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## Effect of pre-cooling methods on shelf life of cold stored tuberose florets

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**Abstract**

Investigation was carried out to evaluate the effect of different pre-cooling methods to extend shelf life of tuberose florets. The harvested flowers were treated with different pre-cooling methods (pre-cooling at 4°C, hydro cooling at 4°C and by use of gel ice packs), followed by treatment with sucrose (10% and 4%) and boric acid (4% and 2%). Analysis was done in terms of physical and chemical characteristics of flowers. Among the three pre-cooling methods, flowers treated with 4% boric and pre-cooled at 4°C showed maximum freshness (4.67) with grade (A) and highest  $L^*$  value (87.23) than the control. Florets treated with 4% boric acid retained moisture content (85.33%) without adversely affecting physico-chemical qualities even up to 4 days of storage. Main objective is to study the response of pre-cooling methods and chemicals (sucrose and boric acid) on extending the marketability of tuberose flowers.

**Keywords:** *Polianthes tuberosa*, boric acid, respiration rate, polyphenol enzyme activity

**1. Introduction**

Tuberose (*Polianthes tuberosa* L.) belongs to the family Asperagaceae native to Mexico. It is being grown in most of the tropical and sub-tropical countries of world (Asif *et al.*, 2001) [1]. Tuberose is an important commercial cut as well as loose flower crop due to its pleasant fragrance, longer vase-life of spikes, higher returns and wider adaptability to varied climate and soil. They are valued much by the aesthetic world for their beauty and fragrance. Tuberose represents sensuality and is used in aromatherapy for its ability to open the heart and calm the nerves, restoring joy, peace and harmony.

Tuberose represents sensuality and is used in aromatherapy for its ability to open the heart and calm the nerves, restoring joy, peace and harmony. Furthermore, essential oil acts as sedative, aphrodisiac, antidepressant, antispasmodic and used to boost blood circulation. Fragrant flowers are added to the favourite beverage prepared from chocolate and served either cold or hot as desired to cure insomnia. In Java, the flowers are eaten along with the juices of the vegetables. Tuberose bulbs are rubbed with turmeric and butter, applied as a paste over red pimples of infants. Dried tuberose bulbs in powdered form are used as a remedy for gonorrhoea.

The post-harvest performance of tuberose that has been transported to far-off markets is worse. Numerous pre- and post-harvest variables, including temperature, relative humidity, irrigation frequency, picking time, nutrition, and handling procedures, have an impact on the quality of tuberose flowers Benschop (1993) [2]. The post harvest behaviour of flowers is an outcome of physiological processes, which may act independently to affect the senescence and shelf life of flowers but most of them are inter-related. Since tuberose originated from the sub-tropics, loss of quality might be due to chilling injury induced by exposure to low but non-freezing temperatures during storage and marketing, alternatively it might be the result of post harvest desiccation or improper temperature management. Flowers are extremely perishable, maintaining their physiological functions and the beginning of their senescence also depends on ethylene (Figueroa *et al.*, 2005) [4].

Proper post harvest treatments can greatly extend the shelf life of tuberose and routinely be carried out with flowers intended for storage and transport. The different post harvest technologies available are pre cooling, use of chemical preservatives and different packaging materials. Further, storage of the flowers at low temperatures enhances the post-harvest life. Adequate packaging protects the produce from physical, physiological and pathological deterioration during transport and marketing which helps in extending shelf life by retaining their attractiveness Krishnamoorthy (1990) [9]. Use of ethylene absorbent in packages helps in delaying senescence of florets by absorbing ethylene produced inside packages. Treating of flower buds with novel chemicals before packaging and transport they acts as barrier for respiration, moisture loss, to extend the shelf life with better retention of colour and turgidity of petals.

However, very few results were documented to prolong the storability and transport of tuberose loose flowers. Therefore, it is a need of the hour to propose proper post harvest handling of tuberose loose flowers both in cold storage as well as ambient condition and for transportation for the benefit of farmers which helps to overcome losses occurred during marketing.

## 2. Materials and methods

### 2.1. Materials

The present investigation was undertaken in the Department of Post-Harvest Technology, University of Horticultural Sciences, Bagalkot, India during the year 2017-18. Prajwal, a commercially important tuberose cultivar grown in this region was used for the study. This cultivar is a single type, having more fragrance, extensively cultivated and used as loose flower. Flower buds were procured from historical place, Badami, near Bagalkot. The unopened flower buds at pin hole stage which were ready to open in subsequent days were harvested in the early morning and they were brought to the laboratory within 2 hours. Three different methods of pre-cooling and two pulsing chemicals were used in the study to evaluate physiological, biochemical and physical characteristics of tuberose.

#### 2.1.1 Pre cooling methods

1. Buds were pre cooled by placing them at 4°C for about one hour
2. Hydro cooling done by using cold distilled water of temperature 4°C was noted by using thermometer and buds were pre-cooled for about 5 min.
3. Buds were placed over the gel ice packs (25% weight of buds).

### 2.2. Methodology

#### 2.2.1 Sucrose and boric acid

Sucrose solution of 4% and 10% were prepared by dissolving 100 gm and 40 gm in 1000 ml of distilled water, respectively. Boric acid of 2% and 4% was prepared by weighing 2 gm and 4 g separately and dissolved in 100 ml of warm water and volume made up to 1000 ml.

The pre-cooled buds were dipped in solution for about 15 minutes and removed to air dry under ceiling fan. The control buds were dipped in distilled water. The treated as well as untreated buds were placed in baskets and kept under storage 10°C for further observations.

#### 2.3. Freshness index

Observations were made at an interval of two days, till these were presentable (depending up on the shelf life of the loose flower). Loose flower were assigned A, B and C grades which were given points as shown below.

Appearance	Grade	Points
Flowers with original bright colour	A	5
Flowers with faded from original colour	B	3
Flowers severly faded from original color	C	1

#### 2.4. Fragrance score

Scale for fragrance

Score	Fragrance
4	Very strong
3	Strong
2	Mild
1	Least and undesirable

### 2.5. Moisture (%)

The moisture of tuberose flowers was measured at regular intervals. Two grams of flower was taken and cut in to small pieces and placed in Sartorius electronic moisture analyzer (Model: MA 35) and the direct reading was noted down from the instrument screen and expressed in per cent.

### 2.6. Instrumental colour values ( $L^*$ , $a^*$ , $b^*$ )

Flower colour was measured using a portable colorimeter (Lovibond LC 100, Model RM200 Portable spectrophotometer, The Tintometer Limited, Salisbury, UK). The colorimeter was calibrated with a white standard calibration plate before measurement and the direct reading was noted down from the instrument screen. As per the Commission on Illumination (CIE)  $L^*a^*b^*$  system of colour representation, the  $L^*$  value corresponds to a dark-bright scale and represents the relative lightness of colors with a range from 0 to 100 (0 = black, 100 = white). Values of  $a^*$  and  $b^*$  represents hue (colour). The  $a^*$  and  $b^*$  values extend from -60 to 60;  $a^*$  negative is for green and  $a^*$  positive is for red and  $b^*$  negative is for blue and positive for yellow.

### 2.7. Enzyme activity

Polyphenol oxidase activity in tuberose was measured following the method of Eason *et al.* (2002)<sup>[7]</sup>.

**Principle:** Phenol oxidizes are copper containing proteins that catalyze the aerobic oxidation of phenolic substrates to quinines, which are auto oxidized to brown pigments known as melanins. These can be estimated spectrometrically at 495nm.

#### Reagents

##### Tris Hcl (50 mM, P<sup>H</sup> 7.2)

- 50 mM of tris HCL buffer with 7.2 p<sup>H</sup> was prepared by dissolving 7.88g of tris HCL in small amount of water and volume made upto 1 litre with distilled water and then P<sup>H</sup> adjusted to 7.2 with 1N NaOH.
- Catechol 0.01M: 0.55g of catechol dissolved in small amount of distilled water and then volume made of 500 ml with distilled water and used immediately after preparation.
- Sorbital (0.4M): 72.87g of sorbital dissolved in small amount of distilled water and then volume made to 500 ml with distilled water and stored in glass bottles.
- Nacl (10 mM): 0.29g of Nacl dissolved in 100 ml of distilled water and volume made to 500 ml with distilled water to get 10 mM of Nacl solution.
- Phosphate buffer (0.1M P<sup>H</sup> 6.5): Add 38.1 ml of 1M dibasic monohydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>) and 61.9 ml of monobasic dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) dilute combined stock solution to 1litre with distilled water to get 0.1M Phosphate buffer with P<sup>H</sup> 6.5.

#### Preparation of enzyme extract

HCL, Sorbital and Nacl) extraction medium centrifuge at 2000rpm for 10 min and supernatant was used for assay of Polyphenol oxidase (PPO) activity.

#### PPO enzyme assay

For measuring the PPO enzyme activity, add 2.5 ml of phosphate buffer and 0.3 ml of catechol solution to cuvette placed in spectrophotometer, then add 0.2 ml of enzyme extractant to it. Change in the absorbance for every 30 seconds upto 5 minutes was recorded at 495nm.

### Calculation

Enzyme units in sample =  $K \times (\text{Absorbance/minute}) \times \frac{\text{U/g}}{\text{FW}}$

Where  $K$  for catechol oxidase = 0.272

One unit of catechol oxidase is defined as the amount of enzyme that transforms 1  $\mu$ mole of dihydrophenol to 1  $\mu$ mole quinine per minute.

### 2.11 Experimental design and data analysis

The experiment was carried out with 11 treatments and the experiment was repeated 3 times and pooled data was subjected to statistical analysis. Flowers were arranged in Complete Randomised Design. Randomly selected fruits were taken to analyse freshness index, fragrance score, moisture content, Instrumental colour values ( $L^*$ ,  $a^*$ ,  $b^*$ ) and enzyme activity. Statistical analyses were performed using Web Agri Stat Package (WASP) Version 2. Significant differences among means at  $P = 0.05$  were determined by post hoc tests using Duncan's multiple range test.

## 3. Results and discussion

### 3.1 Freshness index

Freshness is one of the economic and quality attribute which influence the market price as well extending the enjoyment of flowers by consumers. In an effort to give customers fresh flowers that are as radiant as the moment they were first plucked, florists use an assortment of stay-fresh techniques. During 4<sup>th</sup> day of storage tuberose florets pre cooled at 4 °C showed fragrance index score (2.66) *i.e.* flowers moderately faded from original colour followed by hydro cooling, where as buds pre cooled by gel ice pack showed least freshness index (1) *i.e.* flowers severely faded from original colour which is on par with control. This might be due to pre cooling is one of the techniques used for the rapid removal of the "field heat" which leads to reduction of both the rate of metabolism and prevention of water loss which delays freshness off as reported by (Bhardwaj and Sen, 2003).

### 3.2 Fragrance

Fragrance is an elusive quality, it may be fleeting and change over time. From a long distance, flower fragrances are more effective than visual signals. production of many flower fragrances displays a circadian cycle that is, they are stronger at certain times during the day or night where as tuberose are more fragrant during evening as they are night blooming in nature. Fragrance is negatively correlated with age. Tuberose buds pre cooled at 4 °C retained better fragrance even during 4<sup>th</sup> day of storage with score ranging from (2.3 to 3), whereas least and undesirable fragrance noticed in control (1) which ends the shelf life. The endogenous and exogenous ethylene leads to loss of fragrance (Sexton *et al.*, 2005) [20]. This ethylene action could be delayed by effect of boric acid.

### 3.3 Moisture content

The marked reduction in moisture content indicates the advancement of senescence of flowers thus it is an important factor that affects the shelf life. In present investigation the moisture content decreased from 2<sup>nd</sup> day (84.18%) to 4<sup>th</sup> day (74.83%) of storage. Among all the treatments higher moisture content retained in T<sub>4</sub> (87.74%) followed by T<sub>2</sub> (85.64%) and T<sub>3</sub> (82.91%) and control (66%) moisture content this might be due to the slower loss of moisture from pre cooled commodities during storage, such conditions can easily be achieved by lowering the storage temperature, as the environment tends to be more saturated simply by reduction in temperature which brings down the heat load thus moisture loss is reduced (Lurie and Ben 1990) [12]. In addition to this, the boric acid maintained water balance, similar recordings obtained in tuberose (Sharma *et al.*, 2008) [21].

### 3.4 Colour ( $L^*a^*b^*$ )

Colour is one of the most prominent parameters that reflect tuberose quality and freshness. Colour changes or fading can affect the flower quality and acceptability, rapid senescence of flowers causes yellowing and browning. In this study, change in florets colour is due to decreased  $L^*$  and increased  $a^*$  and  $b^*$  values (Schmitzer *et al.*, 2009). Among the treatments T<sub>4</sub> recorded maximum  $L^*$  (89.74),  $a^*$  (-3.13) and minimum  $b^*$  (14.08) value which indicates maximum brightness, better retention of greenness with less yellowing. This may be attributed to the fact that pre-cooling reduces the production and sensitivity to ethylene that accelerates senescence (Brosan and Sun 2001) [5]. However in addition to pre cooling, boric acid had beneficial effect that reduced solute leakage from florets with better membrane integrity due to which good colour retention is observed. It was reported that combination of pre cooling and boric acid treatment retains better colour in tuberose florets (Chakrabraty *et al.*, 2012) [3].

### 3.5 Enzyme activity

Polyphenol oxidase (PPO) catalyses the oxidation of monophenols and/or *o*-diphenols to *o*-quinones with the concomitant reduction of oxygen to water which results in protein complexing and the formation of brown melanin pigments. Among the treatment T<sub>4</sub> recorded less browning with minimum enzymatic activity whereas untreated florets recorded higher amount of enzyme activity (1.25 U/g FW) on 4<sup>th</sup> day of storage. It is thought that the depletion of carbohydrates may induce hydrolysis of intercellular membranes to supply respiratory substrate and subsequently allow vacuole-sequestered phenols to be oxidised by PPO. Similar findings in rose where boric acid supply the sugars to corolla and acts as a antioxidant, further initial decrease in temperature by pre cooling which delays the enzymatic activities (De and Barman 1998) [6].

**Table 1:** Effect of different pre-cooling methods on fragrance score and freshness index of tuberose cv. Prajwal stored at 10 °C

Treatments	Fragrance score	Freshness index
T <sub>1</sub> : P <sub>1</sub> + 10% sucrose	2.16	4.33
T <sub>2</sub> : P <sub>1</sub> + 2% boric acid	2.66	4.66
T <sub>3</sub> : P <sub>1</sub> + 4% sucrose	2.33	2.66
T <sub>4</sub> : P <sub>1</sub> + 4% boric acid	3.00	5.00
T <sub>5</sub> : P <sub>2</sub> + 10% sucrose	2.33	3.00
T <sub>6</sub> : P <sub>2</sub> + 2% boric acid	2.16	1.33
T <sub>7</sub> : P <sub>2</sub> + 4% sucrose	2.16	1.33
T <sub>8</sub> : P <sub>2</sub> + 4% boric acid	2.83	3.00
T <sub>9</sub> : P <sub>3</sub> + 4% sucrose	1.60	1.33

T <sub>10</sub> : P <sub>3</sub> + 4% boric acid	1.33	2.66
T <sub>11</sub> : Control	1.16	1.00
Mean	2.11	2.75
S.Em ±	0.32	1.40
CD at (0.01)	0.93	4.66

P<sub>1</sub>: Pre-cooled at 4°CP<sub>2</sub>: Hydro-cooled at 4°CP<sub>3</sub>: Gel ice pack**Table 2:** Effect of different pre-cooling methods on moisture (%) in buds of tuberose cv. Prajwal stored at 10 °C

Treatments	Moisture (%)		Mean
	Days of storage		
	2	4	
T <sub>1</sub> : P <sub>1</sub> + 10% sucrose	88.16	80.5	84.33
T <sub>2</sub> : P <sub>1</sub> + 2% boric acid	89.13	82.16	85.64
T <sub>3</sub> : P <sub>1</sub> + 4% sucrose	87.16	78.66	82.91
T <sub>4</sub> : P <sub>1</sub> + 4% boric acid	90.16	85.33	87.74
T <sub>5</sub> : P <sub>2</sub> + 10% sucrose	84.16	74.83	79.49
T <sub>6</sub> : P <sub>2</sub> + 2% boric acid	85.16	77.16	81.16
T <sub>7</sub> : P <sub>2</sub> + 4% sucrose	83.43	73.5	78.46
T <sub>8</sub> : P <sub>2</sub> + 4% boric acid	86.13	78	82.06
T <sub>9</sub> : P <sub>3</sub> + 4% sucrose	79.06	64.5	71.78
T <sub>10</sub> : P <sub>3</sub> + 4% boric acid	81	68.26	74.63
T <sub>11</sub> : Control	72.46	60.26	66.36
Mean	84.18	74.83	
S.Em ±	0.33	0.30	
CD at (0.01)	0.93	0.86	

P<sub>1</sub>: Pre-cooled at 4°CP<sub>2</sub>: Hydro-cooled at 4°CP<sub>3</sub>: Gel ice pack**Table 3:** Effect of different pre-cooling methods on colour L\*, a\* and b\* in buds of tuberose cv. Prajwal stored at 10 °C

Treatments	L*		Mean	a*		Mean	b*		Mean
	Days of storage			Days of storage			Days of storage		
	2	4		2	4		2	4	
T <sub>1</sub> : P <sub>1</sub> + 10% sucrose	91.16	83.2	87.18	-1.46	-0.50	-1.46	13.94	18.35	16.145
T <sub>2</sub> : P <sub>1</sub> + 2% boric acid	90.16	85.16	87.66	-2.06	-1.58	-2.06	13.2	17.34	15.27
T <sub>3</sub> : P <sub>1</sub> + 4% sucrose	88.53	82.2	85.36	-3.08	-3.76	-3.08	14.2	18.16	16.18
T <sub>4</sub> : P <sub>1</sub> + 4% boric acid	92.26	87.23	89.74	-2.61	-2.67	-2.61	12	16.16	14.08
T <sub>5</sub> : P <sub>2</sub> + 10% sucrose	89.16	84.16	86.66	-1.63	-1.63	-1.63	17	19.93	18.46
T <sub>6</sub> : P <sub>2</sub> + 2% boric acid	87.76	82.46	85.11	-1.74	-1.74	-1.74	15.35	21.63	18.49
T <sub>7</sub> : P <sub>2</sub> + 4% sucrose	85.23	80.33	82.78	-1.53	-1.44	-1.53	16.53	21.33	18.93
T <sub>8</sub> : P <sub>2</sub> + 4% boric acid	88.23	83.2	85.71	-1.93	-1.89	-1.93	14.49	19.33	16.91
T <sub>9</sub> : P <sub>3</sub> + 4% sucrose	77.73	72.6	75.16	-1.50	-1.47	-1.50	18.33	22.46	20.39
T <sub>10</sub> : P <sub>3</sub> + 4% boric acid	79.16	74.2	76.68	-1.92	-1.9	-1.92	17.34	23.33	20.33
T <sub>11</sub> : Control	68.26	63.06	65.66	-1.11	-0.20	-1.11	19.33	24.16	21.74
Mean	85.24	79.80	Mean	-2.03	-1.7	Mean	15.6	18.07	
S.Em ±	0.21	0.14		0.21	0.14		0.41	0.43	
CD at (0.01)	0.63	0.44		0.63	0.44		1.18	1.2	

P<sub>1</sub>: Pre-cooled at 4°CP<sub>2</sub>: Hydro-cooled at 4°CP<sub>3</sub>: Gel ice pack**Table 4:** Effect of different pre-cooling methods on polyphenol oxidase activity (U/g FW) in buds of tuberose cv. Prajwal stored at 10 °C

Treatments	Polyphenol Oxidase activity (U/g FW)		
	Days of storage		
	2	4	Mean
T <sub>1</sub> : P <sub>1</sub> + 10% sucrose	0.01	0.30	0.15
T <sub>2</sub> : P <sub>1</sub> + 2% boric acid	0.02	0.40	0.21
T <sub>3</sub> : P <sub>1</sub> + 4% sucrose	0.03	0.50	0.26
T <sub>4</sub> : P <sub>1</sub> + 4% boric acid	0.00	0.10	0.05
T <sub>5</sub> : P <sub>2</sub> + 10% sucrose	0.02	0.60	0.31
T <sub>6</sub> : P <sub>2</sub> + 2% boric acid	0.01	0.50	0.25
T <sub>7</sub> : P <sub>2</sub> + 4% sucrose	0.02	0.45	0.23
T <sub>8</sub> : P <sub>2</sub> + 4% boric acid	0.03	0.39	0.21
T <sub>9</sub> : P <sub>3</sub> + 4% sucrose	0.05	0.72	0.38
T <sub>10</sub> : P <sub>3</sub> + 4% boric acid	0.04	0.63	0.33
T <sub>11</sub> : Control	0.06	1.25	0.65

Mean	0.02	0.49	
S.Em ±	0.001	0.03	
CD at (0.01)	0.02	0.10	

P<sub>1</sub>: Pre-cooled at 4°C

P<sub>2</sub>: Hydro-cooled at 4°C

P<sub>3</sub>: Gel ice pack

#### 4. Conclusion

On the basis of results obtained in the present investigation on different post-harvest treatments used for extending shelf life of tuberose florets during storage and transport, it can be concluded that, pre-cooling at 4°C proved is the method followed by hydro cooling and gel ice pack and with respect vase life solutions, sucrose and boric acid were found effective in maintaining freshness of florets by lowering the rate of weight loss, respiration, moisture and wilting as well as enzyme activity, thus helping in extending the shelf life of tuberose florets.

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