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Pectin changes during the development and ripening of eggplant fruits

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In developing and ripening eggplant fruits (Semi-round striped, Purple long and Black round), the main pectin fractions, water-, ammonium oxalate-, and alkali-soluble, were estimated up to maturity and ripening. The determinations were made using the *m*-hydroxydiphenyl assay, which was automated resulting in a rapid and sensitive method to quantitatively determine the pectic substances. For all cultivars studied, total pectic polysaccharides decreased during the initial growth period and after 15 days showed a slightly increasing trend. The concentration of water-soluble pectic substances decreased until 28 days, as did the oxalate-soluble fraction. These two pectin fractions increased during the development period between 28 and 42 days while the alkali-soluble (protopectin) fraction decreased. The increase of protopectin before physiological maturity, and its subsequent decrease with fruit ripening and softening, indicated the involvement of this fraction in the texture maintenance of eggplant fruits.

INTRODUCTION

A number of workers have shown that, during ripening of various fruits (peach, tomato, mango) changes in pectic substances are produced (Shewfelt & Smith, 1972; Malis-Arad *et al.*, 1983; Tandon & Kalra, 1984), which are correlated to softening and textural modifications of fruits.

The loss of firmness during fruit ripening has been attributed to changes taking place in the parenchymatous cell wall (Thimann, 1980). Thus, the cell wall metabolism and, essentially, the increase of enzymatic activity have been implicated in the overall regulation of the ripening process (Varga & Bruinsma, 1986).

In the parenchyma of fruits and vegetables, pectic polysaccharides are the major polysaccharides. These are held in the wall mainly by calcium bridges to form calcium pectates and by some ester linkages to neutral sugars. Rhamnogalacturonans are the major constituents of pectic substances in which many of the galacturonic acid residues are present as methyl esters. Attached to this main chain are side chains consisting primarily of D-galactose and L-arabinose and lesser amounts of other sugars (Selvendran *et al.*, 1987).

Pectic substances may be analysed colorimetrically after reaction between an appropriate reagent and the anhydrogalacturonic acid residues of pectin. Although the carbazole reaction is often used, it has been known that this method has the disadvantage of neutral sugar interferences. However, the method which uses *m*-hydroxydiphenyl as chromogen is more specific for uronic acids and adequate for their quantitative determination in the presence of neutral sugars.

Little information is available on pectic substances of eggplant fruits (Aubert & Pochard, 1981) and there are no reports on the changes of the different pectic fractions during the development and ripening of these fruits.

This study was undertaken to observe variations in the three major pectin fractions during the development of eggplant fruits. A procedure which uses *m*-hydroxydiphenyl reagent for the colorimetric assay was adopted and it was automated, resulting in a rapid, sensitive and specific method for pectin determinations.

MATERIALS AND METHODS

Eggplant fruits (*Solanum melongena* L.) obtained from an hydroponic culture were used for this study. Samples of three different cultivars (Semi-round striped, Purple long, and Black round) were harvested at various development stages (at 5, 11, 15, 28, 42 and 54 days after fruit set). Determinations were made on fruits from 12 plants of each cultivar. On each sampling day, fruits were selected and sorted carefully in a visual manner for uniformity of size, colour and maturity.

Recovery of alcohol–insoluble solids (AIS) residue

The selected fruits were peeled and sliced. From each sample, three 10 g aliquots were blended with 40 ml of 96% ethyl alcohol. The mixture was triturated in a Polytron homogenizer for 3 min and heated for 10 min

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in a water bath at 95°C. The mixture was then centrifuged for 20 min at 1000 *g* after which the supernatant was decanted and discarded. The leaching was repeated with 70% ethyl alcohol for 10 min at 85°C, thus obtaining the AIS residue.

Fractionation of pectic substances

The AIS residue was subjected to sequential extraction of three distinct classes of pectic substances, following the method of Robertson (1979). The fractionation procedure involves progressive extraction of the alcohol-insoluble solids by a series of solvents; water (pectinic acids or high methoxyl pectins); a solution of ammonium oxalate (pectic acids or low methoxyl pectins and pectates); and cold alkali (protopectin).

From each of the above three extracts, aliquots were taken for analysis by the automated meta-hydroxydiphenyl method (Thibault, 1979) in which we introduced several modifications. Standard solutions containing 0–100 μg/ml galacturonic acid (GA which had been dried for 6 h in a vacuum oven at 6°C) were used.

Automated *m*-hydroxydiphenyl method

A Technicon Autoanalyzer II was used. This system was modified and improved in our laboratory for pectin measurements. Optimal conditions of work are indicated in Fig. 1. The reagents used were a solution of 0·02% of *m*-hydroxydiphenyl (MHDP) and a solution of concentrated sulphuric acid (98%) with 5% of concentrated phosphoric acid (85%), this latter acid acting as a surfactant. Four aliquots from each extract were measured. One of them acted as a blank, without *m*-hydroxydiphenyl reagent, to correct for the slight pink colour produced when neutral sugar-containing materials are heated in sulphuric acid.

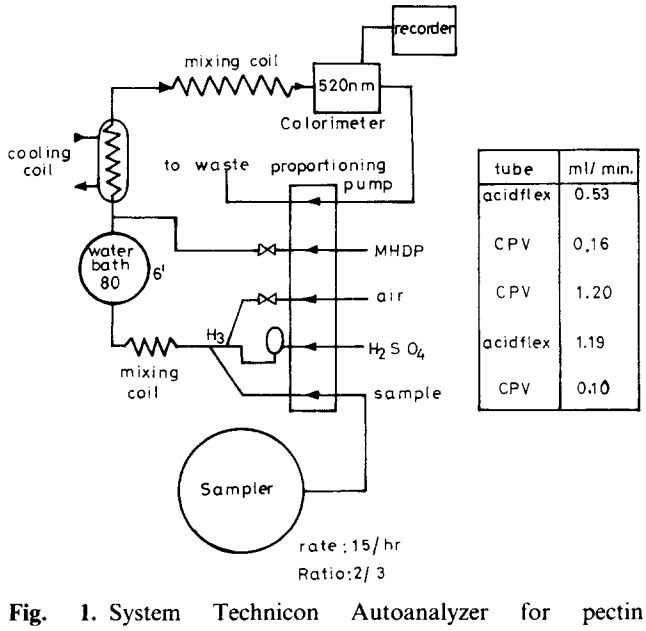

Fig. 1. System Technicon Autoanalyzer for pectin measurements.

Statistical treatment of data

Standard error and linear regression coefficients were calculated from the data corresponding to the validation of the automated colorimetric method.

LSD for cultivars (cv) and samplings (s) were calculated using a two-way analysis of variance ($P < 0.05$) (Snedecor & Cochran, 1967).

RESULTS AND DISCUSSION

Figure 2 represents the standard curve for galacturonic acid, all the results being the average of four determinations. The curve follows the Beer–Lambert law up to 100 μg/ml of galacturonic acid (GA). Method detection limit is 10 μg/ml, thus showing the sensitivity of the method to low concentrations of GA.

Figure 3 shows the control maps of twenty replicate assays. It proves that reproducibility is very good.

A recovery study was carried out adding different amounts of GA to a pectin extract in which the GA concentration was 25 μg/ml. Recovery data are showed in Table 1. The recovery percentage is very high (99·8%).

The development and ripening period of the eggplant fruits extended over a 6-week period from the onset of the fruit until ripening, reaching the maximum fruit growth 2 weeks later (Table 2).

The changes in the different pectin fractions during development of eggplant fruits are shown in Table 3. All contents are expressed as mg of anhydrogalacturonic acid per 100 g fresh weight. Total pectin content was calculated as the sum of the water-, oxalate-, and alkali-soluble fractions.

The concentration of total pectic polysaccharides in the fruit decreased sharply during the initial growth period (about 15 days for Purple long (PL) cv. and 11

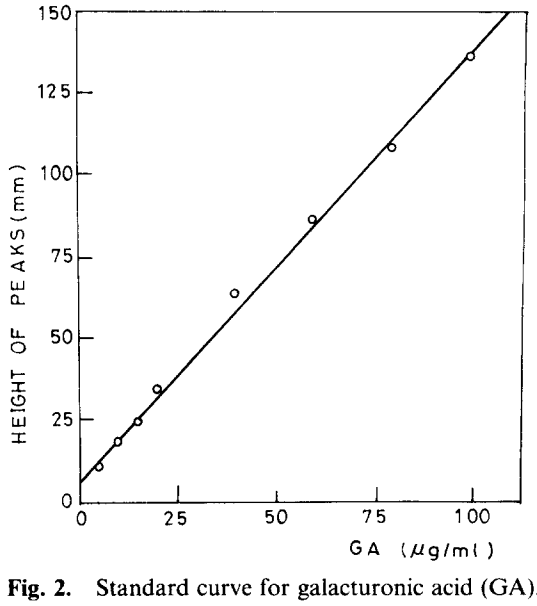

Fig. 2. Standard curve for galacturonic acid (GA).

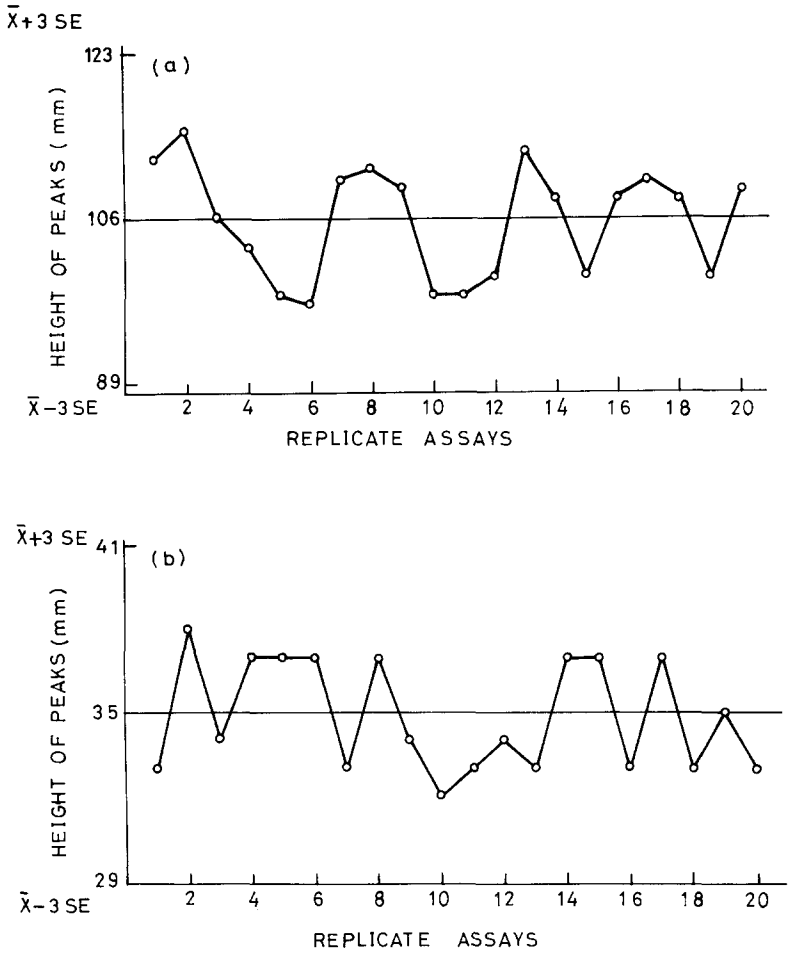

Fig. 3. Control maps for GA solutions: (a) 60 μg/ml; (b) 20 μg//ml (SE = standard error).

days for Black round (BR) and Semi-round striped (SS) cvs.). From these times, the pectic polysaccharide content showed a slightly increasing trend; the rate of increase hastened during the latter part of the development period for BR cultivar.

In addition to changes in pectic polysaccharides, the distribution among the three fractions also changed during fruit development and ripening. The majority of the pectic polysaccharides was in the oxalate-soluble fraction, which contained from 89% to 53% of the total pectic polysaccharides, except for 28-day fruits of BR and SS cultivars (44% and 34%, respectively).

In samples from BR and SS cultivars, there was a decreasing trend in oxalate-soluble pectic polysaccharides until 28 days (commercial harvest) after fruit set, and then a continuous increase, reaching the maximum value at 54 days for BR cultivar. For PL cultivar, this increase was observed from 15 days after fruit set.

Table 1. Recovery assay from eggplant fruit samples

Added GA μg	Recovered GA μg[a]
50	51
100	97
200	198
300	315
400	407
500	493

$y = 1{\cdot}002x + 1{\cdot}309$; $r = 0{\cdot}9989$.
[a] All results are the average of four determinations.

The concentration of alkali-soluble pectin also decreased during the initial 11-day period. However, there was a marked increase in base-soluble pectic polysaccharides during the subsequent stages until 28 days, resulting in an overall increase in the proportion of the total pectic polysaccharides in this fraction. From this development stage, the protopectin content underwent, again, a marked decrease. The concentration of water-soluble pectic polysaccharides decreased until 28 days (commercial harvest stage), as in the case of the oxalate-soluble fraction. From this time, an increase was observed until the full ripening (42 days), followed by a net decrease of this fraction in the overripe fruits. Water-soluble pectin is the minor fraction of pectic polysaccharides in eggplant fruits and accounted for about 3% and 18% depending on the development stage and cultivar. The peak in this latter fraction was observed about 2 weeks later than the maximum in protopectins (alkali-soluble fraction). Tandon & Kalra (1984), in a study of pectic polysaccharides during mango fruit development, observed that water-soluble pectin was maximal in the ripe fruit whereas protopectin increased a few days earlier in respect of maturity. In our case, the protopectin increased until maximum growth rate and then declined.

During the development period between 28 days and

Table 2. Weights (g) and sizes expressed in length and diameter (cm) of the fruit for each sampling period

		Days after fruit set					
	Cultivar	5	11	15	28	42	54
W(g)	SS	11	25·9	51·3	214·9	390·6	446
	PL	13	46·0	86·5	154·2	216·6	217·4
	BR	14·1	48·4	74·5	364·6	479·9	584·7
L(cm)	SS	2·5	5·3	7·5	13·4	15·3	17·5
	PL	4·6	6·6	10·3	18·3	20·8	23·6
	BR	3·7	6·1	6·3	13·7	15·2	18·7
D(cm)	SS	1·7	3·3	4·1	6·9	7·5	8·1
	PL	2·4	3·9	4·1	5·3	5·3	5·5
	BR	2·8	3·8	4·2	7·8	9·7	12·4

42 days after eggplant fruit set, water-soluble (high methoxyl) and ammonium-oxalate-soluble (low methoxyl) pectic polysaccharides increased while the alkali-soluble (protopectin) fraction decreased, as occurs in other fruits (banana, date) (Israeli, 1986; Reuveni, 1986). According to Tandon & Kalra (1984), in a study of mango fruit, as the fruits ripen enzymatic de-esterification and depolymerization of cell-bound pectin might occur, which eventually yield water- and ammonium oxalate-soluble pectins. Protopectin, upon hydrolysis, would yield soluble pectins and their content increases in ripe eggplant fruit. After 42 days, the water-soluble fraction (high methoxyl) started to decrease probably due to a new enzymatic de-esterification that resulted in new increase of the ammonium oxalate-soluble fraction (low methoxyl). According to several authors (Hobson, 1981; Tucker & Grierson, 1982; Varga & Bruinsma, 1986), the enzymatic hydrolysis of insoluble pectin to soluble forms may be due to de novo synthesis of enzymes (polygalacturonase and pectin methylesterase). It seems that protopectin is an important pectin fraction which increases before physiological maturity and then decreases with fruit ripening and softening. This fraction, together with other cementing material, is responsible for the maintenance of texture.

Table 3. Changes in the different pectin fractions during development of eggplant fruits (expressed in mg GA/100 g fresh matter)

		Days after fruit set					
cv.	Fraction	5	11	15	28	42	54
SS	Water	32	52	50	44	58	48
	Oxalate	269	154	162	106	154	218
	Alkali	106	58	87	158	112	93
	Total	407	264	299	308	324	359
PL	Water	53	44	39	33	65	35
	Oxalate	382	303	185	224	274	357
	Alkali	162	106	128	163	78	64
	Total	597	453	352	420	417	456
Br	Water	45	33	27	23	39	18
	Oxalate	166	141	173	121	242	478
	Alkali	102	86	129	152	60	38
	Total	313	260	329	296	341	534

Water: LSD (cv) = 2; Oxalate: LSD (cv) = 7; Alkali: LSD (cv) = 3; LSD (s) = 3; LSD (s)= 10; LSD (s) = 4.

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