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Determination of Polygalacturonic Acid Content in Sweet Cherry (0900 Ziraat) Extracts by Fourier Transform Infrared Spectroscopy and Effects of $CaCl_2$ and $Ca(NO_3)_2$ Foliar and Fruit Sprays Appications on Polygalacturonic Acid

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Polygalacturonic acid (PGA) calibration standards were prepared by being mixed polygalacturonic acid with potassium bromide to cover a range of acid concentrations (10-98 %). A linear relationship between polygalacturonic acid content and carbonyl absorption band area was found ($R^2 = 0.982$). Polygalacturonic acid contents of 0900 Ziraat sweet cherry fruits were calculated from the linear fit equation. For fourteen different sources (1-14) of cherries, the polygalacturonic acid contents determined by FT-IR spectra were 16.68, 15.80, 65.56, 69.21, 78.64, 74.10, 83.66, 91.10, 53.46, 61.32, 48.19, 43.99, 39.62, 36.65 %, respectively. Foliar and fruits of cherry trees were sprayed with containing calcium chloride and calcium nitrate. Polygalacturonic acid content was affected by $CaCl_2$ and $Ca(NO_3)_2$ applications and subsequently increased an average of 474 % with $CaCl_2$ and 290 % with $Ca(NO_3)_2$ compared with the control.

Keywords: Polygalacturonic acid, Sweet Cherry, Calcium chloride, Calcium nitrate.

INTRODUCTION

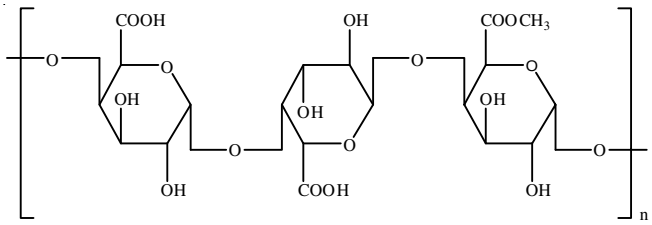
Pectin is composed of polymers of galacturonic acid[1,2]. During senescence polygalacturonic acid can be degraded by enzymes that fragment large pectin polymers. In order to monitor pectin breakdown the uronic acid content was monitored during storage. A high level of polygalacturonic acid is an indication that pectin polymers are being broken down into smaller fragments. This fragmentation of high molecular weight polygalacturonic polymers leads to fruit softening. The textures of fruits and vegetables and hence processing characteristic, are largely influenced by the pectin content. Many food processors and pectin ingredient suppliers need to determine pectin content to control the quality of their products[3]. The pectin content in food products content was determinate as polygalacturonic acid by chemical and instrumental methods[4-7].

FT-IR spectra provides qualitative information on functional groups and may be used to identify and quantify specific compounds. Diffuse reflectance FT-IR spectra (DRIFTS) has been used to determine the degree of pectin esterification[8]. Researchers found that absorbance of the COO-R groups increased with increase in degree of esterification and the band area was linearly related to degree of esterification. The bands were observed at 1760-1745 and 1640-1620 cm^{-1} for COO-R and COO^- groups, respectively[9]. Diffuse reflectance FT-IR spectra (DRIFTS) was developed for pectin measurement[3].

Fruit quality maintenance is strongly influenced by preharvest environmental factors. Preharvest nutritional status of fruit, especially in respect to calcium, is an important factor affecting potential storage life[10]. Calcium plays a very important role in the structure of the cell wall, thus influencing the firmness of fruit tissues[11]. Fruits with a high level of calcium have lower respiration rate and longer potential storage life than fruits containing low calcium. Many physiological disorders in fruits are associated with calcium deficiency. The calcium level of fruit is raised by a foliar spray. Whereas, in many cases, it is very difficult to achieve because of the restricted uptake and penetration of calcium into the fruit and its movement within fruit tissue[12]. In this study, the analysis of polygalacturonic acid content, and acid content exchange by $CaCl_2$ and $Ca(NO_3)_2$ applications of 0900 Ziraat sweet cherry are reported. The polygalacturonic acid (PGA) in the cherry fruit are determined by FT-IR spectroscopy (**Scheme-I**).

EXPERIMENTAL

The infrared absorption spectra were obtained from a Perkin Elmer BX II spectrometer in KBr discs and were reported in cm^{-1} units. Spectra were collected by co-adding 32 scans at a resolution of 0.5 cm^{-1} in 4000-400 cm^{-1} range. KBr



Scheme-I: Chemical structure of polygalacturonic acid (PGA)

and polygalacturonic acid were purchased from Sigma-Aldrich (Steinheim, Germany). They were used as received. High-purity water from a Millipore Simplicity 185 water purification system (Millipore Iberian S.A., Madrid, Spain) was used for all chemical analyses and glassware washing.

A set of 10 calibration polygalacturonic acid standards was prepared by blending polygalacturonic acid with KBr to obtain acid standards with polygalacturonic acid content of 10, 20, 30, 40, 50, 60, 70, 80 and 98 %, respectively. Pectin (PGA) was extracted by literature method[13] from cherry samples (**1-14**). FT-IR spectra and total carbonyl absorption peak area at 1740-1635 cm^{-1}, from free COO- and esterified COO-R groups of polygalacturonic acid samples were obtained using the method described for polygalacturonic acid standards. The polygalacturonic acid contents of 0900 Ziraat sweet cherry samples were calculated from the line fit equation developed from FT-IR spectra.

Cherry fruit were harvested on fourteen different trees from the Çanakkale-Lapseki in Sahinli village in Turkey. $CaCl_2$ and $Ca(NO_3)_2$ were applied four times. The first application was at the March 6, 2010 and the second and the third and the fourth applications were conducted at 1 week intervals. The following treatments were used in the experiment: 1. Control: Trees sprayed with water. 2. Trees sprayed with $CaCl_2$: 2.4, 3.2 and 4 g/L. 3. Trees sprayed with $Ca(NO_3)_2$: 3.4, 4.6 and 5.8 g/L.

RESULTS AND DISCUSSION

The polygalacturonic acid (PGA) in pectin standards is measured by diffuse reflectance FT-IR spectra. It can be used as an alternative method for determining pectin content in commercial pectin samples and pectin extracts. There were no significant differences in the values obtained from the FT-IR and HPLC methods[3]. The FT-IR method favored due to the rapid and cost-effective. The FT-IR spectra of polygalacturonic acid standards are given in Fig. 1. The bands in the 2000-1000 cm^{-1} region are independent of pectin source and may be used to identify galacturonic acid[14,15]. The broad and strong vibration band of the wave numbers of 3450-3435 cm^{-1} was from O-H stretching due to C-OH and adsorbed moisture in the polygalacturonic acid samples. The C-H absorption bands of methyl group of the methyl ester and C-H in the ring structure polygalacturonic acid are observed at 2942-2920 cm^{-1}. The stretching frequency observed at 2758-2650 cm^{-1} in samples shows the presence of O-H....O intramolecular hydrogen bond[16,17]. The strong absorption band between 1234-1100 was from ethereal C-O-C (Csp^3-O-Csp^3) bond the ring structure polygalacturonic acid. The carbonyl absorption bands are observed at 1740-1735 and 1647-1635 cm^{-1} for $COOCH_3$ and COOH carboxyl groups, respectively. It was observed that the total carbonyl absorption area increased as the polygalacturonic acid content increased (Fig. 1). The carbonyl absorption area of the polygalacturonic acid standards is given Table-1. The linear relationship between polygalacturonic acid content and carbonyl absorption band area revealed a high correlation (R^2 = 0.986) (Fig. 2). The fitted model was given by means of the equation: Y = 17.40 + 0.696X (Y is FT-IR peak area and X is polygalacturonic acid content). Student's *t*-test was used to analyze data. LSD values were used to differentiate mean values and significance, as defined $p < 0.05$. The polygalacturonic acid content in the sweet cherry samples was calculated using the equation (Y= 17.40 + 0.696X).

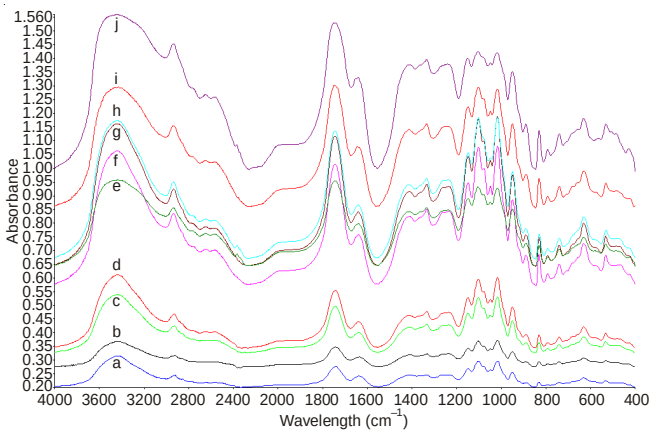


Fig. 1. FTIR spectra of the 4000-400 cm^{-1} region of polygalacturonic acid standards diluted with KBr : (a) 10, (b) 20, (c) 30, (d) 40, (e) 50, (f) 60, (g) 70, (h) 80, (i) 90, (j) 98 %

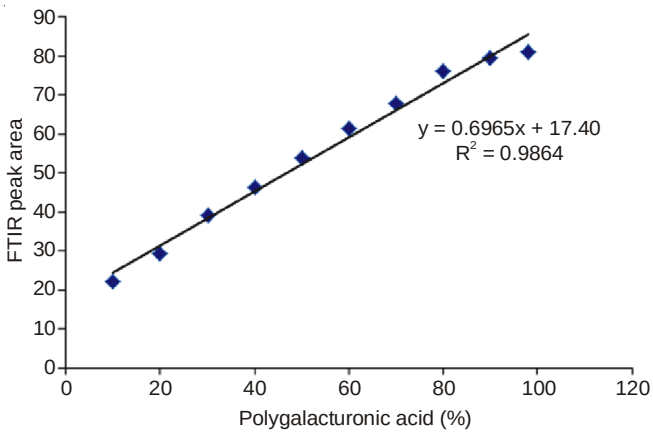


Fig. 2. Regression line of polygalacturonic acid(PGA) content of standards

TABLE-1
THE CARBONYL ABSORPTION AREA OF THE POLYGALACTURONIC ACID (PGE) STANDARDS

Polygalacturonic acid (%)	FTIR carbonyl peak area
10	22.20
20	29.10
30	39.25
40	46.14
50	53.95
60	61.33
70	67.56
80	76.13
90	79.28
98	80.81

The FT-IR spectra of cherry fruit samples compound **1-14** are given in Fig. 3. The calculated polygalacturonic acid (PGA) content is given in Table-2. As clear from Table-2, the samples **7-8** have more polygalacturonic acid than other samples. The total polygalacturonic acid was lowest in samples **1** and **14**. In the literature, the total polygalacturonic acid was reported to be 54.98-88.3, 60.8 and 76.4 % for soy hull, apple and lemon, respectively[9,18]. The amount of polygalacturonic acid found range from 60 to 90 mg/g db in Navel peel[13]. The authors reported that mango peels (49.4 ± 0.5 mg PGA/L juice) are rich in pectins[19,20]. Based on these results, polygalacturonic acid (PGA) contents of sweet cherry samples **3-10** were similar to soy hull, apple and lemon.

TABLE-2
THE CALCULATED POLYGALACTURONIC ACID (PGE) CONTENT OF THE CHERRY SAMPLES

Cherry samples[a]	Polygalacturonic acid (%)	Cherry samples[a]	Polygalacturonic acid (%)
1	16.68	**8**	91.10
2	15.80	**9**	53.46
3	65.56	**10**	61.32
4	69.21	**11**	48.19
5	78.64	**12**	43.99
6	74.10	**13**	39.62
7	83.66	**14**	36.65

[a]Foliar and fruit of cherry trees were sprayed with containing calcium chloride and calcium nitrate. **1-2**: Control, samples **3-4**: 2.4, samples **5-6**: 3.2, samples **7-8**: 4 g/L $CaCl_2$; samples **9-10**: 3.4, samples **11-12**: 4.6, samples **13-14**: 5.8 g/L $Ca(NO_3)_2$)

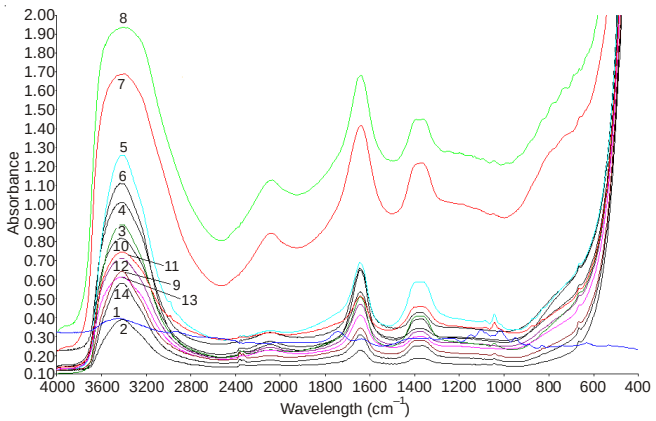


Fig. 3. FTIR spectra of the cherry samples sprayed with $CaCl_2$ and $Ca(NO_3)_2$ (1-2: Control, Samples **3-4**: 2.4, **5-6**: 3.2, **7-8**: 4 g/L $CaCl_2$; Samples **9-10**: 3.4, **11-12**: 4.6, **13-14**: 5.8 g/L $Ca(NO_3)_2$)

Fruit softening during ripening is brought about by a disassembly of the fruit cell wall, the major changes including depolymerization and solubilization of pectins, depolymerization of matrix glycans (hemicelluloses) and loss of pectic galactose side chains[21]. The cell wall-modifying proteins that bring about changes in cell wall structure and its component polysaccharides have not all been identified, but transgenic suppression of ripening-related enzymes involved in pectin metabolism resulted in improvements in various aspects of quality. Transgenic suppression of pectin methylesterase activity had little effect on fruit softening during ripening, but processed juice and paste showed higher soluble solids content and increased viscosity[22-24]. Thus, increased fruit quality can result from reduced cell wall breakdown during ripening, not necessarily an improvement in firmness but an improvement in fruit integrity and in the properties of processed fruit products.

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