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ORIGINAL ARTICLE

Chemical elicitors improve the shelf life of phalsa (*Grewia asiatica* L.) by inducing antioxidants and controlling microbes

Pinal B. Vyas, Venkata Ramana Rao Tadapaneni* and Vasudev R. Thakkar

BRD School of Biosciences, Sardar Patel University, Sardar Patel Maidan, Vadtal Road, Post Box No. 39, Vallabh Vidyanagar, 388120 Gujarat, India

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Abstract – Introduction. Phalsa (*Grewia asiatica* L.) fruit has limited marketability due to its high degree of perishability, which leads to extensive postharvest losses. In view of the short postharvest shelf life and perishable nature of the fruit, this study aimed to determine the efficacy of salicylic acid (SA) or sodium benzoate (SB) alone or in combination with calcium chloride ($CaCl_2$) treatments for improving the shelf life and quality of phalsa fruit. **Materials and methods.** Phalsa fruits dipped for 15 min in solutions of T1 = SA 2 mM, T2 = SA 2 mM + $CaCl_2$ 1%, T3 = SB 0.1% and T4 = SB 0.1% + $CaCl_2$ 1% were stored at two different temperatures, low temperature (10 ± 1 °C) and room temperature (25 ± 1 °C), while the untreated fruit served as control. **Results and discussion.** The treatments of 0.1% sodium benzoate (T3) and salicylic acid 2 mM + calcium chloride 1% (T2) were found to be effective in enhancing antioxidants and bioactive compounds such as ascorbic acid, total anthocyanins, etc. These treatments also increased total phenolics inhibited polyphenol oxidase activity and reduced the microbial load in phalsa fruit stored at low as well as room temperature. **Conclusion.** The treatment of 0.1% sodium benzoate could increase the shelf life of phalsa fruit to 14 days in low temperature storage conditions as compared with only 7 days in the control.

Keywords: India / phalsa / *Grewia asiatica* / fruit quality / phenolics / postharvest management

Résumé – Des éliciteurs chimiques améliorent la conservation des fruits du phalsa (Grewia asiatica L.) par l'induction d'antioxidants et le contrôle des microbes. Introduction. La commercialisation des fruits du phalsa (*Grewia asiatica* L.) est limitée en raison de leur périssabilité qui conduit à de fortes pertes post-récolte. Cette étude a pour but de déterminer l'efficacité de traitements à l'acide salicylique (SA) ou au benzoate de sodium (SB), seuls ou en combinaison avec du chlorure de calcium (CaCl2) pour améliorer la durée de conservation et la qualité des fruits du phalsa. **Matériel et méthodes.** Les fruits du phalsa ont été trempés pendant 15 min dans des solutions de T1 = SA 2 mM, T2 = SA 2 mM + CaC_{l2} 1 %, T3 = SB 0,1 % et T4 = SB 0,1 % + CaCl2 1 %, puis ont été stockés à deux températures différentes, à savoir : à basse température (10 ± 1 °C) ou á température ambiante (25 ± 1 °C), tandis que les fruits non traités ont servi de témoin. **Résultats et discussion.** Les traitements au benzoate de sodium à 0,1 % (T3) et à l'acide salicylique à 2 mM + CaC_{l2} à 1 % (T2) ont amélioré la teneur des fruits en antioxydants et en composés bioactifs tels que l'acide ascorbique, les anthocyanes totales, etc. Ces traitements ont également augmenté la teneur des fruits en composés phénoliques totaux, ont inhibé l'activité de la polyphénol oxydase et ont réduit la charge microbienne des fruits stockés à 10 °C autant ainsi qu'à température ambiante (25 °C). **Conclusion.** Le traitement au benzoate de sodium peut augmenter la durée de conservation des fruits du phalsa jusqu'à 14 jours après récolte à basse température, par rapport à seulement 7 jours pour le contrôle.

Mots clés : Inde / phalsa / *Grewia asiatica* / qualité du fruit / composés phénoliques / gestion post-récolte

1 Introduction

Phalsa (*Grewia asiatica* L.), an exotic bush plant, is considered horticulturally as a small fruit crop but it is used as a folk medicine [1]. It has chromosomal status $2n = 18$ and belongs to the family Tiliaceae [2]. The fruit is a drupe, globose in shape and pea-sized, and it is red and purple in color with one or two seeds in it [3]. According to the "Encyclopedia of World Medicinal Plants", phalsa is astringent, coolant and stomachic. Despite its small size, this mostly unexplored wonder fruit has many health benefits. Phalsa contains high amounts of phosphorous, iron, potassium, sodium, vitamin A and vitamin C The anthocyanins and flavonoids present in phalsa are thought to be protective against cancer. Phalsa originates in

* Corresponding author: tadapanenirao@yahoo.com

Southern India but it is now grown in Pakistan, Bangladesh, Nepal, Sri Lanka, the Philippines, Thailand, Laos, Cambodia and Vietnam. The popularity of phalsa fruit is due to its attractive color, ranging from crimson red to dark purple, and its pleasing taste. The ripened phalsa fruit is consumed fresh as a dessert or processed into refreshing fruit and soft drinks which are enjoyed during the hot summer months in India [4]. In fact, it is said to be the third favorite summer fruit after mango and peach. When consumed during summer, it provides a much-needed cooling effect. The plant genetic resource status of phalsa indicates that there are no distinct cultivars available [2].

Being highly perishable, the fruit must be utilized within 24 h after picking. A very delicate and perishable fruit, phalsa is also difficult to transport. This is one of the reasons why it is not available throughout the country. However, phalsa fruit has a short postharvest shelf life and is considered suitable only for local marketing [5]. Due to lack of preservation and storage facilities, postharvest losses of fruits frequently remain at a high level in India. Use of chemical elicitors is a technology which helps to extend the shelf life and improve the nutritional properties of fruit. Thus, the application of chemical elicitors would be an effective measure for improvement of the postharvest shelf life of phalsa fruit.

Salicylic acid [C6H4 (OH) COOH is a plant hormone inhibiting ethylene biosynthesis and delaying senescence [6]. Salicylic acid has been shown to inhibit the conversion of aminocyclopropane-1 carboxylic acid (ACC) into ethylene by suppressing ACC oxidase activity [7]. Some beneficial effects of salicylic acid treatments in fruit are reported in the literature. For example, application of exogenous salicylic acid at non-toxic concentrations to fruit was shown to delay the ripening and softening of banana [8] and kiwi fruit [9]. Moreover, dietary salicylates from fruit and vegetables are described as bioactive compounds with potential health benefits [10], and considered as Generally Recognized as Safe (GRAS).

An antioxidant such as sodium benzoate (NaC7H5O2) also inhibits the ethylene production, interfering essentially with the conversion of ACC to ethylene [11]. Sodium benzoate is also used as a food preservative, and the mechanism starts with the absorption of benzoic acid into thecell. If the intracellular pH falls to 5 or lower, the anaerobic fermentation of glucose through phosphofructokinase decreases sharply; this inhibits the growth and survival of microorganisms that cause food spoilage [12]. Sodium benzoate is commonly used as a preservative for long-term storage of fruit pulp because of its antimicrobial activity [13]. Addition of calcium improves the rigidity of cell walls and obstructs enzymes such as polygalacturonase from reaching their active sites, thereby retarding tissue softening and ultimately delaying ripening. Thus, postharvest calcium application maintains cell turgor, membrane integrity and tissue firmness and delays membrane lipid catabolism [14].

To the best of our knowledge, no scientific literature is available on extension of the shelf life and improvement of the quality of phalsa fruit. Therefore, the current study was undertaken to evaluate the potential of postharvest treatments of salicylic acid (SA), sodium benzoate (SB) and calcium chloride ($CaCl_2$) on the shelf life and physicochemical characteristics of phalsa fruit during its postharvest storage.

2 Materials and methods

2.1 Chemicals

The chemicals used for the present study were procured from local vendors: SA (M.W. = 138.12) and $CaCl_2$ (M.W. = 147.02) from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India), and SB (M.W. =144.11) from Himedia (Mumbai, India).

2.2 Plant material

The phalsa fruits were collected in the month of May 2013 from seven-year-old tall-type phalsa plants propagated through seeds and growing in the well-drained and loamy soil of an orchard located in the vicinity of Bandhani village, Petlad Taluka, Anand District, Gujarat, India. Flowering of the phalsa started from the months of February-March and continued until May. The period between flowering and fruit maturity was 45−55 days. Fruits started ripening in mid-May and continued up to June with adequate sunlight, dry climatic conditions and a hot temperature (40−45 °C). Phalsa fruits were harvested at their commercial maturity stage; they were immediately transported to the research laboratory and then they were graded for their uniformity in size, shape and color, and fruits free from blemishes were selected.

2.3 Application of chemical elicitor treatments

The experiment was carried out under two different storage conditions (*i.e.* low temperature and room temperature) with 250 g of phalsa fruit (approx.150−200 in number) per set with two replicate sets for each of the storage conditions. From these two experimental sets, one set was used for visual observations, while the other one was subjected to physicochemical, biochemical and microbiological analyses. From each of the treatments a control set of 1 g of phalsa fruit was taken for each parameter at regular intervals and all the analyses were carried out in three replicates. The phalsa fruits were washed with tap water and surface-disinfected with 2% sodium hypochlorite for 2 min, rinsed with tap water and air-dried. The postharvest treatments (T), T1 = SA 2 mM, T2 = SA 2 mM + $CaCl_2$, T3 = SB 0.1% and T4 = SB 0.1% + $CaCl_2$ 1%, were carried out for 15 min by the dipping method. Fruits dipped in distilled water served as control (T5). The fruits were air-dried at ambient temperature for 30 min in an attempt to reduce possible chemical injury. Thereafter, all the phalsa were placed in plastic boxes and stored in two different storage conditions, i.e. one set at low temperature (L.T.) 10 ± 1 °C (set i) and the other set at room temperature (R.T.) 25 ± 1 °C (set ii). The fruits of treated and control sets from both the storage conditions were evaluated for the following quality attributes of phalsa at the beginning of the experiment (*i.e.* day 0), then at regular intervals of 4 days up to 12 days of storage at L.T. and also at regular intervals of 2 days up to 4 days of storage at R.T.

2.4 Determination of physicochemical attributes

The pH of fruit samples was determined as per the method described by the AOAC [15]. The Total Soluble Solids (TSS)

content of fruit was determined by using a refractometer (Atago Co., Tokyo, Japan). A homogeneous sample was prepared by blending the fruit. The sample was thoroughly mixed, a few drops of juicy fruit pulp were placed on the prism of the refractometer and a direct reading was taken by reading the scale in ° Brix as described by the AOAC [15].

2.5 Determination of biochemical attributes

Total sugars were estimated by the phenol-sulfuric acid method cited by Thimmaiah [16]. Estimation of total anthocyanins was carried out as per the method described by Lees and Francis [17]. Estimation of the total phenolic content (TPC) was carried out according to the method described by McDonald *et al.* [18]. The quantitative analysis of ascorbic acid was carried out by using the dinitrophenylhydrazine (DNPH) method described by Roe and Kuether [19].

2.6 FRAP (Ferric Reducing Ability of Plasma) assay

Antioxidant activity (FRAP assay) was determined according to Benzie and Strain [20], with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $CH_3COONa.3H_2O$ and 16 mL CH_3COOH), pH 3.6, 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) solution in 40 mM hydrochloric acid and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37 °C before using. Fruit (1 g) was extracted with 10 mL methanol and centrifuged at 6,000 g for 15 min, using a centrifuge (Rota 4-V/FM, Plasto Crafts). The methanol aliquots of 0.01 mL were mixed with samples and allowed to react with the FRAP solution in the dark for 30 min. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm by spectrophotometer (Elico-SL 207, Hyderabad, India). Results were expressed as μM Fe^{2+} g^{-1}.

2.7 Extraction and assay of polyphenol oxidase (PPO) (EC 1.14.18.1)

The extraction and assay of PPO activity were carried out by using the method of Xiao *et al.* [21]. A fruit sample of 1 g was extracted manually in 0.05 M potassium phosphate buffer (pH 6.8) and centrifuged at 8000 × g, 4 °C for 15 min (model: Eppendorf 5430R, NY, USA). The clear supernatant was taken as an enzyme sample for its assay. The reaction mixture consisted of 3 mL potassium phosphate buffer (0.05 M, pH 6.8), catechol (0.02 M) and 0.1 mL of enzyme solution to a total volume of 3 mL in the cuvette. The initial rate of the enzyme catalyzed reaction was linear over 3 min. In overall determinations, the activity of PPO was assayed in triplicate using a UV-visible spectrophotometer (UV-1800, Shimadzu, USA) and one unit was defined as a change of 0.001 absorbance units min^{-1} at 398 nm.

2.8 Microbiological studies

Microbes on the fruit surface were examined by the following method described by the ICMSF [22]. Treated and untreated phalsa were examined for microbial contamination by enumeration of total aerobic bacteria, yeast and mold colonies. Serial dilutions of samples were prepared by washing 1 g fruit tissue vigorously with 9 mL of sterile buffered peptone water in sterile conditions and inoculated over plate count agar (PCA) at 30 °C for 24 h and potato dextrose agar (PDA) at 27 °C for 5–7 days, respectively, by the spread plate method. After incubation, the number of colonies was counted and expressed as log colony-forming units per gram fresh weight (log CFU g^{-1} FW).

2.9 Postharvest shelf life

The shelf life of phalsa fruits was calculated by counting the days required for them to attain the last stage of ripening, but up to the stage where they remained acceptable for selling and also for consumption.

2.10 Statistical analysis

The study consisted of a randomized block design, with three replicates. The data presented in this paper was statistically analyzed by SPSS 17 software and the mean and standard deviation (SD) were calculated. The statistical significance of the data was assessed by one-way analysis of variance and the LSD test. Mean comparisons were performed using the HSD of Tukey's test to examine whether the differences between treatments and storage times were significant at $P < 0.05$. The overall least significance difference (LSD, $P \leqslant 0.05$) was calculated and used to detect significant differences among all the treatments and control set [23].

3 Results and discussion

3.1 pH and TSS as affected by chemical elicitors

A decrease in pH values was observed in the control set of fruits over a period of time. In fruits treated with T2, T3 and T4, pH did not decrease much, whereas in the T1-treate fruits , the pH was reduced, which may be because SA itself is acidic in nature (*table I*). The initial decrease in pH could be due to the antimicrobial action of treated acids , which would also cause disruption of membrane transport and/or permeability, anion accumulation, or a reduction in internal cellular pH by the dissociation of hydrogen ions from the acid [24]. In all the treatments pH was found to be significantly ($P < 0.05$) increased on the 12th day at L.T., which could be due to a reduction in the microbial load, which would produce organic acids from the sugar present in the fruit. Utilization of organic acids will reduce acidity, so that pH will increase.

In general, a gradual increase occurs in TSS during the storage period and this kind of increase in TSS is attributed

Table I. Effect of chemical elicitors on the pH of phalsa fruit (*Grewia asiatica* L.) stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	pH at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	3.22 ± 0.02[a]	3.00 ± 0[a]	2.40 ± 0[c]	4.00 ± 0.00[b]
T2	3.22 ± 0.02[a]	3.00 ± 0[b]	2.80 ± 0[b]	3.90 ± 0.00[c]
T3	3.22 ± 0.02[a]	3.00 ± 0[d]	3.00 ± 0[a]	4.10 ± 0.00[a]
T4	3.22 ± 0.02[a]	3.00 ± 0[c]	2.60 ± 0[b]	0 ± 0
T5	3.22 ± 0.02[a]	3.00 ± 0[b]	2.90 ± 0[a]	0 ± 0
	pH at room temperature during storage			
	Day 0	Day 2		Day 4
T1	3.22 ± 0.02[a]	3.09 ± 0.02[b]		2.60 ± 0.00[b]
T2	3.22 ± 0.02[a]	3.33 ± 0.02[d]		0 ± 0
T3	3.22 ± 0.02[a]	3.20 ± 0.02[e]		2.90 ± 0.00[a]
T4	3.22 ± 0.02[a]	3.10 ± 0.02[c]		2.90 ± 0.00[a]
T5	3.22 ± 0.02[a]	3.05 ± 0.02[a]		0 ± 0

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

Table II. Effect of chemical elicitors on the soluble solids (TSS, °Brix) of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	TSS at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	1.23 ± 0.06[a]	1.00 ± 0[d]	1.83 ± 0.06[a]	1.00 ± 0.00[a]
T2	1.23 ± 0.06[a]	2.00 ± 0[b]	1.50 ± 0.00[b]	1.00 ± 0.00[b]
T3	1.23 ± 0.06[a]	1.00 ± 0[c]	1.23 ± 0.06[c]	1.00 ± 0.00[b]
T4	1.23 ± 0.06[a]	1.00 ± 0[d]	1.20 ± 0.00[c]	0 ± 0
T5	1.23 ± 0.06[a]	2.00 ± 0[c]	1.30 ± 0.10[c]	0 ± 0
	TSS at room temperature during storage			
	Day 0	Day 2		Day 4
T1	1.23 ± 0.06[a]	1.83 ± 0.06[a]		1.07 ± 0.06[c]
T2	1.23 ± 0.06[a]	0.93 ± 0.06[d]		0 ± 0
T3	1.23 ± 0.06[a]	1.10 ± 0.00[c]		2.30 ± 0.00[b]
T4	1.23 ± 0.06[a]	1.10 ± 0.00[e]		2.20 ± 0.00[a]
T5	1.23 ± 0.06[a]	1.40 ± 0.00[b]		0 ± 0

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

to the fact that the cell walls of fruits contain large amounts of polysaccharides, mainly pectin and cellulose, that digested due to the activity of the cell wall-degrading enzymes, which leads to a significant increase in TSS content [25]. In the present study, the TSS was significantly ($P < 0.05$) higher in the control set of fruit as compared with treated fruits in both the storage conditions. The lowest TSS was recorded in the fruits treated with (T1) and (T4) at L.T., *i.e.* 10 ± 1 °C on the 2nd day of the storage period. Besides, the results of the present study also showed that SA treatment also lowers the TSS value as compared with control fruits, which indicates that SA delays the fruit softening process as well as starch degradation. Similar results were reported by Rao *et al.* [26] in capsicum treated with SA and $CaCl_2$. The reduction in the TSS of calcium-treated fruit was probably due to the slowing down of respiration and metabolic activity; hence, it retards the ripening process. In this regard, Ali *et al.* [27] stated that the slower respiration also slows down the synthesis and use of metabolites, resulting in lower TSS due to the slower change from carbohydrates to sugars. According to Mirdehghan and Ghotbi [28], the cell walls contain large amounts of polysaccharides, mainly pectins and cellulose, that are digested due to the activity of the cell wall-degrading enzymes, leading to a significant increase in TSS content: perhaps this may be the reason for the significant accumulation of TSS content in the treated phalsa fruit of the current study (*table II*). The increasing trends in TSS content in the initial days of storage might be due to ripening of fruit during storage, while reduction in TSS in the later stages may be due to degradation of soluble sugars into alcohols and water [29].

3.2 Total sugars as affected by chemical elicitors

The rate of total sugar utilization was reduced due to treatments of SA and SB in both the storage conditions (*table III*). This might be due to a reduced microbial load because of SA and SB treatments. During the 2nd day of the storage period,

Table III. Effect of chemical elicitors on the total sugars (mg g^{-1}) of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	Total sugars at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	98.92 ± 3.42^{e}	62.43 ± 0.33^{c}	58.37 ± 1.79^{b}	49.50 ± 1.40^{b}
T2	98.92 ± 3.42^{e}	58.59 ± 0.90^{d}	59.94 ± 0.21^{b}	51.50 ± 2.40^{ab}
T3	98.92 ± 3.42^{e}	66.41 ± 0.40^{b}	66.36 ± 3.06^{a}	54.20 ± 1.20^{a}
T4	98.92 ± 3.42^{e}	70.37 ± 1.03^{a}	60.76 ± 0.51^{b}	0 ± 0
T5	98.92 ± 3.42^{e}	74.91 ± 3.27^{e}	48.72 ± 0.08^{c}	0 ± 0
	Total sugars at room temperature during storage			
	Day 0	Day 2	Day 4	
T1	98.92 ± 3.42^{e}	62.78 ± 6.16^{a}	31.99 ± 0.95^{ab}	
T2	98.92 ± 3.42^{e}	59.94 ± 0.21^{a}	0 ± 0	
T3	98.92 ± 3.42^{e}	68.14 ± 3.03^{a}	33.70 ± 1.09^{a}	
T4	98.92 ± 3.42^{e}	60.76 ± 0.51^{ab}	30.98 ± 0.57^{b}	
T5	98.92 ± 3.42^{e}	70.50 ± 0.67	0 ± 0	

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

Table IV. Effect of chemical elicitors on the anthocyanins (μg g^{-1}) of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	Anthocyanins at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	0.19 ± 0.00^{a}	0.482 ± 0.002^{e}	0.81 ± 0.01^{c}	1.03 ± 0.00^{b}
T2	0.19 ± 0.00^{a}	1.273 ± 0.005^{c}	0.76 ± 0.01^{e}	1.14 ± 0.01^{a}
T3	0.19 ± 0.00^{a}	1.566 ± 0.015^{a}	0.84 ± 0.00^{b}	1.02 ± 0.00^{b}
T4	0.19 ± 0.00^{a}	1.368 ± 0.009^{b}	0.77 ± 0.01^{d}	0 ± 0
T5	0.19 ± 0.00^{a}	0.814 ± 0.007^{d}	0.74 ± 0.04^{a}	0 ± 0
	Anthocyanins at room temperature during storage			
	Day 0	Day 2	Day 4	
T1	0.19 ± 0.00^{a}	0.45 ± 0.00^{b}	0.41 ± 0.00^{a}	
T2	0.19 ± 0.00^{a}	0.52 ± 0.00^{a}	0 ± 0	
T3	0.19 ± 0.00^{a}	0.33 ± 0.00^{e}	0.28 ± 0.00^{c}	
T4	0.19 ± 0.00^{a}	0.39 ± 0.00^{c}	0.33 ± 0.00^{b}	
T5	0.19 ± 0.00^{a}	0.37 ± 0.00^{d}	0 ± 0	

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

fruit treated with (T2) exhibited significantly ($P < 0.05$) lower levels of total sugars (59.94 mg g^{-1}) at R.T. as compared with those of the control set of fruit. On the other hand, on the 4th day of storage, the lowest amount of total sugars was noted in the set of fruits treated with T2 (58.58 mg g^{-1}) at L.T. as compared with that of the control set of fruit. Higher sugar contents in the control set of phalsa on the 2nd day at R.T. and the 4th day at L.T. might be due to maximum conversion of complex carbohydrates into simple sugars in both the storage conditions. Gupta *et al.* [30] reported that the increase could be due to the rapid hydrolysis of starch into simple sugars, and consequently the rate of conversion was higher than the utilization during earlier stages, but utilization of sugars in respiration was at a much faster rate during later stages. Similar trends have also been reported by Prashant and Masoodi [31] in peach fruit treated with SA in different storage conditions. The differences in sugar contents of stages related to utilization of simple sugars in treatments suggest slow ripening and the senescence process in the SA-treated experiments.

3.3 Antioxidants as affected by chemical elicitors

3.3.1 Total anthocyanins

Anthocyanins are a group of phenolic compounds responsible for the red–blue color of many fruits and vegetables, and provide beneficial effects for human health [32]. The concentration of anthocyanins in treated and control phalsa in both the storage conditions is presented in *table IV*, which reveals that all the treated phalsa had a progressive and significant ($P < 0.05$) increment in their total anthocyanin concentration

Table V. Effect of chemical elicitors on the ascorbic acid (mg 100 g^{-1}) of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	Ascorbic acid at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	97.71 ± 0.95^{a}	266.30 ± 10.70^{abc}	59.79 ± 2.53^{c}	48.33 ± 5.09^{b}
T2	97.71 ± 0.95^{a}	335.40 ± 4.00^{a}	95.00 ± 10.80^{ab}	56.04 ± 2.19^{a}
T3	97.71 ± 0.95^{a}	301.00 ± 45.80^{ab}	127.20 ± 15.89^{a}	50.63 ± 2.25^{ab}
T4	97.71 ± 0.95^{a}	250.20 ± 36.50^{bc}	120.20 ± 14.60^{ab}	0 ± 0
T5	97.71 ± 0.95^{a}	206.00 ± 2.60^{c}	85.63 ± 15.70^{bc}	0 ± 0
	Ascorbic acid at room temperature during storage			
	Day 0	Day 2		Day 4
T1	97.71 ± 0.95^{a}	311.20 ± 67.40^{d}		44.38 ± 3.12^{b}
T2	97.71 ± 0.95^{a}	370.10 ± 80.30^{b}		0 ± 0
T3	97.71 ± 0.95^{a}	419.80 ± 91.70^{a}		85.83 ± 24.44^{a}
T4	97.71 ± 0.95^{a}	314.20 ± 67.90^{c}		58.96 ± 3.97^{ab}
T5	97.71 ± 0.95^{a}	249.50 ± 54.10^{e}		0 ± 0

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

throughout the storage period, which means the phalsa became darker with storage as ripening progressed. However, the level of anthocyanins was found to be higher in all the treated fruits in both the storage conditions as compared with the control set, which had 0.814 μg g^{-1} total anthocyanins on the 4th day of storage, but eventually it declined to 0.735 μg g^{-1}. The results of the present study demonstrated that during 8 days of storage, all the treated phalsa had a significantly ($P < 0.05$) higher accumulation of anthocyanins. After 12 days of storage, the fruits treated with (T3) retained anthocyanin accumulation at lower levels as compared with the other treated fruit stored at low temperature. These results are supported by the findings of Sidiane *et al.* [33], who reported that anthocyanin content increased in SA-treated strawberry fruit during the storage period as compared with untreated fruits. In this study, it was observed that the incorporation of SA and $CaCl_2$ in the treatment formulation had significant effects on delaying anthocyanin accumulation.

3.3.2 Ascorbic acid content

Application of elicitors comprising SA and SB alone and in combination with $CaCl_2$ had a significant ($P < 0.05$) effect on the ascorbic acid content of the phalsa (*table V*). Generally, it was noted that in fruits, ascorbic acid content increases gradually with increasing SA rates during all periods of storage, while it decreases sharply with increasing time of storage, as reported by Awad [34]. Moreover, Abbasi *et al.* [35] reported that the reason for high ascorbic acid in calcium-treated fruits might be that metabolic activities are not as fast as in untreated fruits. Therefore, in untreated fruits the respiration rate and ethylene production were at a higher rate, due to which ascorbic acid constantly decreased rapidly as compared with calcium-treated fruits. Similarly, in the present study, the contents of ascorbic acid in both treated and control fruit decreased with the increase in the storage time, and all the treatments tested in the current study significantly ($P < 0.05$) inhibited the decrease. The initial ascorbic acid content of phalsa fruit was (97.7 mg 100 g^{-1} FW), while after 4 days of storage, the untreated samples showed significantly lower amounts of ascorbic acid (206.0 mg 100 g^{-1} FW) at L.T. , and the treatments caused a delay in this decrease in ascorbic acid content. The treated fruits retained their ascorbic acid contents for up to 12 days of storage at L.T., but fruits treated with T4 and control were decayed; among the treatments, the highest retention was found in the fruit treated with (T3). Among the tested treatments, SB 0.1% and SA + $CaCl_2$ maintained higher levels of ascorbic acid during the entire storage period. Our results are in conformity with the results of Lu *et al.* [36], who reported that SA delays the decline of ascorbic acid content in winter pineapple fruit.

3.4 Antioxidant activity as affected by chemical elicitors

Fruits act as free radical scavengers by donating a pair of electrons and neutralizing free radicals, which are oxidizing in nature and harm-causing agents [37]. It was observed in the present study that the antioxidant activity in treated phalsa was better than in untreated fruits, in which we noted a rapid loss in antioxidant activity. As anthocyanins were found to increase, the total antioxidant activity was also checked, because of the possibility of increase in other antioxidants as well. The total antioxidant activity of control and treated samples in both storage conditions is presented in *table VI*. The total antioxidant activity level at the beginning of storage was 1.22 μM Fe^{2+} g^{-1} and it increased gradually during storage in both treated as well as untreated phalsa in both the storage conditions. During 8 days of storage, the fruit treated with (T2) exhibited higher activity of total antioxidants (6.43 μM Fe^{2+} g^{-1}), whereas lower activity (2.86 μM Fe^{2+} g^{-1}) of total antioxidants was noted in untreated fruit in low temperature storage conditions. Our results are in agreement with those of Mirdehghan and Ghotbi [28], who stated that calcium

Table VI. Effect of chemical elicitors on the total antioxidant activity (μMFe^{2+} g^{-1}) of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	Total antioxidant activity at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	1.22 ± 0.06^{a}	3.995 ± 0.158^{a}	5.313 ± 0.391^{b}	5.342 ± 0.332^{b}
T2	1.22 ± 0.06^{a}	3.828 ± 0.173^{a}	6.426 ± 0.045^{a}	7.975 ± 0.493^{a}
T3	1.22 ± 0.06^{a}	3.094 ± 0.172^{b}	4.677 ± 0.523^{b}	5.653 ± 0.087^{b}
T4	1.22 ± 0.06^{a}	2.526 ± 0.107^{c}	5.335 ± 0.119^{b}	0 ± 0
T5	1.22 ± 0.06^{a}	2.401 ± 0.024^{c}	2.862 ± 0.555^{c}	0 ± 0
	Total antioxidant activity at room temperature during storage			
	Day 0	Day 2	Day 4	
T1	1.22 ± 0.06^{a}	3.790 ± 0.017^{b}	2.763 ± 0.201^{a}	
T2	1.22 ± 0.06^{a}	4.030 ± 0.162^{ab}	0 ± 0	
T3	1.22 ± 0.06^{a}	3.652 ± 0.099^{b}	2.843 ± 0.554^{a}	
T4	1.22 ± 0.06^{a}	4.434 ± 0.241^{a}	2.337 ± 0.105^{a}	
T5	1.22 ± 0.06^{a}	2.847 ± 0.338^{c}	0 ± 0	

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

treatments at different concentrations could increase and preserve the total antioxidant activity of pomegranate fruit during storage. However, total antioxidant activity increased with prolonged storage time, probably due to the increased anthocyanins as the major phenolic compounds that contribute to total antioxidant activity.

3.5 Changes in activity of PPO and its relation with TPC as affected by chemical elicitors

Enzymatic browning is one of the most important reactions that occur in fruits and vegetables, usually resulting in negative effects on color, taste, flavor and nutritional value. The reaction is a consequence of phenolic compound oxidation by PPO, which triggers the generation of dark pigments [38]. Phenolic compounds in fruits and vegetables may produce beneficial effects by scavenging free radicals [39]. Thus, phenolic compounds may help to protect cells against the oxidative damage caused by free radicals [40]. The variation in the TPC as a function of treatments and storage time in the current investigation is presented in *table VII*. For the treated as well as control sets of phalsa, the TPC progressively decreased over the entire storage period in both the storage conditions, but control fruits had a significantly ($P < 0.05$) greater decrease. During the 4 days of the storage period, the fruit treated with (T1) exhibited higher levels of phenols (2.43 mg g^{-1}), and during the 8 days of storage, the amount of total phenols recorded was 2.64 mg g^{-1} at R.T and L.T.storage, respectively. Incorporation of $CaCl_2$ with the SB and SA maintained higher TPC as compared with that of the control fruit in both storage conditions. These results are in accordance with Vyas *et al.* [41], who noted that application of salicylic acid and calcium chloride improved the TPC in custard apple fruit. Huang *et al.* [42] also noted an increase in the total phenolic content of cara cara 'navel' orange treated with an elicitor compound, SA. The reduction in the TPC during storage of phalsa is probably due to oxidation by PPO. Initially, at day 0, the activity of PPO in phalsa was 31.81 U min^{-1}mg^{-1} protein. The specific activity of PPO increased suddenly in the control set of phalsa in both the storage conditions, whereas lower activity of PPO was noticed in (T3)- and (T1)-treated fruit (*table VII*). Application of sodium benzoate significantly inhibited the PPO activity of phalsa fruit in both the storage conditions. According to Macheix *et al.* [43], the decrease in phenolic compounds at the end of storage might be due to breakdown of the cell structure in the fruit. In this study, the treated fruits retained their phenolic contents for up to 12 days of storage at L.T., but fruits treated with T4 and control decayed. Among them, the highest retention was found in the (T1)-treated fruit.

3.6 Effect of chemical elicitors on the microbial load of phalsa

The results pertaining to the microbial load of phalsa showed an increasing pattern for both bacterial and fungal counts during storage (*figures 1a–1d*). In the study, the decreased level of pH and total sugars led us to check the effect of SA and SB on microbes present on the surface of phalsa fruit. The data revealed that the maximum microbial numbers were found in control fruit, followed by SA in combination with $CaCl_2$ and SB with $CaCl_2$, while the minimum numbers were determined in the (T3) and (T1) treatments during storage at low temperature, with significant differences. The increasing pattern in the number of microbial colonies in the present study was in agreement with previous studies by Hashmi *et al.* [44] and Akhtar *et al.* [45], who also noted increased microbial counts during storage of chemically preserved mango pulp. Microorganisms continue propagation and multiplication as far as a conducive environment is available. Fresh commodities provide an ideal substrate for the microorganisms and thus their number increases during storage. Higher acid contents and increased sugars inhibit the growth of microorganisms. Furthermore, SA and SB treatments have been proved over the years as effective in inducing disease resistance and

Table VII. Effect of chemical elicitors on the total phenolics (TPC, mg g^{-1}) and polyphenol oxidase (PPO, U min^{-1} mg^{-1} protein) activity of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	TPC at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	3.50 ± 0.50^{e}	2.43 ± 0.05^{b}	2.64 ± 0.14^{a}	1.34 ± 0.11^{a}
T2	3.50 ± 0.50^{e}	2.24 ± 0.06^{c}	2.44 ± 0.10^{ab}	0.85 ± 0.02^{c}
T3	3.50 ± 0.50^{e}	1.62 ± 0.04^{d}	1.11 ± 0.09^{d}	1.27 ± 0.06^{b}
T4	3.50 ± 0.50^{e}	1.88 ± 0.12^{e}	1.65 ± 0.09^{e}	0 ± 0
T5	3.50 ± 0.50^{e}	3.37 ± 0.02^{a}	2.35 ± 0.02^{b}	0 ± 0
	TPC at room temperature during storage			
	Day 0	Day 2		Day 4
T1	3.50 ± 0.50^{e}	3.14 ± 0.21^{b}		2.41 ± 0.12^{a}
T2	3.50 ± 0.50^{e}	2.43 ± 0.09^{c}		0 ± 0
T3	3.50 ± 0.50^{e}	3.60 ± 0.06^{a}		2.12 ± 0.01^{b}
T4	3.50 ± 0.50^{e}	1.68 ± 0.04^{d}		1.82 ± 0.56^{c}
T5	3.50 ± 0.50^{e}	2.22 ± 0.01^{b}		0 ± 0
Treatments	PPO at low temperature during storage			
	Day 0	Day 4	Day 8	Day 12
T1	31.81 ± 10.79^{a}	126.4 ± 3.7^{a}	63.51 ± 9.61^{b}	41.90 ± 8.41^{a}
T2	31.81 ± 10.79^{a}	123.9 ± 18.5^{a}	67.23 ± 17.3^{b}	43.16 ± 3.89^{a}
T3	31.81 ± 10.79^{a}	139.4 ± 44.2^{a}	105.1 ± 26.36^{a}	45.48 ± 13.22^{a}
T4	31.81 ± 10.79^{a}	82.9 ± 29.6^{a}	68.32 ± 3.26^{ab}	0 ± 0
T5	31.81 ± 10.79^{a}	135.8 ± 6.9^{a}	29.04 ± 5.95^{b}	0 ± 0
	PPO at room temperature during storage			
	Day 0	Day 2		Day 4
T1	31.81 ± 10.79^{a}	76.90 ± 22.47^{ab}		28.55 ± 14.09^{a}
T2	31.81 ± 10.79^{a}	60.44 ± 8.48^{ab}		0 ± 0
T3	31.81 ± 10.79^{a}	44.06 ± 11.16^{ab}		37.65 ± 10.30^{a}
T4	31.81 ± 10.79^{a}	39.93 ± 6.21^{b}		34.32 ± 4.72^{a}
T5	31.81 ± 10.79^{a}	81.29 ± 18.49^{a}		0 ± 0

Different letters in the same column indicate significant differences at $P \leqslant 0.05$.

defense mechanisms in plants. Microorganism-induced decay has been reported to be reduced in SA-treated fruits of sweet cherry [46], kiwi fruit [47], pears [48] and strawberry [49]. The growth rate of the microorganisms that cause postharvest rots is controlled by temperature. These disease-causing organisms grow faster at warmer temperatures. Therefore, if storage temperatures are low, the rate of disease development can be considerably reduced and the storage life and quality of the fresh product can be assured [50]. In the present study, a similar trend was noted; the fungal count and bacterial count was higher in phalsa fruits stored at R.T. as compared with those of the fruits stored at L.T.

Table VIII. Effect of chemical elicitors on the shelf life of phalsa fruit stored at low (10 ± 1 °C) or room (25 ± 1 °C) temperature. Treatments: T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control.

Treatments	Shelf life (in days)	
	Low temperature	Room temperature
T1	11	4
T2	10	3
T3	14	5
T4	9	4
T5	7	2

3.7 Effect of chemical elicitors on postharvest shelf life

The shelf life of phalsa fruits was extended enormously with all the currently tested postharvest treatments in both the storage conditions as compared with the control fruits. During storage at 25 ± 1 °C, the fruits treated with the T3 treatment had a longer shelf life, *i.e.* 5 days, while the shortest shelf life (2 days) was noted in the control fruits. The phalsa fruits treated with the T3 treatment had their shelf life extended to the maximum duration of 14 days at 10 ± 1 °C (*table VIII*). A similar explanation was given by Srividya *et al.* [11] that antioxidants such as SB and benzyl adenine also inhibit the ethylene production, interfering, essentially, with the conversion of aminocyclopropane-1 carboxylic acid (ACC) into ethylene, and thereby increasing the shelf life of tomato. The results

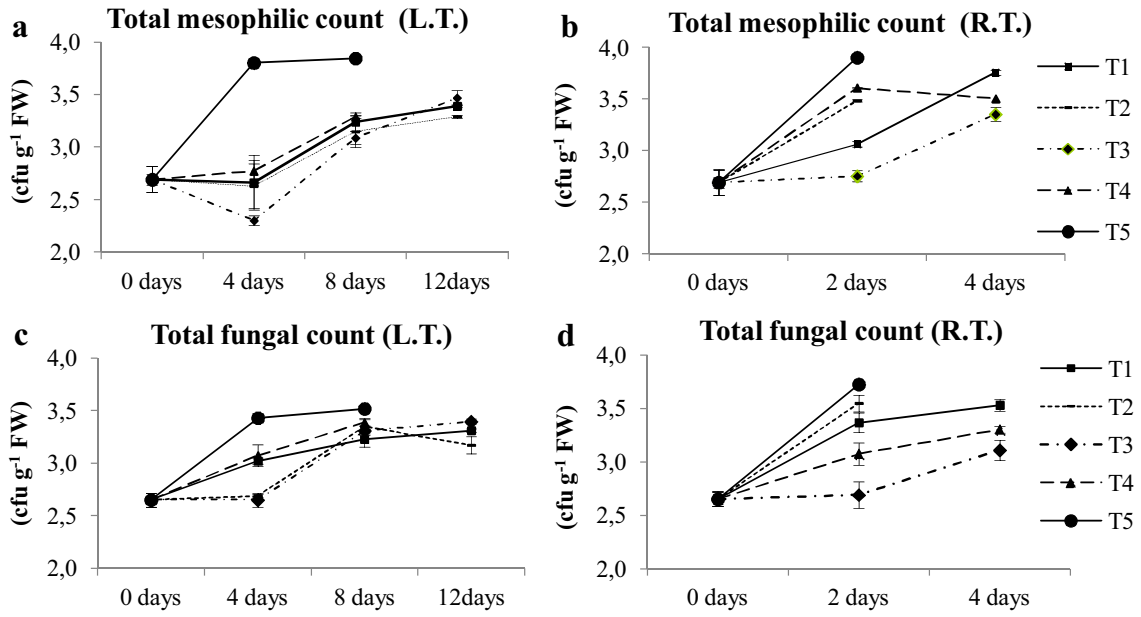

Figure 1. Effect of chemical elicitors on (a) the total mesophilic count of phalsa fruit stored at low temperature (L.T.), (b) the total mesophilic count of phalsa fruit stored at room temperature (R.T.), (c) the total fungal count of phalsa fruit stored at low temperature, (d) the total fungal count of phalsa fruit stored at room temperature. T1: Salicylic acid (2 mM), T2: Salicylic acid 2 mM + $CaCl_2$ (1%), T3: Sodium benzoate (0.1%), T4: Sodium benzoate (0.1%) + $CaCl_2$ (1%), T5: Control. Vertical bars represent ± standard deviations of means for three replicates (FW: fresh weight).

are supported by the findings of Cheour *et al.* [51], who reported that the application of calcium prolonged the storage life of strawberries, as measured by a delay in accumulation of sugars, decrease in organic acids, increase in the color saturation index and mold development. Further, Lam *et al.* [52] stated that SA, as an antitranspirant chemical, can retard moisture loss associated with pericarp browning of fruit. Senescent changes resulting in losses in physicochemical changes and nutritional quality can also be inhibited. Consequently, fruit storage life could be prolonged. Krishna *et al.* [53] also reported that the application of salicylic acid and calcium chloride as bioregulators extended the shelf life of apples up to 60 days.

4 Conclusion

The present study has revealed the efficacy of sodium benzoate, SA and $CaCl_2$ treatments on phalsa fruit quality in both the storage conditions, *i.e.* 25 ± 1 °C and 10 ± 1 °C, with a better effect on the fruits stored at 10 ± 1 °C. Implications of these treatments showed improved characteristics such as the shelf life and antioxidant compounds of the phalsa fruits. The current study also revealed that treatment with SB (0.1%) was effective in reducing the microbial load of phalsa. In light of these results, the treatments of SB (0.1%), SA (2 mM) and SA (2 mM) with the combination of 1% $CaCl_2$ can be considered in the order of their sequence for effective quality maintenance and the extension of the shelf life of phalsa fruit during postharvest storage at low temperature for their marketing. Therefore, the work presented here indicates that the postharvest application of chemical elicitors such as sodium benzoate, $CaCl_2$ and salicylic acid, which promote the antioxidant potential of phalsa fruit and control its exogenous microbial load, may be useful as an effective method for controlling postharvest decay and improvement of the shelf life of perishable horticultural produce on a commercial scale.

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