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Effect of GRAS chemicals on quality of pear fruits under low temperature storage

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Abstract
The aim of study was to investigate the effect of GRAS chemicals on physiochemical and enzymatic changes of 'Patharnakh' pear fruits stored at low temperature. Pear fruits were dipped in different concentrations of boric acid (0.0, 1.0, 2.0 and 3.0%), sodium bicarbonate (0.0, 1.0, 2.0 and 3.0%), and sodium benzoate (0.0, 1.0, 2.0 and 3.0%) stored at 0-1^0 C and 90-95% RH for 70 days. Physical and biochemical attributes of the fruits were determined at 0, 20, 40, 60 and 70 days during storage. Exogenous applications of GRAS chemicals reduced TSS/acid ratio, SOD enzyme activity, pH, lower value of reducing sugar and non-reducing sugar as compared to control. Boric acid @ 3% maintained fruit colour for longer time. Thus, boric acid treatment@ 3% was found to be most effective for extending the shelf life and maintaining quality of pear fruits during low temperature storage.

Keywords: Pear, GRAS, storage, fruit quality, enzyme activity

Introduction

Patharnakh is the most important pear cultivar grown in the northwestern arid irrigated parts of India, due to its high yield potential, low chill requirement, adaptation to various biotic and abiotic stresses. The fruits of 'Patharnakh' pear are harvested during the hot and humid monsoon season and are liable to post harvest losses. Under ambient conditions, fruit rapidly lose their market value and shelf-life is limited. After harvesting, biochemical changes underlying quality declines encompass free radical injury, cell wall degradation, oxidative damage, membrane lipid peroxidation, high metabolic activity, moisture loss, high respiration rate and loss of nutritional value. Thus, it is imperative to develop alternative postharvest technologies to maintain overall better pear quality for longer time during transport chain. Globally, a number of postharvest technologies have been developed to control postharvest diseases, maintaining quality of fruits and minimizes the losses. Various attempts have been made to improve the postharvest life of pear and to store the surplus fruit under low temperature for a longer period to increase its marketability.

Benzoic acids and its salts are among the most commonly used antimicrobial agents for improving storage ability of fruits. Their wide usage is due to their broad-spectrum activity against yeasts, moulds and bacteria, as well as their non-alteration of food flavor [5]. Those that have been approved and widely used in food are generally recognized as safe (GRAS). Sodium bicarbonate is a novel antifungal agent, inexpensive, readily available and poses lower toxicological risk, plays an important role in controlling the post harvest decay in many fruits. De Costa *et al.* reported that spore production and germination, mycelia growth of *Colletotrichum musae* can also be inhibited by sodium bicarbonate [4]. This may be due to change of pH of growth environment for microbes by sodium bicarbonate [11]. Boron is an essential nutrient element and its deficiency reduces pollen dermination. Exogenous application of boron was shown to reduce the occurance of browning injuries in pear during cold storage [23]. Therefore, considering these effects of GRAS chemicals on fresh horticultural commodities, the present research was conducted to study the effects of post-harvest treatments of GRAS chemicals on physico-chemical and enzymatic changes in pear fruits *cv.* Patharnakh during cold storage.

Singh *et al.* reported that fruits of strawberry cv. Chandler which received Ca or Ca + B had higher values of L, a, b than those, which received boron alone or those in control [18].

2. Materials and methods

For the experiment, fruits of pear *cv.* Patharnakh were harvested from the Fruit Research Farm, Department of Fruit Science, Punjab Agricultural University, Ludhiana (India) in the year 2017. The harvested fruits were sorted on the basis of uniformity in size, colour and absence of visible injury. Selected fruits were dipped for 5 min. in solution of GRAS chemicals (boric acid, sodium bicarbonate and sodium benzoate) at different concentrations (1, 2 and 3%). Control fruits were dipped in water only. The experiment comprised of four treatments with four replications in each treatment. One kg of fruit from every replication of each treatment was packed in corrugated fibreboard boxes (5% perforation) with paper lining and kept at 0–1 °C and 90–95 % RH for 70 days. Fruit samples were analysed after 0, 20, 40, 60 and 70 days of storage for various physico-chemical characteristics. Pear skin colour was recorded from opposite positions of each fruit in Commission International de L'Eclairage (CIE) units by using a ColorFlex 45°/0° spectrophotometer (HunterLab ColorFlex, Hunter Associates Inc., Reston, VA, USA, Hunter 1975)'and expressed' as *L**, *a**, *b** hunter 'colour values [6]. Before taking down observation, the instrument was calibrated 'with a standard white ceramic tile and black' tile and 'set up for D65 as illuminate and' a 10°C observer angle. Sampling was carried out by loading the quartz cuvettes with fruit sample. Chromaticity L* represents the lightness of the fruit colour, which ranges from 0 (black) to 100 (white); a* indicates the redness (+a*) or greenness (-a*) and b* indicates the yellow (+b*) or blue (-b*) colour of fruit skin. TSS/Acid'ratio was estimated by dividing the total soluble solids'with the total titratable acidity of fruit juice. The juice of ten randomly selected mature fruits was extracted and strained through muslin cloth. Fruit juice pH was determined with the help of digital pH meter (Labindia pH analyser). Reducing and non-reducing was determined by lane Eynon method [1]. SOD activity was estimated as method reported by Marklund and Marklund [12]. The protein content in enzyme extract was estimated with method given by Bradford (3). The experiment was set up as per completely randomized design and the data analysis was performed using the Statistical Analysis Software System SAS version 9.3 (SAS Institute, Inc, 1992; Cary, NC, USA).

3. Results and discussion

The pH is an important factor to measure the free acid content in any commodity indicating the degradation of organic acids into sugar [2]. In the present study, pH of all treated fruits and control were measured during 70 days of cold storage (Table 1). All the treatments were found to be effective in maintaining the pH throughout the storage interval with gradual increase in pH. The lowest mean pH was recorded in boric acid @ 3% treated fruits with value 4.04 indicating slowing down the ripening process followed by sodium benzoate @ 2% that is 4.08. The mean maximum pH was recorded in control fruits. In comparison the pH of chemically treated fruit was found to be lower than that of the pH in control fruits, which might be due to differences in atmosphere created by different treatments. The increase in pH may be due to the breakup of acids with respiration during storage. It has been suggested that juice pH increases in fruits as a result of breakup of acids to sugars during respiration. These findings are in accordance of Neeraj and Bhatia in Kinnow mandarin [13]. Studies performed by Togrul and Arslan [20] and Maftoonazad *et al.* with coating of whole peaches showed similar results obtained in the present work as there was increase in the pH during storage [10]. SOD enzyme activities of pear fruits stored for 70 days were varied among treatments and storage durations as shown in table 1. SOD activities reduced with storage time. The pear fruits treated with boric acid @ 3% had higher SOD enzyme activity followed by sodium benzoate @ 2 % then those of control fruits. The activity of SOD in the pulp exhibited significant interaction between GRAS chemicals treatments as well as storage period. SOD enzyme, a class of metal containing proteins that catalyze the dismutation reaction of superoxide radical anions into H_2O_2 and molecular oxygen [16]. Our findings of SOD activity are agreement with Shaham *et al.* [11] who reported decrease in SOD activity in pome fruits especially in apple during storage. TSS/acid ratio can easily be accessed from the table 3 that sugar to acid ratio increased with progress in ripening. At end of storage mean maximum TSS/acid ratio (84.79 %) was observed in control fruits and mean minimum TSS/acid ratio in boric acid @ 3% (31.90 %) treated fruits followed by sodium benzoate @ 2% (33.90). Postharvest treatment of GRAS chemicals significantly delayed the increase in TSS: acid ratio as compared to control fruits. This was due to the highest total sugars and low acid contents of the fruits. Sugar to acid ratio is an important factor for determining the taste of fruits. Interaction between treatments and storage period was found to be significant. Results were similar to the findings of Kaur *et al.* [8] in baramasi lemon fruits after 60 days of storage when fruits were treated with boric acid and packed in modified atmosphere packaging material.

Various treatments showed significant effect on reducing sugars of fruits. The storage period showed a significant effect on the reducing sugars of pear fruits. It is evident from the data that reducing sugars content showed a progressive increase up to 60 days of storage followed by a decline. Among all different treatments mean minimum reducing sugars were significant lower (5.65%) in fruits treated with boric acid @ 3%, which was statistically at par with sodium benzoate @ 2% (5.68%). The control fruits recorded mean highest non-reducing sugars content of 5.84%. The interaction between storage and treatments found to be significant. After 20 days of cold storage, the minimum reducing sugars content (5.38%) was recorded in boric acid @ 3% treated fruits and control fruits showed maximum reducing sugars content (5.72%). Similar trend was followed after 40 and 60 days of storage. The total sugars content was considerably improved in all the treatments up to 60 days of storage. At the end of storage, a decline in level of total sugars was recorded in all treatments. After 70 days of cold storage, it was observed that boric acid @ 3% treated fruits retained the maximum reducing sugars content (5.79%) followed by sodium benzoate @ 2% (5.76%) and the minimum value was observed in control (5.36%). The increase in sugars during storage may be due to the breakdown of complex organic metabolites into simple molecules or due to the hydrolysis of starch into sugars. The gradual increase in reducing sugars might be due to hydrolysis of polysaccharides, dehydration as a result of moisture loss and decrease in acidity by physiological changes during storage. These results are in accordance with the findings of Hussain *et al.* [7] who found that reducing and total sugars increased in Golden Delicious apple during storage. The results are similar to the findings of Kaur *et al*. [9] in Baramasi lemon fruits after 60 days of storage when fruits were treated with boric acid and packed in modified atmosphere packaging material.

The storage period showed a significant effect on the non-reducing sugars of pear fruits. The non-reducing sugars content showed a progressive increase up to 60 days of storage followed by a decline. The mean non-reducing sugars content recorded was 2.08, 2.22, 2.28 and 1.90% after 20, 40, 60 and 70 days of storage, respectively. The data reveals that average maximum non-reducing sugars (2.28%) content was recorded after 60 days of storage and mean minimum reducing sugars content (1.90%) was recorded after 70 days of storage. Various treatments showed significant effect on non-reducing sugars of fruits. Among all different treatments, boric acid @ 3% treated fruits showed lower mean non-reducing sugars of 2.02% which was statistically at par with sodium benzoate @ 2% (2.04%). The control fruits recorded mean highest non-reducing sugars content (2.22%). The interaction between storage and treatment found to be significant. After 20 days of cold storage, the minimum reducing sugars content (1.95%) was recorded in boric acid @ 3% treated fruits and control fruits showed maximum reducing sugars content (2.25%). Similar trend was followed after 40 and 60 days of storage. The total sugars content was considerably improved in all the treatments up to 60 days of storage. At the end of storage, a decline in level of total sugars was recorded in all treatments. After 70 days of cold storage, it was observed that boric acid @ 3% treated fruits retained the maximum reducing sugars content (2.03%) followed by sodium benzoate @ 2% (2.00%) and the minimum value was observed in control (1.71%). The increase in sugars during storage may be due to the breakdown of complex organic metabolites into simple molecules or due to the hydrolysis of starch into sugars. The decline in sugar content at a later stage may be attributed to the fact that after the completion of hydrolysis of starch, no further increase in sugars occurs and instead there is a decline in sugars. This decline in sugars is probably due to the consumption of sugars along with organic acids in respiration [15]. The results are similar to the findings of Kaur *et al.* [9] in Baramasi lemon fruits after 60 days of storage when fruits were treated with boric acid and packed in modified atmosphere packaging material.

The data pertaining to the effect of post-harvest application of boric acid, sodium bicarbonate, sodium benzoate on fruit colour is given in table 5. The *L** was increased with advancement of storage period. The mean fruit colour after 20, 40, 60 and 70 days were 63.82, 64.94, 67.42 and 69.33, respectively. The mean maximum *L** value (69.33) was reported after 70 days of storage and mean minimum fruit colour (63.82) was observed after 20 days. However, the variation between first and second storage interval was statistically at par. There was a significant effect of treatments on *L** value of fruit skin. Data shows that application of boric acid @ 3% delayed the loss of green colour in Patharnakh pear fruits. The fruits treated with boric acid @ 3% showed minimum *L**(64.34) value followed by sodium benzoate @ 2% (64.92), while the maximum (68.22) was recorded in control fruits. The interaction between storage interval and treatments were also found to be significant. All treatment showed a continuous increase in *L** with increase in storage period and attained the maximum value (69.33) at the end of studies. After 20 days of storage, the maximum value for *L** (64.77) was noticed in control fruits and minimum *L** (61.20) was recorded in fruits treated with boric acid @ 3% followed by sodium benzoate @ 2% treatments. A similar trend was followed after 40, 60 and 70 days of storage. The *L** value indicates that brightness has increased gradually during end of storage life. Loss of green colour pigment of fruit peel might be due to degradation of chlorophyll pigments of fruits and increased synthesis of careteniods [21, 22]. Similarly application of chemicals delayed the loss of green colour in pear fruits. The negative *a** value shows that greenness and positive value indicates red colour. The mean fruit colour after 20, 40, 60 and 70 days were -4.58, -2.87, -1.55 and 1.63, respectively. The mean maximum *a** value (1.63) was reported after 70 days of storage and mean minimum *a** value (4.58) was observed after 20 days.

All treatments significantly influenced *a** value of the pear fruits. The average maximum value of *a** value was noted in the untreated fruits (-1.07) during storage. The average minimum *a** values were registered in boric acid @ 3% (-2.32) treated fruits during storage which is statistically at par with sodium benzoate @ 2%(-2.25), respectively. In the untreated fruits rapid loss of green colour was observed than the treated fruits in cold storage condition. The colour development was increased with advancement of storage period. The interaction between storage and treatments were also found to be significant. All the treatments showed a continuous increase in *a** value with increase in storage period and attained the mean maximum value at end of 70 days of storage. After 20 days of storage, the maximum value for *a** (-3.48) was noticed in control fruits and minimum *a** value (-4.92) was recorded in fruits treated with boric acid @ 3% followed by sodium benzoate @ 2% treatments. A similar trend was followed after 40, 60 and 70 days of storage. At the end of storage negative values changed into positive values. The positive *b** value indicates yellowness of the fruits and it increased gradually from the day of harvest till the end of the storage life. Storage interval had a significant effect on *b** value of pear fruits. Mean minimum *b** value was recorded after 20 days of storage and maximum *b** value was recorded after 70 days of storage. All the treatments had significant effect on *b** value. Mean maximum *b** value was recorded in control fruits and mean minimum *b** value was recorded in boric acid @ 3% treated fruits which is statistically at par with sodium benzoate @ 2% treatment. The *b** values of pear fruits increased both in untreated and treated fruits with the advancement of the storage period but boric acid @ 3% had less (43.82) *b** value during storage condition as compared to the other treatments. However, *b** value higher (52.15) in control fruits during storage conditions. After 20 days of storage, the maximum value for *b** (45.57) was noticed in control fruits and minimum *b** value (40.07) was recorded in fruits treated with boric acid @ 3% followed by sodium benzoate @ 2% treatments. A similar trend was followed after 40, 60 and 70 days of storage. The *b** value of fruit color changes because of chlorophyll breakdown and formation of carotenoid pigments. These results showed that all the post harvest treatments delayed the ripening and thus retaining the colour of the pear fruits. Singh *et al.* (2013) observed the reduction in colour development in ber fruits during ambient storage with pre-harvest spray of boric acid @ 1.0 percent followed by post-harvest packaging with poly bags. These results are line with the findings of Singh *et al.* [18] who reported strawberry fruits receiving Ca + B were brighter (higher L, a, b values) and have better quality attributes. Kaur *et al.* [9] also reported that post-harvest applications of boric acid delayed the change in colour of Lemon fruits stored at ambient conditions. Also the results are similar to the findings of Pizato *et al.* [14] in minimally processed peach fruits after 12 days of storage when fruits were treated with different edible coatings and packed in PET packaging.

Conclusion

From this present study, it can be concluded that post-harvest applications of boric acid, sodium bicarbonate, sodium benzoate proved to be effective in delaying the ripening in Patharnakh pear fruits as compared to control. However, boric acid @ 3% was found most effective in maintaining reducing sugar and non reducing sugar content, juice pH, TSS/acid ratio, delay in loss of green colour and SOD activity up to 70 days of cold storage period.

Storage (days)	Treatment	pH	TSS/acid ratio	Reducing sugar	Non reducing sugar
0		3.39	18.63	5.13	1.73
20	Boric acid @ 1%	3.92	37.48	5.67	2.16
	Boric acid @ 2%	3.86	35.09	5.55	2.09
	Boric acid @ 3%	3.79	27.66	5.38	1.95
	Sodium Bicarbonate @ 1%	3.87	36.04	5.61	2.11
	Sodium Bicarbonate @ 2%	3.84	34.25	5.52	2.07
	Sodium Bicarbonate @ 3%	3.82	31.46	5.45	2.02
	Sodium benzoate @ 1%	3.89	36.21	5.65	2.13
	Sodium benzoate @ 2%	3.81	29.05	5.42	2.00
	Sodium benzoate @ 3%	3.83	33.54	5.50	2.06
	Control	3.96	40.26	5.72	2.25
40	Boric acid @ 1%	4.19	40.39	5.86	2.35
	Boric acid @ 2%	4.14	38.09	5.79	2.24
	Boric acid @ 3%	4.05	29.29	5.62	2.05
	Sodium Bicarbonate @ 1%	4.17	39.59	5.82	2.27
	Sodium Bicarbonate @ 2%	4.13	36.62	5.76	2.21
	Sodium Bicarbonate @ 3%	4.10	34.42	5.70	2.12
	Sodium benzoate @ 1%	4.18	40.03	5.84	2.30
	Sodium benzoate @ 2%	4.08	31.67	5.64	2.07
	Sodium benzoate @ 3%	4.11	35.54	5.74	2.19
	Control	4.21	41.83	5.97	2.39
60	Boric acid @ 1%	4.29	48.96	6.22	2.40
	Boric acid @ 2%	4.26	46.07	6.09	2.31
	Boric acid @ 3%	4.13	34.15	5.83	2.07
	Sodium Bicarbonate @ 1%	4.27	47.21	6.19	2.34
	Sodium Bicarbonate @ 2%	4.13	44.71	6.08	2.29
	Sodium Bicarbonate @ 3%	4.23	39.00	6.04	2.14
	Sodium benzoate @ 1%	4.26	47.52	6.21	2.37
	Sodium benzoate @ 2%	4.18	36.46	5.88	2.09
	Sodium benzoate @ 3%	4.24	42.75	6.06	2.23
	Control	4.32	55.56	6.32	2.53
70	Boric acid @ 1%	4.47	50.88	5.42	1.81
	Boric acid @ 2%	4.37	45.85	5.58	1.91
	Boric acid @ 3%	4.19	36.49	5.79	2.03
	Sodium Bicarbonate @ 1%	4.41	45.63	5.54	1.87
	Sodium Bicarbonate @ 2%	4.34	44.36	5.59	1.93
	Sodium Bicarbonate @ 3%	4.29	40.45	5.74	1.96
	Sodium benzoate @ 1%	4.44	49.12	5.45	1.85
	Sodium benzoate @ 2%	4.24	38.42	5.76	2.00
	Sodium benzoate @ 3%	4.31	43.03	5.69	1.94
	Control	4.50	57.52	5.36	1.71
	Treatment	0.18	0.75	0.09	0.06
	Storage interval	0.11	0.47	0.05	0.03
	Interaction	0.36	1.50	0.18	0.12

Storage (days)	Treatment	SOD	*L**	*a**	*b**
0		0.50	60.43	-6.32	40.92
20	Boric acid @ 1%	0.33	65.29	-4.41	45.04
	Boric acid @ 2%	0.36	64.25	-4.52	44.05
	Boric acid @ 3%	0.45	61.20	-4.92	40.07
	Sodium Bicarbonate @ 1%	0.35	64.48	-4.44	44.29
	Sodium Bicarbonate @ 2%	0.38	63.83	-4.59	44.04
	Sodium Bicarbonate @ 3%	0.41	63.14	-4.82	43.59
	Sodium benzoate @ 1%	0.34	64.52	-4.43	44.38
	Sodium benzoate @ 2%	0.43	62.51	-4.88	41.34
	Sodium benzoate @ 3%	0.39	64.16	-4.78	40.07
	Control	0.32	64.77	-3.98	45.57
40	Boric acid @ 1%	0.30	66.07	-2.07	46.16
	Boric acid @ 2%	0.33	64.99	-2.92	45.55
	Boric acid @ 3%	0.39	63.71	-3.44	44.18
	Sodium Bicarbonate @ 1%	0.33	65.34	-2.81	45.79
	Sodium Bicarbonate @ 2%	0.34	64.28	-2.95	45.46
	Sodium Bicarbonate @ 3%	0.36	64.10	-3.10	45.18
	Sodium benzoate @ 1%	0.32	65.52	-2.78	45.98
	Sodium benzoate @ 2%	0.37	63.45	-3.32	44.71
	Sodium benzoate @ 3%	0.35	64.28	-3.20	45.38
	Control	0.28	67.63	-2.10	46.78
60	Boric acid @ 1%	0.26	69.25	-1.41	46.87
	Boric acid @ 2%	0.30	68.57	-1.57	47.22
	Boric acid @ 3%	0.38	65.10	-1.80	45.26
	Sodium Bicarbonate @ 1%	0.29	68.76	-1.51	47.37
	Sodium Bicarbonate @ 2%	0.32	67.19	-1.62	46.94
	Sodium Bicarbonate @ 3%	0.34	65.88	-1.69	46.84
	Sodium benzoate @ 1%	0.27	67.19	-1.42	48.02
	Sodium benzoate @ 2%	0.36	65.82	-1.72	46.29
	Sodium benzoate @ 3%	0.33	66.73	-1.64	46.89
	Control	0.25	69.66	-1.09	49.78
70	Boric acid @ 1%	0.20	70.69	1.41	51.56
	Boric acid @ 2%	0.26	69.78	1.24	47.95
	Boric acid @ 3%	0.35	67.33	0.88	45.75
	Sodium Bicarbonate @ 1%	0.25	70.40	1.31	48.76
	Sodium Bicarbonate @ 2%	0.28	69.29	1.12	47.95
	Sodium Bicarbonate @ 3%	0.3	68.13	0.99	47.03
	Sodium benzoate @ 1%	0.23	70.42	4.42	48.99
	Sodium benzoate @ 2%	0.34	67.89	0.94	46.32
	Sodium benzoate @ 3%	0.29	68.56	1.09	47.20
	Control	0.18	70.80	2.89	52.15
	Treatment	0.04	2.15	0.49	2.13
	Storage interval	0.02	1.36	0.31	1.34
	Interaction	0.08	4.31	0.99	4.26

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