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ORIGINAL RESEARCH ARTICLE

Influence of Different Concentrations of Calcium Pectate on Ripening of Tomato (*Lycopersicon esculentum* L.)

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ABSTRACT

The detached fruit of *Lycopersicon esculentum* L. has been selected for the present ripening study. *Lycopersicon esculentum* L. seeds were obtained from Tamil Nadu Agricultural University, Coimbatore. *Lycopersicon esculentum* L. was grown in the green house of the Botany Department of Annamalai University. The mature green fruits were harvested whenever required for the experimental study. The unripe mature green fruits were stored in the laboratory of Botany Department at room temperature of 28 ± 2°C with a humidity of 85 per cent. The mature green fruits took about five days for their complete ripening. The fruits were treated with Calcium pectate of different micro molar concentration (50, 70, and 100 µM). All the experiments were conducted daily with seven replicates. The pericarp of the fruit was used to study the ripening process. The present investigation was aimed to study the effect of calcium pectate on the ripening of *Lycopersicon esculentum* L. The fruits were treated with different micro molar concentration of (50, 70 and 100 µM) calcium pectate. The colour changes from green to yellow with in the 4 day the 5th day the fruit got split into several valves with over ripening.

Key words: *Lycopersicon esculentum* , Calcium pectate, Tomato, Medicinal plant , Fruit ripening.

1. INTRODUCTION

Lycopersicon esculentum L. is a tropical fruit belongs to the family Solanaceae. It is commonly called as Tomato, It is an important medicinal plant of India. Its each and every part is almost nutritive and has some medicinal significance. In many Asian countries such as the Philippines, Thailand, China and India, the mature-green fruit is a popular vegetable preferred for its distinctive bitterness (Julia, 1967). Fruit ripening is a highly co-ordinate, genetically programmed, and an irreversible phenomenon involving a series of physiological, biochemical, and organoleptic changes that lead to the development of a soft and edible ripe fruit with desirable quality attributes. A wide spectrum of biochemical changes such as increased respiration, chlorophyll degradation, biosynthesis of carotenoids, anthocysanins, essential oils, and flavor and aroma components, increased activity of cell wall-degrading enzymes, and a transient increase in ethylene production are some of the major changes involved during fruit ripening (Brady, 1987).

Fruit ripening is classified in to two types namely aerobic ripening (Natural) and anaerobic ripening (Artificial). The aerobic fruit ripening is regulated in plants by hormones namely ethylene, while the anaerobic ripening is generally regulated by application of chemicals for commercial purpose. The following chemicals are used for anaerobic ripening purpose, namely acetylene (gas) calcium carbide, Ethel (2-chloro ethyl phosphonic acid) and ethephon. Hence in the present investigation an attempt has been made to study the effect of calcium pectate on the ripening of *Lycopersicon esculentum* L. fruit. The possible participation of calcium in senescence in plants may be inferred from the fact that calcium is widely known to play a major role in membrane structure and function (Jones and Lunt, 1967). Its importance in the regulation of ion transport is well established (Epstein, 1961). Its effects on the maintenance of RNA and protein levels have been described by Trewavas (1970, 1972), and these components are considered to be central indices of senescence.

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Each of the parameters of senescence, such as chlorophyll loss, protein decrease, and increases in apparent free space and hydraulic conductivity, is affected by changes in the calcium status of the tissue (Poovaiah and Leopold, 1973; Poovaiah, 1987) and role of calcium in fruit repining has studied of Ferguson (2001) and. Tingwa and Young (1974) has been studied the effect of calcium on the ripening of Avocada (*Persea Americana* Mill.) fruits.

The disappearance of chlorophyll-a and b during the maturation of pears passé-creassane was found to be a reaction of the first order. In the process, chlorophyll-a decreased more rapidly than chlorophyll-b (Laval-Martin, 1969). In the flavor of Citrus fruits, Hamlin Oranges, Robinson tangerines and marsh grape fruits, the total chlorophyll content decreased and the ratio of chlorophyll a/b decreased as well (Jahn, 1973). The same trend has been observed in pummelo (Gross *et al.,* 1983). On the other hand, in some fruits it has been shown that chlorophyll-b is rapidly destroyed (Gross, 1981).

2. MATERIALS AND METHODS

The detached fruit of *Lycopersicon esculentum* L. Var. CO 1 has been selected for the present study. *Lycopersicon esculentum* L. seeds were obtained from Tamil Nadu Agricultural University, Coimbatore. *Lycopersicon esculentum L.* was grown in the green house of the Botany Department of Annamalai University. The mature green fruits were harvested whenever required for the experimental study. The unripe mature green fruits were stored in the laboratory of Botany Department at room temperature of 28 ± 2°C with a humidity of 85 per cent. The mature green fruits took about five days for their complete ripening. The fruits were treated with Calcium pectate of different micro molar concentration (50, 70, and 100 μM). All the experiments were conducted daily with seven replicates. The pericarp of the fruit was used to study the ripening process.

2.1 BIOCHEMICAL STUDIES

(a) Estimation of chlorophylls

Hundred milligram of fruit material was ground in a mortar and pestle with 20 ml of 80 per cent acetone. The supernatant was saved. The pellet was re-extracted with 5 ml of 80 per cent acetone each time, until it became colorless. All the supernatants were pooled and utilized for chlorophyll determination. The chlorophyll content in the 80 per cent acetone extract was determined by Arnon's method (1949); Absorbance was read at 645 nm and 663 nm in a Spectronic 20.

Chlorophyll a (mg/1) : 12.7 A_{663}-2.69 A_{645}
Chlorophyll b (mg/1) : 22.9 A_{645}-4.68 A_{663}
Total Chlorophyll (mg/1) : 20.2 A_{645}+8.02 A_{663}

(b) Estimation of Carotenoids (Carotenes and Xanthophylls)

Carotenoids were isolated and estimated by the method of Davies (1965). Aqueous acetone extracts were shaken thrice with an equal volume of hexane in separating funnel and the combined hexane fractions were washed with equal volumes of water. To separate carotenes from xanthophylls, the hexane fraction containing the carotenoids was extracted repeatedly with 90 percent methanol. The hexane fraction containing carotenes and methanol fraction containing xanthophylls was measured by utilizing the values of absorbance at 424 and 450 nm respectively.

(c) Estimation of Total Anthocyanins

Anthocyanins were estimated, following the method of Fuleki and Francies (1968).Hundred grams of the fruit material was blend with 100 ml of ethanolic HCl in a blender at full speed. The extract was transferred to a 500 ml glass stopper bottle and it was stored overnight in a refrigerator at 4°C. The extract was transferred to 500 ml volumetric flask and was made up to the volume. The extract was prepared for spectro photometric measurement. 25 ml of extract was filtered through a fine porous, sintered glass funnel small aliquot of the filtrate was diluted with ethanolic HCl to yield optical density (OD) and was stored in the dark for 2 hours and the colour of the extract was read in a Spectronic 20 at 535nm.

(d) Estimation of proteins

Protein content was estimated following the method of Lowry *et al.* (1951).Hundred milligram of the fruit material was macerated with a mortar and pestle with 10 ml of 20 per cent Trichloro acetic acid (TCA). The homogenate was centrifuged for 15 minutes at 600 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged. The supernatant was taken and made up to 5 ml with 0.1 N NaOH. This extract was used for the estimation of total protein. To 0.5 ml of protein extract, 5 ml of the reagent 3 was added, and this was allowed to stand for 10 minutes at 28°C. 0.5 ml of Folinphenol reagent was added to this solution and kept at room temperature (28°C) for 10 minutes. The absorbance was read at 600 nm in Spectronic 20.

(e) Estimation of starch

Starch was extracted and estimated, using the method of Clegg (1956).The residue left behind after the alcoholic extract of the material was taken for starch extraction and estimation. Starch was solubilized with 52 per cent perchloric acid for 50 minutes, filtered, and was made up to 100 ml in a volumetric flask, with distilled water. One to two ml of the perchloric acid extract was diluted with 5 ml of deionised water in test tube and 10 ml of anthrone reagent was added in cold. The contents were heated for 7.5 minutes at 100°C in a boiling water bath. The test tubes were cooled rapidly and the colour intensity was read at 630 nm in a Spectronic 20. The starch content was calculated, using a standard graph prepared with glucose.

(f) Estimation of soluble sugars

Soluble sugars, reducing and non-reducing sugars, were estimated following the method of Nelson (1944).

Extraction

Two g of fruit material was macerated in a mortar and pestle with 80 per cent ethyl alcohol. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved and made up to 20 ml with 80 per cent ethyl alcohol. This extract was used to estimate both reducing and non-reducing sugars.

(g) Estimation of reducing sugars

To 1 ml of ethanolic extract, 1 ml of fresh Nelson's reagent (prepared by mixing copper tartrate solution and copper sulphate solution 25:1 (v/v) was added. The mixture was heated in boiling water for 20 minutes, and then cooled. To the cooled mixture, 1 ml of Nelson's Arseno molybdate reagent was added. The solution was diluted to 25 ml with distilled water. The intensity of the resulting blue colour was read at 520 nm in a Spectronic 20. The content of the reducing sugar was calculated from glucose standard graph.

(h) Estimation of non-reducing sugars

Non-reducing sugars were hydrolyzed to reducing sugars, and the total sugar was estimated.

Hydrolysis

One ml of ethanolic extract was evaporated to dryness in a boiling water bath. To the residue, 1 ml of distilled water and 1 ml of concentrated H_2SO_4 were added. The mixture was hydrolyzed by incubating in an oven at 50°C for 30 minutes. The solution was neutralized with 1N NaOH.

(i) Estimation of Total sugar

Total sugar of the hydrolyzed sample was estimated by using Nelson's Arsenomolybdate method. Non-reducing sugar content was calculated by subtracting the value of reducing sugar from the total sugar. The various results obtained from the experimental study were statistically analysed and are presented in Tables.

3. RESULTS AND DISCUSSION

In the present investigation the mature detached fruit of *Lycopersicon esculentum* L. was used to study the ripening process and the fruits were treated with different micro molar concentration of (50, 70 and 100 μM) calcium pectate. The colour changes from green to yellow with in the 4 day the 5th day the fruit got split into several valves with over ripening.

3.1 Changes in the physio-chemical characters

(a) Fruit firmness and total soluble solid

The results on the fruit firmness and the total soluble solid changes are presented. The fruit firmness gradually decreased while the total soluble solids increased during ripening both in the control and treated fruits. The level of increase/decrease in the content was slow in 70 μM treated fruits.

(b) Titratable acidity and pH

The results in the titratable acidity and pH changes are presented. The titratable acidity gradually increased on the other hand the pH gradually decreased both in the control and treated fruits during the ripening. The level of the increase/decrease in the content was slow in 70 μM in treated fruits.

Biochemical changes

(a) Chlorophyll

The results on the chlorophyll pigment changes are presented in (**Table 1**). The chlorophyll a, b and total chlorophyll gradually decreased during the ripening in the control and treated fruits the process of decrease was slow in the fruits treated with 70 μM calcium pectate compare to that of other treated fruits and control. The content of chlorophyll 'a' was more than that of chlorophyll 'b' both in the control and treated fruits during the ripening process.

(b) Carotenoid

The results on the carotenoid changes are presented in (**Table 2**). The content of carotenoid gradually increased throughout the ripening period both in the control and treated fruits. The slow increase was found in 70 μM treated fruits compare to that of other treated fruits and control.

(c) Anthocyanins

The results on the content of anthocyanins changes are presented in (**Table 3**). The anthocyanins gradually increased both in the control and treated fruits during the ripening process. The slow increase was found in 70 µM treated fruits compare to that of control and other fruits

(d) Proteins

The results on the total protein content changes are presented (**Table 4**). The protein content gradually decreased during ripening in control and treated fruits. The decrease was slow in 70 µM treated fruits.

(e) Starch

The results on the starch content changes are formed (**Table 5**). The content of starch decreased throughout ripening period both in the control and treated fruits. The decrease was slow in 70M treated fruits.

(f) Sugar

The results on the sugar content changes are presented (**Table 6**). The sugar content gradually increased in control and treated fruits. The increase was slow in 70 µM treated fruits. The reducing sugar content was more than that of non-reducing sugar in control and treated fruit throughout the ripening process.

Table 1: Influence of calcium pectate on the changes in the chlorophyll 'a', chlorophyll 'b' and total chlorophyll content during the ripening of fruit of bitter gourd (*Lycopersicon esculentum* Linn.)

Days	Control			50 µM Calcium pectate			70 µM Calcium pectate			100 µM Calcium pectate		
	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1	0.125 ± 0.010	0.031 ± 0.002	0.156 ± 0.012	0.117 ± 0.009	0.029 ± 0.002	0.146 ± 0.012	0.111 ± 0.009	0.027 ± 0.002	1.138 ± 0.011	0.111 ± 0.009	0.027 ± 0.002	0.138 ± 0.011
2	0.103 ± 0.007	0.025 ± 0.001	0.128 ± 0.008	0.105 ± 0.007	0.026 ± 0.002	0.131 ± 0.009	0.107 ± 0.007	0.026 ± 0.001	0.133 ± 0.008	0.094 ± 0.007	0.023 ± 0.002	0.117 ± 0.009
3	0.098 ± 0.005	0.024 ± 0.001	0.122 ± 0.007	0.094 ± 0.005	0.023 ± 0.001	0.117 ± 0.007	0.096 ± 0.006	0.024 ± 0.001	0.120 ± 0.007	0.073 ± 0.004	0.018 ± 0.001	0.091 ± 0.005
4	0.092 ± 0.005	0.023 ± 0.001	0.115 ± 0.005	0.090 ± 0.005	0.022 ± 0.001	0.112 ± 0.006	0.074 ± 0.004	0.028 ± 0.001	0.102 ± 0.005	0.040 ± 0.002	0.010 ± 0.000	0.050 ± 0.002
5	0.068 ± 0.05	0.019 ± 0.001	0.087 ± 0.006	0.071 ± 0.004	0.017 ± 0.001	0.088 ± 0.005	0.072 ± 0.002	0.020 ± 0.000	0.092 ± 0.002	0.030 ± 0.002	0.007 ± 0.000	0.037 ± 0.002

(Values are expressed in mean ± SE of seven samples expressed in mg/g fr. wt.)

Table 2: Influence of calcium pectate on the changes in the carotenoid content during the ripening of fruit of (*Lycopersicon esculentum* Linn.)

Days	Control			50 µM Calcium pectate			70 µM Calcium pectate			100 µM Calcium pectate		
	Carotene	Xanthophyll	Carotenoid	Carotene	Xanthophyll	Carotenoid	Carotene	Xanthophyll	Carotenoid	Carotene	Xanthophyll	Carotenoid
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1	0.010 ± 0.008	0.004 ± 0.003	0.014 ± 0.011	0.010 ± 0.008	0.004 ± 0.004	0.014 ± 0.011	0.010 ± 0.008	0.013 ± 0.007	0.002 ± 0.007	0.002 ± 0.007	0.004 ± 0.032	0.006 ± 0.039
2	0.013 ± 0.009	0.004 ± 0.002	0.017 ± 0.011	0.010 ± 0.008	0.005 ± 0.003	0.015 ± 0.011	0.015 ± 0.010	0.025 ± 0.017	0.010 ± 0.001	0.010 ± 0.001	0.005 ± 0.003	0.015 ± 0.004
3	0.015 ± 0.009	0.010 ± 0.006	0.025 ± 0.015	0.013 ± 0.007	0.006 ± 0.003	0.019 ± 0.010	0.018 ± 0.010	0.031 ± 0.017	0.011 ± 0.006	0.011 ± 0.006	0.006 ± 0.003	0.017 ± 0.009
4	0.020 ± 0.001	0.017 ± 0.008	0.037 ± 0.018	0.025 ± 0.012	0.008 ± 0.004	0.033 ± 0.016	0.020 ± 0.011	0.036 ± 0.018	0.013 ± 0.006	0.013 ± 0.006	0.015 ± 0.008	0.028 ± 0.014
5	0.024 ± 0.014	0.019 ± 0.011	0.043 ± 0.025	0.014 ± 0.066	0.019 ± 0.011	0.033 ± 0.077	0.031 ± 0.018	0.058 ± 0.034	0.024 ± 0.014	0.024 ± 0.014	0.021 ± 0.012	0.045 ± 0.026

(Values are mean ± SE of seven samples expressed in mg/100 g fr. wt.)

Table 3: Influence of calcium pectate on the changes in the anthocyanin content during the ripening of fruit of (*Lycopersicon esculentum* Linn.)

Days	Control	50 µM Calcium pectate	70 µM Calcium pectate	100 µM Calcium pectate
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1	0.033 ± 0.026	0.038 ± 0.030	0.038 ± 0.030	0.048 ± 0.038
2	0.043 ± 0.030	0.048 ± 0.033	0.043 ± 0.030	0.058 ± 0.040
3	0.048 ± 0.028	0.053 ± 0.031	0.053 ± 0.031	0.063 ± 0.037
4	0.058 ± 0.029	0.068 ± 0.034	0.063 ± 0.031	0.067 ± 0.036
5	0.068 ± 0.040	0.073 ± 0.043	0.067 ± 0.056	0.073 ± 0.006

(Values are mean ± SE of seven samples expressed in mg/100 g fr. wt.)

Table 4: Influence of calcium pectate on the changes in the protein content during the ripening of fruit of (*Lycopersicon esculentum* Linn.)

Days	Control	50 µM Calcium pectate	70 µM Calcium pectate	100 µM Calcium pectate
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1	0.055 ± 0.044	0.058 ± 0.046	0.059 ± 0.047	0.065 ± 0.052
2	0.053 ± 0.037	0.052 ± 0.036	0.050 ± 0.035	0.061 ± 0.042
3	0.051 ± 0.030	0.049 ± 0.029	0.048 ± 0.028	0.047 ± 0.028
4	0.050 ± 0.035	0.048 ± 0.024	0.047 ± 0.023	0.045 ± 0.022
5	0.049 ± 0.029	0.047 ± 0.028	0.046 ± 0.027	0.043 ± 0.025

(Values are mean ± SE of seven samples expressed in mg/g fr. wt.)

Table 5: Influence of calcium pectate on the changes in the starch content during the ripening of fruit of (*Lycopersicon esculentum* Linn.)

Days	Control	50 µM Calcium pectate	70 µM Calcium pectate	100 µM Calcium pectate
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1	0.073 ± 0.058	0.067 ± 0.053	0.069 ± 0.055	0.070 ± 0.056
2	0.065 ± 0.045	0.057 ± 0.039	0.054 ± 0.037	0.051 ± 0.035
3	0.058 ± 0.034	0.051 ± 0.030	0.047 ± 0.028	0.042 ± 0.025
4	0.049 ± 0.024	0.031 ± 0.020	0.038 ± 0.019	0.033 ± 0.016
5	0.030 ± 0.032	0.030 ± 0.022	0.032 ± 0.025	0.028 ± 0.022

(Values are mean ± SE of seven samples expressed in mg Glucose equivalent/g fr. wt.)

Table 6: Influence of calcium pectate on the changes in the reducing sugar, non-reducing sugar and total sugar content during the ripening of fruit of Tomato (*Lycopersicon esculentum* Linn.)

Days	Control			50 µM Calcium pectate			70 µM Calcium pectate			100 µM Calcium pectate		
	Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar	Total sugar	Reducing sugar	Non-reducing sugar	Total sugar
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
1	0.108 ± 0.008	0.038 ± 0.003	0.146 ± 0.011	0.103 ± 0.008	0.037 ± 0.002	0.140 ± 0.010	0.102 ± 0.008	0.034 ± 0.002	0.137 ± 0.010	0.107 ± 0.006	0.031 ± 0.002	0.140 ± 0.008
2	0.114 ± 0.007	0.043 ± 0.003	0.157 ± 0.010	0.112 ± 0.007	0.042 ± 0.002	0.154 ± 0.009	0.109 ± 0.006	0.035 ± 0.002	0.143 ± 0.008	0.112 ± 0.006	0.033 ± 0.002	0.143 ± 0.008
3	0.118 ± 0.007	0.044 ± 0.002	0.162 ± 0.010	0.123 ± 0.007	0.043 ± 0.002	0.166 ± 0.009	0.116 ± 0.006	0.037 ± 0.002	0.153 ± 0.008	0.116 ± 0.007	0.035 ± 0.002	0.151 ± 0.009
4	0.123 ± 0.006	0.053 ± 0.002	0.176 ± 0.008	0.132 ± 0.006	0.051 ± 0.002	0.183 ± 0.008	0.127 ± 0.007	0.048 ± 0.002	0.175 ± 0.009	0.123 ± 0.007	0.042 ± 0.002	0.167 ± 0.009
5	0.132 ± 0.007	0.057 ± 0.003	0.189 ± 0.011	0.139 ± 0.008	0.052 ± 0.003	0.191 ± 0.011	0.129 ± 0.008	0.044 ± 0.002	0.171 ± 0.010	0.135 ± 0.008	0.049 ± 0.002	0.184 ± 0.010

(Values are mean ± SE of seven samples expressed in mg Glucose equivalent/g fr. wt.)

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