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Research Article

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Application of Nitric Acid, Oxalic Acid and Ascorbic Acid to Reduce Browning and Post-Harvest Loses of Litchi (*Litchi Chinensis*) Fruit Under Cold Storage

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Abstract

Litchi is a climacteric fruit that exhibits faster rate of repair and keeps getting riper even after being harvested. The increasing water content also renders litchi fruit more susceptible to post-harvest deterioration and disease attack, shortening the fruit's shelf life. The Present research titled "Application of Nitric acid, Oxalic acid and Ascorbic acid to reduce browning and post-harvest loses of litchi (Litchi Chinensis) fruit under cold storage" was carried out to examine the combine application of Nitric acid, Oxalic acid and Ascorbic acid on pericarp browning of litchi cultivar 'Bedana' under cold storage. The study was conducted at Horticulture laboratory in 'The University of Haripur' during 2021. Litchi fruits cultivar 'Bedana' were dipped for 15 minutes in each treatment 0.5% nitric acid+1% oxalic acid + 1% ascorbic acid, 1% nitric acid + 2% oxalic acid + 2% ascorbic acid and 1.5% nitric acid + 3% oxalic acid + 3% ascorbic acid, stored for 12 days under cold storage at 4±1 ^oC. Data on different parameters was recorded after each 3 days' interval. Results demonstrated that combine application of 1.5% nitric acid + 3% oxalic acid + 3% ascorbic acid decreased POD (2.5 U mg^{-1} protein) and increased in total antioxidants (52.0 mg 100 mL^{-1}), CAT (39.2 U mg^{-1} protein) and SOD (48.7 U mg^{-1} protein) while, the untreated fruits (control) showed an increased in TPC (14.2 mg GAE/100g), and POD (2.5 U mg-1 protein) and decreased CAT (36.5 U mg^{-1} protein) and SOD (43.5 U mg^{-1} protein) therefore, combine application of 1.5% nitric acid + 3% oxalic acid + 3% ascorbic acid is recommends to enhance litchi storage life and to reduce the pericarp browning and fruit decay of litchi under cold storage.

Keywords: Litchi, Anti-Oxidants, Post-Harvest, Cold Storage.

1. Introduction

Litchi (*Litchi chinensis Sonn*) is a tropical fruit belongs to the family Sapindaceae [1]. It has been cultivated since 1766 B.C [2]. A large brown seed, enclosed in the fruit aril, holds the fruit [3]. The litchi fruit is a rich source of antioxidant-active ascorbic acid and phenolic compounds [4,5]. It also contains essential elements such as calcium, magnesium, phosphorus, vitamin A and B. Litchi comprises 0.98% oil, 1.3% acid, 78.3% moisture, 0.95% protein, 6.88% sugar reduction, 13.76% total sugars, 0.68% ash and 6.69% hydrolysable sugars [6]. It reduces the level of blood sugar and controls elevated blood pressure, anti-asthmatic and cardiovascular function [7].

Litchi is a climacteric fruit and shows higher rates of reparation and continues to ripen after harvesting [8]. The higher water content also makes litchi porn to postharvest decay and diseases attack which makes storage duration of litchi very short [9]. The estimated post-harvest losses during storage are up to 25 to 35% and can reach above 50% before reaching to consumers [10]. The concentration of bioactive compounds decreases rapidly after harvesting [11]. Another problem is the browning of litchi fruits after harvest in which fruit peel start to change colour with in the first 24 hours and fruit peel begins to turn from red to brown [12]. The litchi red peel colour is one of the key features for determining the commercial output of litchi [13,14]. Rapid, humidity-related pericarp browning decreases value, and creates severe transportation and marketing problems for litchi fruits, as pericarp transforms it entirely from round to brown within days of harvest [15]. Browning of litchi has primarily been attributed to red pigment loss (anthocyanin) and the undermining of polyphenol oxidase (PPO) and peroxidase compounds. Due to unique physiology of litchi fruit the lack of water supply to pericarp from pulp also plays a role in browning of litchi fruit. The water loss also reduces antioxidant potential and increases phenolic oxidation [16]. The cell's pH, which influences the structure, stability and colour of anthocyanin enzymes and thus contributes to pericarp browning is also enhanced by water loss [17]. Meanwhile, micro cracking happened on the thin skin surface with fruit maturation and senescence, which also sped up depletion of water [18].

Many different method and new techniques are being implemented and tried by scientists to reduce the post-harvest damages and to prevent pericarp browning in litchi fruit [19]. Use of human and environment friendly chemicals like Oxalic acid, Nitric acid and Ascorbic acid is one of them. These chemicals naturally occur as organic acid and preserves membrane integrity and slows fruit to ripen [20]. Post-harvest use of oxalic acid has been shown to postpone ripening and preserve post-harvest consistency of different fruits and vegetables by slowing the development, respiration and output of active oxygen species, thereby enhancing antioxidant capacity [21]. It also reduces chilling injury in peach, mango and tomato, regulates browning of litchi fruit and reduces decay in jujube and mango [22-24]. Nitric oxide is mainly described as free radical gas that play a role as a various functional signalling molecule in both animals and plants [25]. It is used to regulate a range of developmental and physiogical processes in plants, it is anti-sensitivity and anti-ripening by action and controls respiration rate, disease occurrence, ethylene biosynthesis, delayed rind colour changes, and turnout in minimization of enzymatic activities [26,27]. Vitamin C or Ascorbic acid (AA), an antioxidant, is beneficial for inhibiting browning reactions [28]. By forming ascorbyl, it directly stores harmful radicals and decreases o-quinones formed by Polyphenol oxidase to phenolic substrates [29]. Being an antioxidant, it regulates the development of micro-organisms that render food spoilage [30].

Keeping in mind the economic and market value of litchi fruit and its cultivation on limited area due to specific climatic requirements, it is of utmost importance to preserve the post-harvest quality and appeal of litchi fruit during storage and marketing in order to do so; many methods and techniques are being implemented by scientist which includes use of various human-friendly chemicals. The practice of using these chemicals are gaining immerse acceptance in scientific studies and by the growers. Among these chemicals the effectiveness of Oxalic Acid, Nitric acid and Ascorbic Acid has been studied extensively on various fruits and vegetables, but major limitation of these studies is that they only used either one of the above mentioned chemical. Hence this study was designed to study the combine effects of Oxalic Acid, Nitric acid and Ascorbic Acid on litchi fruit during storage and marketing. Hence this study was undertaken to study the effect of different storage durations (Cold storage) and to investigate the effectiveness of different combinations of Oxalic acid, Nitric acid and Ascorbic acid to enhance shelf life and post-harvest quality of litchi fruits.

2. Materials and Methods

The current study "Management of Litchi (Litchi Chinensis) fruit pericarp browning by application of different anti-browning agents under cold storage" was carried out in the Horticulture Lab, Department of Horticulture, University of Haripur, from August to September 2021. Total Randomized Design (CRD) was used for the experiment. Anti-browning chemicals and storage time were also factors to consider. The following materials and methods were utilized in the study:

2.1 Collection of Fruits

Ripped and unripe fruits of litchi cultivar Badana were harvested and collected from orchards of Khanpur region. The collected fruits were wrapped in cardboard boxes and kept at temperature of 11±3°C and washed vigorously with purified water to eliminate staining particles and microbial load from fruits.

2.2 Treatment Application

The washed and dried fruit was then treated with anti-browning agents, consisting of nitric acid, oxalic acid and ascorbic acid combinations. The following four therapies were used in these

combinations;

T0= Control

T1= 0.5% nitric acid + 1% oxalic acid + 1% ascorbic acid

T2= 1% nitric acid + 2% oxalic acid + 2% ascorbic acid

T3= 1.5% nitric acid + 3% oxalic acid + 3% ascorbic acid

In each treatment, the Litchi fruit was then dipped at ambient temperature for 15 minutes. For any duplication, a fresh solution was prepared. Fruit treated was separated from the solvent and kept on rough drying pages. The weight of each duplication was observed after drying.

2.3 Storage Duration

Litchi fruits were preserved at cold storage at temperatures of 2±4°C during 3, 6, 9 and 12 days after application of treatments. Three replications of the handled fruits. 21 fruits as the care unit being taken. Data were obtained at daily intervals of 3 days with respect to various parameters. The pulp and peel samples for examination of overall anti-oxidant, total phenolic material, pH have been preserved directly after each deletion. The physico-chemical characteristics of the fruit have been established. Although catalase (CAT), peroxidase (POX), and dismutase (SOD) enzymes activity were observed in peel samples.

2.4 Phytochemical Screening

2.4.1 Total Phenolic Contents (mg GAE/100g)

Total phenolic contents (TPC) were computed by with some amendments using the Folin Ciocalteu reagent technique [31]. The 10ml FC-reactive was dissolved to 100 ml of solution in distilled water. FC-reagent (200µL) and vortex were extensively added in each sample (100ml). In each batch, the 700 mM Na_2Co_3 (800µL) is inserted and incubated for 2 hours. Sample (200µL), each estimated at 765 nm, was then transferred to a transparent 96-pit plate. TPC quantity was measured with a Gallic acid reference curve. Gallic acid was the equivalent of the findings.

2.4.2 Total Antioxidants (% DPPH inhibition)

Absolute plant-based antioxidant activities were tested in samples using the scavenger capability for, spectrophotometrically performed 2, 2-diphenyl-1-picrylhydrazyl stable radicles outlined [32]. In a solution of DPPH, 5 mL 0.004 percent methanol is applied to aliquots (50µL) at varying concentrations (50-150 µg mL-1) of peeling and pulp extraction. The absorption was observed at a blank distance of 517 nm after a 30-minute incubation time at room temperature Inhibition DPPH (%) = 100 × (Ablank = Asample/ Ablank). A blank is the absorbance of the control reaction (containing DPPH without sample) A sample is the absorbance of DPPH later addition of the sample.

2.4.3 Catalase Determination (U mg^{-1} protein)

The activity of the catalase in litchi peel has been calculated by the process with certain improvements [33]. A newly-created 5.9 mM 100 µL H_2O enzyme extract (100 µL) has been combined for the inducement of enzyme reactions. ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) has been used to create catalytic activity at 240 nm and express it as U mg^{-1} Protein. One unit of Catalysis was well-defined as 0.01 units per minute absorbance shifts.

2.4.4 Peroxidase Determination (U mg^{-1} protein)

Peroxidase activity with a certain alteration has been studied using the approach [33]. The inclusion of a phosphate buffer (pH 5) of 50 mM 800 µL in 40 mM 100 µL H_2O_2 and guaiacol of 20 mM 100 µL was a fresh reaction mechanism. Enzyme extract absorption (100 µL) was then mixed to reaction mixtures (100 µL) and registered as U mg^{-1} protein at 470 nm with the Microplate Reader (ELX800) from Winooski, VT, USA. The absorbent shift in 0.01 units per minute was described as one unit of peroxide action.

2.4.5 Superoxide Dismutase Determination (U mg^{-1} protein)

The superoxide dismutase analysis was conducted using the process defined for calculating the 50 percent photochemical reduction in nitro blue tetrazolium (NBT) [34]. A 500 µL phosphate buffer (50 mM, pH5), 200 µL (22 µm) of methione (12 µM) and a mixture of 100 µL of purified water with 100 µL enzyme extract were included in each of the test tubes. Each test tube included 500 µL of phosphate buffer (50 mM, pH5). Test tubes with fluorescent lamps were stored in a 15-minute enclosure. The absorbance was reported at the level of 560 nm by ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, U). An enzyme sum which inhibited 50 percent of NBT photo decrease was described as one unit of SOD activity.

2.4.6 Statistical Analysis

Statistic 8.1 was used for two-factor (treatments and storage days) factorial structures for window software, including treatments and fruit storage time. The experimental findings were analyzed for variation (ANOVA). Two fruit with three replicates have been considered per experimental device. The results of counseling have been measured from the (Fisher, s) LDC test at $p < 0.05$ with a significant f test [35].

3. Results and Discussion

Source	DF	TPC	TAO 50µ	TAO 100µ	TAO 150µ	CAT	POX	SOD
Replication	2	13.118	5.374	8.793	2.808	1.41	2.517E-12	1.15
Treatment	3	65.372*	61.586*	19.964*	6.511*	22.96*	4.35E-11*	66.93
Storage Duration	4	572.42*	346.808*	294.079*	345.414*	3429.94*	1.94E-07*	3039.71
T*SD	12	12.906*	3.218*	1.820^{NS}	0.429^{NS}	4.69*	2.80E-11*	6.51
Error	38	2.491	0.654	3.912	1.045	1.09	6.71E-12*	0.45
Total	59							

** Indicates the statistical significant difference at p≤0.05* ***TPC****=Total phenolic content,* ***TAO****=Total antioxidants,* ***CAT****=Catalyze Determination,* ***POX****= Peroxidasedetermination,* ***SOD****= Super oxidase determination*

Table 1: ANOVA table showing level of significance

3.1 Total Phenolic Contents (mg GAE/100g)

Analysis of variance regarding total phenolic contents mg GAE/100g showed that highly significant difference (p<0.01) exists between applied treatment, storage durations and their interaction (Table-1).

A decreasing trend was recorded for total phenolic contents (%) for storage duration. The greater total phenolic contents (19.36 mg GAE/100g) were recorded at 0 storage day, while least reduction in total phenolic contents (4.37 mg GAE/100g) was recorded in 12th storage day. Data regarding total phenolic contents reveals that highest fruit total phenolic contents (14.80mg GAE/100g) was recorded in T1 where lowest fruit total phenolic contents (10.56 mg GAE/100g) was recorded in T2 (Table-2). The interaction of treatment and storage showed that highest total phenolic contents (23.35mg GAE/100g) was recorded in storage day0 in fruits which were kept under control, while least reduction in total phenolic contents (4.28 mg GAE/100g) at T2 and Storage day5 (Figure-1). Litchi fruits exhibited lowest total phenolic contents later 12 days of cold storage duration. The decline of TPC in pulp tissues is recognized due to PPO enzyme who involved in the higher rate of oxidation of phenolic compound with the evolution of cold storage duration. These discoveries were maintained by Altunkaya and Gokmen 2008 and Ali et al., 2021; where they observed reduction in TPC of lettuce was revealed is due to the PPO oxidation [36,37]. T1 who was the combination of Nitric Acid 0.5%, Oxalic Acid 1% and Ascorbic Acid 1% showed the higher TPC on 1st storage duration as compared with control, T2 and T3 treated fruits by decreasing the rate of oxidation of phenolic contents through POD and PPO enzymes activities [38].

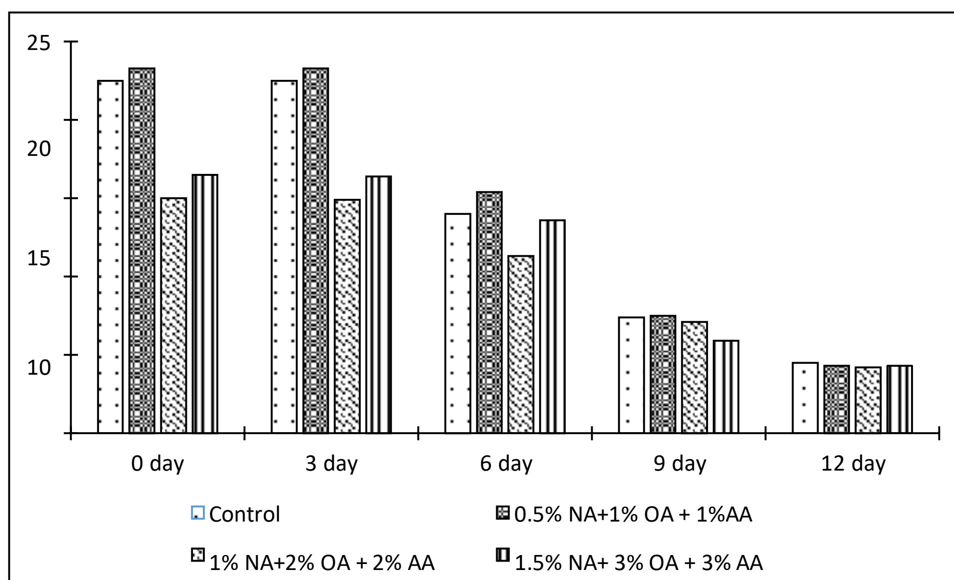

Figure 1: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Total phenolic contents of Litchi fruit

3.2 Total Antioxidants 50 µ (mg 100 mL^{-1})

Analysis of variance regarding Total antioxidants mg 100 mL^{-1} indicated that highly significant difference (P<0.01) exists between applied treatment, storage durations and their interaction (Table-1). A reduction in total antioxidants was observed during the storage of litchi fruits. The maximum total antioxidants (55.99 mg ml-1) were recorded at 0 storage day, while least total antioxidants (43.45 mg ml-1) were recorded in 12thstorage day. Data regarding total antioxidants reveals that highest total antioxidants (54.39 mg ml-1) were recorded in T0 where lowest total antioxidants (50.06 mg ml-1) were recorded in T2 (Table-2). Interaction of treatment and storage durations indicated that highest total antioxidants (58.04mg ml-1) was observed in 0 storage day in those litchi fruits which were not treated with any chemical, while least reduction (41.77mg ml^{-1}) was observed in T3 and 12th Storage day (Figure-2). Reduction of total antioxidant in the pulp of litchi was little in T2 who was the combination of (1% nitric acid, 2% oxalic acid 2% and 2%Ascorbic Acid) as compared to control fruits. It might be happened by the post-harvest application of oxalic acid who declined the total phenolic losses which may resulting raised in antioxidant potential of litchi stored for 84 days at 2 °C [39].

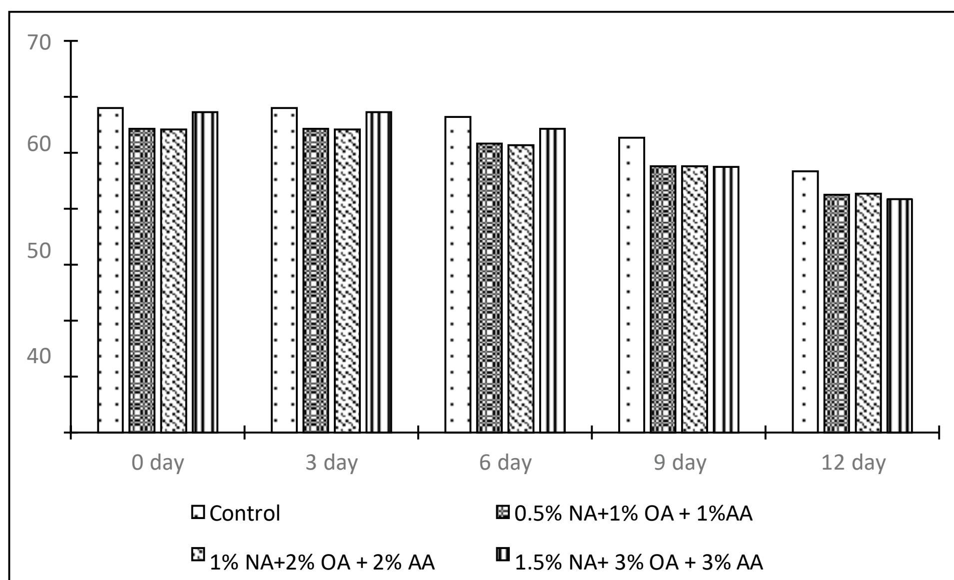

Figure 2: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Total antioxidants 50 µ of Litchi fruit

3.3 Total Antioxidants 100 µ (mg 100 mL^{-1})

Analysis of variance regarding Total antioxidants mg 100 mL^{-1} presented that highly significant difference ($P<0.01$) exists between applied treatment and storage durations, whereas the interaction of treatment and storage durations were non-significant ($p>0.05$) (Table-1). A decreasing trend in total antioxidants was recorded for storage duration during the experiment. Highest total antioxidants (55.48 mg ml^{-1}) were noted during 0 storage day, while least reduction in total antioxidants (43.78 mg ml^{-1}) was recorded in 12th storage day. Treatment of T2 produced highest total antioxidants (52.82 mg ml^{-1}) whereas untreated litchi fruits showed lowest values of total antioxidants (50.15%) (Table-2). Interaction of applied treatments and storage duration presented that highest quantities of total antioxidants (56.77mg ml^{-1}) were observed during 0 storage day in untreated litchi fruits, while least reduction in total antioxidants (42.76 mg ml^{-1}) was observed in T1 at 12thStorage day (Figure-3). Decline in total antioxidant of the juice of litchi was minimum in control and T1 (0.5% nitric acid, 1% oxalic acid and 1%ascorbic acid) as compared to T2 (1% nitric acid, 2% oxalic acid 2% and 2%Ascorbic Acid) and T3 (1.5 % nitric acid, 3% oxalic acid and 3% ascorbic acid). This may be due to the post-harvest application of oxalic acid who reduced the total phenolic losses which may resulting raised in antioxidant potential of litchi stored for 84 days at 2 °C [39,40].

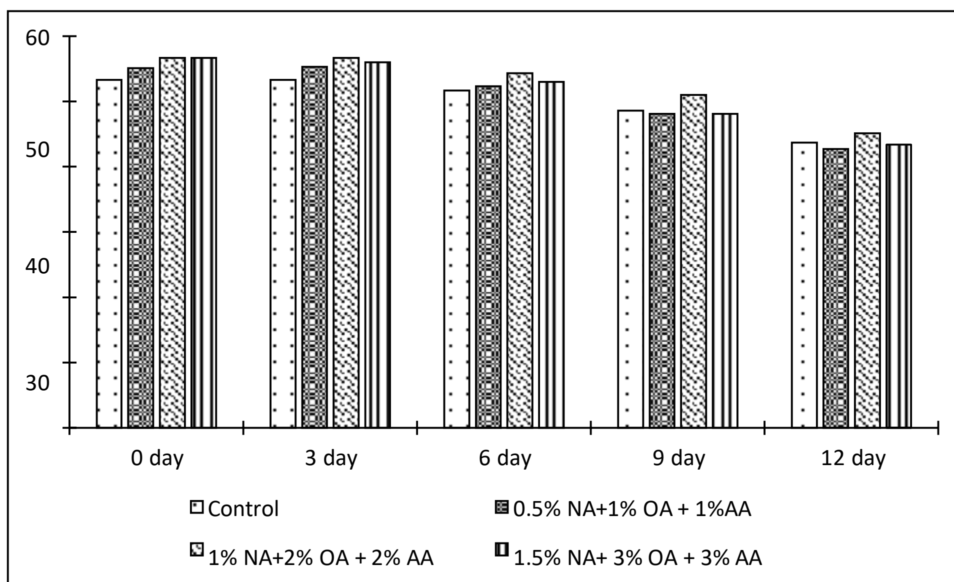

Figure 3: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Total antioxidants 100 µ of Litchi fruit

3.4 Total Antioxidants 150 µ (mg 100 mL-1)

Analysis of variance regarding Total antioxidants mg 100 mL^{-1} showed that highly significant difference ($p<0.01$) exists between applied treatment and storage durations, whereas the interaction of treatment and storage durations were non-significant (Table-1). A decreasing trend was recorded for total antioxidants for storage duration. Maximum total antioxidants (56.01 mg ml^{-1}) were recorded at 0 storage day, while least concentrations of Total antioxidants (43.55 mg ml^{-1}) were recorded on 12thstorage day. T1 produced highest concentrations of total antioxidants (52.22mg ml^{-1}) while lesser antioxidants (50.76 mg ml^{-1}) were recorded in untreated fruits (Table-2). The interaction of treatment and storage days indicated that greater concentrations of total antioxidants (56.51mg ml^{-1}) was recorded in 0 storage day in those litchi fruits which were untreated, while untreated litchi fruits stored for 12th days showed least concentrations of Total antioxidants (42.32 mg ml^{-1}) (Figure-4). Decreased in total antioxidant of the juice of litchi was small in control and T2 (1% nitric acid, 2% oxalic acid and 2% ascorbic acid) as compared to T1 (0.5 % nitric acid, 1% oxalic acid and 1% ascorbic acid) and T3 (1.5 % nitric acid, 3% oxalic acid and 3% ascorbic acid). Indicated that this may be happened by the post-harvest application of oxalic acid who declined the total phenolic losses which may resulting raised in antioxidant potential of litchi stored for 84 days at 2 °C [41,42].

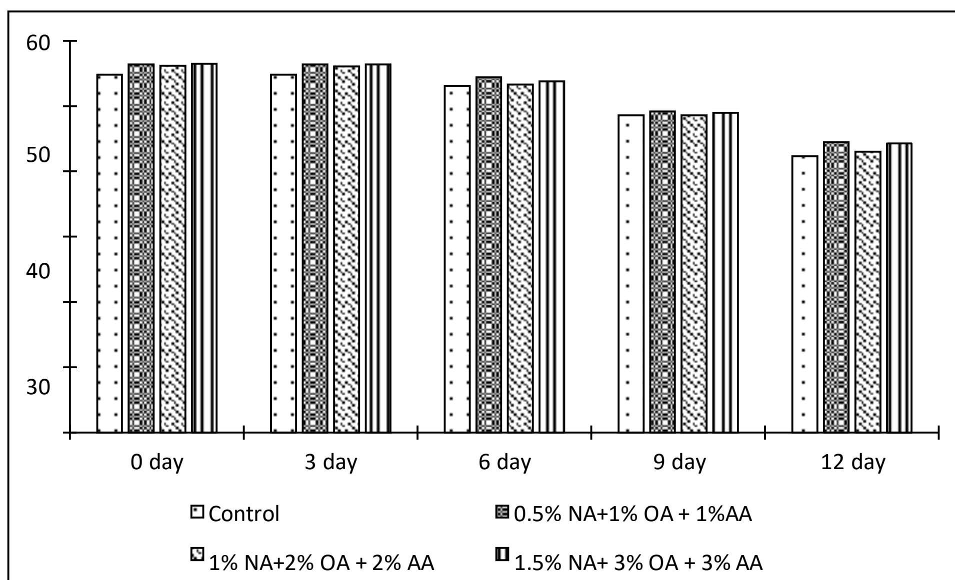

Figure 4: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Total antioxidants 150 µ of Litchi fruit

3.5 Catalase Determination (U mg-1 protein)

Analysis of variance regarding Catalase U mg^{-1} protein showed that highly significant difference (p<0.01) exists between applied treatment, storage durations and their interaction (Table-1).A decreasing trend was recorded for catalyst during storage duration. The maximum catalase (58.75 U mg^{-1} protein) were recorded at 0 storage day, while least catalase (17.80 U mg^{-1} protein) was recorded on 12th storage day. Highest Catalase (39.24 U mg^{-1} protein) was observed in fruits which were treated with T3 whereas least catalase (36.53 U mg-1protein) was noted in control fruits (Table-2). The interaction of treatment and storage showed that highest catalase (59.50 U mg^{-1} protein) was recorded in storage day 0 in those litchi fruits which were left untreated, while least reduction in catalase (15.50 U mg^{-1} protein) at T0 on 12th Storage day (Figure-5). Related results were also reported by who noticed that the litchi fruit treated with oxalic acid hold maximum catalase role in the pericarp tissue of litchi fruit on the time of cold storage [43].

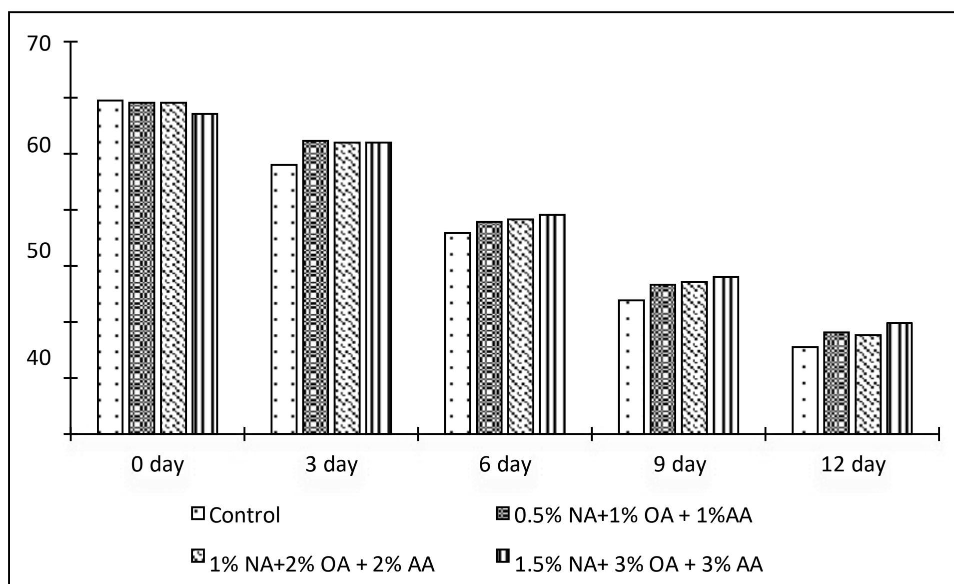

Figure 5: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Catalase Determination of Litchi fruit

3.6 Peroxidase Determination (U mg^{-1} protein)

Analysis of variance regarding Peroxidase U mg^{-1} protein showed that highly significant difference (p<0.01) exists between applied treatment, storage durations and their interaction (Table-1).Data regarding peroxidase observed that total peroxidase (2.62 U mg^{-1} protein) was recorded in T2 where lowest peroxidase (2.59 U mg-1 protein) was recorded in T0, T1 and T3. An increasing trend was recorded for peroxidase for storage duration. The maximum

peroxidase (4.01 U mg^{-1} protein) were recorded at 12thstorage day, while least reduction in peroxidase (1.38 U mg^{-1} protein) was recorded in 0 storage day (Table-2). The interaction of treatment and storage showed that highest peroxidase (4.03 U mg^{-1} protein) was recorded in storage day 12th in fruits which were kept under control, while least reduction in peroxidase (1.36 U mg^{-1} protein) at T3 and Storage day0 (Figure- 6). Pericarp tissue of litchi fruit contains the activity of peroxidase which results with addition due to increase in cold storage duration. also observed the related result in Logan fruit [44]. Reported that peroxidase enzyme in litchi pericarp tissue is also recognized as a browning rendering enzymes [45]. Though, T1 the combination of (0.5% nitric acid, 1%oxalic acid and 1% ascorbic acid) minimized the role of peroxidase enzyme in pericarp tissue of litchi fruit. Also asserted these results and stated less peroxidase role in T1 and T3 as compared to control and T2 the combination of (1% nitric acid, 2% oxalic acid, 2% ascorbic acid 2%) [12]. Due to the loss of moisture the pH changes from acidic to alkaline, the peroxidase enzyme might have taken important role [46].

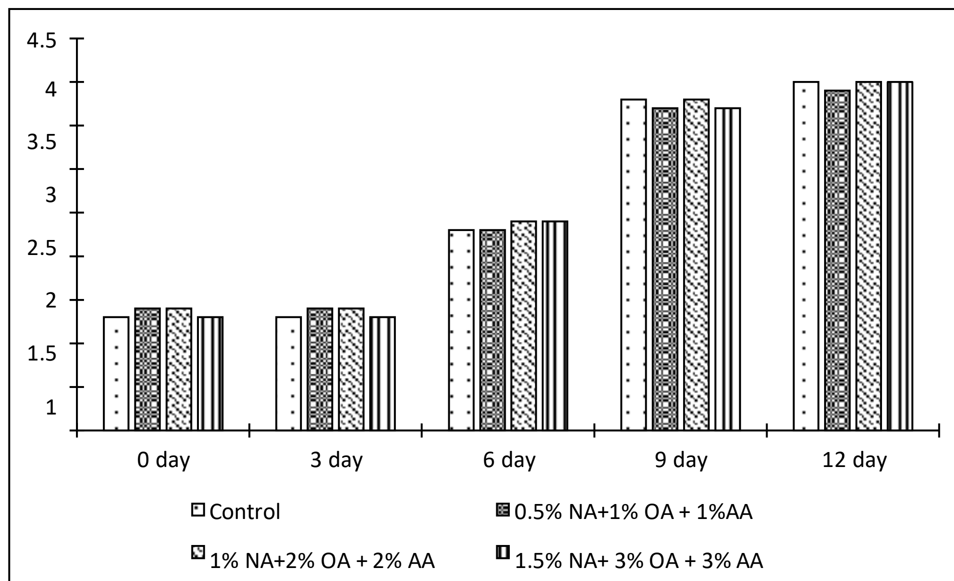

Figure 6: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Peroxidase Determination of Litchi fruit.

3.7 Superoxide Dismutase Determination (U mg^{-1} protein)

Analysis of variance regarding Superoxide Dismutase U mg^{-1} protein showed that highly significant difference ($p<0.01$) exists between applied treatment, storage durations and their interaction (Table-1).A decreasing trend was recorded for superoxide dismutase for storage duration. The maximum superoxide dismutase (67.25 U mg^{-1} protein) were recorded at 0 storage day, while least reduction in superoxide dismutase (27.16 U mg^{-1} protein) was recorded in 12th storage day. Data regarding superoxide dismutase reveals that total superoxide dismutase (48.73 U mg^{-1} protein) was recorded in T3 where lowest superoxide dismutase (43.56 U mg^{-1} protein) was recorded in T0 (Table-2). The interaction of treatment and storage showed that highest superoxide dismutase (67.66 U mg^{-1} protein) was recorded in storage day 0 in fruits which were kept under control, while least reduction in superoxide dismutase (24.50 U mg^{-1} protein) at T0 and Storage day 12th (Figure-7). By maintaining the ROS well below the defined threshold level, these enzymes share a vital role in anti-oxidant defense system [47]. At the time of storage duration, the superoxide dismutase work as a front line defense mechanism and retard the oxidative stress [48]. In current study the superoxide dismutase activity in pericarp tissues was more in T3 (1.5% Nitric Acid, 3% Oxalic Acid and3% Ascorbic Acid) treated fruit, as related to control fruit that was also observed [23].

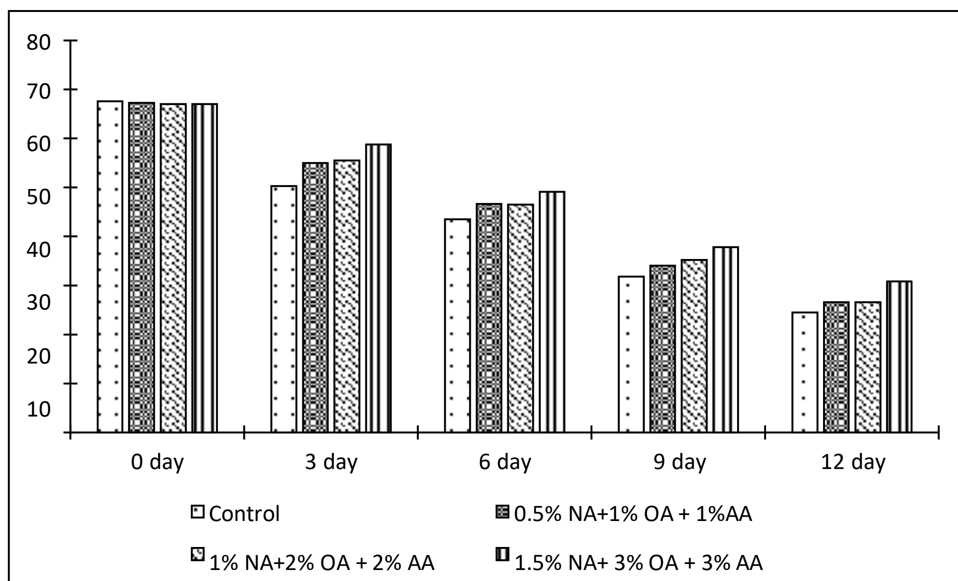

Figure 7: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on Superoxide Dismutase Determination of Litchi fruit

Treatments	TPC	TAO 50µ	TAO 100µ	TAO 150µ	CAT	POX	SOD
Control	14.2 a	54.3 a	50.1 b	50.7 b	36.5 b	2.5b	43.5 c
T1	14.8 a	50.1 c	50.7 b	52.2 a	38.8 a	2.5 b	45.9 b
T2	11.3 b	50.0 c	52.8 a	51.4 ab	38.8 a	2.6a	46.1 b
T3	10.5 b	51.6 b	51.4 ab	52.0 a	39.2 a	2.5b	48.7 a
LSD	1.1667	0.5977	1.4620	0.7557	0.7731	1.915	0.4983
Storage Duration							
SD1	19.3 a	55.9 a	55.4 a	56.0 a	58.7 a	1.3 d	67.2a
SD2	19.3 a	55.9 a	55.3 a	55.9 a	51.0 b	1.4 d	54.9 b
SD3	13.6 b	53.4 b	52.8 b	53.6 b	37.7 c	2.3 c	46.4 c
SD4	7.0 c	48.9 c	49.0 c	48.9 c	26.4 d	3.8 b	34.7 d
SD5	4.3 d	43.4 d	43.7 d	43.5 d	17.8 e	4.0 a	27.1 e
LSD	1.3045	0.6682	1.6346	0.8449	0.8643	2.141	0.5571
LSD* T×SD	2.6089	1.3364	3.2692	1.6898	1.7287	4.282	1.1142

Similar letters in same column showes statistical non-significance at $p \leq 0.05$ TPC=Total phenolic content, TAO=Total antioxidants, CAT=Catalyze Determination, POX= Peroxidase determination, SOD= Super oxidase determination

Table 2: Effect of Oxalic Acid, Nitric Acid and Ascorbic Acid treatments and cold storage on phytochemical and enzyme activity of Litchi fruit

4. Conclusion

It is concluded from the experiment that combined application of (1.5 % nitric acid, 3% oxalic acid and 3% ascorbic acid) on post-harvest of litchi efficiently decreased the pericarp browning and prolonged the shelf life of litchi fruit up to 12 days under cold storage. This treatment preserved (fruit weight loss, fruit decay, pericarp browning) & health related beneficial bioactive compound such as pH, total soluble solid and Peroxidase. It also maintained the total anti-oxidants, ascorbic acid, total phenolic contents, catalase, treatable acidity and superoxide dismutase. Hence; It is recommended to apply the 1.5 % nitric acid, 3% oxalic acid and 3 % ascorbic acid in combination to prolong the shelf life and to delay the pericarp browning of litchi fruit. It is also suggested to evaluate by further increase in the level of nitric acid, oxalic acid and ascorbic acid for the reduction of browning and prolong the shelf life of litchi fruit.

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