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RESEARCH THE INFLUENCE OF ETHEPHON ON THE RIPE AMBARELLA (*SPONDIAS DULCIS* L.)

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Abstract
These days, there are many types of chemicals which have ability to hasten the ripe fruit and improve the quality of fruits like ethylene, calcium carbide, ethephon (ethrel). The goal of this research determines the influence of ethephon (2-chloroethyl phosphonic acid) on the ripe ambarella (Spondias dulcis L.). Ambarella was a tropical fruit originating in the South Pacific region, made a healthy addition to diet, helping meet daily fiber, iron and vitamin C needs. The samples were soaked into ethephon solution at concentrations of 0, 0.3, 0.6, 0.9 and 1.2 % (v/v), then preserved in plastic basket, covered with cloth, temperature 30±2.3 ^{o}C, humidity 71±11.37 % (room condition). The use of specifications for evaluating were the percentage of ripe fruits, weight loss, the content of reducing sugar, total acidity and sensory evaluation of ambarella. The results showed that fruits soaked into concentration of 0.9 % (v/v) ethephon have stimulated fruits that were the quick and uniform maturity. Consumers are not unpleasant with the sensory evaluation quality of fruits soaked with ethephon. Currently, in Vietnam, we have not a regulation for allowing the maximum content of ethephon in ambarella. We hope that the Ministry of public Health will issue and adding the maximum amount permitted ethephon residues in ambarella. So, the ripe ambarella with ethephon is applied widely.

Keywords: 2-chloroethyl phosphonic acid, fruit, ethephon, glucose, preservation.

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1. INTRODUCTION

Ambarella (*Spondias dulcis* L.) was cultivated widely in the South Vietnam. It has an oval-shaped or an egg-shaped, contain a lot of water. The dimension and quantity of fruit is not equal, the length of fruit is of 6-10 cm, and diameter of fruit is of 4-8 cm, the peduncle match to pip through the fruit's pulp, 2-10 fruits/bunch. Peel is not really smooth, thick and green with brown tiny spots. It is an acidic fruit with a crunchy texture that tastes like a cross between a mango and pineapple. They can eat the fruit fresh or use it to produce jellies and juice processing (Hau V.C., 1996). Mature fruits produce a tiny amount ethylene, not as high as the ripe fruit by ethephon. So that it does not make uniform maturation in the batch. At the present, ethephon has been interesting in many fields, it is known as a substance which regulates the growth of plants, is able to release ethylene, stimulating the defoliation, making fruits ripe in order to help fruits ripe uniform, decreasing preservation time and reducing post harvest losses (Con P.V., 2005*)*. In recent times, there have been many opposite opinions about toxicity of ethephon that make worriment to consumers (Thach N.Q. *et al.,* 1999*),* for instance ethephon is irritant to the skin or the eyes but is not a skin sensitizer, it was not a carcinogen and is classified by IARC (International Agency for Research on Cancer) as group D (not carcinogenic to humans) and FAO pointed out a maximum allowable daily intake for ethephon at 0.05 mg/kg body weight/day (Quang B.Q., 2007). Using ethephon in Vietnam is quite new and an unpromulgated law about the impact of chemicals residues on ripening ambarella. Thus, the purpose of this research is to survey the ripening process of ambarella by ethephon.

2. MATERIALS AND METHODS

Materials.

Ambarella (*Spondias dulcis* L.) is harvested in An Giang province, Vietnam. It was harvested after 6 months since the three bore fruit. Harvest fruits are 6-10 cm in length, 4-8 cm in diameter and with weight 105-110 g/fruit.

Ethephon are liquid form, light yellow, transparent and from China.

Methods

Ripe ambarella processing

Ambarella → Shorting → Washing → Soaking → Keeping dry → Preserving

Ambarella is kept in plastic baskets covered with canvas at storage temperature 30±2.3 ^{o}C, humidity 71 ± 11.37 %

Analysis methods

The percentage (%) of weight loss was used by electronic scales; the mass loss rate based on the volume of the preservation process results over the volumes initially.

The glucose examination was determined by a glucometer Cleverchek (Germany).

$$\% \text{ Glucose} = x * 10^{-3} * 180 * \frac{V}{1000 * m} * 100$$

x: The concentration of glucose displays on the glucometer (mmol/L); 180: Molecular mass of glucose; m: Mass of fruit (g); V: The dilution of solution (ml)

The total acidity value was determined by (AOAC 942.15, 2007). Titration acidity was performed by NaOH 0.1 N with phenolphthalein 0.1% as an indicator and expressed in grams (g) of total acidity per 100g of fruit.

Residue examination of ethephon on ambarella was determined by gas chromatography-mass spectrophotometer (GC–MS) (AOAC 2007.01, 2007). It was expressed in milligrams (mg) of ethephon per 1 kg of fruit.

Different testing on sensory evaluation: pair comparison test – the simple difference test (or the same/different test) (Lawless *et al.*, 2010).

Data analysis. Each sample of ambarella had weight of 2.1-2.2 kg, corresponding to 20 fruits; repeated 3 times. Data would be analyzed by Statgraphics software (Centurion XV) with confidence interval p_{value}=0.05.

3. RESULTS AND DISCUSSION

Effect of ethephon concentration solution on ambarella maturity and weight loss versus preservation time

In table 1, the ripen rate of ambarella in the different time are increase due to the time of ripening.

In which, the samples soaked in chemical with the high concentration has a ripen rate higher and quicker than the samples that soaked in chemical with the low concentration or without soaking. At the time of 80 hours, sample in 0.9 % of ethephon solution reached the highest ripening rate (93.3 %).

Ambarella at these concentrations ripened quite equally, has a specific aroma and yellow peel. It shows that ethephon released to create ethylene so as to stimulate fruit ripe, amount of ethylene was released more and more will help fruit ripe quickly (Phuc T.H., 2000). The ripenrate of fruits in the different concentration are increase due to the time of soaking. The samples soaked in chemical with the high concentration has a ripen rate with a quick speed appropriately which compares to the samples soaked in chemical with the low concentration or without soaking, at the time of 100 hours, the sample that did not soak in chemical achieve the highest rate of ripen fruit is 70%. The ethephon treatment samples achieved the highest ripen rate with preservation time from 70 to 90 hour. After that, all samples decreased due to the appearance of damages

During post harvest ripening, the weight loss of fruits increased steady over the preservation time (Table 2), the respiration of vegetable or fruit produced carbon dioxide, water, it created the high energy… and consumed amount of glucose and oxygen (aerobic respiration process).

Beside, ambarella had a high water content 59.65 - 85.47 %, thus the evaporation process was also the main reason to decrease the natural weight of fruit and vegetable (Nguyet T.N.M. *et al.,* 2008; Thuyet H.V. *et al.,* 2000). At first, the weight loss change insignificantly, it is slowly about 0-2 %.

Starting at 30 hours, the weight loss raises nearly twice beside to the period of 20 hours. In the term from 30 to 140 hours, all samples had the same weight loss but it increases slowly and steady.

Table 1. Percentage of ripe ambarella (%) versus the preservation time

Hours	Conc. (%,v/v)				
	0	**0.3**	**0.6**	**0.9**	**1.2**
0	-	-	-	-	-
10	-	-	-	-	-
20	-	-	-	-	-
30	-	1.7±2.9 Aa	-	1.70±2.9 Aa	-
40	3.3±5.8 Aa	13.3±11.5 ABb	23.3±7.6 Bb	21.7±8.7 Bb	21.7±6.0 Bb
50	3.3±5.8 Aa	40.0±5.0 Bd	50.0±5.0 Bc	43.3±10.4 Bc	43.3±8.0 Bc
60	6.7±7.6 Aa	45.0±5.0 Bd	73.3±2.9 Cd	68.3±7.6 Ce	70.0±5.0 Cd
70	35.0±5.0 Ab	86.7±2.9 Bf	90.0±8.0 Be	90.0±10.0 Bf	86.7±10.0 Be
80	48.3±7.6 Ac	83.3±11.5 Bf	91.7±7.6 Be	93.3±5.8 Bf	86.7±19.0 Be
90	50.0±5.0 Aa	85.0±13.2 Bbc	90.0±7.6 Ba	90.0±2.9 Ba	90.0±2.9 Ba
100	71.7±7.6 Ad	66.7±7.6 Ae	80.0±10.0 Ade	83.3±7.6 Af	73.3±5.8 Ad
110	71.7±10.4 Ad	46.7±5.8 Ad	60.0±20.0 Ac	55.0±13.2 Ad	45.0±5.0 Ac
120	46.7±11.5 Ac	25.0±8.7 Ac	33.3±7.6 Ab	31.7±10.4 Ab	20.0±8.7 Ab
130	75.0±5.0 Ad	9.7±7.6 Bf	10.0±0.0 Be	11.7±5.8 Bf	10.0±2.9 Be
140	6.7±2.9 Aa	1.7±0.0 Ba	1.7±0.0 Ba	-	-

Various capital letters in the same row are significant difference at the level of p=5%.
Various lowercase letters in the same column are significant difference at the level of p=5%.

Table 2. Weight loss (%) according to storage time

Hours	Conc. (%, v/v)				
	0	**0.3**	**0.6**	**0.9**	**1.2**
0	0.0±0.0 Aa	0.0±0.0 Aa	0.0±0.0 Aa	0.0±0.0 Aa	0.0±0.0 Aa
10	2.05±0.28 Bb	2.14±0.28 Bb	1.48±0.06 Ab	1.59±0.13 Ab	1.3±0.31 Ab
20	2.27±0.24 Ab	2.29±0.25 Ab	2.03±0.17 Ab	2.02±0.12 Ab	2.22±0.28 Ac
30	3.67±0.19 Ac	3.38±0.79 Ac	3.41±0.53 Ac	3.19±0.28 Ac	3.25±0.13 Ad
40	4.73±0.46 Ad	4.48±0.49 Ad	4.3±0.47 Acd	4.15±0.28 Ad	4.05±0.24 Ae
50	5.54±0.47 Ad	5.02±0.37 Ade	4.7±0.53 Ade	4.94±0.28 Ae	4.92±0.28 Af
60	6.08±0.51 Ae	5.91±0.50 Aef	5.63±0.56 Aef	5.58±0.14 Af	5.55±0.27 Ag
70	6.91±0.22 Aef	6.91±0.32 Afg	6.52±0.64 Afg	6.38±0.28 Ag	6.35±0.55 Ah
80	7.41±0.24 Afg	7.38±0.43 Ag	6.84±0.78 Afg	7.1±0.61 Ah	6.98±0.27 Ai

90	8.04±0.46 Ag	7.91±0.47 Ag	7.73±0.84 Agh	7.34±0.28 Ah	7.62±0.48 Aj
100	8.91±0.34 Ah	7.65±0.52 Ag	8.53±0.56 Ahi	8.29±0.28 Ai	8.57±0.48 Ak
110	9.62±0.5 Ai	9.88±0.47 Ah	9.18±0.83 Aij	9.25±0.28 Aj	9.52±0.48 Al
120	10.57±0.49 Aj	10.71±0.27 Ahi	10.14±0.84 Ajk	10.21±0.28 Ak	10.32±0.28 Am
130	11.04±0.49 Aj	11.53±0.25 Aij	11.27±1.11 Akl	11.32±0.28 Al	11.27±0.72 An
140	11.99±0.49 Ak	12.56±0.31 Ajk	12.0±1.33 Alm	12.12±0.28 Am	12.22±0.28 Ao

Various capital letters in the same row are significant difference at the level of p=5%.
Various lowercase letters in the same column are significant difference at the level of p=5%.

Table 3. Amount of glucose (%) of ambarella versus the preservation time.

Hours	**Conc. (%, v/v)**				
	0	**0.3**	**0.6**	**0.9**	**1.2**
0	2.53±0.01 Aa	2.53±0.02 Aa	2.53±0.02 Aa	2.53±0.01 Aa	2.53±0.03 Aa
10	3.41±0.17 CDde	3.57±0.05 Dde	2.4±0.35 Aa	2.54±0.41 ABa	2.99±0.39 BCb
20	2.74±0.03 Bab	2.93±0.02 Cbc	2.75±0.06 Bab	2.65±0.02 Aa	2.62±0.05 Aa
30	3.49±0.37 Cde	3.81±0.09 CDe	2.73±0.11 Aab	3.11±0.12 Bbc	3.86±0.09 Dcde
40	3.35±0.06 Acd	3.69±0.26 Bde	3.51±0.21 ABde	3.5±0.06 ABcd	4.71±0.08 Ch
50	4.4±0.20 BCghi	3.79±0.21 Be	3.04±0.02 Abc	4.24±0.27 Cef	4.68±0.19 Dh
60	4.19±0.05 Bhi	5.16±0.12 Ch	3.4±0.49 Acd	5.59±0.14 Dh	5.0±0.07 Ch
70	4.33±0.15 Cij	4.13±0.15 Cf	3.75±0.07 Bde	3.35±0.08 Ac	4.18±0.22 Cefg
80	4.13±0.04 Bhi	4.65±0.07 Cg	4.19±0.26 Bf	4.38±0.14 BCf	3.83±0.17 Acd
90	3.86±0.10 Afgh	4.49±0.46 Ag	4.56±0.19 Af	4.82±0.68 Ag	4.26±0.07 Afg
100	3.95±0.38 Afgh	4.79±0.27 Bg	5.01±0.22 Bg	4.45±0.36 ABfg	4.12±0.40 Adefg
110	3.74±0.18 Cefg	2.75±0.10 Aab	3.37±0.39 Bcd	2.8±0.07 Aab	3.03±0.10 ABb
120	3.64±0.07 Aedef	3.47±0.23 Ad	3.8±0.22 ABe	4.14±0.21 BCef	4.34±0.25 Cg
130	3.03±0.44 Abc	2.62±0.02 Aab	3.71±0.06 Bde	2.61±0.12 Aa	3.58±0.28 Bc
140	4.67±0.21 Cj	3.08±0.15 Ac	4.48±0.18 Cf	3.83±0.17 Bde	3.93±0.01 Bdef

Various capital letters in the same row are significant difference at the level of p=5%.
Various lowercase letters in the same column are significant difference at the level of p=5%.

Effect of ethephon concentration solution on reducing sugar and total acidity versus in preservation time

In general, content of glucose increased during preservation time due to the starch metabolized into glucose that created a big energy and a part of sugar accumulated inside ambarella, it was the main reason that makes to increase the reducing sugar. Afterwards, when the fruit became the maturity, glucose was used in the aerobic respiration process. Therefore, content of sugar declined in storage time (Peter G. *et*

al., 2002; Tan L.V. *et al.,* 2008). Content of sugar increased slowly from 2.5 % to 5.6 % during 0 to 60 hours. It achieved a peak 5.6 % in 0.9 % (v/v) ethephon solution (Table 3). After this period, content of sugar reduced and fluctuated according to storage time. At this time, the ripening fruit rate started to raise, appeared damages and the ferment process occurred at same time. For this reason, the content of sugar change significantly at p_{value}=0.05.

The total acidity value in ambarella to fluctuated and decreased during storing time. The total acidity was main substance for respiration process, gained the upper hand over other substances and could decrease up to 50% during the existing time of fruits because there was the osmosis of acid through the cell membrane and it created a lot of soluble substance such as salt, sugar… (Tan L.V. *et al.,* 2008). From 70 to 140 hour, the sample without ethephon treatment was had the significant difference with non ethephon treatment samples almost time (Table 4). The internal enzyme systems of fruit along with the development of microbial fermentation takes place strongly, combined with the aerobic respiration and under the effect of ethylene from ethephon, the fruit will ripen quickly (Nguyet T.N.M. *et al.,* 2008). During the research, the sample with ethephon treatment 0.9 % (v/v) has a high rate of ripen fruit, high reducing sugar content, low weight loss and fruit ripe uniform and has a good taste in the preservation time about 70 – 80 hours.

Table 4. Variation of the total acid content (g/l) over time preserved

Hours	Conc. (%, v/v)				
	0	0.3	0.6	0.9	1.2
0	6.105±0.065 Ade	6.068±0.128 Ae	5.993±0.064 Acd	6.105±0.065 Acd	6.105±0.065 Ae
10	6.477±0.112 Cf	7.147±0.112 Dg	6.105±0.065 Bcd	7.407±0.129 Ef	5.583±0.00 Acd
20	5.285±0.065 Aa	5.470±0.00 ABab	5.508±0.171 Bb	6.514±0.064 De	5.769±0.129 Cde
30	5.620±0.065 Ac	5.620±0.065 Abc	5.434±0.359 Ab	5.211±0.065 Ab	5.211±0.171 Ac
40	5.955±0.065 Ad	5.993±0.129 Ade	5.918±0.193 Ac	6.030±0.335 Ac	8.076±0.233 Bh
50	5.360±0.112 Aab	5.285±0.129 Aa	5.06±0.129 Aa	5.473±0.297 Ab	5.248±0.224 Ac
60	6.253±0.112 Ae	6.588±0.295 Af	6.291±0.562 Ade	6.328±0.065 Ade	6.626±0.171 Af
70	6.700±0.00 Bg	6.961±0.129 Bg	6.775±0.065 Bfg	7.296±0.233 Cf	6.142±0.296 Ae
80	6.142±0.224 Bde	5.771±0.281 Acd	7.183±0.065 Ch	6.067±0.171 ABcd	7.072±0.065 Cg
90	7.593±0.00 Di	6.142±0.00 Be	6.923±0.224 Cgh	6.105±0.065 Bcd	5.846±0.062 Ade
100	7.370±0.00 Bh	6.514±0.171 Af	6.551±0.258 Aef	6.588±0.193 Ae	6.812±0.112 Afg
110	5.173±0.065 Aa	5.211±0.065 Aa	4.839±0.064 Aa	6.514±0.171 Ce	5.844±0.718 Bde
120	6.030±0.112 Dd	5.734±0.131 Cbcd	6.067±0.065 Dcd	5.211±0.065 Bb	4.315±0.125 Ab
130	6.143±0.293 Cde	5.211±0.341 Ba	4.988±0.065 Ba	4.839±0.233 Ba	3.793±0.225 Aa
140	5.509±0.233 Bbc	5.918±0.193 Cde	4.950±0.17 Aa	5.394±0.174 Bb	6.849±0.171 Dfg

Various capital letters in the same row are significant difference at the level of p=5%.
Various lowercase letters in the same column are significant difference at the level of p=5%.

Testing the composition of ambarella

In table 5, content of glucid decrease because there are the hydrolysis starch into glucose and content of protein increased slightly. Vitamin C declined extremely, over 40 %; it was oxidized and become dehydroascorbic acid under the effect of temperature and light. (Tan L.V. *et al.,* 2008). After the preservation time about 80 hours, the ethephon residue in ambarella was 5.7 mg/kg. The concentration of ethephon residues in fruits will decrease over preservation time because ethephon could be hygroscopic and released ethylene (Phuc T.H., 2000). At the present in Vietnam, there are not any regulations for allowing the maximum content of ethephon in ambarella. Consequently, we had to rely on the standard of FAO, it pointed out a maximum allowable daily intake (ADI) for ethephon at 0.05 mg/kg body weight/day (Quang B.Q., 2007).

Table 5: The composition of ambarella

Compound	Unripe ambarella	Ripe ambarella*
Glucide (%)	13.4	9.42
Protein (g/100g)	0.88(Nx6.25)	1(Nx6.25)
Ethephon (mg/kg)	0	5.7
Vitamin C (mg/kg)	153.24	89.77

(*): Sample with ethephon treatment 0.9 %, after 80 hours

Fig 1. Ambarella before soaking in ethephon solution

Fig 2. Ambarella after soaking in 0.9 % ethephon solution

Sensory evaluation of ambarella after soaking in ethephon solution

Using 24 participants who have evaluated the similarities and differences of the samples of ambarella with ethephon 0.9 %, preservation time 80 hours and ambarella which was bought in the local market and have the similar ripen

Table 6: Responding summary of participants

Sample order	Quantity of samples	Response	
		Similar	Different
AA	6	4	2
AB	6	5	1
BB	6	2	4
BA	6	4	2

A: samples soaked into ethephon solution
B: samples did not soaked into ethephon solution (sample from local market)
$\chi^2 = 1.6$; $\chi^2_{cri} = 2.71$
$\chi^2 < \chi^2_{cri}$, consumers can not recognize the difference between the soaked samples and unsoaked samples into ethephon solution. In other words, two samples (A and B) are similar at the level of p=5%.

4. CONCLUSIONS

The concentration of ethephon at 0.9 % (v/v) can stimulated to ripe ambarella in about 70–80 hours. The ripe fruit was uniform, the weight loss and total acidity changed insignificantly, high content of reducing sugar and the rate of ripe fruits get more over 90%.
Consumers can not recognize the difference between soaked samples and unsoaked samples into the ethephon solution. The soaking samples at 0.9 % (v/v) can make the fruit keeps uniform, yellow, glossy peel, characteristic odour and good flavour. The ethephon residues was 5.7 mg/kg after 80 hour preservation time, it can affect to the consumer's health. Then we propose the Vietnam government which defined the rules about ethephon residues on ambarella.

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