

Pradhan RC, Said PP and Singh S (2013). Physical properties of bottle gourd seeds. *Agric Eng Int : CIGR J.*, 15(1): 106-113.

Sinha AK (1972). Colorimetric assay of catalase. *Anal. Biochem.,* 47: 389-395.

Sigaud-Kutner TCS, Neto AMP, Pinto E and Colepicolo P (2005). Diel activities of antioxidant enzymes, photosynthetic

pigments, and malondialdehyde content in stationary-phase cells of *Tetraselmis gracilis* (Prasinophyceae). *Aqu. Bot.,* 82: 239–249.

Sun X, Wang Z, Kadouh H and Zhou K (2014). The antimicrobial, mechanical, physical and structural properties of chitosan-gallic acid films. *LWT–Food Sci. Technol.,* 57(1): 83–89.

Ullah J, Shah A, Ahmad T and Qazi IM (2007). Effect of $CaCl_2$ coating on the sensory quality cv. Kingstar stored at ambient conditions. *Sarhad J. Agri.,* 23: 775-779.

Verbruggen N and Hermans C (2008). Proline accumulation in plants: a review. *Amino Acids,* 35(4): 753–759.

Wang CY and Ji ZL (1989). Effect of low-oxygen storage on chilling injury and polyamines in zucchini squash. *Sci Hort..,* 39: 1–7.

Wang Y (2005). Food chemistry effects of high oxygen concentration on pro- and anti–oxidant enzymes in peach fruits during postharvest periods. *Food Chem.,* 91: 99-104.

Xing Y, Li X, Xu Q, Yun J, Lu Y and Tang Y (2011). Effects of chitosan coating enriched with cinnamon oil on qualitative properties of sweet pepper (*Capsicum annuum* L.). *Food Chem.,* 124: 1143-1450.

Xu WT, Peng Xl, Luo YB, Wang J, Guo X and Huang KL (2009). Physiological and biochemical responses of grape fruit seed extract dip on ‘Redglobe’ grape. *LWT-Food Sci. Techno.,* 42: 471–476.