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Effect of calcium pectate on the biochemical and pigment changes during the ripening of bitter gourd fruit (*Momordica charantia* L Var-Co-1)

A. Anbarasan and C. Tamilmani

Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

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ABSTRACT

The present investigation was aimed to study the effect of calcium pectate on the ripening of Momordica charantia L. Var-Co-1 fruits. 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. All the studies were carried out using the fruit pericarp tissue individually both in the treated and control fruits and the following results were obtained during the ripening process. The chlorophyll content protein and starch decreased while the carotenoid, anthocyanins and sugars increased during ripening. Among the different concentration of calcium pectate (50, 70 and 100 µM). The 70 µM concentration treated fruits alone had more delaying effect then that of other treatment during the ripening process.

Keywords: Calcium pectate, *Momordica charantia*, Biochemical and pigment Parameters.

INTRODUCTION

The calcium ion is known to be involved in many fundamental physiological plant processes involving cell walls, membranes, chromosomes, and enzyme activation. In post-harvest physiology, disorders such as bitter pit in apples have been directly linked to low Ca content of the fruits. It has been suggested that such disorders result from increased respiration rate following membrane permeability changes. Higher Ca levels are seen in the fresh depressed preclimacteric, climacteric, and post climacteric respiration of apples. The same inhibitory effect was observed on respiration of apple Mitochondria [1].

A fruit is the product of determinate growth from an angiospermous flower of inflorescence [2]. Fruits like all other plant parts develop and grow, become mature, then old and finally die. The most obvious visual symptom of fruit ripening is the colour change. Fruit colours are produced by three main classes of pigments – the green chlorophylls, the yellow to red carotenoids, and the red, blue and violet anthocyanins [3]. The colour changes during ripening of fruits results largely from the loss of chlorophyll, the synthesis of carotenoids and the synthesis of pigmented phenolic compounds such as anthocyanins. In any one commodity, the typical colour change in ripening may result from only one or from any combination of these process [4]. 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 [5]. The carotenoid pigments are widely distributed among living organisms, both animal and vegetable. As the carotenoids are synthesized only in plants (a part from certain bacteria). Their levels in vegetable matter are much higher than in animal matter [6]. The external expression of anthocyanin pigmentation depends on pH, and it gives red colour in acid medium and blue in neutral and alkaline medium, but it seems unlikely that localized pH is a major factor in determining the colour of anthocyanin

containing fruits [7]. Anthocyanins are located mainly in the skin of the fruits as in plums, apples, pears, grapes and American Cranberries. In other fruits, they are found both in skin and flesh, predominating in the skin as in some sweet cherries or more evenly distributed as in sour cherries [8]. The total anthocyanin content increased fourfold in ripe fruits [9]. Antioxidant activities and anthocyanin content of fresh fruits of common Fig. (*Ficus carica* L.) have been studied by [10]. A decrease in total protein was found in assays of four different batches of Haas and Fuerte avocados [11]. During ripening almost entire starch is converted into simple sugars, such as sucrose, fructose and glucose. Only 1-2 per cent of starch remain in the ripe fruits [12] and [13]. The flavour of fruit is compounded mainly of its content of sugars, of acids and of numerous volatile aroma components, which are present in very small quantities but elicit a considerable olfactory response. Changes of flavour during post-harvest ripening typically result from an increase in sugar at the expense of reserve carbohydrate, a decrease in acids, which may be respired and considerable increase in the production of volatile aroma components [4]. The above review of literature clearly reveals that the studies on ripening of *Momordica charantia* fruit is very meager. Hence in the present investigation, an attempt has been made to study the effect of calcium pectate on the biochemical and pigment changes during ripening of fruit of *Momordica charantia.*

MATERIALS AND METHODS

The detached fruit of *Momordica charantia* L. Var. CO 1 has been selected for the present ripening study. *Momordica charantia* L. var. CO 1 seeds were obtained from Tamil Nadu Agricultural University, Coimbatore. *Momordica charantia* 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 micromolar 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.

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 colourless. All the supernatants were pooled and utilized for chlorophyll determination. The chlorophyll content in the 80 per cent acetone extract was determined by [14] 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) : $20.2\ A_{645}+8.02\ A_{663}$

Estimation of Carotenoids (Carotenes and Xanthophylls)

Carotenoids were isolated and estimated by the method of [15]. 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 xanthophyll, 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.

Estimation of Total Anthocyanins

Anthocyanins were estimated, following the method of [16]. 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 stoppered bottle and it was stored overnight in a refrigerator at 4°C. The extract was transferred to 500 ml volumetric flask and was made upto the volume. The extract was prepared for spectrophotometric measurement. 25 ml of extract was filtered through a fine porous, sintered glass funnel. A 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.

Estimation of proteins

Protein content was estimated following the method of [17]. Hundred milligram of the fruit material was macerated with a mortar and pestle with 10 ml of 20 per cent Trichloroacetic 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 upto 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.

Estimation of starch

Starch was extracted and estimated, using the method of [18]. 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 upto 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.

Estimation of soluble sugars

Soluble sugars, reducing and non-reducing sugars were estimated following the method of [19].

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 upto 20 ml with 80 per cent ethyl alcohol. This extract was used to estimate both reducing and non-reducing sugars.

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 Arsenomolybdate 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.

Estimation of non-reducing sugars

Non-reducing sugars were hydrolysed 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 hydrolysed by incubating in an oven at 50°C for 30 minutes. The solution was neutralized with 1N NaOH.

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.

Observation and results

In the present investigation the mature detached fruit of *Momordica charantia* L. Var-Co -1 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.

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.

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.

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.

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

Starch
The results on the starch content changes are formed in Table-5. The content of starch decreased throughout ripening period both in the control and treated fruits. The decrease was slow in 70 µM treated fruits.

Sugar
The results on the sugar content changes are presented in 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.

RESULTS AND DISCUSSION

Calcium is an element that differs from others by being imported into fleshy fruit only in small amounts, much less than into leaves. Although Ca is sufficiently available in the soil of most orchards, localized Ca deficiency may become a problem in several fruit and vegetable crops, with the risk of large economic losses. The Calcium content of fruit is usually expressed as a percentage of either fresh mass (FM) or dry mass (DM). In apple, the maximum concentration of Ca in the fruit is usually reached shortly after flowering [20], [21]. Therefore, it drops rapidly, beginning with the initial stage of rapid fruit growth this concerns with the onset of rapid cell enlargement, the decrease of the Ca concentration with increasing fruit mass continues until harvest, reaching very low levels in the fruit flesh, in apples in average 3-6 mg 100 g^{-1} FM, or 25-35 mg 100 g^{-1} DM, with great variations for reasons to be outlined later, this Ca concentration is usually taken as an estimate to make commercial decisions relating to storage and marketing [22].

Changes in colour, from green to red are consequence of chlorophyll degradation and accumulation of large amount of carotenoids within the plastids as the chloroplast present in the mature green fruit are transformed into chromoplasts [23]. This transition seem to be a controlled process rather than a degenerative one as regreening is possible in some fruits as in valancia organs [24]. 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 (Table-1). The content of chlorophyll ‘a’ was more than that of chlorophyll ‘b’ both in the control and treated fruits during the ripening process.

Chlorophyll is present in unripe fruits, which at the beginning of their development contain chloroplast in high amount in peel than in pulp. During ripening the chloroplast are gradually disorganized as the thylakoids are destroyed and chloroplast broken down, although this phenomenon is almost ubiquitous occurring in the great majority of fruits, there are some exceptions; fruits which retain chlorophyll at the ripe stage, like apple, pear and goose berry cultivars [3]. Chlorophyll degradation occurs widely in nature as part of the chlorophyll molecules is not known, however [25]. In an early study of colour changes in two apple cultivars, chlorophyll was globally assessed during fruit development. In both apple cultivars, chlorophyll continuously decreased during ripening [26].

Table - 1. Effect of calcium pectate on the changes in the chlorophyll 'a', chlorophyll 'b' and total chlorophyll content during the ripening of fruit of bitter gourd (*Momordica charantia* Linn.)
(Values are expressed in mean ± SE of seven samples expressed in mg/g fr. wt.)

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

Table - 2. Effect of calcium pectate on the changes in the carotenoid content during the ripening of fruit of bitter gourd (*Momordica charantia* Linn.)
(Values are mean ± SE of seven samples expressed in mg/100 g fr. wt.)

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

Table - 3. Effect of calcium pectate on the changes in the anthocyanin content during the ripening of fruit of bitter gourd (*Momordica charantia* Linn.)
(Values are mean ± SE of seven samples expressed in mg/100 g fr. wt.)

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

Table -4. Effect of calcium pectate on the changes in the protein content during the ripening of fruit of bitter gourd (*Momordica charantia* Linn.)
(Values are mean ± SE of seven samples expressed in mg/g fr. wt.)

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

Table -5. Effect of calcium pectate on the changes in the starch content during the ripening of fruit of bitter gourd (*Momordica charantia* Linn.)
(Values are mean ± SE of seven samples expressed in mg Glucose equivalent/g fr. wt.)

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

Table - 6. Effect of calcium pectate on the changes in the reducing sugar, non-reducing sugar and total sugar content during the ripening of fruit of bitter gourd (*Momordica charantia* Linn.)
(Values are mean ± SE of seven samples expressed in mg Glucose equivalent/g fr. wt.)

Days	Control			50µM Calcium pectate			70 µM Calcium pectate			100µM 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

The content of carotenoid gradually increased throughout the ripening period of both in the control and treated fruits the slow increase was found in 70 µM treated fruits compare to that of treated fruits (Table-2). The carotenoid composition of *Momordica charantia* fruit (pericarp) at four levels of maturity was investigated by [27] 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 (Table-3). Anthocyanins are located mainly in the skin of the fruit as in plums, apples, pear and grapes. In other fruits they are found both in the skin and in the flesh, predominating in the skin as in some sweet-cherries or more evenly distributed as in sour cherries [8]. In pomegranate during ripening six anthocyanin pigments were found to be responsible for the red colour of the fruits [28]. The protein content gradually decreased during ripening in control and treated fruits the decrease was slow in 70 µM treated fruits (Table-4). A similar decrease in total protein was found in assays from different batches of Haas and Fuerte avocados [11]. But a different trend was observed in apples [29] and in cantaloupe [30] where there was a net increase in protein during ripening. Certain proteins which are synthesized at a high rate easily in the climacteric, are synthesized at a lower rate as ripening proceeds while for other protein the reverse is true [31], the increased degradation or decreased protein synthesis or both may be responsible for the decreased protein.

The content of starch decreased throughout ripening period both in the control and treated frits the decrease was slow in 70 µM treated fruits (Table-5). Starch, which in the main storage polysaccharide in many unripe fruits, is degraded during ripening, resulting in sweetness and textural changes in fruits. Guava fruits also exhibited a decrease in starch and an increase in the content of reducing and non-reducing sugars during ripening starch content decreased significantly from 3.42% at mature green stage to 0.90% at overripe stage. Starch content has also been reported to decrease in fruits like papaya [32]. As fruit begin to soften starch deposits are degraded and sugar and flavour components are accumulated [33]. Similar trend was observed in *Momordica charantia* as starch degraded the total soluble solid was proportionately increased. The sugar content gradually increased in control and treated fruits. The increase was slow in 70 µM treated frits (Table-6). The total sugars increased from 4.76% at mature green stage to 8.96% at over ripening stage. Both reducing and non-reducing sugars were present at the same concentration (2.38%) at mature green stage. Reducing sugars increased substantially to 5.60% at over ripening stage, whereas non-reducing sugars increased slightly only between ripe and overripe stages. This increase is mainly due to degradation of starch [32]. The reducing sugar content was more than that of non-reducing sugar in control and treated fruit throughout the ripening process. In the soft fruits reducing sugar content consists of glucose and fructose [7]. In *Momordica charantia* the reducing sugar predominate the non-reducing sugar and hence it is a soft fruit. As the content of starch decreased, the level of reducing and non-reducing sugar increased.

CONCLUSION

In these studies among the different concentration of calcium pectate treatment, 70µM alone had more delaying effect than that of other concentration and control.

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