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Method Development for a Consumer Safe Application of Ethephon in the Artificial Ripening of *Embul* Banana (*Musa* spp.)

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Abstract- Artificial ripening is a requirement to manage market demand and supply in banana value chain. Fruit traders use synthetic chemicals to speed up the ripening process, and it creates problems when exposing over doses and inappropriate application methods. Therefore, the study was conducted to find out the optimum combination of artificial ripening agents and their application in the ripening induction process at ambient conditions (30°C ± 2). The Embul bananas were taken at appropriate maturity level and the treatments were applied in different concentrations such as 100, 150, and 200 ppm with exposure time of 12, 18, and 24 hours. The treated bananas were stored at 30°C ± 2 at ambient condition for 6 days. The quality parameters such as total soluble solids (TSS), colour, pH, Fruit firmness, chlorophyll content (TCC), and total degree of ripeness were assessed. The consumer preference was evaluated using 5 point hedonic scale. The bananas were treated with 200ppm of ethephon for 24 h and 18 h were shown the lower firmness such as 7.39N ±0.73 and 12.43N ± 3.06, respectively at the 2nd day of storage, compared with control (38.47N ± 4.17). uring the physiological ($p< 0.05$) higher in 200 ppm treated for 24 h (24.80±0.44) followed by 200 ppm treated for 18 h (21.73 ± 1.32) at the 2nd day of storage. Therefore, 200 ppm ethephon exposed for 18 hours could be recommended for artificial ripening of Embul bananas by extending the shelf life for 6 days.

Keywords: artificial ripening, ethephon, shelf life, banana

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1. Introduction

Fruits provide many essential nutrients in the human daily diet, such as vitamins, minerals, fibre, antioxidants, and phytonutrients. During the physiological development of fruits, such as fruit set, fruit development, and fruit ripening, and are three distinct stages involved in producing quality fruit for consumption. Using genetic and breeding process it is needed to pay attention more attention on fruit ripening, as this important process activates a whole set of biochemical pathways that make the fruit attractive, desirable, and edible for consumers (Bouzayen et al., 2010). Fruit ripening is a process in which the biochemical and physiological characters of the organ are developed on its physical and chemical nature (Giovanonni, 2001). Edible ripe stage is more suitable for consumption of fruits and after attaining a physiological maturity the activities such as fruit growth, development, and senescence are take place. During the ripening, change in colour, softening of the pericarp, and changes in sweetness and flavor and attractive to eat can be identified (Seymour et al., 1993; Brady, 1987). Ethylene regulates the fruit ripening by controlling a series of chemical and biochemical activities. Physiological changes such as flesh firmness, pulp moisture, TSS, and pH can be enhanced using ripening inducers by altering the process of ripening. The activity of compounds such as antioxidants, flavonoids and phenolics, in banana flesh were also increased by ethephon (Maduwanthi and Marapana, 2021). With the advancement of technology, different artificial ripening methods have been introduced to meet consumer demand and address various economic issues. Natural fruit ripening has not create any harmful effects and there is no any abnormalities in their metabolic reactions. (Hewajulige and Premadasa, 2020). Due to various applications of fruit ripening agents, the fast deterioration of fruits leads to economic losses during the supply chain. The health hazarders such as dizziness, weakness, skin ulcers, and heart-related diseases can be happened due to regular consumption of artificially ripened fruits (Fattah and Ali 2010). Therefore, it is important to identify the most convenient dosing protocols for artificial ripening of fruits for commercial production. Therefore, the study was conducted to optimize the safer dosing protocol of ripening agents for the artificial ripening of banana fruits.

2. Materials and Methods

A. ***Sample Collection and Preparation***

All the experiments and analyses were carried out at the laboratory of the National Institute of Post-Harvest Management, Anuradhapura, Sri Lanka.

Properly matured, green coloured *Embul* banana (*Musa* spp.) bunches indicated by the disappearance of angularity in cross section as described in Siddiq *et al*. (2012) were harvested in March 2019 from a commercial orchard in the North Central part of Sri Lanka. Soon after harvesting, banana bunches were carefully packed in ventilated plastic containers and transported to the laboratory. After reaching the fruit to the laboratory, de-handing was done and allowed to remove the latex. Bananas at maturity by disappearance of angularity in cross-section, were selected. The selected bananas were washed with chlorinated (200 ppm), followed by surface drying. Selected banana fruits were exposed to 100 ppm, 150 and 200 ppm ethephon concentrations for 12 h, 18 h, and 24 h, respectively. 1 ml, 1.5 ml, and 2 ml of ethephon were dissolved in 1000 ml of distilled water, respectively, for preparation of 100,150, and 200 ppm ethephon solutions. Calcium hydroxide solution was mixed with the prepared ethephon solution to catalyze the release of ethylene gas from ethephon. The ripening chamber was sealed immediately after mixing the prepared solutions together. The remaining was allowed to ripen naturally and kept as a control. Each lots were packed in small-sized ventilated plastic containers and placed inside the ripening chamber (30°C ± 2 and 85% RH). The ethylene treatment was provided for 12h, 18h, and 24h, and thereafter, fruits were kept at normal environmental condition for another 48 h for ripening.

B. Quality Evaluation of Fruits during Storage

Fruit firmness, pH, Total soluble solids (TSS), peel colour and total chlorophyll content of the *Embul* banana were measured after treatment within a one-day interval during 6 days of storage. And the degree of ripeness was evaluated. Sensory qualities were evaluated at the table-ripening stage of bananas.

1) Total Soluble Solids (TSS):

Total soluble solids was measured using a handheld digital refractometer (ATAGO, Model HR-5, Japan). A small amount of chopped banana was collected on a piece of clean cloth and the juice was squeezed into a beaker. The extract was placed on the prism surface of the refractometer, and readings were reported as % Brix.

2) Firmness:

The firmness of the bananas were measured using a penetrometer (Model CS 1-2, Italy). The finger was punched by the probe to a constant pressure by lowering the handle gently according to Liew and Lau, 2012.

3) pH:

The pH was determined using a pH meter (Thermo Orion - 420). Banana fruits were chopped, and extract was collected by pounding chopped banana in a clean cloth using a mortar and pestle. The juice was collected by squeezing the cloth, and the extract was collected to determine the pH of the treated banana fruits.

4) Peel Colour:

The peel colour was determined using the colorimeter (Konica Minolta CR 400, Japan). Three measurements from each sample were taken at the distal end and, the middle part the stem end. L*, a*and b* values were recorded.

5) Chlorophyll Content:

The chlorophyll content of the peel of the ethephon treated *Embul* banana was determined. Banana peel (2.5 g) was scraped, and chlorophyll was extracted into an 85% acetone solution by placing the scraped peel on muslin cloth and extracting the juice using a mortar and pestle (Ranganna (1995).

The chlorophyll extract was volume up to 50 ml in volumetric flask in an 85% acetone solution. Chlorophyll content was measured by using a spectrophotometer (DR 6000, USA) at 660 nm and 642 nm wave lengths.

Total chlorophyll (mg/L) = (7.12 × OD at 660 nm) + (16.8 × OD at 642 nm)
Chlorophyll a (mg/L) = (9.93 × OD at 660 nm) + (0.777 × OD at 642 nm)
Chlorophyll b (mg/L) = (17.6 × OD at 642 nm) + (2.81 × OD at 660 nm)

6) Determination of Degree of Ripeness:

The time required for ripening and shelf life of ethephon treated *Embul* banana were observed using a developed ripening colour chart (Fig 2).

7) Sensory Evaluation:

The flesh and peel appearance, , taste, odour and overall acceptability of the ethephon treated *Embul* bananas were evaluated using a five-point hedonic scale; (1= dislike extremely, 2= dislike moderately, 3= neither like nor dislike, 4=like moderately, 5=like extremely) using 30 untrained panelists in 6 days after treatment.

C. Statistical Analysis

Completely Randomized Design (CRD) was used as a statistical design to observe significant differences in quality parameters between treatments by two factor factorials. All parametric data were analyzed using ANOVA in SAS package 9.0 with a confidence interval of 95%, and Tukey's method used for mean separation. Friedman in MINITAB 16 software with a confidence interval of 95% was used for analysis the results of sensory evaluation.

3. Results and Discussion

A. Total Soluble Solids (TSS)

The data on the Total Soluble Solids (TSS) content of *Embul* bananas during the storage period are given in Table 1. TSS was higher at the 2nd day of storage in fruits treated with 200 ppm of ethephon for 24 h followed by 200 ppm treated for 18 h samples significantly (p<0.05). The untreated (control) fruits recorded the significantly (p<0.05) lowest TSS values during the storage periods compared to ethephon treated samples. It was observed the significant increase in TSS content in all treated samples as well as the untreated sample (control) during storage period, except 200 ppm treated for a 24 h and 18 h. The results revealed that TSS of 200 ppm treated for 24 h sample and 200 ppm treated for an 18 h sample found to be increased to the 4th day of storage. With the advancement of ripening, the substantial utilization of sugars and hence reduced the TSS, and the results were confirmed by the findings of Singh, (2012). The increase in TSS during storage may be due to the hydrolysis of starch and other polysaccharides in to soluble forms of sugar. Similar results were observed in mango, papaya, tomato like fruits, and increased in TSS during storage at ambient and low temperatures (Chandra *et al*., 2017).

B. Firmness

Changes of fruit firmness of *Embul* banana were given in Table 2. It is evident from the data that the fruit firmness showed a decreasing trend during storage period. The untreated (control) fruits showed a, significantly (p<0.05) higher mean fruit firmness value during the storage period followed by 100 ppm treatment and exposure for a 12-hour (h) period. Fruits treated with 200 ppm for 24 h and 200 ppm for 18 h showed significantly (p<0.05) lower firmness values on the 2nd day of storage compared to all other treatments. On the other hand, the fruits treated with 200 ppm ethephon concentration for 24 h and 18 h experienced a faster loss of firmness during storage. The decrease in the firmness of fruits may be due to the thinning of the cell walls or cellular disintegration, which leads to membrane permeability. Loss of turgor, degradation of starch, and enzyme catalyzed changes in wall structure lead to fruit softening (Maduwanthi and Marapana, 2017). Anbarasan and Tamilmani (2013) reported that fruit firmness gradually decreased from the initial stage to the final stage of ripening.

C. pH

Table 3 reported the data on the pH of the *Embul* banana during the storage period. Two days after treatments the pH values of 200 ppm treated for 24 h and 200 ppm treated for 18 h were significantly (p<0.05) when compared to the control. It was further observed that at pH values of the control sample and 100 ppm treated for 12 h, 18 h, and 24 h were significantly the same in 2 days and 4 days after treatment. The control sample was recorded the significantly (p<0.05)

highest pH value after 6 days. According to the findings of Nura *et al*. (2018), the pH value of calcium carbide treated banana fruits also decreased with increasing calcium carbide concentration. The same findings were mentioned in Sapota fruit (*Manilkara zapota*) with an increase in ethylene concentration (Vidhya *et al*., 2017). The pH of dwarf Cavendish bananas decreased from 5.4 to about 4.5 during ripening (Ward and Nussinovitch ,1996)

Table 1

Changes in Total Soluble Solids (TSS) of banana during storage (30ºC, 85%RH)
Initial value at 0 day: 5.90^{a} ± 0.77

Treatment	Time (Days After Treatment - DAT)		
	2 DAT	4 DAT	6 DAT
Control	9.32^{d} ± 0.97	9.83^{d} ± 1.27	17.73^{g} ± 2.75
100 ppm for 12 h	10.18^{d} ± 0.56	10.12^{d} ± 0.60	19.97^{fg} ± 0.48
100 ppm for 18 h	9.78^{d} ± 0.36	9.80^{d} ± 0.36	21.47^{ef} ± 0.35
100 ppm for 24 h	9.87^{d}± 0.14	12.65^{cd} ± 3.90	26.65^{abc}±0.28
150 ppm for 12 h	10.38^{d} ± 1.37	18.27^{bc} ± 4.61	23.22^{de} ± 0.95
150 ppm for 18 h	9.38^{d} ± 0.63	28.05^{a} ± 0.28	28.08^{ab} ± 0.64
150 ppm for 24 h	10.02^{d} ± 0.08	27.80^{a} ± 0.25	28.62^{a} ± 0.10
200 ppm for 12 h	13.97^{c} ± 1.59	23.56^{ab} ± 0.17	25.00^{cd} ± 0.00
200 ppm for 18 h	21.73^{b} ± 1.32	25.10^{a} ± 0.65	25.03^{cd} ± 0.23
200 ppm for 24 h	24.80^{a} ± 0.44	25.73^{a} ± 0.61	25.63^{bcd}±0.81

Data presented as Mean ± SD
Means within the same column with different superscripts are significantly different (p<0.05).

Table 2

Changes in fruit firmness of banana during storage (30ºC, 85%RH)

Treatment	Time (Days After Treatment - DAT)		
	2 DAT	4 DAT	6 DAT
Control	38.47^{ab} ± 4.17	37.32^{a} ± 1.14	16.58^{a} ± 0.93
100 ppm for 12 h	37.11^{ab} ± 2.25	35.36^{a} ± 4.86	14.38^{ab} ± 2.98
100 ppm for 18 h	38.47^{ab} ± 2.10	33.09^{a} ± 0.98	12.28^{b} ± 1.12
100 ppm for 24 h	38.77^{a} ± 2.79	26.62^{a} ± 10.91	6.79^{cd} ± 0.64
150 ppm for 12 h	38.07^{ab} ± 2.59	24.87^{ab} ± 15.70	7.43^{c} ± 1.14
150 ppm for 18 h	37.17^{ab} ± 2.70	16.44^{c} ± 0.77	7.48^{cd} ± 0.41
150 ppm for 24 h	36.83^{ab}± 14.77	12.93^{c} ± 0.61	6.03^{d} ± 0.67
200 ppm for 12 h	22.70^{bc} ± 5.19	8.18^{bc} ± 1.67	8.18^{bc} ± 1.67
200 ppm for 18 h	12.43^{c} ± 3.06	7.76^{c} ± 0.71	4.73^{cd} ± 0.41
200 ppm for 24 h	7.39^{c} ± 0.73	5.68^{c} ± 0.07	3.98^{cd} ± 0.32

Table 3

Changes in pH of ambul banana during storage (30ºC, 85%RH)

Treatment	Time (Days After Treatment - DAT)		
	2 DAT	4 DAT	6 DAT
Control	5.33^{a} ± 0.03	5.49^{a} ± 0.58	5.20^{a} ± 0.07
100 ppm for 12 h	5.33^{a} ± 0.04	5.49^{a} ± 0.03	4.88^{b} ± 0.02
100 ppm for 18 h	5.33^{a} ± 0.33	5.46^{a} ± 0.05	4.76^{b} ± 0.01
100 ppm for 24 h	5.37^{a} ± 0.01	5.23^{ab} ± 0.21	4.78^{b} ± 0.04
150 ppm for 12 h	4.92^{b} ± 0.14	4.76^{bc} ± 0.28	4.20^{e} ± 0.04
150 ppm for 18 h	5.10^{ab} ± 0.07	4.38^{c} ± 0.04	4.31^{de} ± 0.07

150 ppm for 24 h	$4.94^{b} \pm 0.15$	$4.41^{c} \pm 0.12$	$4.30^{de} \pm 0.02$
200 ppm for 12 h	$4.45^{c} \pm 0.14$	$4.28^{c} \pm 0.02$	$4.42^{cd} \pm 0.01$
200 ppm for 18 h	$4.08^{d} \pm 0.26$	$4.29^{c} \pm 0.10$	$4.53^{c} \pm 0.06$
200 ppm for 24 h	$4.14^{cd} \pm 0.03$	$4.44^{c} \pm 0.06$	$4.48^{c} \pm 0.06$

Initial value at 0 day:$5.43^{a} \pm 0.31$
Data presented as Mean ± SD
Means within the same column with different superscripts are significantly different (p<0.05).

Table 4
Changes in Total Chlorophyll content of the ambul banana during storage (30ºC, 85%RH)

Treatment	Time (Days After Treatment - DAT)		
	2 DAT	4 DAT	6 DAT
Control	$8.24^{a} \pm 0.00$	$7.50^{a} \pm 0.02$	$5.62^{a} \pm 0.02$
100 ppm for 12 h	$7.48^{b} \pm 0.02$	$7.01^{b} \pm 0.06$	$3.33^{b} \pm 0.00$
100 ppm for 18 h	$7.50^{b} \pm 0.02$	$3.33^{d} \pm 0.00$	$1.70^{c} \pm 0.06$
100 ppm for 24 h	$7.01^{c} \pm 0.06$	$1.70^{e} \pm 0.06$	$0.97^{d} \pm 0.06$
150 ppm for 12 h	$5.13^{d} \pm 0.02$	$3.68^{c} \pm 0.01$	$0.97^{d} \pm 0.01$
150 ppm for 18 h	$4.54^{e} \pm 0.00$	$1.62^{e} \pm 0.00$	$0.86^{de} \pm 0.00$
150 ppm for 24 h	$3.99^{f} \pm 0.00$	$1.62^{e} \pm 0.00$	$0.79^{ef} \pm 0.02$
200 ppm for 12 h	$4.54^{e} \pm 0.00$	$1.62^{e} \pm 0.00$	$0.68^{fg} \pm 0.08$
200 ppm for 18 h	$3.68^{f} \pm 0.01$	$1.62^{e} \pm 0.00$	$0.69^{f} \pm 0.08$
200 ppm for 24 h	$4.09^{f} \pm 0.46$	$1.01^{f} \pm 0.00$	$0.56^{g} \pm 0.03$

Initial value at 0 day: $8.43^{a} \pm 0.39$
Data presented as Mean ± SD
Means within the same column with different superscripts are significantly different (p<0.05).

D. ***Chlorophyll Content***

The total chlorophyll content of the banana is presented in Table 4. Significantly (p<0.05), highest total chlorophyll content was recorded in the control sample throughout the storage period ts. The results indicated that the control sample showed slower ripening when compared to treated samples. The most important compounds responsible for the change in peel colour were chlorophyll and carotenoids. The stability of chlorophyll depends on temperature, pH, surface-active ions enzymes, salts, and (Nisha *et al*., 2004). The chlorophyll content of banana peel decreased and became absent in ripe fruits (Maduwanthi and Marapana, 2017).

E. ***Colour***

Colour development of fruit is an important feature during ripening that is used in determining the quality of fruits for marketing. The peel colour is also being used to characterize the state of ripeness of banana fruit. Hence, it is important to quantify the yellow colour development during ripening. Generally, the colour conversion was from dark green to pale green and then a slight yellow colour from the unripe to the ripe stage. The colour of the banana peel changed from green to yellow. Peel lightness (L*) value, greenness (a*) value, and yellowness (b*) value were useful
approaches to determining of the development of the peel colour of bananas during ripening (Shahir and Visvanathan, 2014).

The data on the colour L* value was graphically presented in figure 1 (a). Fruits treated with 200 ppm for 18h and 24h samples had significantly (p<0.05) higher L* values 2 days after

treatment compared to other treatments. L* value of the control sample was comparatively lowerduring

storage. Peel lightness was indicated by the L* value, which ranged from 0 to 100, where 0 indicates black colour and 100 indicate white colour. The L* value tends to be increased during ripening due to colour conversion from dark green to yellow.

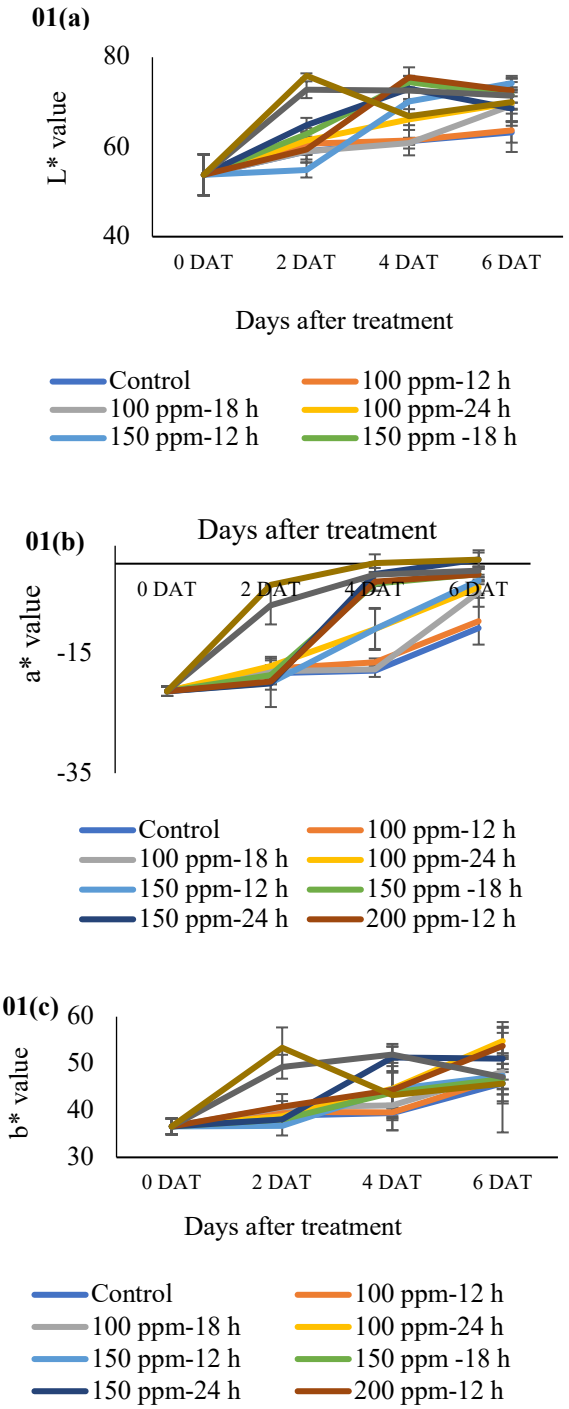

Figure 1. *Changes of (a) L*, (b) a*, (c) b* values of Embul banana during storage (30℃, 5% RH)*

The L* value of 200 ppm treated for 18h and 24h samples was found to be increased up to 2 days after treatment and then started to decrease. The reason explained by Shahir and Visvanathan, 2014 that, development of brown colour patches over the peel which leads to reduction of L* value. The data on colour a* during the storage period was graphically presented in figure 1 (b). The initial a* value was reported as -21.32 ± 0.78 and increased during the storage. Banana fruits treated with 200 ppm ethephon for 18 h and 24 h showed the highest a* value in 2 days after treatment significantly (p<0.05), compared to other treatments. Higher a* values in 6 days after treatment were recorded in control and 100 ppm treated for 12h samples. The colour a* value indicates the greenness to redness, where, negative values (-a*) indicate the greenness and positive values (+a*) indicate redness. The* value tends to be increased during ripening, which denotes the decreasing greenness due to the degradation of chlorophyll pigments. The greenness value of banana fruit peel increased from the negative side to the positive side. This confirms the de-greening of banana peel. The data on the colour b* value during storage period was graphically presented in figure 1 (c). The initial b* value was recorded as 36.60 ± 1.68 in all treatments and found to be increased during the storage period. A similar pattern was observed in the 200 ppm treated for 18h. The colour b* value indicate the yellowness to blueness. The b* value tend to be increase during ripening due to the de-greening process.

F. Time Required for Ripening and Shelf Life

Figure 2. *Ripening colour chart of "Embul" banana*

1- green, 2- green with trace of yellow, 3- green and yellow both occur, 4- yellow with trace of green, 5- green tip, 6- completely yellow colour, 7- clear yellow colour with signs of dark marking.

The bananas treated with 200 ppm ethephon for 18 and 24 h showed a faster rate of ripening compared to other treatments. 100 ppm ethephon treated for 12, 18, and 24 h showed slower ripening rates and the same was observed in control. The marketable ripening stage was identified using a developed ripening chart of the *Embul* banana (Figure 2). Bananas treated with 200 ppm ethephon treated for 18 h were able to extend the shelf life for 4 days after ripening, while samples treated with 200 ppm for 24 h were able to extend a shelf life of 3 days. The marketable ripening stage was identified using the developed ripening chart, where 75% of fruits reached ripeness at the 5th stage (Figure 2). Results revealed that 200 ppm treated for 24 h showed better marketability in 2 days after treatment up to 6 days. 100 ppm treated for 12, 18, and 24 h with control were taken for 5 days to reach optimum marketability, which exhibited a slow rate of ripening. 150 ppm ethephon concentration treated for 12, 18 and 24 h showed faster ripening than control, and 100 ppm treated samples exhibited slower ripening rate than 200 ppm treated samples.

G. Sensory Evaluation

There was a significant (p<0.05) difference in taste, flesh colour, peel colour, odour, and overall acceptability of bananas treated with different ethephon dosages (figure 3). The highest overall acceptability was recorded by a 200 ppm ethephon treated sample for 18 hours. and the lowest

overall acceptability was recorded in the control. The highest acceptance for peel appearance and flesh appearance was recorded by 200 ppm ethephon treated sample for 18 hours. Significantly lower acceptance for peel appearance, flesh appearance, and odour was recorded by the control. Compared with estimated medians of treatments, there were significant differences (p<0.05) for flesh appearance, taste, peel appearance, odour, and overall acceptability of *Embul* bananas which treated with different ethephon dosages. The results were confermation with the findings of Vidhya *et al*. (2017), reported the highest overall acceptability was recorded in the sapota fruits treated with 100 ppm ethylene exposed for 24h rather than 150 ppm ethylene treated samples Gunasekara *et al*. (2015), reported the low sensory scores and low levels of nutritional qualities in *Embul* bananas treated with calcium carbide and ethephon while naturally ripened fruits recorded highest sensory acceptance. Although ethephon treated fruits recorded higher consumer acceptance than calcium carbide treated fruits (Gunasekara *et al*., 2015).

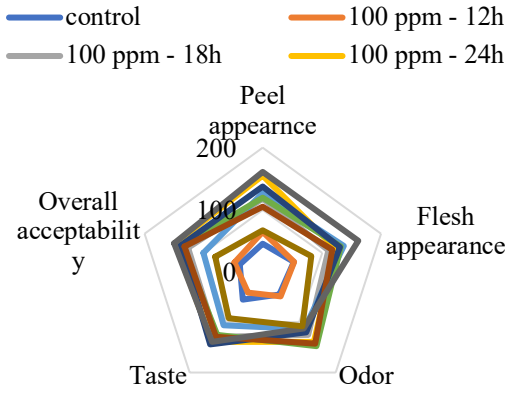

Figure 3: *Sensory properties of ethephon treated Embul banana*

4. Conclusions

The rapid ripening was shown by the banana fruits treated with 200 ppm ethephon for 18 and 24 hours, and the fruit treated with 200 ppm ethephon concentration for 18 hours showed higher consumer acceptance, as most of the other quality attributes, such as fruit firmness, TSS, and sensory properties, were preserved. Therefore, 200 ppm ethephon exposure for 18 hours could be recommended as a more effective dosage for the acceleration of the ripening process of *Embul* bananas by maintaining the shelf life of 6 days.

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