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Physio-Chemical Studies on the After-Ripeng of Hot Pepper Fruit

Part VII. Effects of Ethephon on the Major Compoments

by

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辛味種 고추의 追熟에 關한 生理化學的 硏究

제 7 보 主要成分에 미치는 Ethephon의 效果

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Abstract

The physiological and chemical investigations to explain the after-ripening processes in hot green pepper fruit were carried out by treating the fruit with ethephon either alone or with phenylalanine. The studied metabolic changes in fruit during after-ripening period was carbon dioxide and oxygen concentration in interior of the pepper fruit, total carotenoid, β-carotene, total sugar, and free reducing sugar in pepper fruit. These metabolic changes were explained inrelation to the color enhancement judged by the color score to explain the after-ripening processes.

Ethephon treatment at 500 ppm significantly accelerated color enhancement as compared to the control and further ethephon treatment increased the number by 20 per cent which was not possible in control during same after-ripening period.

The oxygen concentration in interior of the pepper fruit during after-ripening period was increased in control when the color score (color enhancement) increased rapidly. However, with ethephon treatment, the oxygen concentration was decreased when the color score increased.

Although total and free reducing sugar content were decreased during the after-ripening period, total carotenoid and β-carotene content was increased by 50 and 200 per cent, respectively, over control. Also the capsaicin contents was increased by 20 per cent by ethephon treatment over control. Although phenylalanine treatment did not affect the capsaicin content, capsaicin content tended to be increased by phenylalanine treatment when treated with ethephon.

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Introduction

Hot pepper is one of the most important vegetable in Korea for various food preparation, particularly for winter preparation. To evaluate the quality of the hot pepper fruit, various results have been reported by Lee, *et al.* on the physio-chmical and on the the major components changes during maturation [1-5] and after-ripening period [6-11].

Harvestig time of red hot pepper fruit in Korea overlaps with the seeding time of chinese cabbage and raddish. Further, due to the unexpected early frost dangers, farmers are forced to pick the fruit while they are still green. Therefore farmers should dry the unripe pepper fruit to obtain a red-ripe fruit. During this drying period, many fruits become rot and considerably large number remain green which is not suitable for sale and processes.

Ethephon [(2-chloroethyl) phosphonic acid] has been widely used in various horticultural crops [12-22] and particulariy in pepper, ethephon treatment can accelerate color enhancement during maturation[23-25].

These studies were initiated to observe the after-ripening effects of the green pepper fruit by ethephon, and phenylalanine as a precursor of capsaicin.

Materials and Methods

Uniform green pepper fruits after 30-35 days from flowering were harvested from Poonggag-Myun, Chungdo-Kun in 1974. Five hundred fruits in each tretment were soaked in ethephon, phenylalanine, or ethephon and phenylalanine solution (Table 1) for two minutes and then dried under the shade. Color changes were observed and scored at room temperature (20-25°C).

Table 1. Concentration of ethephon and phenylalanine in various treatments.[z]

	Concentration (ppm)	
	Ethephon(Eth)	Phenylalanine(Phe)
Control	0	0
Phe	0	200
Eth	500	0
Eth+Phe	500	200

z: Tricon at 0.1 per cent were used in all treatments as a wetting agent.

1. Color score determination: Changes in color according to the red-ripe degree was scored by the following 0-5 point scale and expreseed as a percentage (0:Completely green, 1:10 per cent red-ripe, 2:30 per cent red-ripe, 3:60 per cent red-ripe, 4:80 per cent red-ripe, and 5: Completely red-ripe).

2. Total carotenoid determinaton: Pigements in the pericarp were extracted to the methods by Curl [26-27] and total carotenoid content was determined by measuring the optical density at 475 mμ as capsanthin $\left(E^{1\%}_{1cm}=1,790\right)$ after petroleum ether elution to the methods by Davies[28].

3. β-Carotene determination: Sample prepared for total carotenoid was spotted in plate coated with Ca $(OH)_2$: silica gel G (6:1). Separation was by developing with petroleum ether: benzene (98:2) and then eluted with hexane according to the methods by Gross, *et al.*[29] and by Bolliger[30]. The concentration of β-carotene was calculated by measuring the optical density at 451mμ $\left(E^{1\%}_{1cm}=2,650\right)$.

4. CO_2 and O_2 determination in interior of the pepper fruit: The concentration of O_2 and CO_2 in interior of the pepper fruit was analyzed by Scholänder micro gas analyzer after collecting gas from the saturated salt solution containing finely cutted pepper fruit.

5. Total and free reducing sugar determination: Somogyi methods were used for free redncing sugar, and total sugar after hydrolysis with HCl.

6. Capsaicin Determination: To the methods by Kosuge[31], capsaicin was extracted with Soxhlet extraction apparatus by ether extraction. Ether partitioned fraction was diluted with CCl_4 and washed with CH_3COOH. This was again partitioned with NaOH containing NaCl and then capsaicin was measured by measuring optical density at 295 mμ.

Results and discussions

1. Changes in color enhancement: Ethephon has been widely used as a germination promotor and also

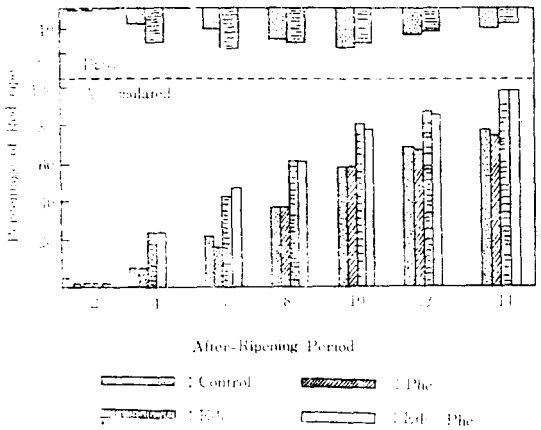

Fig. 1. Changes in Color Score in Each Treatment during After-Ripening Period.

as a growth retardant. Recently, ethephon is being used as a color enhancement promotor in apple, banana, and tomato.

In pepper fruit, color enhancement by ethephon application was successful when sprayed before harvest[21,25,32]. However, no reports have been released on the effect of ethephon treatment on the color changes to the fruits after harvest.

After the 14 days of after-ripening period, control and phenylalanine treated pepper showed 75 per cent of red-ripe fruit, and ethephon with phenylalanine treated peppers showed 95 per cent of red-ripe fruit. Since there was no phenylalanine effects on color changes, it is believed that ethephon is only effective compound for the enhancement in green pepper fruit (Figure 1).

In control, daily maximum increment of color enhancement, peak occurred 10 days after the treatment and ethephon treatment accelerated peak by 4 days as compared to the control. Hahn, *et al.*[24] reported the highest color enhancement effects when ethephon at 1,000ppm concentration was sprayed to fruit before harvest during maturation. Our results indicated 500 ppm concentration treatment during after-ripening period after harvest gave similar responses reported by Hahn, *et al.*[24].

2. **Changes in Total Carotenoid:** Total carotenoid content expressed as contents of capsanthin in pericarp at the 14th day of the after-ripening period are shown in Table 2.

Table 2. Total Carotenoid Content at the 14th Day of After-Ripening Period in Each Treatment (mg%-d.w.).

Fresh	Treatments			
	Control	Phe	Eth	Eth+Phe
7.54	48.20	43.35	78.50	73.50

Total carotenoid contents showed similar pattern discussed in the changes of color enhancement; ethephon treatment alone or in combination with phenylalanine showed higher content than control and phenylalanine treatment. Color enhancement effect expressed by the total carotenoid contents by ethephon and phenylalanine treatment could also be attributed by the ethephon effect, not the phenylalanine effect (Table 2).

3. **Changes in β-Carotene:** β-Carotene, which comprise more than 90 per cent of hydrocarbone compounds in the carotenoid compounds[2] is important in nutrition since it is a provitamin A. β-Carotene content was highest with ethephon treatment which showed similar trends observed in the total carotenoid (Table 3).

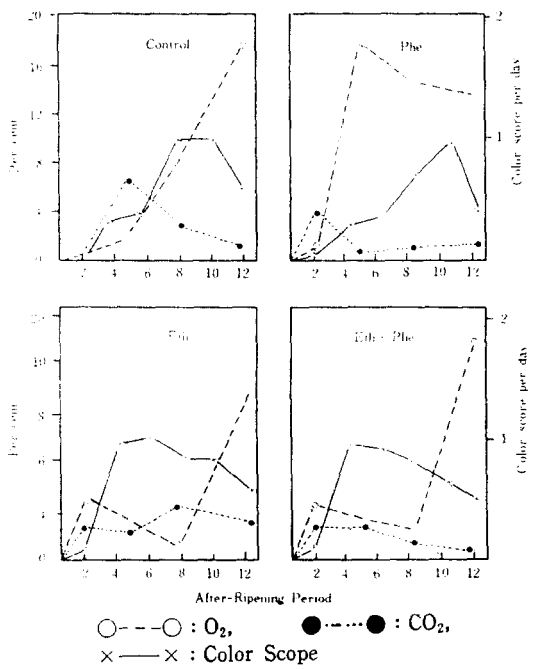

Fig. 2. Changes in CO_2 and O_2 level in Interior of the Pepper Fruit in Each Treatments during After-Ripening Period.

Table 3. β-Carotene Content at the 14th Day of After-Ripening Period in Each Treatment (mg%-d.w.).

Fresh	Treatments			
	Control	Phe	Eth	Eth+Phe
0.35	2.88	3.15	4.88	4.30

4. Changes of CO_2 and O_2 Concentration in Interior of the Pepper Fruit: It is well known that ethylene gas treatment accelerates after-ripening processes in apple and banana. Nowdays, ethylene, a by-product of oil refining, or ethephon, which releases ethylene, treatment are used commercially in lemon and orange for after-ripening[20,21]. However, ethylene treatment does not play any role in ripening processes if concentration of CO_2 and O_2 is very high in the atmosphere[33] and in lemon and orange, less than 1 per cent carbon dioxide concentration is required for after-ripening by ethylene treatment.

Changes of CO_2 and O_2 concentration in interior of the pepper fruit released with the color enhancement (color score) are shown in Figure 2. In control, CO_2 concentration reached to a maximum of 6.5 per cent at 5th day of after-ripening period and decreased thereafter. On the contrary, O_2 concentration rised rapidly at 5th day and so does the color score.

Although the gaseous composition between the environment surrounding the fruit and inside the interior of pepper fruit may be different, color enhancement may be rapid when the carbon dioxide concentration inside the interior of the pepper fruit reached to 2 to 3 per cent. The observed color score and the measured CO_2 and O_2 concentration relationships in control were similar in the phenylalanine treated fruit.

However, ethephon treatment either alone or with phenylalanine did not infuluence the CO_2 concentration level during the whole after-ripening period. On the contrary, oxygen concentration increased at the beginning of the after-ripening period and then decreased at which time the color score increased. Thereafter, O_2 concentration increased with the decreased color score. Therefore it is concluded that color enhancement is closely related with the gaseous composition inside the interior of the pepper fruit.

5. Changes in the Total Sugar and Free Reducing Sugar: At the 14th day of after-ripening period total sugar and free reducing sugar contents were investigated. Total sugar content fell from 28 per cent per dry weight to 16.35 per cent in control and to 12.62, 8.58, and 7.84g% by phenylalanine, ethephon, and ethephon with phenylalanine treatment, respectively (Table 4).

Table 4. Total Sugar(TS) and Free Reducing Sugar(RS) Content at the 14th Day of After-Ripening in Each Treatment(g%-d.w.).

Fresh	Treatments			
	Control	Phe	Eth	Eth+Phe
TS 28.00	16.35	12.62	8.58	7.84
RS 17.10	10.77	7.18	6.18	6.99

6. Changes in Capsaicin: Capsaicin, one of the major component in hot pepper fruit was indentified by Thresh[34] in 1876. Later, it has been considered by Leete and Louden[35] and Bennett and Kirby[36] that phenylalanine is a precursor for the vanillylamine

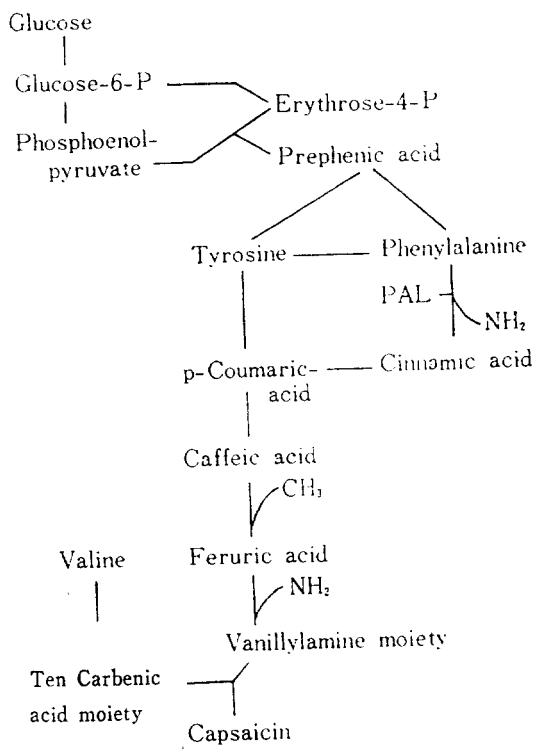

Fig. 3. Proposed Pathway of Capsaicin

moiety, which in turn forms capsaicin with ten carbenic acid moiety (Figure 3).

Byotou[37] reported that ethephon can activate phenylalanine ammonia lyase, which is a key enzyme for vanillylamine moicty fermation from phenylalanine. Therefore it will be interesting to study the effect of the ethephon and phenylalanine treatment on the capsaicin formation.

Table 5. Capsaicin content at the 14th day of after-ripening in each treatment (mg%-d.w.).

Fresh	Treatments			
	Control	Phe	Eth	Eth+Phe
88.2a[z]	96.5[b]	98.3[b]	115.2[c]	120.8[d]

z: Means with different letter are significantly different at 1 per cent level by Duncan's Multiple Range test.

The capsaicin contents in the control increased from 88.2mg at the beginning of the experiment to 96.5 mg at the 14th day of after-ripening. At this time capsaicin contents treated with phynylalanine, which is a substrate for capsaicin, treatment increased to 98.5 mg, which is not significantly different from that by control. However, ethephon treatment increased the contents to 115.3 mg, significantly higher than the control and the phenylalanine treatment. Further ethephon and phenylalanine treatment at the same time increased the capsaicin contents to 120.8 mg, which is significantly different from the control and the ethephon treatment (Table 5).

要 約

在來辛味種 綠熟 고추의 追熟에 따른 2-chloro ethyl phosphonic acid와 capsaicin生合成의 sub strate인 phenylalanine의 處理效果를 檢討코저 color score, 고추 空胞部의 O_2와 CO_2, 果皮部의 total carotenoid, β-carotene, 全糖, 遊離還元糖 및 capsaicin의 含量變化를 測定한 結果는 다음과 같다.

1. ethephon 500ppm의 處理는 control區에 比하여 현저한 着色促進의 效果를 볼 수 있었으며 특히 約 20%의 赤變不可의 고추를 赤變可能케 하였다.
2. 追熟期間中 고추 空胞部의 gas組成은 control區에서는 O_2의 含量이 增加하는 時期에 color score가 增加하였으며, 反對로 ethephon 處理區에서는 color score가 급증하는 時期에 O_2의 含量이 低下하였다.
3. 追熟에 따른 全糖 및 還元糖의 變化는 處理區 다같이 현저히 減少하였고, total carotenoid 含量은 ethephon 處理로서 約 50%가 增加하였으며, β-carotene은 約 2倍의 增加를 보였다.
4. 處理別 capsaicin의 含量變化는 ethephon處理로서 約 20%의 增加를 볼 수 있었고, substrate인 phenylalanine의 단독 處理는 그 效果가 나타나지 않았으나, ethephon과 同時 處理함으로써 多少의 效果를 認定할 수 있었다.

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