CHANGES IN CELL WALL POLYSACCHARIDES DURING SOFTENING OF 'BELLE OF GEORGIA' PEACHES

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CHAPTER I

INTRODUCTION

Ripening of fruits is a complex process which involves changes in firmness, flavor, color and palatability of the fruit. Loss of firmness or softening is one of the most dramatic processes that occur during ripening of fruits. In most of the fleshy fruits, flesh firmness serves as an important determinant of quality. It is probably the most reliable index of maturity and is an economically important attribute, affecting the edible quality and shelf life of the fruit. Softening has the greatest impact on harvest timing due to a related increase in susceptibility to biological and shipping damage. To permit shipment to distant markets, most strategies to control softening involve harvest during the early phases of ripening, thus the edible quality has suffered.

For softening to occur, polymeric interactions between the carbohydrate polymers in the cell walls of adjacent cells must be weakened to allow the cells to move with respect to each other (Huber, 1984; Fischer and Bennett, 1991). The area between the primary cell walls, which forms a rather continuous intercellular matrix is known as middle lamella. This layer is rich in pectic polysaccharides and has received considerable attention as a potential control site of fruit

softening. In tomatoes, loss of middle lamella integrity was implicated as a component process during softening (Crookes and Grierson, 1983).

Pectin is often associated with acidic polysaccharides which consists of a back bone of mainly α (1-4) bound D-galacturonic acid residues with kinks on the chain produced by (1-2) linked L-rhamnose residues. Pectins may be described in terms of 'smooth' and 'hairy' blocks. The dominant feature of smooth blocks is a linear copolymer of α (1-4) linked galacturonic acid and its methyl ester. Inserted within this smooth homogalacturonan polymer are α (1-2) linked rhamnosyl residues. 'Hairy' pectin blocks include rhamnogalactouronans I and II and are complex heteropolymers composed of 12 different sugars. The rhamnogalactouronan polymers are distributed throughout the primary cell wall (McNeil et al., 1984; Fischer and Bennett, 1991). The hairy regions of apple pectins consist of rhamnogalacturonan fragments carrying arabinogalactans and galacturonan fragments carrying single unit xylose side chains (De Vries et al., 1983). The main hemicellulose in apple was a fucogalactoxyloglucan. Efficient extraction of pectins from apple cell walls required both pectolytic and nonpectolytic enzymes (Renard et al., 1991). Rhamnogalacturonase, an enzyme that cleaves the RG1 backbone, has been used to degrade 'modified hairy regions' from apple cell walls. The extracted material was composed of galacturonic acid (42%) and neutral sugars (46%) especially

arabinose and had a high galacturonicacid : rhamnose ratio (Renard et al., 1992).

Hemicelluloses are a wide group of polysaccharides which are soluble in alkali and are attached to cellulose by multiple hydrogen bonds. In most dicots, the principle hemicellulose is xyloglucan, which has a backbone of ß(1-4) linked glucose residues with α(1-6) linked xylose, galactose and fucose residues in various proportions as side chains (Albersheim, 1975; Aspinall, 1981; McNeil et al., 1984). Other hemicellulose components include glucomannans and galactomannans.

Softening associated changes in pectins and hemicelluloses have been studied in a number of fruits. During tomato fruit softening solubilization of polyuronides, galactose and arabinose occurs (Gross and Wallner, 1979; Gross, 1984; Seymour et al., 1990). Cell walls of tomato fruit exhibited increased solubilization of pectin as ripening proceeded (Carrington et al., 1993). High molecular mass hemicellulosic polysaccharides also appeared to be degraded into lower molecular mass polymers during tomato fruit ripening (Huber, 1983; Sakurai and Nevins, 1993; Maclachlan and Brady, 1994).

McCollum et al., (1989) reported that softening of muskmelon was related to modifications of pectic and hemicellulosic polysaccharides. There was an increase in solubility and a decrease in molecular size of polyuronides

during softening. Molecular size of hemicelluloses shifted from larger to smaller polymers and this decrease was accompanied by changes in neutral sugar composition. On a mole percent basis, there were decreases in galactose and glucose in large hemicellulosic polymers with softening. Relative xylose content approximately doubled in large polymers during softening and it was the predominant neutral sugar in the small polymers and remained fairly constant.

Downshifts in polyuronide molecular weight (Huber and O'Donoghue, 1993) and decreases in hemicellulose molecular weight was reported during avocado fruit softening (O'Donoghue and Huber, 1992).

Young persimmon fruits contained a large proportion of pectins, 46% by dry weight of cell walls that decreased to 20% with softening (Cutillas-Iturralde et al., 1993). The amount of total hemicelluloses per unit mass cell wall decreased two fold as fruits softened (Cutillas-Iturralde et al., 1994).

In kiwifruit, cell wall changes during softening are characterized by solubilization of the pectic polymers without changes to their primary structure or degree of polymerization. Following solubilization, the polymers then became susceptible to depolymerization and degalactosidation. It was assumed that polyuronides became soluble by changes in calcium concentration and binding sites within the wall (Redgwell et al., 1991). In pears, cell wall arabinose solubilized during fruit softening was identified as a

component of pectic arabinan, indicating that arabinose loss occurred in unison with pectin degradation (Ahmed and Labavitch, 1980).

Softening of nectarines resulted in solubilization of pectic polymers of high molecular weight from cell wall material. Concurrently, galactan side chains were removed from pectic polymers. Pectic polymers were depolymerized to lower molecular weight during the later stages of softening (Dawson et. al., 1992).

Softening of peaches has long been associated with the conversion of protopectin to soluble forms (Chapman and Horvat, 1990; Postylmayr et al., 1966; Pressey and Avants, 1978; Shewfelt, 1965). Although changes in the other polysaccharides may be involved, the solubilization of pectin has received the most attention because of the preponderance of this polysaccharide in the middle lamella. Most of the research on peach softening has concentrated on endo and exo polygalacturonase and their activity during softening (Callahan et al., 1992; Downs and Brady, 1990; Downs et al., 1992; Lester et al., 1994; Pressey and Avants, 1973, 1978). Pressey and Avants (1978), have demonstrated that peach softening is accompanied by conversion of water-insoluble to water-soluble pectin, and that a higher percentage of water insoluble pectin is solubilized in melting flesh (freestone) than in non-melting flesh (clingstone) peaches. They also noted the occurrence of exo and endo polygalacturonase in

melting flesh peaches, but only exo polygalacturonase in non-melting flesh peaches.

Little is known concerning cell wall modifications during softening of peach cultivars, apart from changes in pectic polymers. Modification of other cell wall components such as hemicelluloses may also occur during softening. The main objectives of this study were to characterize changes in pectin and hemicellulose sugar composition during softening of peach, to determine if pectin and hemicellulose changes during softening represented a conserved event across years, and to determine if apparent molecular size of pectin and hemicellulose change during softening.

Literature Cited

Ahmed, A.E. and J.M. Labavitch. 1980. Cell wall metabolism in ripening fruit. 1. Cell wall changes in ripening 'Bartlett' pears. Plant Physiol. 65:1000-1013.

Albersheim, P. 1975. The walls of growing plant cells. Sci. Amer. 232:80-95.

Aspinall, G.O. 1980. Chemistry of cell wall polysaccharides. pp. 473-500. In: J. Preiss (ed.) The biochemistry of plants. Vol. 3 Carbohydrates: Structure and Function. Academic Press, New York.

Callahan, A.M., P. H. Morgens, P. Wright, and K. E. Nichols. 1992. Comparison of Pch313 (pTOM13 homolog) RNA accumulation during fruit softening and wounding of two phenotypically different peach cultivars. Plant Physiol. 100:482-488.

Carrington, C. M. S., L.C. Greve, and J. M. Labavitch. 1993. Cell wall metabolism in ripening fruit. V1. Effect of the antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes. Plant Physiol. 103:429-434.

Chapman, G.W. and R. J. Horvat. 1990. Changes in nonvolatile acids, sugars, pectin, and sugar composition of pectin during peach (Cv Monroe) maturation. J. Agri. Food Chem. 38:383-387.

Crookes, P. R. and D.Grierson. 1983. Ultra structure of tomato

fruit ripening and the role of polygalacturonase isoenzymes in cell wall degradation. Plant Physiol. 72:1088-1093.

Cutillas-Iturralde, A., I. Zarra, and E.P. Lorences. 1993. Metabolism of cell wall polysaccharide from persimmon fruit. Pectin solubilization occurs in apparent absence of polygalacturonase activity. Physiol. Plant. 89:369-375.

Cutillas-Iturralde, A.,I. Zarra, S. C. Fry, and E. P. Lorences. 1994. Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycosylase. Physiol. Plant. 91:169-176.

Dawson, D. M., L. D. Melton, and C. B. Watkins. 1992. Cell wall changes in nectarines (Prunus persica). Plant Physiol. 100:1203-1210.

De Vries, J. A., F. M. Rombouts, A. G. J. Voragen, and W. Pilnik. 1983. Distribution of methoxyl groups in apple pectic substances. Carbohydrate Polymers. 3:245-248.

Downs, C. G. and C. J. Brady. 1990. Two forms of exopolygalacturonase increase as peach fruits ripen. Plant Cell Environ. 13:523-530.

Downs, C. G., C. J. Brady, and A. Gooley. 1992. Exopolygalacturonase protein accumulates late in peach fruit ripening. Physiol Plant. 85:133-140.

Fischer, R. L. and A. B. Bennett. 1991. Role of cell wall

hydrolases in fruit ripening. Ann Rev. Plant Physiol. Plant Mol Biol. 42:675-703.

Gross, K. C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato. Physiol. Plant. 62:25-32.

Gross, K. C. and S.J. Wallner. 1979. Degradation of cell wall polysaccharides during tomato fruit ripening. Plant Physiol. 63:117-120.

Huber, D. J. 1984. Strawberry fruit softening: The potential role of polyuronides and hemicelluloses. J. Food Sci. 49:1310-1315.

Huber, D. J. and E. M. O'Donoghue. 1993. Polyuronides in avocado (Persea americana) and tomato (Lycopersicon esculentum) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. 102:473-480.

Lester, D. R., J. Speirs, G. Orr, and C. J. Brady. 1994. Peach (Prunus persica) endopolygalacturonase cDNA isolation and mRNA analysis in melting and nonmelting peach cultivars. Plant Physiol. 105:225-231.

Maclachlan, G. and C. Brady. 1994. Endo-1, 4-ß-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. Plant Physiol. 105:965-974.

McCollum, T. G., D. J. Huber, and D. J. Cantliffe. 1989. Modification of polyuronides and hemicelluloses during

muskmelon fruit softening. Physiol.Plant. 76:303-308.

McNeil, M., A. G. Darvill, S. C. Fry, and P. Albersheim. 1984. Structure and function of the primary cell walls of plants. Ann. Rev. Biochem. 53:625-663.

O'Donoghue, E. M. and D. J. Huber. 1992. Modification of matrix polyuronides during avocado(Persea americana) fruit ripening:an assessment of the role of Cx-cellulase. Physiol Plant. 86:3-42.

Postylmayr, H. L., B.S. Luh, and S. J. Leonard. 1966. Characterization of pectic changes in freestone and clingstone peaches during ripening and processing. Food Tech. 10:618-625.

Pressey, R. and J. K. Avants. 1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52:252-256.

Pressey. R. and J. K. Avants. 1978. Differences in the polygalacturonase composition of clingstone and freestone peaches. J. Food Sci. 36:1070-1073.

Redgewell. R. J., L. D. Melton, and D. J. Brasch. 1991. Cell wall polysaccharides of kiwifruit (Actinidia deliciosa): effect of ripening on the structural features of cell wall materials. Carbohydr. Res. 209:191-202.

Renard, C. M. G. C., A. G. J. Voragen, J. F. Thibault and W. Pilnik. 1991. Studies on apple protopectin. 1V. Apple xyloglucans and influence of pectin extraction treatments

on their solubility. Carbohydr. Polymers. 15:387-403.

Renard, C. M. G. C., J. F. Thibault, A. G. J. Voragen, L. A. M. van den Broek and W. pilnik. 1992. Studies on apple protopectin V1: extraction of pectins from apple cell walls with rhamnogalacturonase. Carbohydr. Polymers. 22:203-210.

Sakurai, N. and D. J. Nevins. 1993. Changes in physical properties and cell wall polysaccharides of tomato (Lycopersicon esculentum) pericarp tissues. Physiol Plant. 89:681-686.

Seymour, G. B., I. J. Colquhoun, M S. Dupont, K. R. Parsley and R. R. Selvendran. 1990. Composition and structural features of cell wall polysaccharides from tomato fruits. Phytochemistry. 29:725-731.

Shewfelt, A. L. 1965. Changes and variations in the pectic constitution of ripening peaches as related to product firmness. J. Food Sci. 30:573-576.

CHAPTER II

CHANGES IN SUGAR COMPOSITION DURING SOFTENING FOR EXTRACTS OF PECTIN AND HEMICELLULOSE IN 'BELLE OF GEORGIA' PEACHES.

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Abstract. Changes in cell wall polysaccharide sugar composition associated with peach fruit softening were characterized. Enzymically inactive cell walls were prepared from peach [Prunus persica (L) Batsch] fruit harvested in 1991 and 1992, at three different stages of softening. Pectin associated polysaccharides and hemicellulose associated polysaccharides were extracted from the cell walls sequentially and sugar compositions of the extracts were determined by GLC of trimethyl-silylated derivatives. Pectin extracts from both years were enriched in galacturonic acid, arabinose and rhamnose. Hemicellulose fractions extracted with 1 M potassium hydroxide contained a high mole percentage of xylose, glucose and fucose. Hemicellulose fractions extracted

with 4 M potassium hydroxide contained a substantial amount of pectin associated sugars, in addition to hemicellulose associated sugars. During softening in both the years, hemicellulose associated sugars increased in proportion to pectin associated sugars in this fraction, suggesting a role for both polysaccharide classes in peach fruit softening. The relative stability of changes for pectin and hemicellulose extracts in softening fruit over years, suggests participation of these polymers as a conserved event in peach fruit softening.

Introduction

Fruit softening is one of the most dramatic processes that occur during ripening of fruits. Softening of fruit tissues is thought to be associated with changes in degree of polymerization and sugar composition of polysaccharides, resulting in alterations of cell wall structure and tissue cohesion (Fischer and Bennett, 1991). Particular attention has been given to modification of pectic polysaccharides during softening of fruits. In tomato pericarp, softening involved solubilization of polyuronides, galactose and arabinose (Carrington et al.,1993; Gross and Wallner,1979; Seymour et al.,1990). During softening of many fruits, including muskmelon (McCollum et al., 1989), avocado (Huber and O'Donoghue,1993), persimmon (Cutillas-Iturralde et al.,1993),

kiwifruit (Redgewell et al.,1991) and pear (Ahmed and Labavitch,1980), pectins often become depolymerized and more soluble. During softening of nectarines, pectic polymers were depolymerized and solubilized, and galactan side chains were lost (Dawson et al.,1992). In addition to changes in pectic polymers, softening associated changes in hemicellulosic polymers have been reported. Hemicelluloses have also been shown to change in molecular weight distribution during the softening of tomato (Huber, 1983, Tong and Gross,1988), strawberry (Huber,1984), hotpepper (Gross et al.,1986) and muskmelon (McCollum et al.,1989).

Softening of peaches has long been attributed to the conversion of protopectin to soluble forms (Chapman and Horvat, 1990; Postylmayr et al.,1966; Pressey and Avants,1978; Shewfelt, 1965). Pressey et al. (1971) correlated an appearance of polygalacturonase activity with an increase in water soluble pectin and fruit softening. Pressey and Avants (1973) resolved the peach fruit polygalacturonase into an exo-acting and endo- acting form and in 1978, they noted the occurrence of exo and endo acting enzymes in freestone peaches, but only an exo- acting enzyme in ripe clingstone peaches (Pressey and Avants, 1978). PME activity shows no direct relationship to softening in peaches (Shewfelt,1965).

Peach fruit softening has also been studied by following changes in the sugar composition and apparent molecular weight of pectic substances during on-tree and shelf-ripening. Weight

percentage of cell walls, firmness and pectin content decreased markedly between the 21st and 22nd week after flowering; and between the 3rd and 6th day of storage for melting flesh peaches as compared to non-melting flesh peaches (Fishman et al.,1991). The molecular weight of chelator soluble pectins and alkali soluble pectins in melting flesh peaches was greater than that for non-melting flesh peaches (Fishman et al., 1993). Callahan et al.(1992) compared the RNA accumulation of pch313 (pTOM 13 Homolog, which codes for endopolygalacturonase enzyme) during fruit softening of two phenotypically different peach cultivars. The cultivar that softened faster, 'Bailey', had a significantly higher amount of pch313 RNA accumulate during softening than in the slower softening cultivar 'Suncrest'. Interestingly, peach fruit only accumulated one fiftieth the amount of EPG during ripening, compared to tomato fruit.

Apart from changes in pectic polymers, little is known concerning cell wall modifications during softening of peach fruit. Modifications of other cell wall components such as hemicelluloses may also occur during softening. No information is available on possible year to year variation in cell wall polymer changes during softening of peaches. The objectives of this study were to characterize changes in pectin and hemicellulose sugar composition during softening of peach fruit, and to compare these changes across years.

Materials and Methods

Plant material. 'Belle of Georgia' peach fruit was obtained from the same commercial grower and from the same location within the orchard, in 1991 and 1992. Fruits were harvested from at least 30 trees based on visual indication of ripeness and transported to the laboratory in ice chests. Mesocarp firmness was measured after removal of pericarp on opposite cheeks of each fruit using an Effegi penetrometer (Effegi, Alfonsive, Italy) with a standard 8 mm probe. Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), using the average of both measurements, and utilized immediately for cell wall preparation.

Preparation of fruit cell walls. Enzymically inactive cell walls were prepared essentially as described by Huber (1991). Fruit mesocarp was separated from the pericarp and stone, placed on ice and diced into small pieces. Mesocarp tissues were then homogenized for 6 minutes on ice with Tris saturated phenol [ratio of fruit tissue to tris saturated phenol was 1:3 (w/v)] using an Omni Mixer homogenizer (Omni International, Waterbury, CT) set at speed 6 (three successive bursts of two min each). The homogenate was then filtered through two layers of mira cloth over a buchner funnel, and the initial filtrate was saved for water soluble polysaccharide recovery. The residue was washed with water until the phenol smell was gone, then transferred to a scintered glass funnel and washed with

1 l chloroform:methanol (1:1, v/v) followed by washes with acetone (1 to 2 l) until a fluffy consistency was obtained. Acetone washed cell wall residue was dried in an oven at 60·C and stored in a brown colored bottle.

Water soluble polysaccharide recovery. Polysaccharides solubilized during cell wall preparation were recovered from the aqueous phase of the initial tris saturated phenol filtrate by precipitation with hot ethanol (Dick and Labavitch, 1989). The aqueous phase was boiled with 4 volumes of 95% ethanol, cooled on ice for 30 minutes, and precipitates were recovered by centrifugation at 8000 g for 5 minutes. Supernatant was discarded and the pellet was suspended in 50 mM ammonium acetate, pH 5.2 and stirred for 30 minutes. The supernatant was recovered by centrifugation at 8000 g for 5 minutes, and the pellet was discarded. Four volumes of 95% ethanol was added to the supernatant and stirred overnight at 1·C. The precipitate was recovered by centrifugation at 8000 g for 5 minutes and supernatant was discarded. The pellet was redissolved in 50 mM ammonium acetate, pH 5.2, with stirring, then centrifuged at 8000 g for 5 minutes. The pellet was discarded, and the supernatant was dialyzed against deionized water and freeze dried.

Extraction of cell walls. Cell walls were extracted sequentially to obtain pectin associated polysaccharides and then hemicellulose associated polysaccharides as described by Selvendran et al.(1985) with some modifications. Cell walls (1

g) were hydrated in 100 ml of 500 mM imidazole, pH 7 plus 0.05 % sodium azide *in vacuo* and then incubated for 12 h at 1·C. Samples were centrifuged and washed with 100 ml of distilled water 4 to 5 times. The residue was then re-extracted with 500 mM imidazole plus 0.05 % sodium azide for 2 additional hours at 20-22·C, followed by centrifugation and 4 to 5 distilled water washes. Imidazole and water rinses for both temperatures were combined, dialyzed against deionized water, then freeze dried and weighed. Imidazole was substituted for CDTA or EDTA to extract chelator soluble pectins because imidazole, in contrast to CDTA or EDTA, readily dialyzes away against deionized water (Mort et al., 1991). The imidazole extracted residues (0.85 grams) were then extracted with 100 ml of 0.05 M sodium carbonate plus 20 mM sodium borohydride for 16 h at 1·C. The cell wall material was further extracted with the same concentration of sodium carbonate and sodium borohydride for 3 h at 20-22·C. The supernatants designated as sodium carbonate (cold) and sodium carbonate (warm) were adjusted to pH 5 with acetic acid and dialyzed with 6 changes of deionized water and freeze dried. Extraction with sodium carbonate at 1·C deesterifies the pectins, thus minimizing depolymerization by ß-elimination during subsequent extractions (Selvendran et al., 1985).

The "depectinated" residues were extracted with a graded series of KOH, from 1 M to 4 M, to extract hemicelluloses. In all cases except the final extraction, 10 mM sodium

borohydride was added to convert reducing sugars into alcohols and prevent a peeling reaction. The final extraction medium contained 3-4% boric acid. The "depectinated" residues were extracted with 1 M KOH plus 10 mM sodium borohydride for 2 h at 1·C and then for 2 h at 20-22·C. Further extraction was carried out with 4 M KOH plus 10 mM sodium borohydride for 2 h at 20-22·C and then with 4 M KOH plus 3-4% boric acid for 2 h at 20-22·C. Supernatants designated as 1 M KOH cold, 1 M KOH warm, 4 M KOH and 4 M KOH plus boric acid, were adjusted to pH 5 with acetic acid immediately following supernatant recovery and then dialyzed against deionized water and freeze dried. The final residue was freeze dried and weighed.

Sugar composition analysis. Samples (100 to 200 ug) were methanolyzed and trimethylsilyated for gas liquid chromatography as described by Komalavilas and Mort (1989). One μl of the trimethylsilylated sugars was injected onto a DB-1 fused - silica capillary column (30 m * 0.25 mm i. d., J & W Scientific, Inc., Racho Cordova, CA) installed in a Varian 6000 gas chromatograph (Varian Associates, Walnut Creek, CA) equipped with a cool on- column injector and FID detector, using helium as carrier gas. The sample was injected at 105·C, and the temperature was raised to 160·C and held for 4 min before being raised to 200·C at 1·C per min. Sugars were identified, according to comparison with authentic standards and quantified using inositol as internal standard.

Correlation analysis was done for cell walls, water

soluble polysaccharides, pectin and hemicellulose extracts, to determine whether there was any correlation between firmness and sugar composition.

Results

Sugar composition for cell walls of peach fruits harvested in 1991 and 1992, at three firmness levels is presented in Table 1. In both years, cell walls contained considerable percentages of galacturonic acid, arabinose, galactose and xylose. Firm fruits had a higher mole percentage of galacturonic acid and a lower mole percentage of arabinose, when compared to soft fruits. No consistent trends with softening were observed for other measured sugars.

Polysaccharides solubilized during cell wall preparation, obtained from the aqueous layer of the buffered phenol filtrate, contained substantial portions of galacturonic acid, arabinose and galactose (Table 2). In both years, polysaccharides released from firm fruits had a higher mole percentage of galacturonic acid and lower mole percentage of arabinose when compared to soft fruits. The galacturonic acid:rhamnose ratio decreased as fruits softened. Extraction recovery was higher for firm fruits. In both years, soluble polysaccharides from medium soft and soft fruits had higher mole percentage of galactose compared to firm fruits, and this increase was more pronounced in 1992 than 1991.

Imidazole extracts from both years were enriched in galacturonic acid and arabinose (Table 3). As fruits softened, there was a decrease in mole percentage of galacturonic acid, a substantial increase in mole percentage of arabinose and a slight increase in rhamnose and galactose. The ratio of galacturonic acid : rhamnose decreased as softening progressed.

Sodium carbonate extracts from both years were enriched in galacturonic acid, arabinose and rhamnose (Table 4). Arabinose and rhamnose were particularly enriched in these fractions compared to imidazole extracts. In sodium carbonate cold extracts, firm fruits had higher mole percentage of galacturonic acid and lower mole percentage of arabinose when compared to medium soft and soft fruits. Sodium carbonate cold extracts had a higher ratio of galacturonic acid:rhamnose than warm extracts. Extraction recovery was also higher for cold compared to warm sodium carbonate extracts. In comparison to imidazole extracts, sodium carbonate extracts showed a lower ratio of galacturonic acid : rhamnose and also lower mole percentage of galacturonic acid. Sodium carbonate cold extracts from both years, showed higher mole percentage of galacturonic acid than polysaccharides solubilized during cell wall preparation. In both years, firm fruits contained a higher mole percentage of galacturonic acid in sodium carbonate cold extracts, compared to cell walls.

1 M KOH (1°C and 20-22°C) extracts were enriched in xylose

(13 to 49 mole percent) and glucose (10 to 27 mole percent) in both years compared to imidazole and sodium carbonate extracts (Table 5). Extracts at 1·C had higher mole percentages of xylose and glucose when compared to extracts at 20-22·C. There was not much difference in xylose:glucose ratio between firmness levels or over years. In both years, the ratio of xylose to glucose was greater for cold extracts than warm extracts. Fucose was found at a higher mole percentage in extracts at either temperature when compared to cell walls, imidazole or sodium carbonate extracts. The amount of pectin associated sugars (galacturonic acid, arabinose and rhamnose) versus hemicellulose associated sugars (xylose, glucose and fucose) increased substantially in warm extracts when compared to cold extracts and these differences were stable across years. In 1991, 1 M KOH cold extracts contained 8 to 17 mole percent of pectin associated sugars and 67 to 72 mole percent of hemicellulose associated sugars, in contrast to 1 M KOH warm extracts which contained 43 to 55 mole percent of pectin associated sugars and 27 to 39 mole percent of hemicellulose associated sugars. In 1992, 1 M KOH cold extracts contained 10-14 mole percent of pectin associated sugars, 62 to 65 mole percent of hemicellulose associated sugars, and in 1 M KOH warm extracts pectin associated sugars ranged from 49 to 55 mole percent, and 26 to 33 mole percent of hemicellulose associated sugars. Cold 1 M KOH extracts had lower galacturonic acid:rhamnose ratio when compared to imidazole

and sodium carbonate extracts. Extraction recovery was higher for sodium carbonate and imidazole extracts than 1 M KOH extracts.

Four M KOH plus 10 mM sodium borohydride extracts resembled 1 M KOH warm extracts more than 1 M KOH cold extracts in terms of sugar composition, but extraction yields were higher for 4 M KOH versus 1 M KOH extracts (Table 6). Xylose,glucose and fucose were enriched in this fraction compared to cell walls. Galacturonic acid:rhamnose ratio did not show any variation with respect to difference in firmness levels as well as over years. Soft fruit contained 38-46 mole percent of hemicellulose associated sugars and 35-38 mole percent of pectin associated sugars when compared to medium soft and firm fruits which contained 35-45 mole percent of hemicellulose associated sugars and 35-45 mole percent of pectin associated sugars. This extract contained lower galacturonic acid:rhamnose ratio, compared to imidazole and sodium carbonate cold extracts. In fruits of all firmness groups, xylose:glucose ratio was very close to 1:1. which was closer than xylose:glucose ratio of other pectin and hemicellulose extracts.

Four M KOH plus boric acid extracts also contained considerable amounts of hemicellulose associated sugars, as well as pectin associated sugars (Table 6). The mole percentage of hemicellulose associated sugars was higher for soft fruit (22-28 mole percent) when compared to medium soft

(14-28 mole percent) and firm fruit (13-24 mole percent). This extract contained higher percentage of pectin associated sugars in fruits of all firmness groups, when compared to hemicellulose associated sugars. Soft fruit contained 52-56 mole percent of pectin associated sugars and 13-24 mole percent of hemicellulose associated sugars, whereas medium soft and firm fruits contained 43-56 mole percent pectin associated sugars and 14-28 mole percent of hemicellulose associated sugars. Galacturonic acid:rhamnose ratio did not differ much between 4 M KOH extracts. Extraction recovery was lower for 4 M KOH + boric acid extracts than 4 M KOH +10 mM sodium borohydride extracts.

In both years the insoluble residue from fruits of all firmness groups contained a high mole percentage of arabinose and glucose (Table 7). It exhibited a higher mole percentage of glucose and lower mole percentage of galacturonic acid compared to cell walls. Galacturonic acid:rhamnose ratio was lower in the extraction residue compared to cell walls in both years. Residue recovery was similar between firmness groups in both years, ranging from 22 to 27 percent of starting weight.

Correlation analysis for cell walls did not show any significant correlation between firmness and sugar composition (Table 8). In water soluble polysaccharides, there was negative correlation between firmness and arabinose, rhamnose, fucose, galacturonic acid, but it was not significant. In imidazole extracts, there was significant negative correlation

between firmness and arabinose, rhamnose, xylose and mannose. In sodium carbonate cold extracts, there was significant negative correlation between firmness and xylose, glucose. In sodium carbonate warm extracts, 1 M KOH extracts and 4 M KOH extracts there was no significant correlation between firmness and sugar composition.

Discussion

In this study, we have characterized changes in polysaccharides during softening of peaches for two successive seasons. The rapidly softening peach cultivar 'Belle of Georgia' was used. We anticipated that cell wall changes that accompany softening would occur at a higher magnitude over a shorter duration of time in this cultivar than for slower softening cultivars. Rapid softening should result from rapid cell wall modifications, from extensive preconditioning of cell wall polymers or from both.

We utilized cell wall preparation procedures designed to inactivate bound hydralases, to eliminate the capacity of resident active bound hydrolases to alter cell wall components both during cell wall preparation as well as during subsequent extraction steps. Tris buffered phenol has been proven as an effective agent to inactivate cell wall hydrolases in other fruit (Huber, 1991). In preliminary trials, we confirmed utilization of buffered phenol for inactivation of peach cell

wall hydrolytic activity (data not shown).

As fruits softened, cell walls exhibited an increase in arabinose and a decrease in galacturonic acid on a mole percent basis (Table 1). In agreement with published reports (Fischer and Bennett, 1991) apparently both charged and neutral sugars were subject to modification during fruit softening. Polysaccharides solubilized during cell wall preparation exhibited high amounts of pectin associated sugars (Table 2). This fraction was solubilized during cell wall preparation, and presumably was 'soluble' in fruits *in vivo*. This extract exhibited increased solubilization of arabinose and galactose as fruits softened, suggesting that arabinose and galactose may be selectively cleaved during softening. A decreased galacturonic acid : rhamnose ratio with softening may indicate increased rhamnogalacturonan solubilization, which could account for some of the arabinose and galactose as side chains. When compared to cell walls, polysaccharides solubilized during cell wall preparation exhibited a lower mole percentage of galacturonic acid, and higher mole percentage of arabinose and galactose suggesting that neutral sugars represent a significant proportion of polysaccharides modified during softening.

Calcium is thought to form ionic bridges between unesterified pectic polymers, thereby conferring rigidity to the cell wall. Our cell wall preparation procedure has been shown to extract little calcium from walls (Huber,1991) and

subsequent extraction by chelation of calcium should result in extraction of the ionically bound homogalacturonan polymers representative of those present in the native walls, plus any associated covalently bound polymers. The homogalacturonan region is thought to consist of blocks of consecutive galacturonic acid residues, interrupted at fairly regular intervals by 1, 2-linked rhamnose residues (McNeil et al.,1984). During fruit softening, endopolygalacturonase is thought to function in hydrolysis of the galacturonic acid-galacturonic acid linkages (but not galacturonic acid-rhamnose linkages). Rhamnose residues are reported to occur once in every 25 galacturonic acid residues for apple, citrus and sunflower pectins (Powell et al., 1982), once in every 70 residues for carrots (Konno et al., 1986), or once on every 72 to 100 residues for beet (Thibault et al., 1993). In our study, based on yields from very firm fruit, the interval for peach appears to average 24. Assuming that imidazole extracted pure homogalacturonan region, this interval appeared to decrease for softening fruit, averaging 16 for medium soft and 8 for soft fruit (Table 3). This supports a selective cleavage of the galacturonic acid in the homogalacturonan region during fruit softening, or increased extraction of the rhamnogalacturonan region in softening fruit. The imidazole insoluble pectic polysaccharide was further solubilized by sodium carbonate, by hydrolysis of weak ester cross-links (Waldron and Selvendran, 1992). Extraction with sodium

carbonate at 1°C deesterifies the pectins, thus minimizing the degradation by ß elimination, and extraction at room temperature is known to solubilize certain pectic substances that are in association with hemicellulosic polymers (Selvendran and O'Neil, 1987). This was true in peaches because warm sodium carbonate extracts contained higher mole percentage of hemicellulose associated sugars, glucose, xylose and fucose (extracted along with pectin associated sugars) compared to cold sodium carbonate extracts (Table 4). Similar results have been reported for other fruits (Cutillas-Iturralde et al., 1993; Ryden and Selvendran, 1990; Seymour et al., 1990).

As fruits softened, imidazole and sodium carbonate extracts exhibited a decrease in galacturonic acid and increase in arabinose. Similarly in nectarines, as ripening progressed, uronide content of both the pectic fractions i.e. CDTA and sodium carbonate soluble fractions decreased (Lurie et al., 1994) and arabinose increased (Dawson et al., 1992). If arabinose was present as rhamnogalacturonan side chain, increasing mole percentages of arabinose with softening may have indicated increased rhamnogalacturonan solubility with softening. The concomitant increase of arabinose with a decrease in galacturonic acid : rhamnose ratio tends to support this hypothesis.

'Depectinated' cell wall material was further extracted with increasing concentrations of KOH to solubilize the

hemicelluloses. Alkali solubilizes the hemicellulose associated polysaccharides by disrupting hydrogen bonding between hemicellulose and cellulose microfibrils (Selvendran et al., 1985). More loosely bound hemicellulose extracts (1 M KOH,cold) were particularly enriched in xylose and glucose (Table 5). Similar results were reported with muskmelon (McCollum et al., 1989), tomato (Carrington et al., 1993), nectarines (Dawson et al., 1992), avocado (O'Donoghue and Huber, 1992), olives (Coimbra et al., 1994) and asparagus (Waldron and Selvendran, 1992). Xyloglucan appeared to be enriched in these extracts. One M KOH extracts were also enriched in fucose, in fruits of all firmness groups in both years. High amounts of fucose were present in 1 M KOH extracts in runner bean tissues along with xylose and glucose. Fucose is strongly associated with xyloglucan side chains (Ryden and Selvendran, 1990). Polymers extracted with cold 1 M KOH contained a higher mole percentage of xylose and glucose than those extracted at room temperature. The room temperature extracts were richer in galacturonic acid, arabinose and rhamnose than the cold extracts. Cold 1 M KOH appeared to extract a more highly purified xyloglucan fraction with fucose side chains. Warm 1 M and 4 M KOH extracts extracted both hemicelluloses and pectins. Similar results were found with apples (Stevens and Selvendran, 1984). Pectin associated sugars represented a higher proportion of the total sugars in more tightly bound hemicellulose extracts (extracted with 4 M

KOH) when compared to more loosely bound extracts (extracted with cold 1 M KOH) in fruits of all firmness groups. Pectin and hemicellulose appear to be some way associated in the cell wall of peach fruit. An increase of proportion of hemicellulose associated sugars over pectin associated sugars in soft fruits suggests a cooperative involvement of both polysaccharide classes during softening. Similarly in other fruits like tomato (Carrington et al., 1993; Gross, 1984; Gross and Wallner, 1979; Huber, 1983; Maclachlan and Brady, 1994; Seymour et al., 1990), muskmelon (Mccollum et al., 1989), avocado (O'Donoghue and Huber, 1992; Huber and O'Donoghue, 1993) and persimmon (Cutillas-Iturralde et al., 1993, 1994) softening associated changes in pectin and hemicellulose polymers have been reported.

There is some other evidence from fruits like tomato and apple which suggests that certain hemicellulose fractions are involved in crosslinking of pectins with other cell wall polymers. In tomato, major hemicellulosic polysaccharide was a xyloglucomannan and there was evidence for the occurrence of small amount of xylan - pectic complex (Seymour et al., 1990). In apples a 4 M NaOH extract contained some fucogalactoxyloglucan and a homogalacturonan devoid of arabinose and rhamnose was present, but it was not possible to affirm that they are covalently linked (Renard et al., 1991). Our results support possible association between pectins and hemicelluloses. Further studies are needed to assess the exact

nature of associations between hemicellulose and pectin, and whether these associations are selectively altered during softening.

Polysaccharides of peach fruit consist of a range of structurally related polymers, which differed in their ease of extraction from the cell wall. Arabinose may be present as side chain of rhamnogalacturonan region and may have dissociated as fruits softened. Hemicellulosic polymer such as xyloglucan was present with fucose side chains. The predominance of pectin associated sugars in tightly bound hemicellulose extracts was an evidence that both pectins and hemicelluloses were associated with each other in the cell wall. In the same extract, hemicellulose associated sugars increased in proportion to pectin associated sugars, as softening progressed. This indicates that peach fruit softening involves changes in both pectin and hemicellulose. The relative stability of sugar compositional changes over two successive years supports participation of pectins and hemicelluloses as a conserved event in peach. These results also suggest that a wide range of both pectolytic and nonpectolytic enzymes may be involved in peach fruit softening.

Literature Cited

Ahmed, A.E. and J.M. Labavitch. 1980. Cell wall metabolism in ripening fruit.1.Cell wall changes in ripening 'Bartlett' Pears. Plant Physiol. 65:1000-1013.

Callahan, A.M., P. H. Morgens, P. Wright, and K.E. Nichols. 1992. Comparision of Pch313 (pTOM13 homolog) RNA accumulation during fruit softening and wounding of two phenotypically different peach cultivars. Plant Physiol. 100:482-488.

Carrington, C.M.S., L. C. Greve, and J.M. Labavitch. 1993. Cell wall metabolism in ripening fruit. V1. Effect of the antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes. Plant Physiol. 103:429-434.

Chapman, G.W. and R.J. Horvat. 1990. Changes in nonvolatile acids, sugars, pectin, and sugar composition of pectin during peach (Cv Monroe) maturation. J. Agri. Food Chem. 38:383-387.

Coimbra, M.A., K.W. Waldron, and R.R. Selvendran. 1994. Isolation and characterization of cell wall polymers from olive pulp (Olea europea L.). Carbohydr. Res. 252:245-262.

Cutillas-Iturralde, A., I. Zarra, and E.P. Lorences. 1993. Metabolism of cell wall polysaccharide from persimmon fruit. Pectin solubilization occurs in apparent absence

of polygalacturonase activity. Physiol. Plant. 89:369-375.

Cutillas-Iturralde, A., I. Zarra, S.C. Fry, and E.P. Lorences. 1994. Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycocylase. Physiol. Plant. 91:169-176.

Dawson, D.M., L.D. Melton, and C.B. Watkins. 1992. Cell wall changes in nectarines (Prunus persica). Plant Physiol. 100:1203-1210.

Dick, A.J. and J.M. Labavitch. 1989. Cell wall metabolism in ripening fruit. IV. Characterization of the pectic polysaccharides solubilized during softening of 'Bartlett' Pear fruit. Plant Physiol. 89:1394-1407.

Fischer, R.L. and A.B. Bennett. 1991. Role of cell wall hydrolases in fruit ripening. Annual Rev. Plant Physiol. Plant Mol. Biol. 42:675-703.

Fishman, M.L., D. T. Gillespie, S. M. Sondey, and Y.S. El-Atawy. 1991. Intrinsic viscosity and molecular weight of pectin components. Carbohydr. Res. 215:91-104.

Fishman, M.L., B. Levaj, D. T. Gillespie, and R. Scorza. 1993. Changes in the physico-chemical properties of peach fruit pectin during on-tree ripening and storage. J. Amer. Soc. Hort. Sci. 118:343-349.

Gross, K.C. 1984. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato

fruit. Physiol. Plant. 62:25-32.

Gross, K.C. and S.J. Wallner. 1979. Degradation of cell wall polysaccharides during tomato fruit ripening. Plant Physiol. 63:117-120.

Gross, K.C., A.E. Watada, M.S. Kang, S.D. Kim, K.S. Kim, and S.W. Lee. 1986. Biochemical changes associated with the ripening of hot pepper fruit. Physiol. Plant. 66:31-36.

Huber, D.J. 1983. Polyuronide degradation and hemicellulose modifications in ripening tomato fruit. J. Amer. Soc. Hort. Sci. 108:405-409.

Huber, D.J. 1984. Strawberry fruit softening: The potential role of polyuronides and hemicelluloses. J. Food Sci. 49:1310-1315.

Huber, D.J. 1991. Acidified phenol alters cell wall pectin solubility and calcium content. Phytochemistry 30:2523-2527.

Huber, D.J. and E.M. O'Donoghue. 1993. Polyuronides in avacado (Persea americana) and tomato (Lycopersicon esculentum) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. 102:473-480.

Komalavilas, P. and A.J. Mort. 1989. The acetylation at 0-3 of galacturonic acid in the rhamnose rich portion of pectins. Carbohydr. Res. 189:261-272.

Konno, H., Y. Yamasaki, and K. Katoh. 1986. Enzymic degradation of pectic substances and cell walls purified

from carrot cell cultures. Phytochemistry 25:623-627.

Lurie, S., A. Levin, L. C. Greve, and J. M. Labavitch. 1994. Pectic polymer changes in nectarines during normal and abnormal ripening. Phytochemistry 36:11-17.

Maclachlan, G. and C. Brady. 1994. Endo-1, 4-ß-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. Plant Physiol. 105:965-974.

McCollum, T.G., D.J. Huber, and D.J. Cantliffe. 1989. Modification of polyuronides and hemicelluloses during muskmelon fruit softening. Physiol. Plant 76:303-308.

McNeil, M., A.G. Darvill, S. C. Fry, and P. Albersheim. 1984. Structure and function of the primary cell walls of plants. Ann. Rev. Biochem. 53:625-663.

Mort, A.J., B.M. Moerschbacher, M.L. Pierce, and N.O. Maness. 1991. Problems one may encounter during the extraction, purification and chromatography of pectic fragments, and some solutions to them. Carbohydr. Res. 215:219-227.

O'Donoghue, E.M. and D.J. Huber. 1992. Modification of matrix polysaccharides during avocado (Persea americana) fruit ripening : an assessment of the role of Cx-Cellulase. Physiol Plant. 86:33-42.

Postylmayr, H.L., B.S. Luh, and S.J. Leonard. 1966. Characterization of pectic changes in freestone and clingstone peaches during ripening and processing. Food Tech. 10:618-625.

Powell, D.A., E.R. Morris, M.J. Gidley, and D.A. Rees. 1982. Conformation and interactions of pectins. II. Influence of residue sequence on chain association in calcium pectate gels. J. Mol. Biol. 155:517-531.

Pressey, R. and J.K. Avants. 1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52: 252-256.

Pressey, R. and J.K. Avants. 1978. Differences in the polygalacturonase composition of clingstone and freestone peaches. J. Food Sci. 43:1415-1423.

Pressey, R., D.M. Hinton, and J.K. Avants. 1971. Development of polygalacturonase activity and solubilzation of pectins in peaches during ripening. J. Food Sci. 36:1070-1073.

Redgewell, R.J., L.D. Melton, and D.J. Brasch. 1991. Cell wall polysaccharides of kiwifruit (Actinidia deliciosa): effect of ripening on the structural features of cell wall materials. Carbohydr. Res. 209:191-202.

Renard, C.M.G.C., A.G.J. Voragen, J.F. Thibault, and W. Pilnik. 1991. Studies on apple protopectin. IV: Apple xyloglucans and influence of pectin extraction treatments on their solubility. Carbohydr. Polymers. 15:387-403.

Rydan, P. and R.R. Selvendran. 1990. Structural features of cell wall polysaccharides of potato (Solanum tuberosum). Carbohydr. Res. 195:257-272.

Selvendran, R.R., B.J.H. Stevens, and M.A. O'Neill. 1985. Developments in the isolation of cell walls from edible plants. In C.T. Brett, J.R. Hillman, eds, Biochemistry of plant cell walls. Cambridge University Press, Cambridge, UK. pp 39-78.

Selvendran, R.R. and M.A. O'Neil. 1987. Isolation and analysis of cell walls from plant material. In D. Glick, Methods of Biochemical Analysis, Vol 32. John Wiley Interscience, New York. pp 25-153.

Seymour, G.B., I.J. Colquhoun, M.S. Dupont, K.R. Parsley, and R.R. Selvendran. 1990. Composition and structural features of cell wall polysaccharides from tomato fruits. Phytochemistry 29:725-731.

Shewfelt, A.L. 1965. Changes and variations in the pectic constitution of ripening peaches as related to product firmness. J. Food Sci. 30:573-576.

Stevens, B.J.H. and R.R. Selvendran. 1984. Structural features of cell wall polymers of the apple. Carbohydr. Res. 135:155-166.

Thibault, J.F., C.M.G.C. Renard, M.A.V. Axelos, P. Roger, and M.J. Crepeau. 1993. Studies on the length of the homogalacturonic regions in pectins by acid hydrolysis. Carbohydr. Res. 238:271-286.

Tong, C.B.S. and K.C. Gross. 1988. Glycosyl-linkage composition of tomato fruit cell wall hemicellulosic fractions during ripening. Physiol. Plant. 74:365-370.

Waldron, K.W. and R.R. Selvendran. 1992. Cell wall changes in immature asparagus stem tissue after excision. Phytochemistry 31:1931-1940.

Table 1. Sugar composition from cell walls of peach fruit differing in firmness

Resistance to puncture (N)[z]	Sugar Composition (Mole Percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
			1991						
47	22	4	1	9	14	3	5	38	4
30	29	5	2	13	10	3	3	31	4
15	31	4	2	12	13	4	5	24	5
			1992						
47	34	5	2	12	12	2	3	28	3
30	32	4	1	10	9	2	5	35	2
15	38	4	2	12	14	3	4	22	2

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.

Table 2. Sugar composition of water soluble polysaccharides for cell walls of peach fruit differing in firmness

Resistance to puncture (N)[z]	Extraction recovery (percent)[y]	Sugar composition (Mole Percent)								
		Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
						1991				
47	6	24	3	1	5	17	2	6	38	4
30	3	29	4	1	7	23	2	7	24	3
15	3	43	5	2	6	19	3	6	16	1
						1992				
47	5	34	5	1	5	17	2	3	29	4
30	1	30	2	1	10	27	7	8	10	4
15	1	36	3	1	7	32	4	5	8	4

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Extraction recovery was the percentage of the weight recovered from the aqueous phase of the buffered phenol filtrate, to the initial weight of cell wall obtained.

Table 3. Sugar composition of imidazole extracts for cell walls of peach fruit differing in firmness

	Resistance to puncture (N)[z]	Extraction recovery (percent)[y]	Sugar composition (Mole Percent)								
			Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
						1991					
500 mM	47	11	15	4	1	2	8	2	3	62	3
Imidazole	30	5	24	5	1	3	5	2	3	55	2
	15	5	30	6	1	3	10	4	5	38	2
						1992					
500 mM	47	6	12	2	1	3	8	2	5	64	4
Imidazole	30	7	16	3	1	3	6	2	4	64	2
	15	6	25	4	1	4	12	3	5	43	4

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Extraction recovery was the percentage of the weight of freeze dried supernatant to initial weight of cell wall used for extraction.

Table 4. Sugar composition of sodium carbonate extracts for cell walls of peach fruit differing in firmness

	Resistance to puncture (N)[z]	Extraction recovery (percent)[y]	Sugar composition (Mole Percent)								
			Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
						1991					
50 mM Na_2CO_3	47	7	29	6	1	3	8	1	2	49	2
+ 20 mM $NaBH_4$	30	10	48	10	1	5	6	1	3	24	3
1 · C	15	6	40	7	1	5	9	1	4	32	2
50 mM Na_2CO_3	47	5	36	9	2	6	13	1	4	28	1
+ 20 mM $NaBH_4$	30	2	41	8	1	7	7	2	6	26	2
20-22 ·C	15	2	46	8	1	5	9	1	3	27	2
						1992					
50 mM Na_2CO3	47	5	19	3	1	2	9	1	2	61	3
+ 20 mM $NaBH_4$	30	6	23	4	1	1	9	1	2	58	2
1·C	15	14	37	5	1	7	10	1	2	24	3
50 mM Na_2CO_3	47	5	44	6	1	2	10	1	2	33	2
+ 20 mM $NaBH_4$	30	6	50	7	1	2	19	1	2	28	1

20-22·C	15	3	50	8	1	4	13	1	5	21	2

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Extraction recovery is the percentage of the weight of the freeze dried supernatant to initial weight of cell wall used for extraction.

Table 5. Sugar composition of 1 M potassium hydroxide extracts for cell walls of peach fruit differing in firmness

	Resistance to puncture (N)[z]	Extraction recovery (percent)[y]	Sugar composition (Mole Percent)								
			Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
							1991				
1 M KOH +	47	2	4	1	5	47	15	7	19	1	1
10 mM $NaBH_4$,	30	2	7	1	4	41	10	5	22	9	1
1°C	15	3	4	1	3	49	11	8	20	3	1
1 M KOH +	47	4	27	6	2	15	13	2	10	22	3
10 mM $NaBH_4$,	30	4	24	5	3	21	12	4	14	14	2
20-22°C	15	3	26	5	3	25	12	4	11	13	1
							1992				
1 M KOH +	47	3	5	1	1	36	15	9	27	4	1
10 mM $NaBH_4$,	30	3	6	1	3	33	16	8	26	7	1
1°C	15	4	5	1	5	37	15	8	23	6	1
1 M KOH +	47	3	34	4	2	13	13	3	11	17	2
10 mM $NaBH_4$,	30	2	30	4	2	13	15	3	14	17	3

20-22·C	15	5	32	4	3	18	14	4	12	13	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Extraction recovery is the percentage of the weight of the freeze dried supernatant to initial weight of cell wall used for extraction.

Table 6. Sugar composition of 4 M potassium hydroxide extracts for cell walls of peach fruit differing in firmness

	Resistance to puncture (N)[z]	Extraction recovery (percent)[y]	Sugar composition (Mole Percent)								
			Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
							1991				
4 M KOH +	47	9	24	4	4	20	11	4	21	8	1
10 mM $NaBH_4$,	30	14	20	3	4	18	18	6	23	12	2
20-22·C	15	13	22	3	4	23	13	5	19	10	1
4 M KOH +	47	5	34	5	2	9	12	11	13	13	2
3-4%boric acid	30	2	35	4	2	10	13	12	16	7	1
20-22·C	15	7	31	4	2	10	14	14	16	8	1
							1992				
4 M KOH +	47	7	30	4	4	15	16	5	16	11	1
10 mM $NaBH_4$,	30	9	28	3	3	15	16	5	17	12	1
20-22·C	15	10	27	3	3	17	16	7	18	8	1
4 M KOH +	47	3	39	4	1	6	24	2	6	13	2
3-4%boric acid	30	2	32	6	1	6	15	5	7	18	2

20-22·C	15	2	33	5	2	8	16	9	12	13	2

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Extraction recovery is the percentage of the weight of the freeze dried supernatant to initial weight of cell wall used for extraction.

Table 7. Sugar composition of insoluble residue for cell walls of peach fruit differing in firmness

Resistance to puncture (N)[z]	Residue recovery (percent)[y]	Sugar composition (Mole Percent)								
		Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
						1991				
47	22	29	5	3	9	8	2	34	8	1
30	27	37	5	1	10	10	2	26	9	1
15	26	29	4	3	8	10	2	34	9	2
						1992				
47	24	32	4	1	9	13	2	31	8	1
30	23	26	4	1	9	15	3	32	9	1
15	25	28	3	1	6	11	2	44	4	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effei penetrometer.
[y]Extraction recovery is the percentage of the weight of the freeze dried residue to the initial weight of cell wall used for extraction.

Table 8. Correlation Analysis for Peach Cell Walls, Water Soluble Polysaccharides, Pectin Extracts and Hemicellulose Extracts

	Cell Wall	Water Soluble Polysc.	Imidazole Extracts	Na_2CO_3 Extracts (cold)	Na_2CO_3 Extracts (warm)	1 M KOH Extracts (cold)	1 M KOH Extracts (warm)	4 M KOH + $NaBH_4$ Extracts	4 M KOH + Boric Acid Extracts
Ara	0.32	-0.40	-0.63*	-0.09	-0.26	0.14	0.13	-0.08	0.30
Rha	0.69	-0.73	-0.58*	-0.05	-0.30	-0.03	-0.02	-0.5	0.32
Fuc	0.24	-0.42	-0.53	-0.46	0.30	-0.15	-0.35	-0.13	0.01
Xyl	0.44	0.00	-0.66*	-0.52*	-0.28	-0.00	-0.44	-0.30	-0.13
Gal	0.44	0.24	-0.47	-0.20	-0.05	0.09	-0.09	-0.06	-0.13
Man	0.68	0.12	-0.60*	-0.31	-0.36	-0.00	-0.46	-0.39	-0.55
Glc	0.69	0.05	-0.42	-0.58*	-0.06	0.04	-0.45	-0.24	-0.33
GalA	0.64	-0.26	0.22	0.50	0.23	-0.27	0.41	-0.10	0.28
GlcA	0.46	0.12	0.04	0.14	0.11	0.04	-0.05	0.32	0.38

* Significant at 95%

CHAPTER III

CHANGES IN APPARENT MOLECULAR SIZE OF PECTINS AND HEMICELLULOSES DURING SOFTENING OF 'BELLE OF GEORGIA' PEACHES

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Abstract. Pectin and hemicellulose were solubilized from cell walls of peach [Prunus persica (L) Batsch] fruit differing in firmness by extraction with imidazole and sodium carbonate (Pectin extracts) and a graded series of potassium hydroxide (hemicellulose extracts). They were subjected to size exclusion chromatography using a column (1 cm * 50 cm) packed with Toyopearl HW55S with 300 mM ammonium acetate, pH 5.2 as eluent. In imidazole extracts, as fruits softened, there was an increase in a large apparent molecular size peak, which was enriched with arabinose and rhamnose. The small apparent molecular size peak was enriched with galacturonic acid and

probably represented a broad polydisperse peak derived from the homogalacturonan region. For sodium carbonate cold extracts, apparent molecular size distribution changed from a single broad peak (firm) to two distinct peaks (medium soft) and then three distinct peaks (soft) as fruits softened. In sodium carbonate warm extracts, which separated into three apparent molecular size classes in fruits of all firmnesses, as fruits softened there was an increase in large apparent molecular size peak and a decrease in two small apparent molecular size peaks. In cold 1 M KOH extracts three peaks predominated. Xyloglucan appeared to elute predominantly in the second apparent molecular size peak and fucose was strongly associated with it. In 4 M KOH extracts (tightly bound hemicellulose) a higher apparent molecular size peak was predominantly acidic and presumably of pectic origin. Two smaller apparent molecular size peaks were composed of neutral sugars, the second peak became smaller and the third peak larger as fruits softened. Apparent molecular size of both pectins and hemicelluloses changed as peach fruits softened.

Introduction

Ripening of most fleshy fruits is often characterized by softening of edible tissues. It is assumed that modification of cell wall components underlie the process of softening. Pectic polysaccharides are a major constituent of primary cell

walls, co-existing with other polysaccharides such as hemicellulose and cellulose, forming a cross linked matrix network. Physical interconnections between adjacent cells are thought to occur primarily through interlocking of the pectin originating from adjoining cells, and the degree of interconnection affects the rigidity of interaction, or firmness, on a tissue wide basis (McCann et al., 1990). It is thought that the breakdown of the bonds holding this structure leads to loosening of the stability of this network, which results from degradation of cell wall components, causing loss of tissue firmness. Ripening associated modifications in sugar composition and apparent molecular size of pectins and hemicelluloses have been reported in many fruits.

In avocado, molecular sizes of pectic polymers and hemicelluloses extracted from fruit mesocarp cell walls shifted from larger to smaller polymers during ripening (Ranwala et al.,1992). In another study with avocado, hemicelluloses (4 M alkali-soluble) exhibited a very broad distribution of polymer sizes and an overall decrease in apparent molecular weight during softening (O'Donoghue and Huber,1992). Huber (1983) observed a marked change in molecular weight distribution of cell wall hemicelluloses of tomato fruit. During ripening, it was further shown that a progressively lower proportion of high molecular weight polysaccharides coincided with a higher proportion of low molecular weight polymers. Similarly, a hemicellulose fraction

extracted from hot pepper fruit cell walls was modified during maturation and ripening, resulting in a shift from higher to lower molecular weight (Gross et al.,1986). McCollum et al (1989) reported an increase in solubility and a decrease in molecular size of polyuronides in the cell walls of muskmelon fruit as softening progressed. In another study with muskmelon, molecular sizes of pectin and hemicellulose polymers extracted from fruit mesocarp cells shifted from larger to smaller polymers during ripening (Ranwala et al., 1992). In kiwifruit, three distinct molecular size classes of hemicelluloses were observed, with a proportional increase in the smaller polymers with fruit ripening (Redgewell et al.,1991). Ripening of nectarines resulted in solubilization of pectic polymers of high molecular size class and concurrently galactan side chain removed from pectic polymers. Solubilized pectic polymers were depolymerized to lower molecular size class as ripening progressed (Dawson et al., 1992).

Peach softening during ripening has been attributed to the enzymic degradation of pectic polymers (Pressey, 1977). In melting flesh peach 'Redskin' fruit mesocarp, molecular weight of chelator soluble pectin (extracted with CDTA) and alkaline soluble pectin (extracted with sodium carbonate plus CDTA) increased considerably during ripening and storage. In nonmelting flesh peach 'Suncling' fruit mesocarp, molecular weight of chelator soluble pectin and alkaline soluble

fraction was relatively constant during on-tree ripening and storage (Fishman et al., 1993). Although changes in other polysaccharides may be involved , the solubilization of pectin has received the most attention, because of the preponderance of this polysaccharide in the middle lamella. From our previous studies with peach cell walls, we know that both pectin and hemicellulose sugar composition changes during fruit softening (Maness et al., 1993). This study was undertaken to develop an understanding of how the apparent molecular size of these polymers are modified during peach fruit softening. The main objective was to determine if apparent molecular size of pectins and hemicelluloses change during peach fruit softening.

Materials and Methods

Plant material. 'Belle of Georgia' peach fruit was obtained from a commercial grower in 1992. Fruits were harvested from at least thirty trees based on visual indication of ripeness and transported to the laboratory in ice chests. Mesocarp firmness was measured after removal of pericarp on opposite cheeks of the fruit using an Effegi penetrometer (Effegi, Alfonsive, Italy) with a standard 8 mm probe. Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), using the average of both measurements,

and utilized immediately for cell wall preparation.

Preparation of fruit cell walls. Enzymically inactive cell walls were prepared essentially as described by Huber (1991). Fruit mesocarp was separated from the pericarp and stone, placed on ice and diced into small pieces. Mesocarp tissue was then homogenized for 6 minutes on ice with Tris saturated phenol [ratio of fruit tissue to tris saturated phenol was 1:3 (w/v)] using an Omni Mixer homogenizer (Omni International, Waterbury, CT) set at speed 6 (three successive bursts of 2 min each). The homogenate was then filtered through two layers of mira cloth over a buchner funnel, and the initial filtrate was saved for water soluble polysaccharide recovery. The residue was washed with water to remove phenol smell, then transferred to a scintered glass funnel and washed with 1l chloroform:methanol (1:1, v/v) followed by washes with acetone (1 to 2l) until a fluffy consistency was obtained. Acetone washed cell wall residue was dried in an oven at 60·C and stored in a brown colored bottle.

Extraction of cell walls. Cell walls were extracted sequentially to obtain pectin associated polysaccharides and then hemicellulose associated polysaccharides as described by Selvendran et al. (1985) with some modifications. Cell walls (1 g) were hydrated in 100 ml of 500 mM imidazole, pH 7 plus 0.05 % sodium azide *in vacuo* and then incubated for 12 h at 1·C. Samples were centrifuged and washed 4-5 times with 100 ml of distilled water. The residue was then re-extracted with 500

mM imidazole plus 0.05 % sodium azide for 2 additional hours at 20-22·C, followed by centrifugation and 4 to 5 distilled water washes. Imidazole and water rinses for both temperatures were combined, dialyzed against deionized water, then freeze dried and weighed. Imidazole was substituted for CDTA or EDTA to extract chelator soluble pectins because imidazole, in contrast to CDTA or EDTA, readily dialyzes away against deionized water (Mort et al., 1991). The imidazole extracted residues (0.85 grams) were then extracted with 100 ml of 0.05 M sodium carbonate plus 20 mM sodium borohydride for 16 h at 1·C. The cell wall material was further extracted with the same concentration of sodium carbonate and sodium borohydride for 3 h at 20-22·C. The supernatants designated as sodium carbonate (cold) and sodium carbonate (warm) were adjusted to pH 5 with acetic acid and dialyzed with 6 changes of deionized water and freeze dried. Extraction with sodium carbonate at 1·C deesterifies the pectins, thus minimizing depolymerization by ß-elimination during subsequent extractions (Selvendran et al., 1985).

The "depectinated" residues were extracted with a graded series of KOH, from 1 M to 4 M, to extract hemicelluloses. In all cases except the final extraction, 10 mM sodium borohydride was added to convert reducing sugars into alcohols and prevent a peeling reaction. The final extraction medium contained 3-4% boric acid. The "depectinated" residues were extracted with 1 M KOH plus 10 mM sodium borohydride for 2 h

at 1°C and then for 2 h at 20-22°C. Further extraction was carried out with 4 M KOH plus 10 mM sodium borohydride for 2 h at 20-22°C and then with 4 M KOH plus 3-4% boric acid for 2 h at 20-22°C. Supernatants designated as 1 M KOH cold, 1 M KOH warm, 4 M KOH and 4 M KOH plus boric acid, were adjusted to pH 5 with acetic acid and then dialyzed against deionized water and freeze dried. The final residue was freeze dried and weighed.

Size exclusion chromatography. Size exclusion chromatography was carried out on a column (1 cm * 50 cm) of Toyopearl HW55S (Supelco, Inc., Supelco Park, Bellefonte, PA) with 300 mM ammonium acetate, pH 5.2 as eluent. Samples (10 mg) were dissolved in 1 ml of 1 M imidazole, pH 7.0, prior to injection. Four hundred μl of the sample (4 mg) was injected and samples were eluted at a flow rate of 0.5 ml per minute. Peaks were detected using a Waters R401 refractive index detector (Waters Associates, Inc., Framingham, MA) at an attenuation of 8x. Fractions were collected at 1 min intervals and pooled based on the elution time of peaks of interest. Pooled samples were dried under vacuum and rinsed thoroughly with water to remove volatile elution buffer salts. Fractions were analyzed for sugar composition by gas chromatography. The column was calibrated with pullulan standards (Polymer Laboratories Inc. Amherst, MA) of molecular weight 853,000, 380,000, 186,000, 100,000, 48,000, 23,700, 12,200, 5800, 738 and 180, and with pecticacid (Aldrich Chemical CO.,Milw.,WI).

Anion exchange chromatography. Anion exchange separations were conducted for 4 M KOH plus 10 mM sodium borohydride extracts, using Waters (Waters Associates, Milford, MA) accell plus QMA Sep- Pak cartridge. The Sep-Pak column was preconditioned using four volumes of 1 M ammonium acetate pH 5.2, followed by four volumes of 50 mM ammonium acetate, pH 5.2. Five mg of the sample was suspended in 250 µl of 1 M imidazole, pH 7.0. When the samples were completely suspended, 250 µl of 1 M ammonium hydroxide was added to completely solubilize the sample. Then 100 µl of the supernatant was taken and diluted to 1 ml with 50 mM ammonium acetate and applied to preconditioned Sep-Pak cartridges. The effluent (neutral sugar fraction) was recovered and 2 ml of 50 mM ammonium acetate was rinsed through Sep Pak on to the effluent. Bound sugars were recovered by elution with 2 ml of 1 M ammonium acetate (acidic sugar fraction). The nonbound and bound fractions were utilized for further analyses.

Sugar composition analysis. Pooled fractions were methanolyzed and trimethylsilyated for gas liquid chromatography as described by Komalavilas and Mort (1989). One µl of the trimethylsilylated sugars was injected onto a DB-1 fused - silica capillary column (30 m * 0.25 mm i, d., J & W Scientific, Inc., Racho Cordova, CA) installed in a Varian 6000 gas chromatograph (Varian Associates, Walnut Creek, CA) equipped with a cool on- column injector and FID detector, using helium as carrier gas. The sample was injected at 105·C,

and the temperature was raised to 160·C and held for 4 min before being raised to 200·C at 1·C per min. Sugars were identified, by comparison with authentic standards and quantified using inositol as internal standard.

Results

Imidazole soluble fractions separated into two apparent molecular size peaks, a large apparent molecular size peak, represented by peak 1, and a broad polydisperse peak, represented by peak 2 (Figure 1). Peak 1 increased in prominence as fruits softened. Peak 1 also contained an increased mole percentage of arabinose and rhamnose and a decrease in mole percentage of galacturonic acid as fruits softened (Table 9). Galacturonic acid : rhamnose ratio decreased as fruits softened from 20:1 for firm fruit to 2:1 for soft fruit. In peak 2 there was also an increase in mole percentage of arabinose and rhamnose and a decrease in mole percentage of galacturonic acid as fruits softened. Galacturonic acid : rhamnose ratio decreased as fruits softened from 85:1 for firm fruit to 21:1 for soft fruit. In fruits of all firmness groups, peak 1 had higher mole percentage of neutral sugars and a lower mole percentage of galacturonic acid compared to peak 2.

In sodium carbonate cold extracts, as for imidazole

extracts, peak 1 was more prominent in soft fruits compared to medium soft and firm fruits (Figure 2). Peak 1 was enriched with galacturonic acid, arabinose, galactose and rhamnose (Table 10). As fruits softened, there was a decrease in mole percentage of galacturonic acid and glucose, and an increase in mole percentage of galactose. Peak 2 was also enriched with galacturonic acid, arabinose, rhamnose and galactose. Peak 2 contained a higher mole percentage of galacturonic acid compared to peak 1. In soft fruit, peak 2 separated into two size classes.

Sodium carbonate warm extracts separated into three apparent molecular size groups i. e. one large apparent molecular size peak, represented by peak 1, and two small apparent molecular size peaks represented by peak 2 and peak 3 (Figure 3). Consistent with results from imidazole and cold sodium carbonate extracts, peak 1 was more prominent in soft fruit, compared to medium soft and firm fruits. Peaks 2 and 3 were more prominent in firm and medium soft fruits compared to soft fruits. In peak 1, firm fruits and medium soft fruits contained a higher mole percentage of arabinose and galactose, and a lower mole percentage of galacturonic acid, compared to soft fruits (Table 11). In peaks 2 and 3, fruits of all firmness groups showed high mole percentage of arabinose, rhamnose and galactose compared to peak 1. As described for peak 1, these peaks exhibited a higher mole percentage of galacturonic acid in soft fruits, compared to medium soft and

firm fruits. Peak 2 had higher mole percentage of galactose compared to peak 3.

One M KOH cold extracts separated into three apparent molecular size peaks, one large apparent molecular size, represented by peak 1, and two small apparent molecular size peaks represented by peaks 2 and 3 (Figure 4). In contrast with the pectin extracts, peak 1 was more prominent in firm fruits compared to medium soft and soft fruits. Peaks 2 and 3 was more distinct in firm and medium soft fruits, compared to soft fruits. Peak 1 was enriched in xylose, glucose and mannose (Table 12). Firm and medium soft fruits had a high mole percentage of xylose compared to soft fruits. Xylose : glucose ratio approached one in all the fractions in soft fruits. Soft fruit contained a higher mole percentage of galactose, mannose and galacturonic acid than medium soft and firm fruits. Peaks 2 and 3 were enriched in xylose, glucose, galactose and fucose. Fucose was particularly enriched in peak 2 compared to peaks 1 and 3. Galacturonic acid was found at a higher mole percentage in peak 3 for medium soft and soft fruits compared to firm fruits. In comparison to peak 1, firm and medium soft fruits contained lower mole percentage of xylose, and soft fruits contained about the same mole percentage of xylose. In fruits of all firmness groups, glucose was found at a high mole percentage in peak 2 compared to peaks 1 and 3.

One M KOH warm extracts separated into three apparent

molecular size peaks, one large apparent molecular size peak, represented by peak 1 and two small apparent molecular size peaks, represented by peaks 2 and 3 (Figure 5). Similar to pectin extracts, and in contrast to cold 1 M KOH extracts, peak 1 was more prominent in soft fruits compared to medium soft and firm fruits. In fruits of all firmness groups, peak 1 was enriched in xylose, glucose and arabinose (Table 13). There was an increase in mole percentage of arabinose and rhamnose and a decrease in mole percentage of galactose and galacturonic acid, as fruits softened. Peaks 2 and 3 were enriched in xylose, glucose, galactose, arabinose and galacturonic acid. In these peaks, mole percentage of galacturonic acid increased as fruits softened. Peak 2 contained high mole percentage of fucose, xylose and galactose compared to peak 3. Galacturonic acid was present at higher mole percentage in peak 3 than peak 2.

Four M KOH plus sodium borohydride extracts separated into three apparent molecular size peaks, one large apparent molecular size peak, represented by peak 1, and two small apparent molecular size peaks represented by peaks 2 and 3 (Figure 6). Peak 1 was enriched in galacturonic acid, arabinose, xylose, glucose and galactose (Table 14). As fruits softened, there was a decrease in mole percentage of arabinose, rhamnose, galactose, galacturonic acid and an increase in mole percentage of xylose and glucose in peak 1. Peaks 2 and 3 were enriched in glucose, xylose, galactose and

mannose. They contained a lower mole percentage of galacturonic acid and a higher mole percentage of mannose than peak 3. Peak 3 was particularly enriched in mannose. compared to other peaks.

Four M KOH plus sodium borohydride extracts were separated into acidic and neutral fractions using QMA anion exchange Sep Paks. In fruits of all firmness groups, the acidic fraction eluted as peak 1 (Figure 7a). As the original extract, peak 1 was enriched in galacturonic acid and arabinose (Table 15). In soft fruits, galacturonic acid : rhamnose ratio was closer to 1:1. Xylose : glucose ratio was closer to 1:1 in soft fruit. Arabinose and rhamnose was found in higher mole percentage in soft fruits compared to firm fruits. The neutral fraction separated into two apparent molecular size peaks, corresponding to peak 2 and peak 3 for the original extract, with a redistribution from large to small size peak as fruits softened (Figure 7b). As in the original extract, peaks 2 and 3 were enriched in glucose, xylose, mannose and galactose (Table 16). Similar to the original extracts, peak 2 had a higher mole percentage of glucose and lower mole percentage of mannose than peak 3.

Four M KOH plus boric acid extracts separated into three apparent molecular size peaks, one large apparent molecular size peak represented by peak 1, and two small apparent molecular size peaks represented by peaks 2 and 3 (Figure 9). As fruits softened, peak 1 became sharper and peaks 2 and 3

became prominent. Peak 3 was particularly increased in size as fruits softened. Peak 1 was enriched in arabinose, galacturonic acid and galactose (Table 17) and as fruits softened there was an increase in mole percentage of galacturonic acid. Peak 2 was enriched in galacturonic acid, arabinose and galactose. In fruits of all firmness groups, peak 3 had lower mole percentage of galacturonic acid and a higher mole percentage of mannose compared to peaks 1 and 2.

Discussion

Imidazole extracts separated into large and small apparent molecular size peaks. As fruits softened there was a proportional increase in the large apparent molecular size peak but there was not much difference during softening for the small apparent molecular size peak (Figure 1). The large apparent molecular size peak contained a higher mole percentage of arabinose than the small apparent molecular size peak, but in soft fruits, arabinose was found at a much higher mole percentage than in medium soft and firm fruits (Table 9). Galacturonic acid : rhamnose ratio decreased substantially as fruits softened from 20:1 in firm fruit, 13:1 in medium soft fruit to 2:1 in soft fruit, but the ratio of arabinose : rhamnose remained the same, averaging 6:1. Imidazole is thought to extract calcium and solubilize the calcium crosslinked homogalacturonan region. The degree of decrease in

galacturonic acid : rhamnose ratio in peak 1 for softening fruit, along with a relatively constant rhamnose : arabinose ratio, supports a progressive cosolubilization of a relatively large rhamnogalacturonan region during peach softening, with little degradation of putative arabinan sidechains. In other fruits like pears, two major pectic polysaccharides obtained from progressively ripening fruit were a homogalacturonan region and a rhamnogalacturonan 1 like polymer with a high arabinose content. The two pectic polymers were apparently not linked. RG1 in ripening pears appeared to be degraded with the initial loss of much of its arabinose (Dick and Labavitch, 1989). Our data for peach supports increased solubility of RG1 with softening, with little selective loss of putative arabinose side chains. The small apparent molecular size peak exhibited a higher mole percentage of galacturonic acid than the large apparent molecular size peak, and probably represented a broad polydisperse peak of homogalacturonan region. Galacturonic acid : rhamnose ratio decreased with softening in peak 2 from 85:1 for firm fruit, 33:1 in medium soft fruit to 21:1 in soft fruit. Arabinose contents of peak 2 were one half to one quarter of the contents found in peak 1. Some redistribution of homogalacturonan region may have occurred during softening, as indicated by a slight increase in the galacturonic acid : rhamnose ratio. If RG1 was present in peak 2, it probably was much less prominent than in peak 1, as indicated by a higher galacturonic acid : rhamnose ratio

and lower arabinose content of peak 2 compared to peak 1. Softening muskmelon EDTA extracts exhibited a decrease in a large molecular size class and a concomitant increase in small molecular size class peak (Ranwala et al.,1992). While we did not observe a distinct increase in proportion of the small apparent molecular size peak as compared to the large apparent molecular size peak for softening peaches, the reduction in galacturonic acid : rhamnose during softening is consistent with redistribution of larger homogalacturonan regions into the smaller peak. In persimmon fruit, the EDTA soluble extract separated into large and small molecular size classes. As fruits softened, there was a decrease in proportion of the large molecular size class peak, but there was no change in the small molecular size class peak (Cutillas-Iturralde et al.,1993). In nectarines, ripening resulted in solubilization of pectic polymers of high molecular weight from cell wall material, and these solubilized pectic polymers were depolymerized to low molecular weight as ripening progressed (Dawson et al.,1992). EDTA soluble polyuronides from avocado and tomato fruit exhibited downshifts in molecular weight during ripening, but downshifts in molecular weight of tomato polyuronides were less extensive and less rapid than avocado polyuronide downshift (Huber and O'Donoghue, 1993).

In sodium carbonate cold and warm extracts, as in imidazole extracts, the higher apparent molecular size peak was more prominent in soft fruits compared to medium soft and

firm fruits (Figure 2 and 3). In sodium carbonate cold extracts, both higher and lower apparent molecular size peaks were enriched with pectin associated sugars like galacturonic acid, arabinose and rhamnose (Table 10). Apparent molecular size distribution changed from a single broad peak (firm) to two distinct peaks (medium soft) to three distinct peaks (soft). Galacturonic acid eluted predominantly in the higher apparent molecular size group (Table 11). Arabinose and rhamnose eluted with galactose in the two small apparent molecular size groups. Pectin constituents in sodium carbonate extracts appeared to differ from imidazole extracts. A higher galacturonic acid : rhamnose ratio and lower arabinose : rhamnose ratio for the large peak compared to the smaller peaks for sodium carbonate (warm) extracts is consistent with extraction of homogalacturonan-like polymers into large apparent molecular size, and extraction of rhamnogalacturonan-like polymers with lower apparent molecular size. Sodium carbonate extracts may also be extracting RG1 region with arabinose and galactose associated as side chains predominantly with the lower apparent molecular size peak. The backbone of rhamnogalacturonan region is rich in galacturonic acid and rhamnose, and bears numerous side chains rich in arabinose and galactose (McNeil et al., 1984). In other fruits like persimmon, the pectic fraction extracted with sodium carbonate (warm) was composed of high molecular mass polymers, and showed a considerable shift towards the low molecular mass

region as fruits softened. This shift was observed in both uronic acids and non cellulosic neutral sugars (Cutillas-Iturralde et al.,1993). Unlike peaches and persimmon, in nectarines, molecular size of sodium carbonate extracts did not change appreciably during ripening (Dawson et al.,1992).

As fruits soften, there was an increase in the large apparent molecular size peak and a decrease in small apparent molecular size peaks in 1 M KOH (cold) extracts (Figure 4). In these extracts, high amounts of xylose, glucose and mannose eluted in the higher apparent molecular size group, and soft fruit contained a lower xylose : glucose ratio (1:1) compared to medium soft (1:3) and firm fruit (1:2.4) (Table 12). In the middle apparent molecular size peak, xylose and glucose was associated with higher percentages of fucose and galactose than in other two fractions. Xylose : glucose ratio was close to 1:1 in the middle apparent molecular size peak for fruits of all firmness groups. Peak 2 appeared to be most xyloglucan - like and fucose seemed to be highly associated with it. This peak decreased in proportion to other peaks as fruits softened. Unlike peaches, in persimmon fruit, the average molecular mass for xyloglucan present in the 1 M KOH hemicellulose fraction increased up to a certain stage and then decreased in the last stage of fruit softening (Cutillas-Iturralde et al,.1994).

4 M KOH plus sodium borohydride extracts also separated

into a large apparent molecular size peak and two smaller apparent molecular size peaks (Figure 6). This tightly bound hemicellulose extract contained considerable percentage of pectin associated sugars in peak 1 (Table 14). As fruits softened there was a decrease in mole percentage of galacturonic acid, arabinose and rhamnose in this peak. In the two smaller apparent molecular size peaks, there was a proportional redistribution from medium to small apparent molecular size as fruits softened with a concurrent redistribution of mannose from peak 2 to peak 3. Anion exchange separation for this extract revealed that the larger apparent molecular size peak was particularly enriched in galacturonic acid, representative almost exclusively of the charged fraction (Table 15). In the acidic fraction soft fruits exhibited galacturonic acid : rhamnose ratio closer to 1:1 and had high mole percentage of arabinose which indicates rhamnogalacturonan-like region in soft fruit with arabinose side chain. Xylose : glucose ratio is closer to 1:1 in soft fruit indicating the presence of xyloglucan. 4 M KOH is known to extract hemicelluloses. Co-extraction of RG like region with xyloglucan further supports our data that pectins and hemicelluloses are associated with each other. Putative arabinan side chains may be a cross link between RG and xyloglucan. The neutral fraction separated on the size exclusion column (Figure 7b) as peaks 2 and 3 of the native extract (Figure 6) and was mainly composed of neutral sugars

like mannose, glucose and xylose (Table 16). In agreement with apparent molecular size shifts for the native 4 M KOH fraction (Figure 6), there was a reduction in higher molecular size polymers and a proportional increase in lower molecular size polymers. 4 M KOH plus boric acid extracts also separated into a large apparent molecular apparent molecular size peak and two small apparent molecular size peaks. Similar to 4 M KOH plus sodium borohydride extract, this extract also contained high mole percentage of pectin associated sugars in peak 1 and neutral sugars predominated in smaller apparent molecular size peaks. In other fruits, like tomato (Huber, 1983; Tong and Gross, 1988), hot pepper (Gross et al., 1986), strawberry (Huber, 1984) and muskmelon (McCollum et al., 1989), 4 M alkali soluble polysaccharides fractionated into two molecular size groups, and during ripening there was a shift from higher to lower molecular size group. In kiwifruit, three distinct molecular size classes of hemicellulose was reported, with a proportional increase in the smaller polymers with fruit ripening (Redgewell et al., 1991). Avocado fruit exhibited a range of hemicellulose sizes, which underwent a collective shift to a lower average molecular size during ripening (O'Donoghue and Huber, 1992). Peach hemicellulose associated sugars, represented by the neutral fractions, appeared to follow a similar pattern to other fruits, a general redistribution from higher to lower apparent molecular size during softening.

Peach fruit softening appears to be associated with changes in apparent molecular size of both pectins and hemicelluloses. A putative rhamnogalacturonan rich region elutes at a higher apparent molecular size group in chelator soluble extracts, and as the smaller apparent molecular size groups in sodium carbonate extracts, and apparently becomes more soluble in soft fruits. Homogalacturonan region appears primarily as a polydisperse apparent molecular size class in chelator soluble extracts, which does not change in magnitude much as fruits soften. Some evidence suggests that there may be a minor redistribution of homogalacturonan from larger to smaller size classes associated with softening in peaches. Hemicellulosic xyloglucan was most concentrated as an intermediate apparent molecular size peak in 1 M KOH (cold) extracts which decreased in proportion to other peaks as fruits softened. Analysis of tightly bound hemicellulose extracts suggest that pectins and hemicelluloses are in some way associated with each other. Co-extraction of RG-like region with xyloglucan further supports this concept. The ability to separate charged sugars as one peak and neutral sugars as two other separate peaks suggests that the nature of the pectin-hemicellulose interaction is probably not principally by covalent crosslinking between the polysaccharide classes in peach, but some crosslinking may occur. The complexity of apparent molecular size changes in pectin and hemicellulose extracts for peaches appears to

implicate the action of a number of different enzymes capable of degrading specific cell wall components during softening and lends further evidence that both pectin and hemicellulose are involved in softening of peaches.

Literature Cited

Cutillas-Iturralde, A., I. Zarra, and E.P. Lorences. 1993. Metabolism of cell wall polysaccharide from persimmon fruit. Pectin solubilization occurs in apparent absence of polygalacturonase activity. Physiol. Plant. 89:369-375.

Cutillas-Iturralde, A., I. Zarra, S.C. Fry, and E.P. Lorences. 1994. Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycocylase. Physiol. Plant. 91:169-176.

Dawson, D.M., L.D. Melton, and C.B. Watkins. 1992. Cell wall changes in nectarines (Prunus persica). Plant Physiol. 100:1203-1210.

Dick, A.J. and J.M. Labavitch. 1989. Cell wall metabolism in ripening fruit. IV. Characterization of the pectic polysaccharides solubilized during softening of 'Bartlett' Pear fruit. Plant Physiol. 89:1394-1407.

Fishman, M.L., B. Levaj, D. T. Gillespie, and R. Scorza. 1993. Changes in the physico-chemical properties of peach fruit pectin during on-tree ripening and storage. J. Amer. Soc. Hort. Sci. 118(3):343-349.

Gross, K.C., A.E. Watada, M.S. Kang, S.D. Kim, K.S. Kim, and S.W. Lee. 1986. Biochemical changes associated with the ripening of hot pepper fruit. Physiol. Plant. 66:31-36.

Huber, D.J. 1983. Polyuronide degradation and hemicellulose modifications in ripening tomato fruit. J. Amer. Soc. Hort. Sci. 108:405-409.

Huber, D.J. 1984. Strawberry fruit softening: The potential role of polyuronides and hemicelluloses. J. Food Sci. 49:1310-1315.

Huber, D.J. 1991. Acidified phenol alters cell wall pectin solubility and calcium content. Phytochemistry 30:2523-2527.

Huber, D.J. and E.M. O'Donoghue. 1993. Polyuronides in avacado (Persea americana) and tomato (lycopersicon esculentum) fruits exhibit markedly different patterns of molecular weight downshifts during ripening. Plant Physiol. 102:473-480.

Komalavilas, P. and A.J. Mort. 1989. The acetylation at 0-3 of galacturonic acid in the rhamnose rich portion of pectins. Carbohydr. Res. 189:261-272.

Maness, N.O., D. Chrz, S. Hegde, and J. C. Goffreda. 1993. Cell wall changes in ripening peach fruit from cultivars differing in softening rate. Acta Horticulturae 343:200-203.

McCann, M. C., B. Wells, and K. Roberts. 1990. Direct visualization of cross-links in the primary plant cell wall. J. Cell Sci. 106:1347-1356.

McCollum, T.G., D.J. Huber, and D.J. Cantliffe. 1989. Modification of polyuronides and hemicelluloses during

muskmelon fruit softening. Physiol. Plant. 76:303-308.

McNeil, M., A.G. Darvill, S. C. Fry, and P. Albersheim. 1984. Structure and function of the primary cell walls of plants. Ann. Rev. Biochem. 53:625-663.

Mort, A. J., B. M. Moerschbacher, M. L. Pierce, and N. O. Maness. 1991. Problems one may encounter during the extraction, purification and chromatography of pectic fragments, and some solutions to them. Carbohydr. Res. 215:219-227.

O'Donoghue, E.M. and D.J. Huber.1992. Modification of matrix polysaccharides during avocado (Persea americana) fruit ripening : an assessment of the role of Cx-Cellulase. Physiol Plant. 86:33-42.

Ranwala, A. P., C. Suematsu, and H. Masuda. 1992. The role of ß-galactosidases in the modification of cell wall components during muskmelon fruit softening. Plant Physiol. 100:1318-1325.

Redgewell, R.J., L.D. Melton, and D.J. Brasch. 1991. Cell wall polysaccharides of kiwifruit (Actinidia deliciosa): effect of ripening on the structural features of cell wall materials. Carbohydr. Res. 209:191-202.

Selvendran, R.R., B.J.H. Stevens, and M.A. O'Neill. 1985. Developments in the isolation of cell walls from edible plants. In C.T. Brett, J.R. Hillman, eds, Biochemistry of plant cell walls. Cambridge University Press, Cambridge,

UK. pp 39-78.

Tong, C.B.S. and K.C. Gross. 1988. Glycosyl-linkage composition of tomato fruit cell wall hemicellulosic fractions during ripening. Physiol. Plant. 74:365-370.

Table 9. Sugar composition of imidazole extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	17	3	1	5	8	1	3	61	1
Peak 2	5	1	1	1	3	1	1	85	2
Medium soft fruit (30 N)[z]									
Peak 1[y]	17	3	1	4	26	1	1	40	7
Peak 2	10	2	1	1	10	1	4	66	6
Soft fruit (15 N)[z]									
Peak 1[y]	46	8	1	3	16	1	1	18	6
Peak 2	13	3	1	1	8	1	4	63	6

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24-32 min) and peak 2 (33-54 min) were the apparent molecular size peaks of the imidazole extracts separated on Toyopearl HW55S column.

Table 10. Sugar composition of sodium carbonate (cold) extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	21	4	3	4	9	5	7	46	1
Peak 2	25	5	1	1	9	1	1	56	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	17	4	1	5	16	9	5	42	1
Peak 2	24	4	1	2	17	1	4	46	1
Soft fruit (15 N)[z]									
Peak 1[y]	31	2	1	3	18	8	3	33	1
Peak 2	23	6	1	1	15	2	1	50	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (20-26 min) and peak 2 (27-42 min) were the apparent molecular size peaks of sodium carbonate (cold) extracts separated on Toyopearl HW55S column.

Table 11. Sugar composition of sodium carbonate (warm) extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	19	2	3	6	19	9	7	34	1
Peak 2	48	7	1	1	28	1	1	12	1
Peak 3	69	6	2	4	7	4	1	6	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	24	6	1	10	18	7	16	17	1
Peak 2	71	8	1	2	10	1	1	5	1
Peak 3	70	9	1	2	8	5	2	2	1
Soft fruit (15 N)[z]									
Peak 1[y]	5	2	1	5	8	11	17	50	1
Peak 2	44	7	1	5	20	3	3	16	1
Peak 3	35	5	1	13	15	1	6	23	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24-32 min), peak 2 (33-42 min) and peak 3 (43-54 min) were the apparent molecular size peaks of sodium carbonate (warm extracts) separated on Toyopearl HW55S column.

Table 12. Sugar composition of 1 M potassium hydroxide (cold) extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	5	1	1	46	7	12	19	9	1
Peak 2	3	1	6	36	13	3	34	3	1
Peak 3	2	1	2	12	57	1	19	5	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	3	1	1	47	9	13	16	9	1
Peak 2	6	1	6	36	17	2	27	4	1
Peak 3	2	1	1	17	19	1	23	35	1
Soft fruit (15 N)[z]									
Peak 1[y]	9	1	2	18	17	17	17	18	1
Peak 2	4	1	7	20	29	4	29	5	1
Peak 3	4	1	3	21	22	4	26	18	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (22-30 min), peak 2 (31-40 min) and peak 3 (41-52 min) were the apparent molecular size peaks of 1 M potassium hydroxide (cold) extracts separated on Toyopearl HW55S column.

Table 13. Sugar composition of 1 M potassium hydroxide (warm) extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	13	2	1	21	25	4	24	9	1
Peak 2	13	2	9	34	11	2	21	7	1
Peak 3	12	1	1	13	9	1	16	46	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	13	3	12	16	24	3	21	7	1
Peak 2	19	3	4	23	13	2	9	26	1
Peak 3	17	3	1	11	8	1	11	47	1
Soft fruit (15 N)[z]									
Peak 1[y]	18	4	8	30	8	3	22	6	1
Peak 2	17	3	5	13	16	1	14	30	1
Peak 3	11	3	2	11	5	1	13	53	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (24-31 min), peak 2 (32-42 min) and peak 3 (43-54 min) were the apparent molecular size peaks of 1 M potassium hydroxide (warm) extracts on Toyopearl HW55S column.

Table 14. Sugar composition of 4 M potassium hydroxide plus sodium borohydride extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	20	3	4	17	17	2	17	19	1
Peak 2	7	1	1	6	12	6	57	9	1
Peak 3	9	1	1	8	14	42	15	9	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	14	2	1	29	15	1	24	13	1
Peak 2	16	2	6	29	16	3	20	7	1
Peak 3	4	1	1	4	12	59	14	4	1
Soft fruit (15 N)[z]									
Peak 1[y]	10	1	6	29	13	1	26	13	1
Peak 2	8	1	9	25	17	3	32	4	1
Peak 3	3	1	1	5	12	56	20	1	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (22-30 min), peak 2 (31-40 min) and peak 3 (41-54 min) were the apparent molecular size peaks of 4 N potassium hydroxide plus sodium borohydride extracts separated on Toyopearl HW55S column.

Table 15. Sugar composition of acidic fraction of 4 M potassium hydroxide plus sodium borohydride extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	16	1	1	10	14	14	15	28	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	32	9	1	6	6	2	12	31	1
Soft fruit (15 N)[z]									
Peak 1[y]	34	20	1	3	17	3	4	17	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 ( ) is the apparent molecular size peak acidic fraction of 4 M potassium hydroxide plus sodium borohydride extracts separated on Toyopearl HW55S column.

Table 16. Sugar composition of neutral fraction of 4 M potassium hydroxide plus sodium borohydride extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 2[y]	3	1	7	25	2	14	48	0	0
Peak 3	3	2	5	16	19	20	34	0	0
Medium soft fruit (30 N)[z]									
Peak 2[y]	2	2	6	22	15	10	40	2	0
Peak 3	4	1	6	23	14	22	27	3	0
Soft fruit (15 N)[z]									
Peak 2[y]	4	2	7	25	20	5	38	0	0
Peak 3	2	2	6	25	23	8	34	0	0

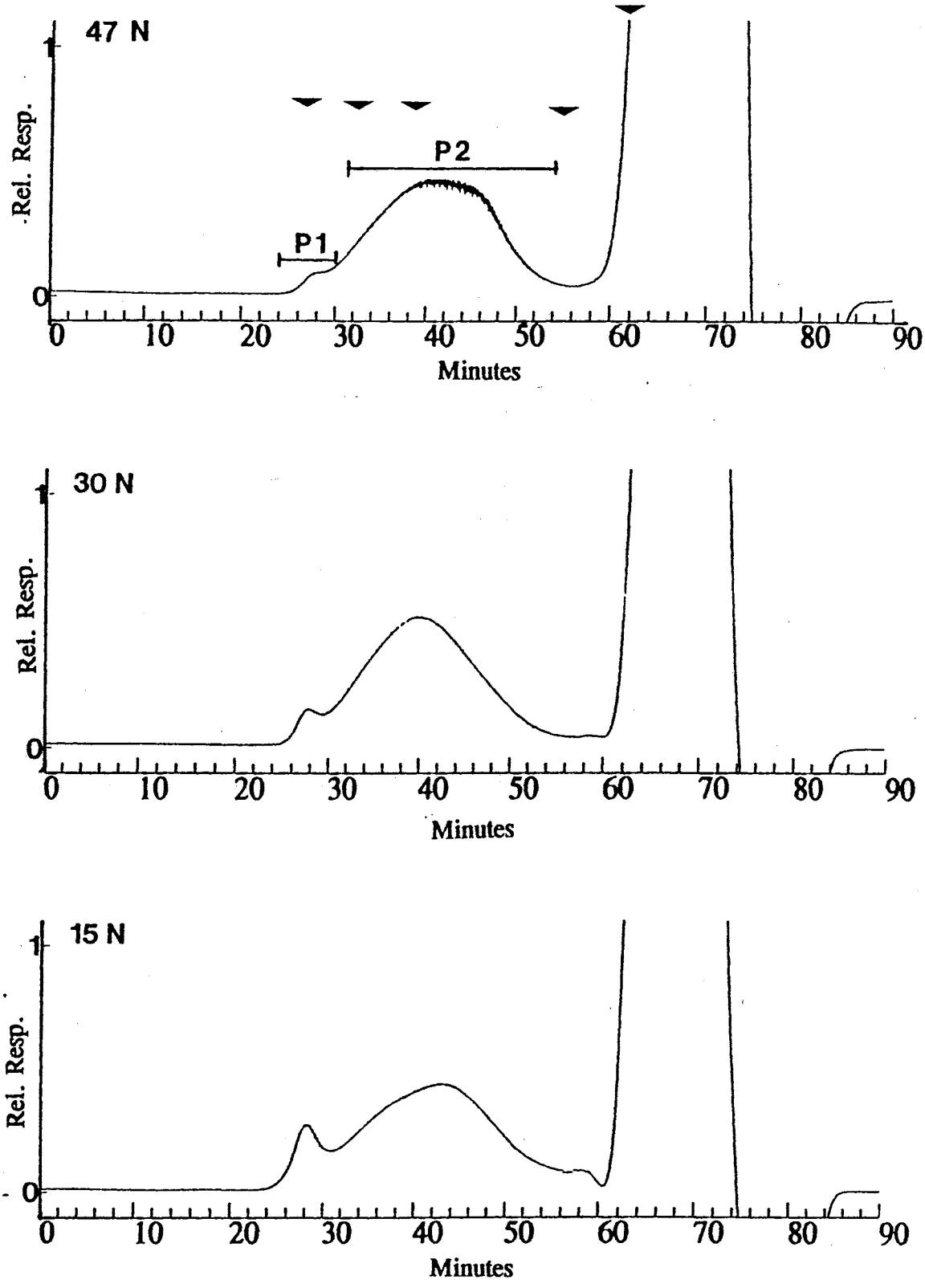
[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 2 (31-40) and peak 3 (41-54) were the apparent molecular size peaks of neutral fraction of 4 M potassium hydroxide plus sodium borohydride extracts separated on Toyopearl HW55S column.

Table 17. Sugar composition of 4 M potassium hydroxide plus boric acid extracts on size exclusion column, for cell walls of peach fruit differing in firmness.

	Sugar composition (mole percent)								
	Ara	Rha	Fuc	Xyl	Gal	Man	Glc	GalA	GlcA
Firm fruit (47 N)[z]									
Peak 1[y]	22	3	1	7	20	19	11	16	1
Peak 2	28	8	2	5	16	9	6	25	1
Peak 3	20	2	1	2	38	21	9	6	1
Medium soft fruit (30 N)[z]									
Peak 1[y]	33	1	6	9	17	6	9	18	1
Peak 2	30	5	1	6	14	16	9	18	1
Peak 3	11	5	4	4	8	52	9	6	1
Soft fruit (15 N)[z]									
Peak 1[y]	28	4	2	10	19	5	11	20	1
Peak 2	21	4	4	11	16	11	18	14	1
Peak 3	48	1	1	3	14	18	12	2	1

[z]Fruits were sorted into firmness groups of 47 N (firm), 30 N (medium soft) and 15 N (soft), by measuring mesocarp firmness on opposite cheeks of the fruit using an Effegi penetrometer.
[y]Peak 1 (22-31 min), peak 2 (32-41 min) and peak 3 (42-54 min) were the apparent molecular size peaks of 4 M potassium hydroxide plus boric acid extracts separated on Toyopearl HW55S column.

Figure 1.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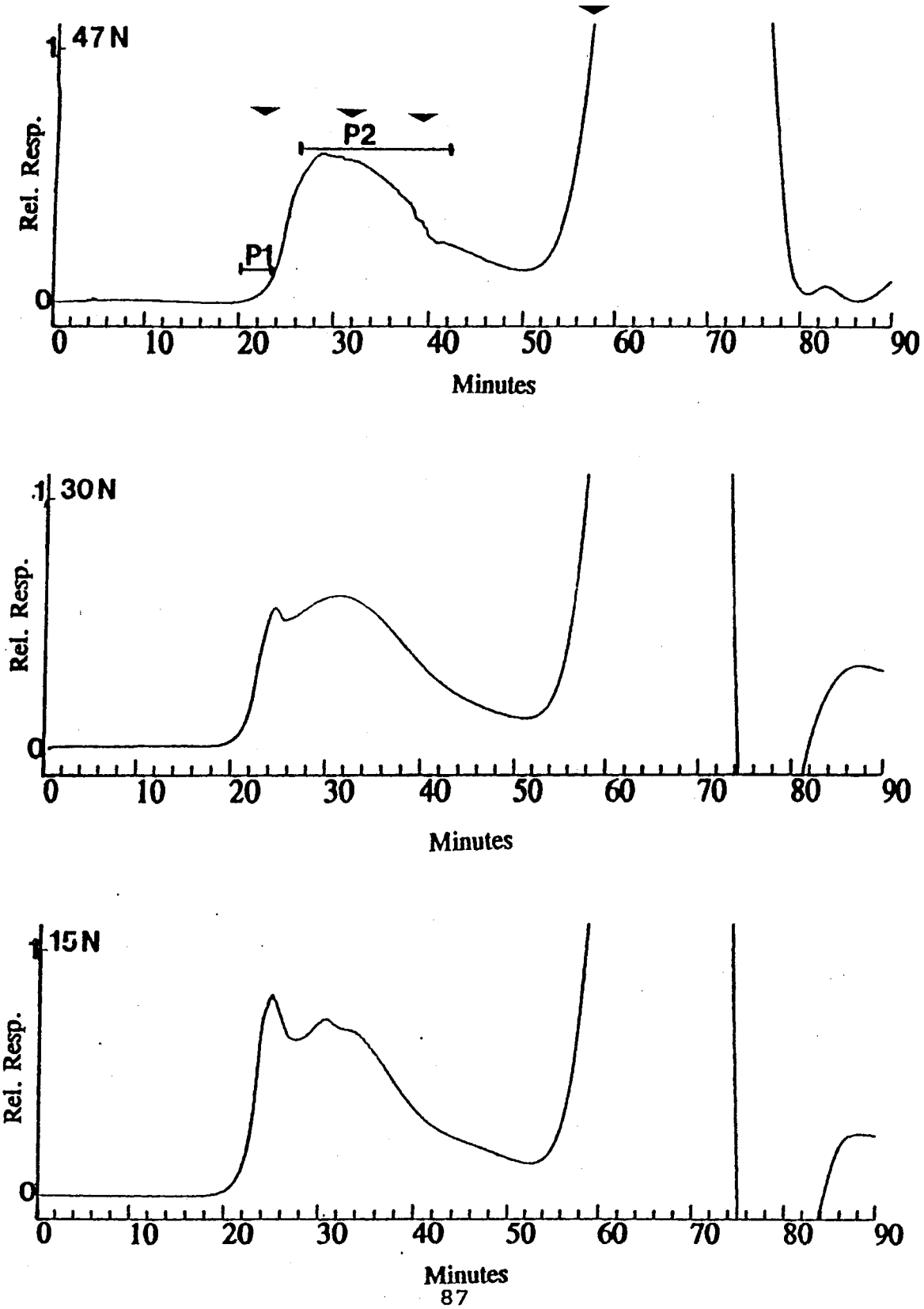
Minutes

Figure 2.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Minutes
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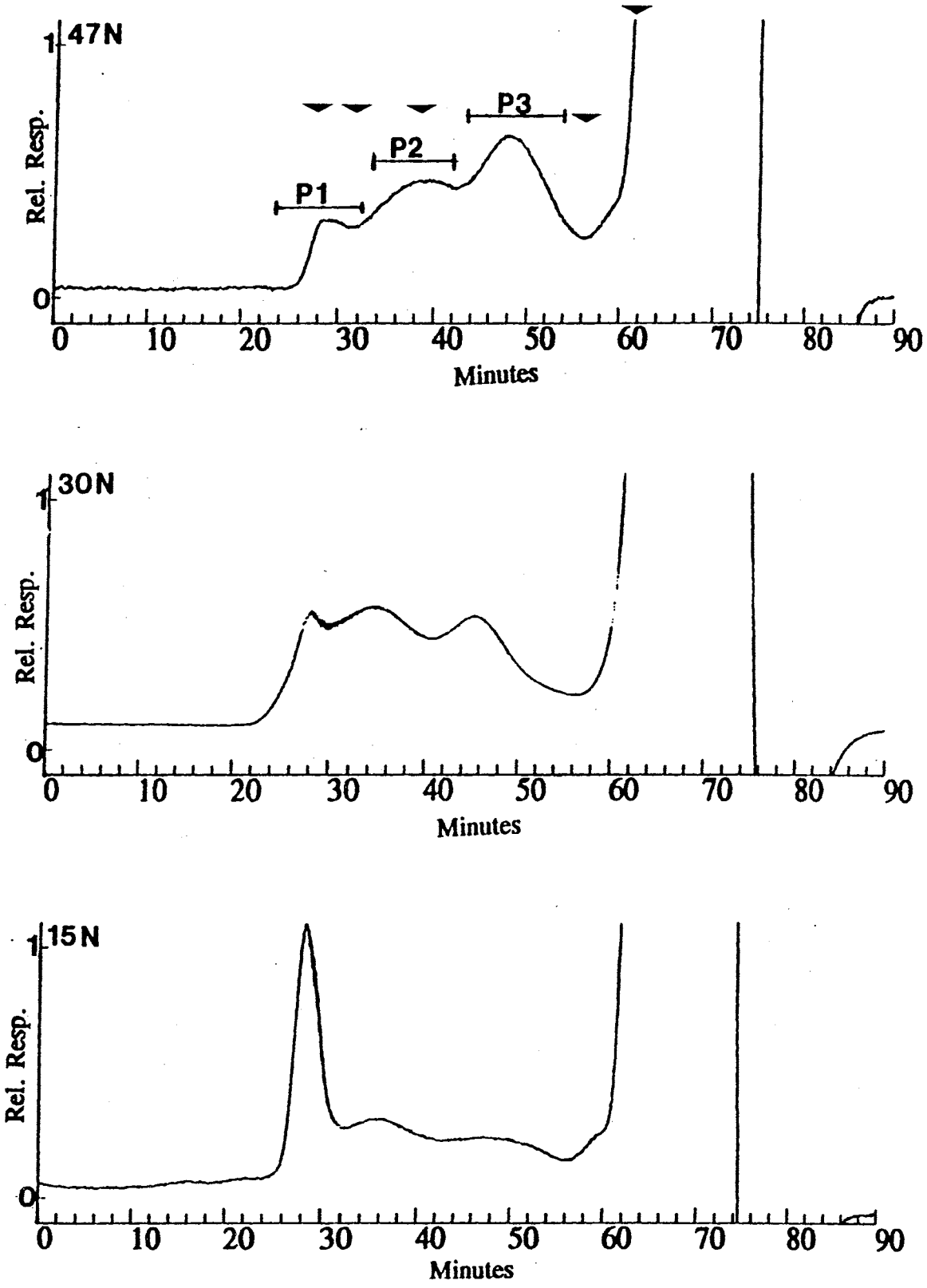
Minutes

Figure 3.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15N
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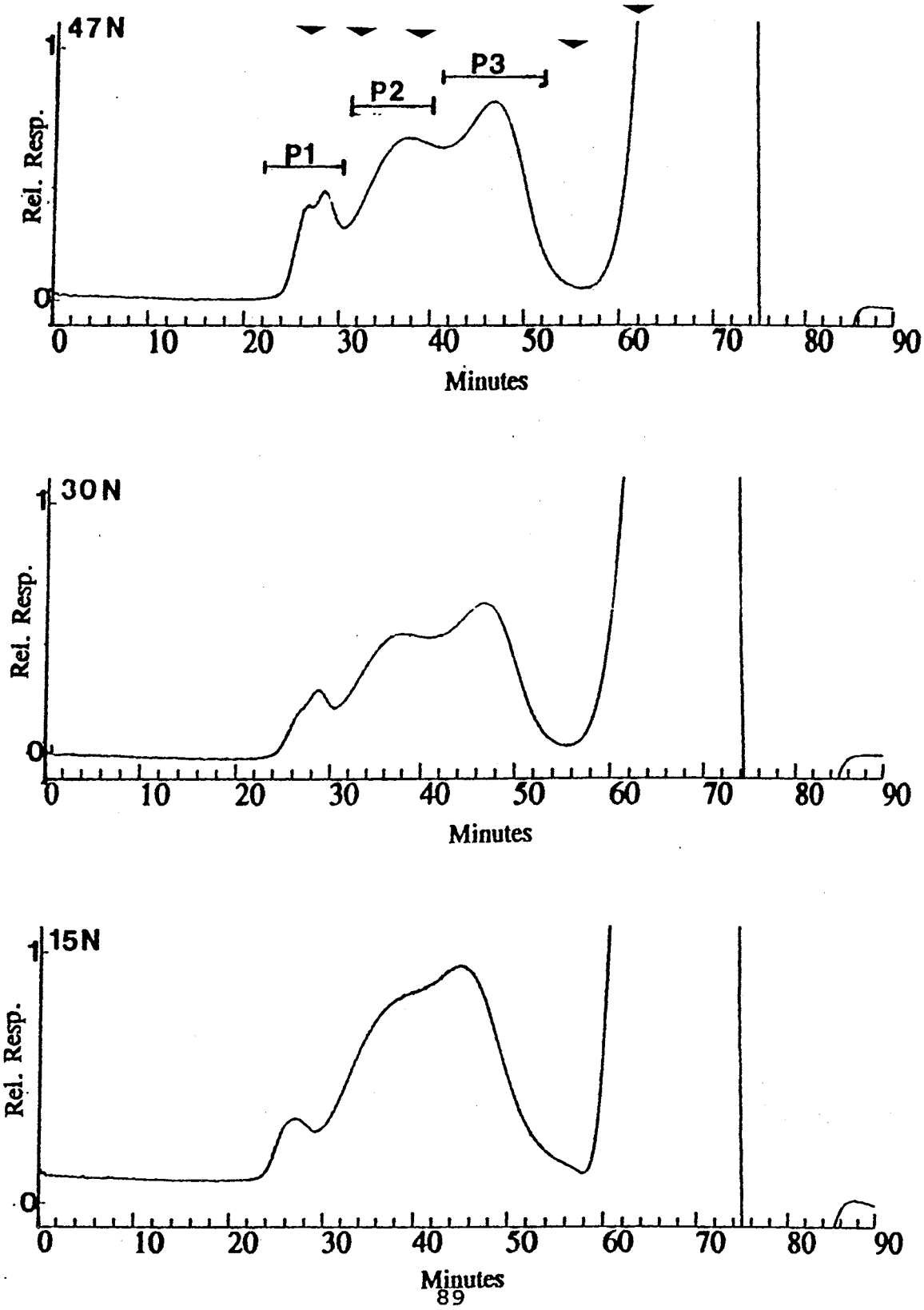
Minutes

Figure 4.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15N
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Minutes

Figure 5.

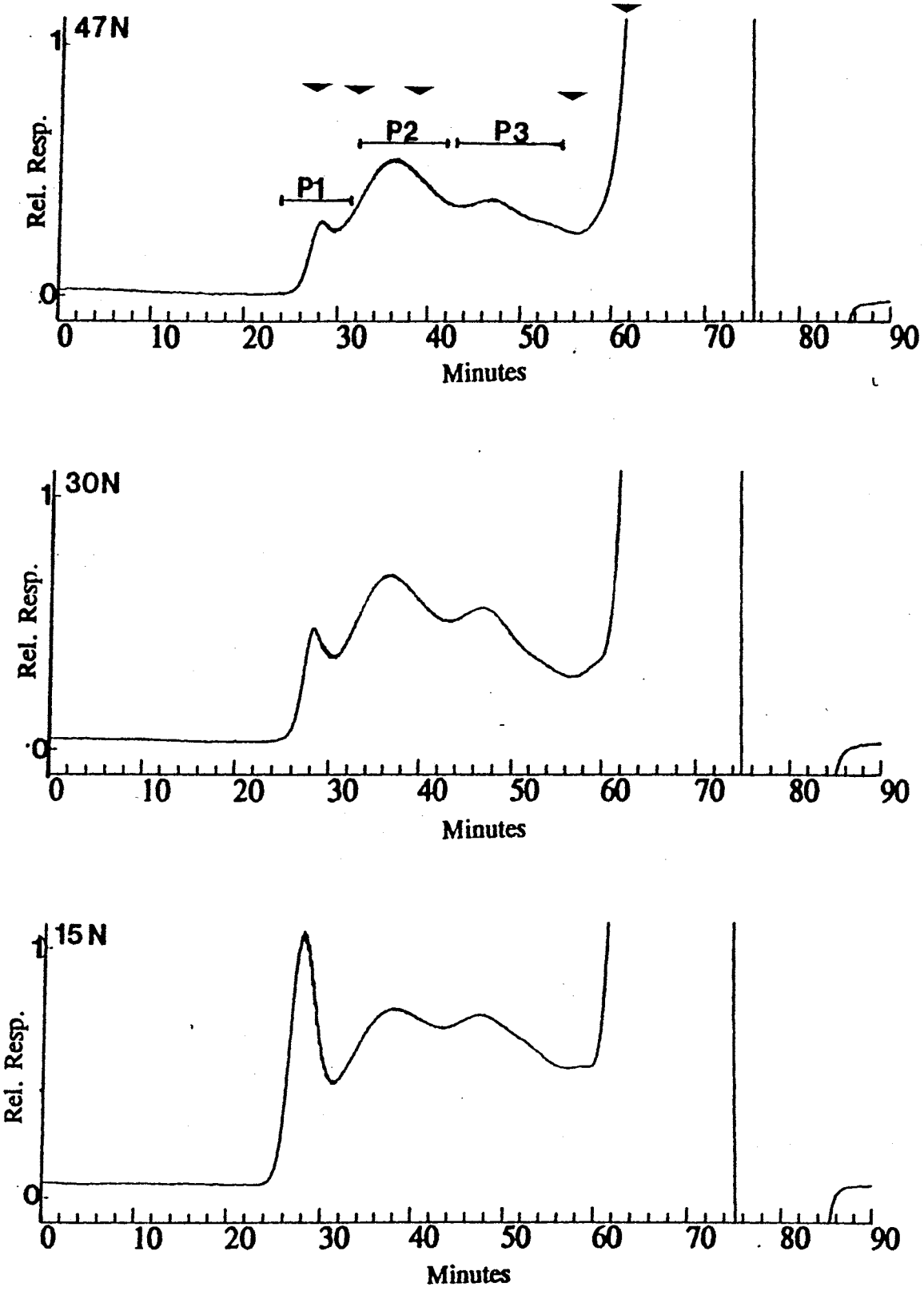
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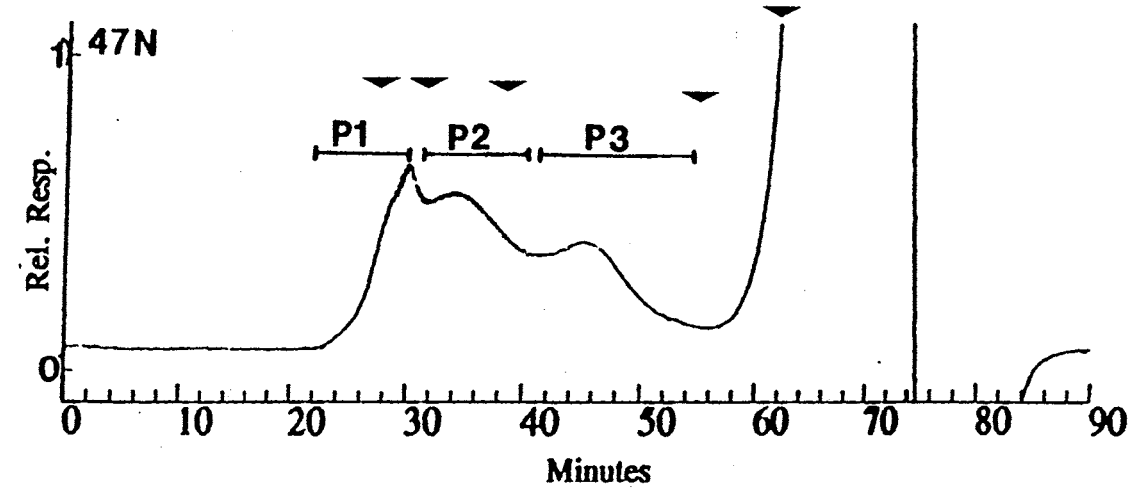
Figure 6.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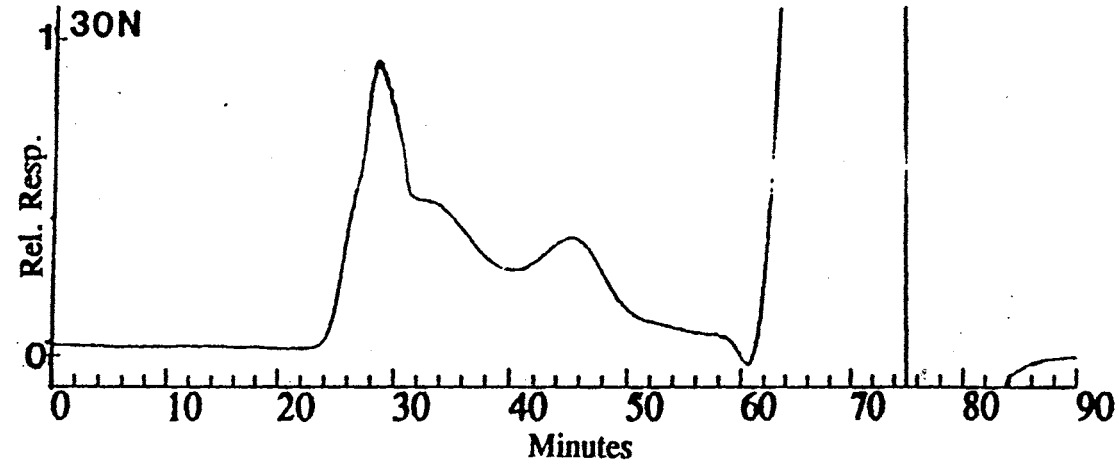
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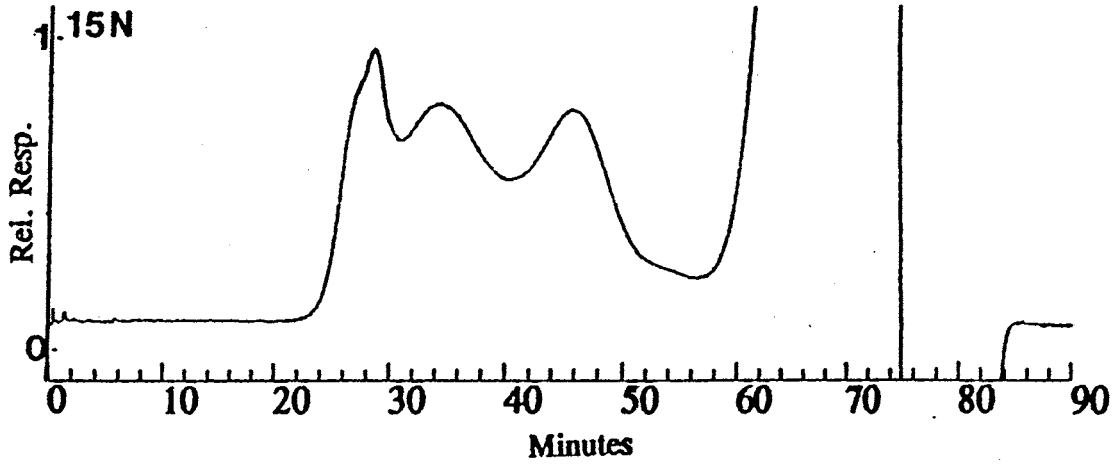
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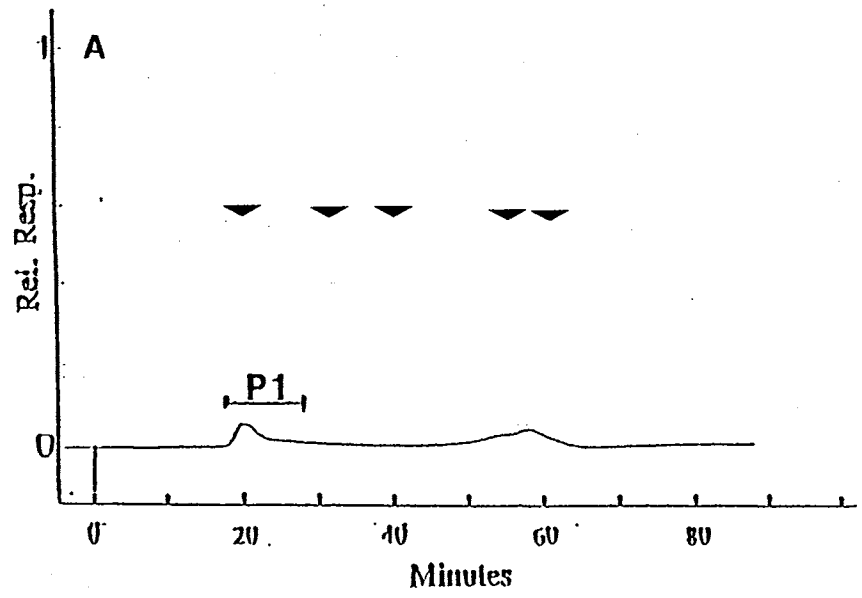
Figure 7.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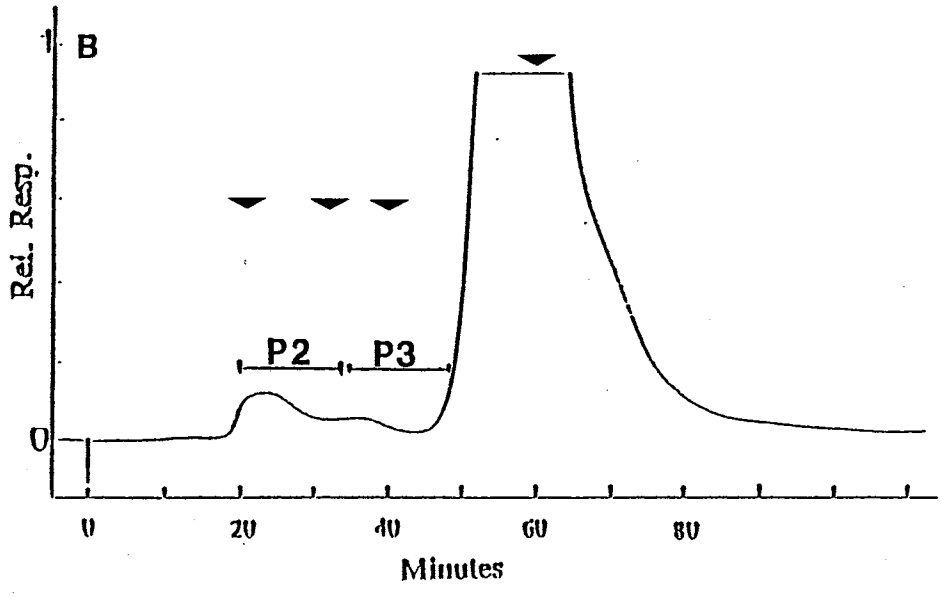
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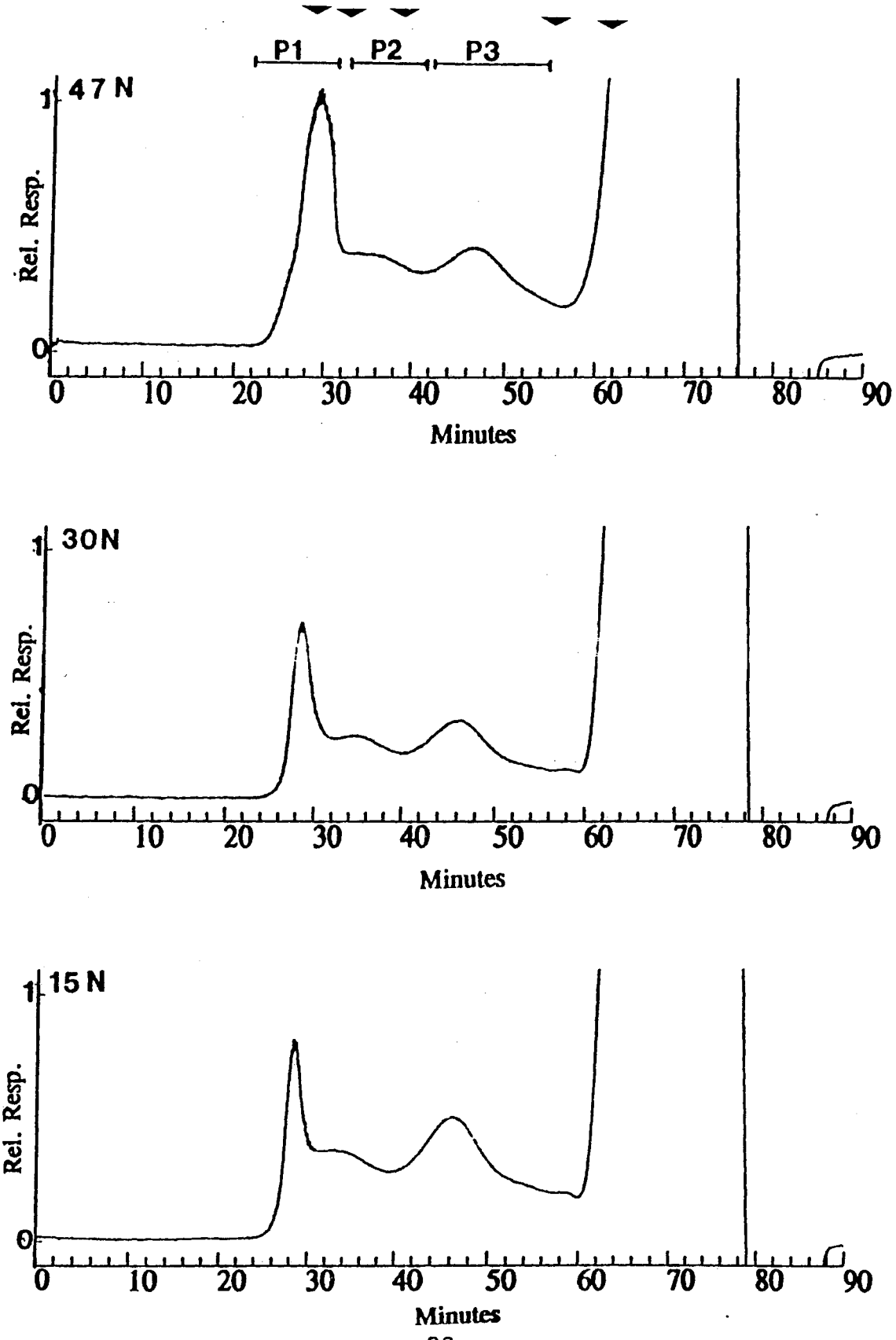
Figure 8.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30N
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0 10 20 30 40 50 60 70 80 90
Minutes
15 N
Rel. Resp.
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0 10 20 30 40 50 60 70 80 90
Minutes

VITA

Supreetha Hegde

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