

## Total Oxalate-Soluble Pectin Concentration in Asian Pear (*Pyrus serotina* Rehd) Fruit in Relation to Ripening, Storage and Internal Browning Disorder

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### ABSTRACT

Oxalate Soluble Pectin (OSP) is related to fruit firmness with respect to the chelation of calcium ions with carboxyl groups of adjacent polyuronide chains. This study was carried out to explore the effect of time of fruit harvest, foliar spray with CaCl<sub>2</sub> during growing season on OSP, Polygalacturonase Activity (PGA), fruit firmness and the effects on fruit shelf life, quality and Internal Browning (IB) disorder. Fruit samples were collected from Asian pear (*Pyrus serotina* Rehd.) trees 'KS', and 'KS'<sub>13</sub> on European pear (*Pyrus communis* L.) seedling rootstocks. Fruits were picked on the 1<sup>st</sup> and 15<sup>th</sup> August, 2006 and stored at 1°C and 80-85% relative humidity (RH). Five stages of extraction were performed on fruit samples including one pre-harvest, one at the time of harvest, and three following fruit harvest. Results indicated that OSP increased slightly during fruit ripening as well as during storage. In both the studied cultivars, the level of OSP in fruit harvested fifteen days after Optimum Time for Harvest (OTH) was higher than that harvested at OTH. The level of OSP in fruit softened after harvest significantly increased starting from the time of harvest. The relationship between flesh firmness, IB and OSP concentration showed the highest correlations in the both of the studied cultivars among PGA. In conclusion, flesh firmness and IB were correlated with the extent of OSP concentration. In addition, fruit, sprayed with CaCl<sub>2</sub> and harvested early, contained less OSP, PGA and IB following long-term storage.

**Keywords:** Firmness, Polygalacturonase activity, Softening, Soluble solid concentration, Storage, Titratable acids.

### INTRODUCTION

One of the major disorders that develop during storage in pear, including Asian pears is internal browning (IB) of the flesh around core regions. It has been reported that mature fruits are more susceptible to senescence IB than immature ones. A determination of the optimum harvest time is an important task for preservation of the quality of Asian pear during the storage

period. European and Asian pears that are picked either before or after the optimum harvest dates develop an inferior taste and are more sensitive to IB disorder, never developing the characteristics such as juicy texture of ripe fruit (Crisosto *et al.*, 1994; Murayama *et al.*, 1998; Arzani *et al.*, 2008; 2009).

Pectin is one of the major components of the primary cell wall and of middle lamella in plant tissues. Pectic matrix provides an

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environment for the deposition, extension of the cellulosic-glycan network and is the major adhesive material among cells (Willats *et al.*, 2001). Pectin degradation plays an important role in fruit ripening, which leads to destruction of cellulose and hemicellulose and is accompanied by a decrease in fruit firmness (Duan *et al.*, 2008). It has been reported that secretions of pectin-degrading enzymes, for example, endo- and exo-polygalacturonase accelerate scab and IB disorder in Asian pear (Faize *et al.*, 2003). It seems that Asian and European pear fruit softening is related to changes in cell wall structure. These changes are related to an increase in soluble polyuronides accompanied by a decrease in insoluble polyuronides (Murayama *et al.*, 2002). It has been reported that chelate-soluble pectin and sodium carbonate-soluble pectin are both closely related to fruit texture (Yang *et al.*, 2006). Although, chelate-soluble pectin is much connected with the textural difference among the different fruit stages of the same cultivar, sodium carbonate-soluble pectin is more related to the textural difference among different fruit cultivars (Yang *et al.*, 2009).

Quality Asian pears develop a buttery and juicy texture, associated with a reduction in extractable juice. In addition, the chelation of calcium ions with carboxyl groups of adjacent polyuronide chains is a key factor of fruit firmness with calcium deposition occurring in the junction zones in the middle lamella (Knee, 1993). Any increase in free pectin carboxyl groups might be expected to enhance the importance of calcium as a firmness-increasing agent (Villalobos and Mitcham, 2008).

Such calcium-binding agents as solutions of oxalate, Ethylene Diaminetetra Acetic Acid (EDTA) or polyphosphate have been employed to extract pectic acid from fruits (Pilnik and Voragen, 1971). For example, solutions of oxalic acid and ammonium oxalate are used to extract pectic acid in the form of oxalate-soluble pectin. Oxalate-soluble pectin exists as pectic acid that can bind calcium and form a cross-link structure

(Yu *et al.*, 1996; Aileen *et al.*, 2008; Yadav *et al.*, 2009). Wide variations in extraction times (10 min to 24 hours) and temperatures (0°C to boiling point) have been employed (Pilnik and Voragen, 1971). Oxalate-soluble pectin has been extracted from plant tissue (Aileen *et al.*, 2008), and residue obtained from water-soluble pectin extracts of dry cell wall (Bouranis and Nivavis, 1992), and alcohol-insoluble solids of fruits and vegetables (Ketsa *et al.*, 1999; Panmanas *et al.*, 2001; Yadav *et al.*, 2009) under different conditions. These have caused the difference or misinterpretation of the data such as different correlation between the pectin concentration and firmness of the fruit, the contrasting comparison of solubilization of different kinds of pectin. Moreover, the time required to completely extract pectin was considerably long and might be unattainable.

The objective of the present research was to evaluate the effect of storage durations,  $\text{CaCl}_2$  application and duration of postharvest on OSP concentration, pectin-degrading enzyme (polygalacturonase), internal browning (IB), firmness and the other related quality attributes of 'KS' and 'KS'<sub>13</sub> Asian pears.

## MATERIALS AND METHODS

### Samples

Fruit samples were collected from Asian pear (*Pyrus serotina* Rehd.) trees 'KS' and 'KS'<sub>13</sub> on European pear (*Pyrus communis* L.) seedling rootstocks, at the Research Orchard, Department of Horticultural Science, Tarbiat Modares University (TMU), Tehran, Iran. Preharvest foliar spray with calcium chloride ( $\text{CaCl}_2$ ) at the concentration of 5 g L<sup>-1</sup> was applied by 2 week intervals from 30 days after full bloom (DAFB) until the time of fruit harvest. Asian pear fruits were picked on the 1<sup>st</sup> and 15<sup>th</sup> August, 2006 and stored at 1°C plus 80-85% relative humidity (RH). Samples were taken from 3 fruits and combined to form the substance for each replication.

### Oxalate-Soluble Pectin (OSP) Extraction

Oxalate-soluble pectin (OSP) extraction was performed according to the method described by Panmanas *et al.* (2001). OSP was extracted from the alcohol-insoluble solids (AIS). The residue was Water Soluble Pectin (WSP) extract, so it was stirred in a solution of 0.25% ammonium oxalate and 0.25% oxalic acid for 1 minute. The OSP was extracted using a hot water bath at  $95\pm 2^{\circ}\text{C}$ . The extraction times employed consisted of: 1 to 15 hours. (Figure 1). Three fruits (at each picking date) were used for each sample extract. OSP concentration was assessed and expressed as pectin concentration in AIS ( $\mu\text{g mg}^{-1}\text{AIS}$ ).

### Internal Browning (IB) Evaluation in the Fruit

Internal browning (IB) in the fruit, including flesh and heart browning was assessed by counting the number of damaged fruits and expressed as percentage.

In addition, in order to determine the intensity of IB incidence according to the percentage of brownish area, affected fruit was cut into four equal longitudinal parts and scored as incidence intensity using the scale of 0 to 5. The nominated scores were: (0) without visible IB damage; (1) slightly visible IB damage with 1 to 10% of the area showing browning; (2) moderately visible IB damage with 11 to 33% of the area showing browning; (3) severe injury with 34 to 66% browning and (5) extreme injury with 67 to 100% browning (Ju *et al.*, 2000).

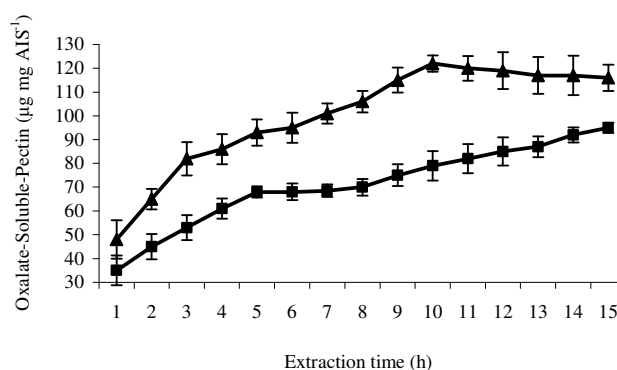
### Determination of Polygalacturonase (PG) Activity

Polygalacturonase (PG) activity was assessed through 2-cyanoacetamide spectrophotometric method (Gross, 1982). Fruit tissue (150 g) was placed in 300 ml of distilled water and homogenized in a Waring

blender for 1 min. The homogenate was filtered through 6 layer cheesecloth and the residue resuspended in 300 ml of distilled water. The suspension was filtered and the residue resuspended in 150 ml of 1M NaCl. The acidity (pH) was adjusted to and maintained at 6.0 with 1N NaOH while the slurry slowly stirred at  $4^{\circ}\text{C}$ . After 3 hours the slurry was filtered through cheesecloth and the filtrate centrifuged at  $9000\times g$  for 15 minutes. Supernatant (1.5 ml) was de-salted on a sephadex G-25 column (10 ml bed volume) which was equilibrated in 50 mM Na-acetate (pH 4.4). The de-salted extract was used for polygalacturonase assay. The extraction procedures were carried out at  $4^{\circ}\text{C}$ . The assay of polygalacturonase activity was based on the hydrolytic release of reducing groups of galacturonic from polygalacturonic acid. Reaction mixture (0.2 ml of total volume) containing 37.5 mM Na-acetate (pH 4.4), 0.2% polygalacturonic acid (washed with 80% ethanol prior to use), and 50  $\mu\text{l}$  of enzyme extract were incubated at  $37^{\circ}\text{C}$  for up to 3 hours. For quantifying the released reducing groups with 2-cyanoacetamide, reactions terminated with 1 ml of cold 100 mM of borate buffer (pH 9). Then 0.2 ml of 1% 2-cyanoacetamide were added, and the samples were mixed and immersed in boiling water bath for 10 minutes. After equilibration to  $25^{\circ}\text{C}$  the absorbance at 274 nm was determined using UNICAM 8620 UV-Vis spectrophotometer. A unit of PG activity is defined as the level of enzyme that can release 1 nmol (galacturonic acid) of reducing groups  $\text{h}^{-1}$  at pH 4.4 and at  $37^{\circ}\text{C}$ .

### Fruit Firmness, Soluble Solids Concentration (SSC) and Titratable Acids (TA) Measurements

Fruit firmness, SSC, and TA were assessed both at the time of fruit harvest and after cold storage (at weekly intervals). Firmness was evaluated through a penetrometer (CNS FARNELL) equipped with a 12 mm plunger at two locations per



**Figure 1.** Extraction curve of oxalate-soluble pectin concentration of Asian pear ('KS'<sub>13</sub>) extracted through hot water (95±2°C). Data are the means (3 fruits) with the error bar indicating the standard error.

fruit. Soluble solid content (SSC) was measured by a hand refractometer (Atago, NSG Precision Cells, Inc., Hicksville, USA). TA was determined by titrating 10 ml of fruit juice to pH 8.3 using 0.1N NaOH and calculated as mmol l<sup>-1</sup> (Chen and Mellenthin, 1981).

### Statistical Analysis

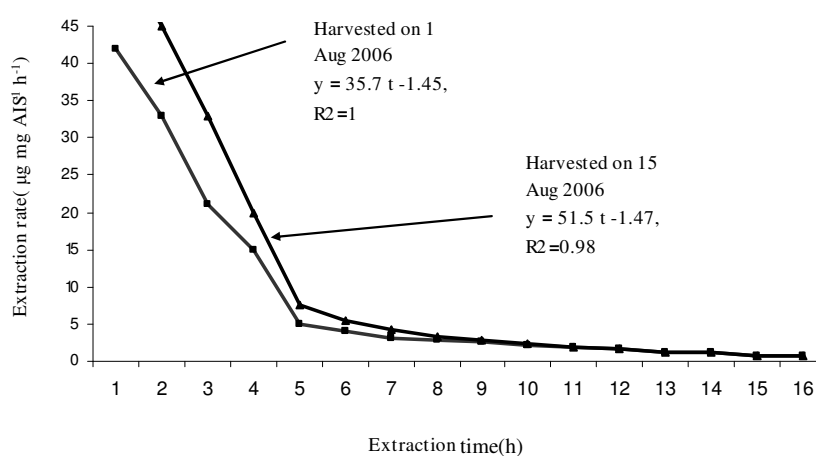
The results were statistically evaluated through analysis of variance (ANOVA) and expressed as mean±standard error (SE). Mean comparisons were made using Duncan's multiple range test (DMRT);

differences were considered statistically significant at  $P \leq 0.05$ . The REG procedure (SAS Institute) was used for multiple regression and correlation analysis.

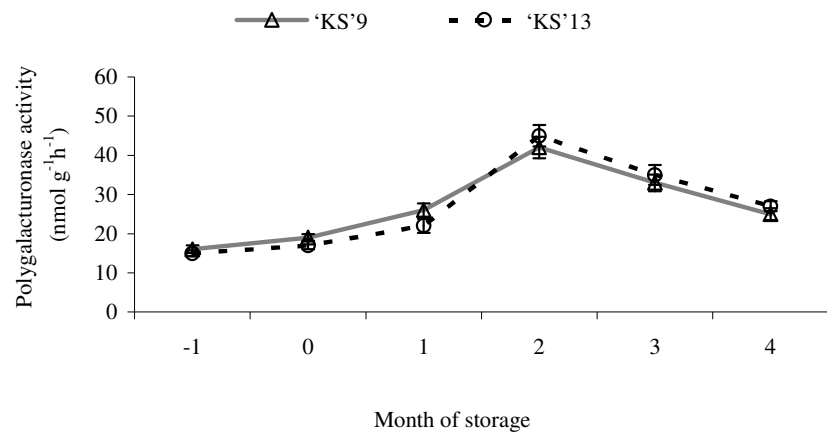
## RESULTES AND DISCUSSION

### Changes of OSP and Fruit Firmness

The changes in OSP from the fruit harvested at different stages are shown in Figure 2. The trend of extraction rates, changed with extraction time is shown in Figure 3. In the case of Asian pear, the power equation pattern best fitted the



**Figure 2.** Extraction rate of oxalate-soluble pectin of Asian pear ('KS'<sub>13</sub>) extracted through hot water (95±2°C).



**Figure 3.** Polygalacturonase activity in Asian pear cv. 'KS'9 and 'KS'13 during preharvest, at harvest and during postharvest: -1 (1 month before harvest) and 0 (harvest time, 1st Aug. 2006).

extraction rates curve. The power equation is defined by  $y = at^b$ , where  $y$  is the extraction rate,  $t$  the extraction time, with  $a$  and  $b$  as constants. Though, the OSP concentration extracted increased considerably with extraction time and picking dates. The OSP extraction rate was highly affected by extraction time, although the picking dates did not affect the extraction rates. These data indicate that the AIS of the fruit at any developmental stage could be used as a determination of half-extraction time. Panmanas *et al.* (2001) reported that to obtain the total OSP concentration, the extraction could be done until half-

extraction time was reached. To get the total concentration, the OSP concentration at half-extraction time needs to be simply multiplied by two. According to the present data, the half-extraction time of Asian pear was 2.5 hours while total OSP concentrations were 115.62 ('KS'9) and 106.56  $\mu\text{g mg}^{-1}\text{AIS}$  ('KS'13) for the fruits harvested on 1<sup>st</sup> August 2006 and 123.26 ('KS'9) and 115.42  $\mu\text{g mg}^{-1}\text{AIS}$  ('KS'13) for the fruits harvested on 15<sup>th</sup> August 2006 respectively (Table 1). In both cultivars, late-harvested fruits on (15<sup>th</sup> August) contained more OSP than the early-harvested (1<sup>st</sup> August) fruit. In fruits picked

**Table 1.** Extracted total oxalate-soluble pectin concentration at different extraction times (preharvest, harvest and postharvest) of two Asian pear 'KS'9 and 'KS'13 fruits.

Stage of storage	Total oxalate-soluble pectin ( $\mu\text{g mg}^{-1}\text{AIS}$ )			
	'KS'9		'KS'13	
	1 August	15 August	1 August	15 August
1 (1 month before HT <sup>a</sup> )	96.32d*	105.15e	87.52d	98.30d
2 (HT)	Control (without IB incidence)	115.62c	123.26c	106.56c
	CaCl <sub>2</sub> foliar spray	102.50	109.70	101.20
	Control (11–33% IB <sup>b</sup> )	146.34	154.21	139.50
	Control (34–66% IB)	142.60	139.74	136.20
3 (1 month after HT)	135.36a	142.30a	132.94a	138.70a
4 (2 months after HT)	126.84b	129.54b	123.61b	131.00b
5 (3 months after HT)	119.20c	113.70d	122.30b	118.64c

<sup>a</sup> Harvest Time; <sup>b</sup> Internal Browning.

\* Mean separation with columns by Duncan's multiple range tests at 1% level.



from trees that were sprayed with  $\text{CaCl}_2$ , OSP was lower than those picked from non sprayed trees in both of the studied cultivars, so such fruits exhibited higher firmness during the storage period. In addition, it was found that the low damaged fruits (11–33% IB) had a more OSP content than the sound fruits and than fruits with a high intensity of IB incidence (more than 33% IB) (Table 1).

Fruits in both cultivars softened during storage. A relatively high level of firmness was observed in 'KS'<sub>13</sub> cultivar after a long-term storage. Also, 'KS'<sub>9</sub> fruit softened faster than fruits of 'KS'<sub>13</sub> cultivar fruit (Table 3). Fruits all softened during ripening, independent of cultivar, harvest time and storage period. The rate of softening was dependent on harvest date as well as on cultivar. In both of the studied cultivars, the later harvested fruits (15<sup>th</sup> August) suffered from a lower firmness than the early-harvested (1<sup>st</sup> August) ones and tended to soften faster. There was a relationship observed between flesh firmness and the extent of OSP in fruits before and after storage in both of the studied cultivars ( $R^2 = 0.98$ ). For OSP concentration in Asian pears, lower flesh firmness correlated with higher levels of OSP after short-term storage (before 3 months of storage) and correlated with lower degree of OSP following long-term storage

(after 3 months of storage) (data not shown). After short-term storage, fruit softened and reached a buttery and juicy texture during ripening in both cultivars and was not buttery along with a juicy when ripened at 20°C after long-term storage.

As shown in Table 1, 2, and 3 and as well as in Figure 2, in both of the studied cultivars, the later harvested fruit tended to soften faster. There was a relationship observed between flesh firmness and the extent of OSP in fruit before and following storage in both the studied cultivars. It has been reported that in pears, the level of OSP generally increases during normal ripening (Yoshioka *et al.*, 1992; Murayama *et al.*, 1998). Ahmed and Labavitch (1980) reported that galacturonic acid in the 80% ethanol perceptible portion of the supernatant fraction of pear fruit homogenate actually increased during fruit softening. Kim *et al.* (1991) also reported that a steady decline in cell wall galactosyl residues was accompanied by an increase in soluble galactose during tomato fruit ripening. In any case, OSP concentration increased when fruits were transferred to 20°C following a short-term storage. After long-term storage, fruits (in both cultivars) also softened during ripening. It has been reported that (Duan *et al.*, 2008) fruit firmness of banana decreased rapidly from

**Table 2.** Polygalacturonase activity (PGA) in Asian pear cv. 'KS'<sub>9</sub> and 'KS'<sub>13</sub> in preharvest, harvest and postharvest duration and the effect of  $\text{CaCl}_2$  foliar spray and internal browning on PGA.

Stage of storage	PGA in 'KS' <sub>9</sub> (nmol g <sup>-1</sup> h <sup>-1</sup> )		PGA in 'KS' <sub>13</sub> (nmol g <sup>-1</sup> h <sup>-1</sup> )	
	Harvest Time		Harvest Time	
	1 August	15 August	1 August	15 August
1 (1 month before HT <sup>a</sup> )	16±1e*	21±1.4e	15±0.7e	19±0.7e
Control (without IB incidence)	19±0.8d	24±1.6d	17±1e	22±0.9d
0	17	21	15	18
(HT) Control (11–33% IB <sup>b</sup> )	26	25	24	22
Control (34–66% IB)	24	23	20	18
1 (1 month after HT)	26±1.7c	33±1.9c	22±1.8d	28±0.9c
2 (2 months after HT)	42±2.7a	39±1.9a	45±2.8a	47±1.8a
3 (3 months after HT)	33±2.1b	35±1.5b	35±2.6b	35±1.7b
4 (4 months after HT)	25±1.2c	24±1.1d	27±1.3c	23±1.2d

<sup>a</sup> Harvest Time; <sup>b</sup> Internal Browning.

\* Mean separation with columns by Duncan's multiple range tests at 1% level.

**Table 3.** Changes of fruit physicochemical properties of two Asian ('KS' 9 and 'KS' 13) pear cultivars during five months of storage.

Cultivar	Properties	Harvest time	Time (Month of storage)					Significance		
			0 <sup>a</sup>	1	2	3	4	5	Cultivar	Cultivar × Time of storage
'KS' 9	Firmness (N)	1Aug	44±1.2a <sup>b</sup>	40±1.8ab	35±1.5b	31±2.0bc	26±1.4c	23±0.8d	*	**
		15Aug	37±0.5a	35±2.1a	32±1.9ab	25±2.0b	23±1.4b	18±0.8c	NS	**
	SSC (°Brix)	1Aug	13.2±0.5a	14.4±0.4a	13.3±0.7ab	12.4±0.6b	11.5±0.4c	9.8±0.3d	NS	**
		15Aug	14.1±0.3ab	15.2±0.2a	12.6±0.5b	11.1±0.4b	10.2±0.3c	8.2±0.3c	NS	**
	TA (mg.100g <sup>-1</sup> FW)	1Aug	33±0.6a	24±0.7b	21±0.5c	19±0.6c	17±0.3d	16±0.6d	*	**
		15Aug	25±0.3a	18±0.5b	16±0.7bc	14±0.6c	14±0.4c	14±0.4c	*	*
'KS' 13	Firmness (N)	1Aug	46±2.3a	42±1.7ab	37±0.9b	33±1.4c	31±1.2c	27±0.6d	*	**
		15Aug	34±1.2a	28±0.9ab	27±0.9ab	24±1.1b	23±1.4b	20±0.9c	NS	**
	SSC (°Brix)	1Aug	14.5±0.5a	15.8±0.5a	14.5±0.7ab	13.0±0.6b	11.7±0.3c	10.8±0.4c	NS	**
		15Aug	15.1±0.3ab	17.2±0.5a	14.3±0.5ab	11.1±0.3b	10.40±0.4c	9.70±0.4c	NS	**
	TA (mg.100g <sup>-1</sup> FW)	1Aug	28±0.7a	24±0.7b	22±0.6c	21±0.5c	19±0.5c	16±0.6d	**	**
		15Aug	22±0.4a	20±0.6ab	19±0.3ab	18±0.8b	15±0.5c	13±0.9d	*	*

<sup>a</sup> Time of fruit harvest.

<sup>b</sup> Mean separation within columns by Duncan's multiple range tests at  $P \leq 0.01$ . Mean values±standard errors. NS, \* and \*\* ; Nonsignificant or significant at  $P \leq 0.05$  or 0.01, respectively.



the initial 11.6 to 1.8 N after 20 days of storage at 25°C.

Fruit softening is generally attributed to cellular wall structure, particularly due to solubilisation of pectin (Lohani *et al.*, 2004). As shown in Table 1, OSP content increased significantly from fruit ripening (stages 1 to 3), indicating that solubilisation might occur. Also a number of reports indicate the conversion of water-insoluble to water-soluble pectin (Majumder and Mazumdar, 2002; Manrique and Lajolo, 2004; Yashoda *et al.*, 2005), similar to the present research results during fruit ripening.

Wang *et al.* (1985) reported that 'Eldorado' pears usually remained firm and dry when transferred to 20°C after 36 weeks of storage at 0°C. Chen and Borgic (1985), reported that pear fruits did not ripen normally, never developing the buttery and juicy texture of ripe fruit with the OSP concentration of the fruit not changing, during 9 days of ripening after fruits were transferred to 20°C environment after prolonged storage at -1 or 0°C. Nevertheless, changes in OSP and softening during ripening and storage in the present research are in agreement with results from other works on pears (Ben-Arie and Sonego, 1979; Yoshioka *et al.*, 1992).

### Changes of Polygalacturonase Activity

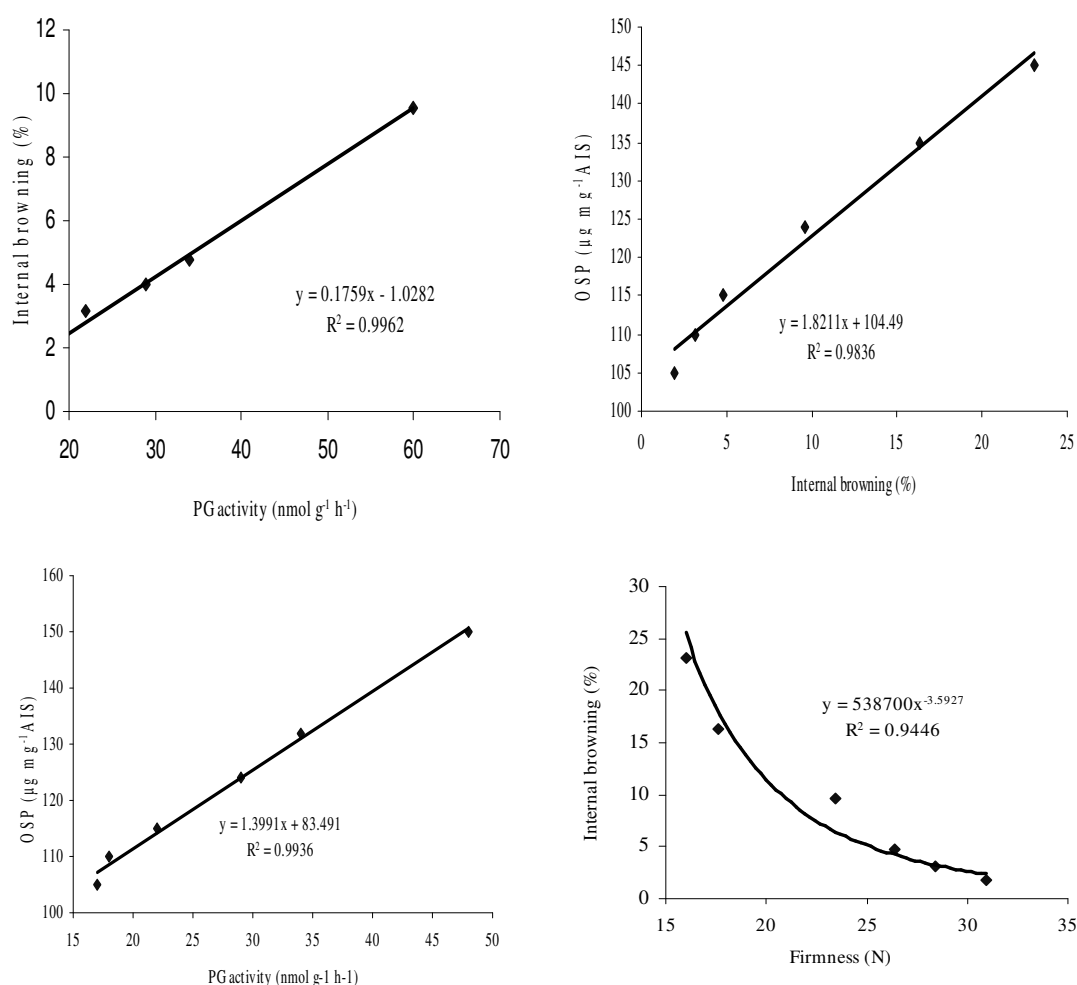
Figure 3 and Table 2 show the changes in polygalacturonase (PG) activity during storage in two Asian pear cultivars determined by measuring the release of reducing sugars from polygalacturonic acid. In both the studied cultivars, PG activity increased from 1 month before to 2 months after harvest then decreased slightly in the early (1<sup>st</sup> August) and the late (15<sup>th</sup> August) harvested fruits. There was a relationship observed between OSP and polygalacturonase activity (PGA). For OSP concentration, higher OSP correlated with higher PG activity in Asian pears before and after storage (Figure 4). The degradation of pectic polysaccharides well corresponded

with softening behaviors in the two Asian pears. Solubilization of pectic polysaccharides was observed in 'KS'<sub>9</sub> and 'KS'<sub>13</sub> Asian pears before 1 to 2 months after harvest (Table 1).

Involvement of polygalacturonase (PG) and/or Pectin Methyl Esterase (PME) in enzymatic disassembly of pectin polysaccharides, during fruit ripening, has been extensively investigated (Nikolic and Mojovic, 2007; Prasanna *et al.*, 2007). Polygalacturonase and PME cooperatively regulate the pectin disassembly. Polygalacturonase hydrolyses α-1, 4-linked D-galacturonic acid, following de-esterification of pectin through PME. In addition, Pectate Lyase (PL) (Payasi *et al.*, 2006) and β-galactosidase (Lazan *et al.*, 2004) may play a combined effect on the disassembly of pectin polysaccharides during fruit ripening/softening. Non-enzymatic action might mediate in the polysaccharide solubilisation in plant tissues (Dumville and Fry, 2003). It has been reported that hydroxyl radical (-OH) was capable of cleaving polysaccharides present in cellular walls in a site-specific reaction, which led to cellular wall loosening and elongation of living coleoptile or hypocotyls in maize and in soybean. Enzymatic and non-enzymatic effects on pectin degradation are associated with modification of wall structure, including the modification in the composition, molecular weight, and structural characteristics of the pectin (Schweikert *et al.*, 2002).

There are some reports on modification of cellular wall polysaccharides in apple (Scalzo, *et al.*, 2005), papaya (Manrique and Lajolo, 2004) and other fruits (Sakurai and Nevins, 1997; Rose *et al.*, 1998). However, variations in cell wall compositions could lead to differences in the softening-associated chemical modification for each fruit species. Huber and O'Donoghue (1993) have demonstrated that depolymerization of pectic polysaccharides in avocado is more extensive than that in tomato fruits, and correlates with the differences in flesh texture between these fruits. Similar





**Figure 4.** The relationship between PG (Polygalacturonase) activity, OSP (Oxalate Soluble Pectin), IB (Internal Browning) and firmness of 'KS'₉ and 'KS'₁₃ Asian pears during storage.

relationships between modes of pectin degradation and particular textural properties have also been revealed in other species (Yoshioka *et al.*, 1992; Redgwell *et al.*, 1997). These findings may suggest that the mode of pectin disassembly is one of the important factors determining fruit firmness and texture. Endo-PG and Exo-PG act on pectin-degrading enzymes for adjacent polyuronide chains that are a key factor for fruit firmness (Knee, 1993). In the present research, as with the rate of softening, the rate of increase in PG activity was greater in late-harvested than in the early-harvested fruits. PG activity determined through

reducing sugar assay increased with ripening in the two Asian pears. Endo-PG activity has been identified in a number of ripening fruits and has been shown to correlate with an increase in soluble pectin during ripening (Hadfield and Bennett, 1998).

The present study demonstrates that PG can lead to significant solubilization of pectic polymers leading to an increase in OSP concentration. The diffusion freedom or mobility of wall-associated proteins has also been implicated as a means of regulating the activity of PG being released during the *in vitro* catalysis (Chun and Huber, 1997), with sustained pectin



solubilization requiring continued provision of fresh enzyme (Rushing and Huber, 1990), consistent with a relatively immobile status of the enzyme. Also The parallel interruption of PG deposition and pectin solubilization in silver-thiosulfate-treated tomato fruit reported Smith *et al.*, 1989.

The biochemical bases for ripening and senescence associated with fruit softening are not yet fully understood. However, dissolution of the middle lamella and cell wall separation due to depolymerization and solubilization of pectin by PG as well as a loss of  $\text{Ca}^{2+}$  have been suggested to contribute to fruit softening (Fischer and Bennett, 1991). The role that calcium plays in increasing cell rigidity has been related to its ability to bind with mid lamella pectate (Moraga *et al.*, 2009). The firmness of the flesh of fresh-cut fruit products may be improved if treated with calcium compounds. Spraying of fruits with solutions of calcium chloride is very effective in maintenance of product firmness (Moraga *et al.*, 2009). Calcium lactate has recently been shown to be as effective as the chloride form. On the other hand, a decrease in the respiration rate has been observed in samples treated with calcium. This may be related to the increase in the membrane rigidity which blocks the gas interchange to a delay in the arrival of the senescence or to the level of active water transport inhibition (Moraga *et al.*, 2009). The results showed that in fruits sprayed with  $\text{CaCl}_2$ , PG activity was lower than in non sprayed trees in both cultivars, so such fruits benefited from a lower OSP and a higher firmness during storage period.

Conceivably the loss of bound  $\text{Ca}^{2+}$  and the ability to form  $\text{Ca}^{2+}$  cross-bridges might have neutralized any effects of reduced depolymerization of pectin on ripening-associated softening of transgenic fruits. Depending on the concentration of  $\text{Ca}^{2+}$  ions, pectin has been suggested to form different types of aggregates (Aileen *et al.*, 2008). Under low  $\text{Ca}^{2+}$  levels, polygalacturonates have been suggested to form primary units of two chains in

antiparallel configuration with about 50% of the carboxyl groups neutralized with  $\text{Ca}^{2+}$ . The excess  $\text{Ca}^{2+}$  is weakly bound to sheet like aggregates formed with several primary units, which add little strength to the polygalacturonate gels (Aileen *et al.*, 2008). This may explain why even after a significant loss of bound  $\text{Ca}^{2+}$  detectable changes in fruit softening were not observed. Reduced PME and PG exhibit opposite effects on firmness during senescence of other fruits. An increase in fruit integrity has been shown in fruits with low PG activity. It is likely that the continuous action of several cell wall hydrolyses, including PG, on structurally altered cell walls results in the loss of fruit integrity during fruit senescence. An increase in PG activity due to reduced bound  $\text{Ca}^{2+}$  levels may be partly responsible for the loss of tissue integrity (Fischer and Bennett, 1991).

PG activity in damaged fruits varied with the intensity of the damage. Slightly affected fruits (index 1) showed a significant increase in PG activity. Later, as the damage progressed (index 2 to 4), PG activity decreased (Table 2). In peach, a remarkable increase in PG activity during ripening was detected in some cultivars (Pressey and Avants, 1978). In European pear, activities of both exo and endo-PG were identified and characterized in ripening fruit (Pressey and Avants, 1978), although the relationship between each type of PG activity and softening was not established.

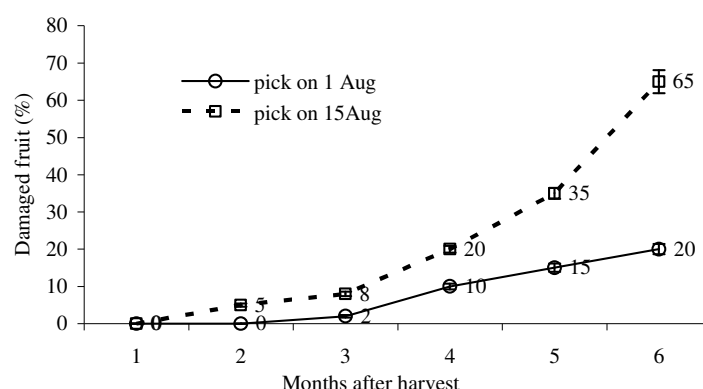
It has been reported that in the high-methoxyl (HM) form of pectin, more than 50% of the carboxyl groups are methylated, while in the low-methoxyl (LM) form less than 50% are methylated. The degree of methylation affects the gelling properties of pectin. High-methoxyl pectin gelation requires an acidic environment,  $\text{pH} < 3.5$  and the presence of  $> 55\%$  co-solute, usually sugar, whereas LM pectin gels over a wide range of pH values, either with or without sugar, and in the presence of  $\text{Ca}^{2+}$  ions. The network structure of HM pectin depends on hydrophobic interactions and hydrogen bonds. In the present research it was found

that sprayed fruits with solutions of calcium chloride were very effective in maintaining product firmness, low OSP, a decrease of PG activity and finally an increase of postharvest longevity in the studied Asian pear fruits.

### Changes of SSC, TA and Internal Browning

Table 3 shows changes of physicochemical properties of the early (1<sup>st</sup> August) and late (15<sup>th</sup> August) harvested 'KS'<sub>9</sub> and 'KS'<sub>13</sub> Asian pears including firmness, SSC and TA. Relatively high levels of SSC were observed in 'KS'<sub>13</sub> cultivar (Table1). It is noteworthy that 'KS'<sub>13</sub> showed the higher SSC but contained a lower acid content. Asian pear SSC developed with fruit ripening, increasing slowly from harvest time (14.1 and 15.1° Brix in 'KS'<sub>9</sub> and 'KS'<sub>13</sub> harvest on 15<sup>th</sup> August) to 1 month after storage (15.2 and 17.2° Brix in 'KS'<sub>9</sub> and 'KS'<sub>13</sub> harvest on 15<sup>th</sup> August) and then decreased only slightly (Table 3). In fruits harvested on 1<sup>st</sup> August after 2, 3, 4 and 5 months of storage SSCs were higher in both cultivars. In addition, SSC decreased significantly from 1 month of storage to 5 months after storage in early and late harvested fruits. IB damaged fruits were higher in fruits harvested on 15<sup>th</sup>

August during storage of either cultivar (Figure 5). In, 'KS'<sub>13</sub>, fruits harvested on 15 August showed 5, 6, 10, 20 and 45% IB symptoms more than those harvested on 1<sup>st</sup> August after 1, 2, 3, 4 and 5 months of storage, respectively. IB can be avoided by early harvesting of Asian pear fruits (Figure 5). Crisosto *et al.* (1994) reported Asian pears grown under California conditions and picked 180 days after full bloom are likely to develop IB during storage. During storage, browning symptoms increased sharply in the more mature fruits (Veltman *et al.*, 2000). In general, the browning tendency of Asian pear fruits increased with advancing maturity (Veltman *et al.*, 2000). Those changes in IB values during storage are in agreement with the present results. In addition, our results indicated that fruit sprayed with CaCl<sub>2</sub> showed a lower IB value than the fruit of non sprayed trees in either cultivar. It has been reported that the chelation of calcium ions with carboxyl groups of adjacent polyuronide chains is a key factor of fruit firmness with calcium deposition occurring in the junction zones in the middle lamella (Knee, 1993). Any increase in free pectin carboxyl groups might be expected to increase the impact of calcium as a firmness-increasing agent (Van Buren, 1979). In the present research, there was a high correlation observed between IB value and the level of OSP in fruits in either



**Figure 5.** Effect of picking date (1 and 15 August) on Internal Browning (IB) extent in Asian pear 'KS'<sub>13</sub> fruit.



one of the studied cultivars during storage (Figure 4). IB incidence was higher in fruits that contained a higher OSP prior to 2 months of storage.

In this study, relationships between SSC, TA as well as between internal browning and OSP concentration were investigated. Pectin is a naturally abundant polysaccharide, and an essential component in initial cell growth as well as in the ripening process. Holm *et al.* (2009) reported that sweetness and thickness of strawberry increased with increasing pure low-methoxyl pectin concentration. Usually, pectin compound contains a large level of sugar, sweetness with SSC differences due to varying sugar content. In the present research, the relative increase in SSC during ripening may account for the decrease in galactose and mannose contents and also relative increase in fructose content during ripening and as well a decrease of SSC during storage may account for the decrease of OSP. Similarly, this release of galactose from pectin fraction and/or the decreased pectin level have been reported during storage of tomato, apple, strawberry and peach (Duan *et al.*, 2008).

In the present work, there existed a relationship among SSC, TA and IB. Higher SSC and TA correlated with lower IB in Asian pears before and after storage. Veltman *et al.* (2003) reported that such fruit disorders as internal browning in pears may develop because Adenosine Triphosphate (ATP) levels drop below levels needed for such cell maintenance activities as membrane repair. Pear cells subjected to anoxia exhibited an ATP synthesis threshold below which membrane lipids hydrolysed. The main source of ATP in fruits comes from sugars and organic acids. Some of such organic acids as Ascorbic and citric play an antioxidant effect on polyphenol oxidase activity (one of the most important factors affecting IB), which act against reactive O<sub>2</sub> (Jimenez *et al.*, 1997). In European pears, a relationship was found between AA content, harvest date and the susceptibility to browning during the storage period

(Veltman *et al.*, 2003). It has been reported that in 'Conference' pear there is a tendency of IB development when AA levels drop below a certain level.

In conclusion, the results indicated that the level of fruit OSP (softened after harvest) significantly increased with time elapsed from harvest. In fruits sprayed with CaCl<sub>2</sub> and harvested earlier (1<sup>st</sup> August), OSP was lower than in those of non sprayed trees and harvested late (15<sup>th</sup> August) in both of the studied cultivars. Fruits in both cultivars softened during storage and the fruits harvested later (15<sup>th</sup> August) suffered from a lower fruit firmness than the early-harvested (1<sup>st</sup> August) ones. Higher OSP correlated with higher PG activity in Asian pears both prior to, and after storage. Nevertheless, in fruits harvested from sprayed trees with CaCl<sub>2</sub>, PG activity was lower than that in the fruits harvested from non sprayed trees in both of the studied cultivars. In addition, fruits picked from sprayed trees with CaCl<sub>2</sub> and harvested on 1<sup>st</sup> August showed a lower percentage of IB incidence in comparison with those harvested from non-sprayed trees with CaCl<sub>2</sub>.

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### غلظت پکتین اغزالات محلول کل در میوه گلابی آسیایی (*Pyrus serotina* Rehd.) در ارتباط با رسیدگی، انبارداری و عارضه قهوه ای شدن داخلی میوه

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#### چکیده

پکتین اغزالات محلول کل با سفتی بافت میوه ارتباط دارد و این همبستگی با توجه به کلات یون های کلسیم با گروه کربوکسیل مجاور زنجیره پلی یورونید بوجود می آید. این آزمایش به منظور بررسی اثر زمان برداشت، محلول پاشی کلرور کلسیم در طول فصل رشد بر پکتین اغزالات محلول (OSP)، فعالیت آنزیم پلی گالاکتروناز (PGA)، سفتی میوه و تاثیر آن ها بر انبارداری، کیفیت و عارضه قهوه ای شدن داخلی میوه (IB) انجام شد. بدین منظور میوه از درختان گلابی آسیایی (*Pyrus serotina* Rehd.)



ارقام 9 'KS'، 13 'KS' که بر روی پایه گلابی اروپایی (*Pyrus communis* L.) پیوند شده بودند جمع آوری گردید. میوه ها در دو تاریخ ۲۰ مرداد و ۵ شهریور ۱۳۸۵ برداشت و در دمای ۱°C و رطوبت نسبی ۸۵-۸۰ درصد نگهداری شدند. بر روی میوه های برداشت شده پنج مرحله استخراج شامل یک مرحله قبل از برداشت معمول میوه، یک مرحله در زمان برداشت و سه مرحله در طول دوره انبارداری انجام شد. نتایج نشان داد که OSP در طول زمان رسیدن و انبارداری به میزان کمی افزایش یافت. در هر دو رقم مورد مطالعه، میزان OSP در میوه هایی که پانزده روز دیر تر از زمان مناسب برداشت، جمع آوری شده بودند بیشتر از میوه هایی که در زمان مناسب برداشت شده بودند بود. همچنین میزان OSP در میوه هایی که بافت آن ها در بعد از برداشت نرم شده بود بطور معنی داری از زمان برداشت بیشتر بود. در هر دو رقم مورد مطالعه همبستگی بالایی بین سفتی بافت میوه، IB، OSP و PGA مشاهده گردید. چنین می توان نتیجه گیری نمود که سفتی بافت میوه و عارضه قهوه ای شدن داخلی میوه به میزان OSP بستگی دارد. همچنین میوه هایی که با کلرور کلسیم محلول پاشی شده و زودتر برداشت شده بودند پس از مدت طولانی انبارداری از میزان کمتری OSP، PGA و IB برخوردار بودند.