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Title: Composition of Pectic Substances in Selva Strawberry (Fragaria x ananassa Duch.)

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Pectic substances in the cell wall have long thought to be responsible for the textural changes in fruit during ripening and senescence. Compositional variation within the pectic macromolecules could affect the external characteristics of the fruit. Strawberries undergo adverse quality changes such as softening and juice loss after freezing and subsequent thawing. To obtain a desirable cultivar that retains sound textural qualities after processing has long been an objective of both strawberry breeders and food processors.

The fruit of Selva is exceptionally firm and some may consider it to be too firm. The purpose of this study was to investigate the compositional factors that would account for the unusual textural qualities of the Selva strawberry. Two major Oregon-grown commercial cultivars, Totem and Benton, were also included to facilitate comparisons between pectic substances and textural profiles of strawberry fruits.

Fruit firmness was measured by the resistance of berry halves to shear and the amount of juice released after thawing. Very firm characteristic of Selva fruit was documented in that the shear value of thawed Selva was equal to fresh Totems and even greater than that of fresh Bentons. Juice-release after thawing was lowest in Selva samples. The quantity of acetone-insoluble solids obtained from freeze-dried powders of strawberry fruits was high for Selva and Benton, and low for Totem. Selva contained higher amounts of total soluble polyuronides and lower quantities of hemicellulose fraction as compared to Totem and Benton. Elution profiles of water-soluble polyuronides and chelator-soluble polyuronides from DEAE ion-exchange chromatography showed that Selva samples had a longer retention time and wider peak distribution, suggesting that its pectin species were of higher molecular weight and/or charge than Totem or Benton samples. The elution profile of ripe Selva was similar to that of underripe Totem and Benton. Ratios of uronic acid to rhamnose, indicating the extent of kinking and branching, were high for Selva and Totem in the water-soluble fraction and for Selva in the chelator-soluble fraction. Arabinose and galactose levels were relatively high in Selva base soluble polysaccharides, indicating that an arabinogalactan may be present in the hemicellulose fraction. This arabinogalactan may play a role in connecting the rhamnogalactan to the glycoprotein

in the cell wall.

Composition of Pectic Substances in Selva Strawberry (*Fragaria x ananassa* Duch.)

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Introduction

Pacific Northwest strawberries are noted for their superior quality because of their distinctive flavors and color intensity. There are many problems confronting the competitive strawberry industry related to yield, quality, and economic factors. The susceptibility to winter injury in the Willamette valley has been overcome by the widespread planting of Totem cultivar. Deficiency in yields has been improved by the adoption of Benton cultivar. With increased competition from California and Mexico, the prices of strawberries for processing are lower than ever before. (Sjulin, 1985).

During the past 15 years, adoption of the Totem cultivar may be a major reason for the lack of yield improvement in Oregon and Washington. The increase planting of Benton cultivar in Oregon during the past decade has led to a problem in processing quality due to its variable internal color and poor texture. For both the fresh and frozen market, berry wholeness is one of the factors that determine the customer's satisfaction. One of the major hurdles in developing the fresh berry market in Oregon is the high perishability of the product (Moulton,

1985). Thus, the development of a firmer cultivar needs to be studied.

Dramatic changes in berry flesh and juice-holding occur after fruit freezing and subsequent thawing. From the perspective of the Oregon berry industry, development of a desirable cultivar which can maintain berry wholeness and juice-holding for both fresh and frozen use will be an immediate and worthwhile goal for the breeding programs.

California day-neutral cultivars are characterized by their prolonged fruiting season (high yields) and extended shelf-life. Currently, there are some day-neutral cultivars in field trials under the breeding program of Agricultural Experimental Station at Oregon State University. Included trial cultivars are Selva, Tilikum, Sakuma, Tribute and Tristar. Selva was developed by Dr. Royce Bringhurst at the University of California, Davis, California. It is a relatively weak day-neutral type that behaves differently than any other University of California cultivars (Bringhurst and Voth, 1983). The fruit of Selva is exceptionally firm and some may consider it to be too firm. Concerning the problems facing the Oregon strawberry industry, Selva is a very interesting cultivar to study because of its unusual textural properties.

Fruit softening is often related to the changes in the cell wall structure during ripening. Several mechanisms of strawberry fruit softening during ripening and senescence

have been proposed, such as the involvement of enzyme polygalacturonase (Gizis, 1964), the changes of degree esterification of polyuronides (Neal, 1965), the increased synthesis of soluble polyuronides (Woodward, 1972; Knee et al., 1977; Huber, 1984), and increased levels of non-covalently bound neutral polysaccharide in the polyuronides (Wesche-E. et al., 1986). As the maturation proceeds, an increase of rhamnose content in relation to uronic acid content was reported (Huber, 1984; Wesch-E. et al., 1986). Rhamnose-induced kinking was thought to be preventing the interpolymeric association of neighboring pectin chains.

Little is known concerning the varietal differences of the strawberry cell wall in relation to the textural profiles. It is not yet clear whether varietal differences in cell wall fractions follow the same trends as changes related to maturity and softening (Buerger, 1986). The aim of this study was to investigate the compositional factors which could account for the unusual textural qualities of the Selva strawberry.

Literature Review

Pectic substances in strawberries

Pectic substances in the cell wall

The pectic substances are a group of closely associated polysaccharides from the primary cell walls and intercellular regions of higher plants. The term pectic substances is commonly used to encompass the methyl ester (pectin), the de-esterified pectic acid and its salts (pectate), and certain neutral sugars that are often attached to the galacturonan backbone (Aspinall, 1980). About one third of the cell wall polysaccharides are composed of pectic substances. (Roelofsen, 1959). Talmadge et al., (1973) reported that the primary cell wall of suspension-cultured sycamore cells contained 34% pectin, 38% hemicellulose, and 26% cellulose.

Pectic substances are mainly pectins that contain alpha 1-4 linked D-galacturonan backbone with L-rhamnose insertion and different neutral sugar side chains. The galacturonan backbone is often esterified in the carboxylic group at C-6 and occasionally acetylated in the hydroxyl group at C-3 or C-4. Structural variations are affected by the rhamnose insertion, the attachment of the neutral

sugars, the esterification of the galacturonic acid residues, and the acetylation of the hydroxyl groups. Each of these additional structural features changes the physical shape of the molecule and reduce the tendency to form the ordered conformations, which indirectly changes the physical properties of the pectins (Rees and Wight. 1971).

Rees (1969) developed the concept that carbohydrate gels were formed by adhesion between neighboring chains at a "junction zone". The kinks introduced by rhamnose insertion to the galacturonan backbone greatly decreased the length of junction zones between adjacent galacturonan chains. A dramatic increase of rhamnose content during the development of strawberry fruit has been reported (Huber, 1984, Wesche-E. et al., 1986). Three structurally distinct pectic polymers were isolated from sycamore cell walls: rhamnogalacturonan 1, rhamnogalacturonan 2, and homogalacturonan (Darvill et al., 1980). Rhamnogalacturonan 1 was rich in rhamnose, arabinose and galactose. Rhamnogalacturonan 2 was the most structurally complex plant polysaccharide of the three containing the rarely observed sugars apiose, 2-O-methylxylose,and 2-O-methylfucose. Homogalacturonan consisted mainly of unbranched alpha 1-4 linked galacturonic acid residues.

Neutral sugar polymers that are often associated with the galacturonan backbone are arabinan, galactan, and arabinogalactan (Darvill et al., 1980). An increase of arabinose of polyuronide origin with the ripening of strawberry was reported by Wade (1964) and Wesche-E. et al., (1986) but not by Neal (1965) and Huber (1984). Galactan was believed to be the bridge between the pectic substances and hemicelluloses in the sycamore primary cell walls (Talmadge et al., 1973). In examining cell wall metabolism of developing strawberry, Knee et al., (1977) found that galactose residues were metabolized from the cell wall into soluble fractions. The galactose in the whole cell wall fraction (ethanol or acetone insoluble solids) are fairly constant during the ripening of strawberry (Neal, 1965; Wesche-E. et al., 1986); the galactose that is associated with soluble polyuronides increases during the ripening of strawberry (Neal, 1965; Huber, 1984; and Wesche-E. et al., 1986). Arabinogalactans are present either as separate neutral polysaccharides or as subunits attached to the rhamnogalacturonan chains in pectins. Arabinogalactans of different origin are well documented by Darvill et al., (1980). The differences in the glycosyl linkages reflect the differences in the neutral sugar compositions of arabinogalactans of different crops.

The degree of esterification determines the pectin behavior in the cell wall and its ion exchange ability (Demarty et al., 1978; Morvan et al., 1979). Since plants also contain pectin-methylesterase, changes in pectin esterification can occur in plants during development, in response to injury, or during processing and storage of fruits and vegetables (McFeeters and Armstrong, 1984). Strawberry pectin-methylesterase is found to increase from the green to early ripe stages but decrease during the senescence (Barnes and Patchett, 1976). Lower degree of esterification increases the chance of polygalacturonic acid binding with cell wall calcium. It is proposed that calcium links the pectic chains at the junction zones in the manner of eggs in an eggbox (Rees, 1975). During the later stage of strawberry ripening, Wade (1964) found no changes of degree of esterification. However, a close interdependence of the degree of esterification of cell wall polysaccharides and the firming of strawberry fruits was reported by Neal (1965). The esterified polysaccharides would facilitate the sliding of the neighboring chains due to the free adhesion to one another.

The presence of relatively low levels of acetyl groups in the pectic substances will hinder the jellification of the pectin, but with more extensive acetylation a new surface of hydrophobic acetyl groups will be formed which

favors gel formation (Doesburg, 1965). The acetyl groups in strawberry polyuronide would hinder the interchain bonding (McComb and McCready, 1957). Wade (1964) found that the acetyl content of total cell wall polysaccharides was constant during ripening of fruit and decreased during storage of sulphited strawberry. Knee et al., (1977) reported that constant quantities of acetyl groups were present throughout strawberry fruit development. The distribution of the acetyl groups on the rhamnogalacturonan chain is unknown (Jarvis, 1984).

Physiological changes during ripening of strawberry

A fruit is said to be physiologically mature when ripening occurs. Ripening brings about physical as well as chemical changes. The strawberry, unlike apple, does not exhibit a respiration climacteric due to the increased production of ethylene during ripening (Woodward, 1972). Before, as well as after harvest, fruits undergo a complex series of biochemical reactions, such as conversion of starch to sugars, change in the form of sugars, use of sugars in respiration, decrease in organic acids, changes in pectic components, and production of volatile compounds (Westwood, 1978). In the study of post-harvest physiology of fruits, softening has long been a major commercial

concern. Softening results from changes in the pectic substances binding cells together (Doesburg, 1965).

Fruit weight and cell morphology:

As the fruit ripens, strawberries show an increase in the mean weight per berry and a decrease of solvent (acetone or ethanol) insoluble cell wall material per gram fruit (Wade, 1964; Neal, 1965; Woodward, 1972). Fruit growth was not exponential and in later stages of growth the falling survival rate was correlated to fruit softening (Woodward, 1972). Microscopic examination showed that parenchyma cells enlarged considerably with a decrease in the contact area between adjacent cells (Wade, 1964; Neal, 1965). By selective staining of pectin with Ruthenium red, it was found out that the parenchymatous cells were surrounded by a continuous layer of pectinous material, even after the extensive cell separation during ripening (Neal, 1965). The cells treated with EDTA show the same staining pattern with Ruthenium red, which is also similar to the natural ripening cells with a complete layer of pectinous material. After petal fall, cell number continues to decrease logarithmically with time and cell dimension increases logarithmically (Knee et al., 1977). As the time after petal fall extended, starch in the plastid diminished concurrently with extreme tubular

proliferation of the tonoplast (double membranes that surround the vaculoe) along with decreased staining of outer cell walls. During cell expansion the main increase is in vacuolar volume; protein synthesis and cell wall polysaccharide synthesis fail to keep pace with cell expansion.

Enzymes and ripening:

A critical role in the initiation of ripening has been focused on the activity of pectolytic enzymes, which are known to be involved in the fruit softening. In climacteric fruit, such as tomato, ripening is associated with ethylene synthesis, which occurs at about the same time as the onset of the respiratory climacteric (Rhodes, 1980). Strawberries are generally considered to be a non-climacteric fruit, between 21 and 28 days after petal fall receptacles become red and softened but there is no change in the rate of ethylene production (Knee et al., 1977).

Polygalacturonase synthesis occurs at the onset of ripening and the degradative action of this enzyme on the fruit cell wall releases wall-bound enzymes which bring about ethylene synthesis and other ripening events (Tigchelaar et al., 1978). Polygalacturonase is found in many fruits (Pilnik and Voragen, 1970) and its activity has

been shown to correlate with the loss of firmness in peaches (Pressey et al., 1971) and tomatoes (Huber, 1983). Most polygalacturonase are of the "endo" type, randomly hydrolyzing the polysaccharide chains into oligmer units. The presence of polygalacturonase in strawberry fruit is doubtful, having been reported by some workers (Gizis, 1964) but not by others (Neal, 1965; Barnes and Patchett, 1976; Huber, 1984). Woodward (1972) suggested that the enzymatic activity was involved in the decline of the polyuronide viscosity but provided no evidence for the detection of enzymes. Pectin-methylesterase is more widespread (Pilnik and Voragen, 1970) but its role in ripening is uncertain. It has been suggested that pectin-methylesterase is a prerequisite for the action of polygalacturonase through the removal of the ester unit at the C-6 carboxylic group. Pectin-methylesterase has definitely been detected in strawberry by Neal (1965) and Barnes and Patchett (1976). The drop in pectin-methylesterase activity during the later stage of ripening (Barnes and Patchett, 1976) is consistent with the suggestion by Neal (1965) that cell wall separation at the middle lamella could be brought about by methylation of polyuronides in this region. A Cx-type cellulolytic activity was observed in ripe strawberry fruit and increased in the overripe fruit (Barnes and Patchett, 1976). This enzyme is unlikely to be a true cellulase as

it does not attack insoluble cellulose. However, its activity is used to provide a possible explanation for the loss of firmness in strawberry fruit. No polygalacturonase and polymethylgalacturonase activity was detected in strawberry fruit (Barnes and Patchett, 1976). The majority of current evidence points toward that the loss of firmness in senescent strawberries is not due to an enzymatic degradation of the pectic material of the fruit cell wall. The elution pattern of polyuronides from Ultrogel AcA 34 during ripening did not reveal any sign of molecular weight changes indicative of polygalacturonase modification (Huber, 1984). The author detected the alteration of hemicellulose fractions with ripening, but no hemicellulase was found. The shifting of high molecular weight to low molecular weight in hemicellulose fractions occurred independently of polyuronide degradation.

Polyuronides and ripening:

The clearest change in cell wall composition that accompanies fruit softening is a decrease in wall-bound uronic acids (pectin) that is closely matched by an increase in soluble uronides (Labavitch, 1981). Wade (1964) extracted cell wall insoluble polysaccharides and noticed a significant drop in anhydro-uronic acid for both fresh fruit during ripening and preserved fruit with

increase of storage time. A reexamination of Wade's extraction procedure shows that the water soluble polysaccharide fraction was discarded during extraction to prevent masking of any changes occurring in the insoluble material. This discarded fraction may have an important role in the texture changes during ripening. Neal (1965) found that no major changes occurred in the overall cell wall compositions and the total polyuronides remained constant over the maturation period of strawberry. However, maturation is accompanied by an increase in the amount of water soluble polyuronides and also of arabinans and galactans. Almost all of the polyuronides can be rendered soluble at all stages of ripeness by EDTA, which emphasizes the importance of cations in the solubilization of pectin. It is suggested that the polyuronides become water soluble as maturation proceeds, and the changes in EDTA-soluble polyuronides is associated with the separation of the cell walls. This again emphasizes that the changes lie within the subdivision of the cell wall rather than in the total cell wall composition. A net synthesis of polyuronides after 20 days of petal fall consisted mainly of soluble polyuronides (Woodward, 1972). The amount of insoluble polysaccharides is small and relative constant during the development of fruits as compared to soluble polysaccharides. The increase in water soluble polyuronides that results from the activation of

polygalacturonase was reported in peach (Yamaki et al., 1979) and in tomato (Brady et al., 1982). Knee et al., (1977) reported that the wall bound polyuronide decreased as freely soluble polyuronide increased during the development of strawberry fruit, although there was no increase in total polysaccharide. The high proportion of freely soluble polyuronide at petal fall might be derived from the cell plates of dividing cells and the young primary walls in which polyuronides are relatively unentangled with other polysaccharides. Huber (1984) obtained soluble polyuronides from ethanol insoluble powder of strawberry and found that a modified, more freely soluble form was synthesized during ripening though total polyuronides as a percentage of ethanol powder remained constant. Wesche-E. et al., (1986) reported that the water soluble polyuronides increased as chelator soluble polysaccharides decreased during ripening. The increased levels of water soluble polyuronides may alternatively be due to solubilization of chelator soluble polyuronides during expansion of the cell wall as the fruit ripens.

Neutral sugars and ripening:

The most apparent change accompanied with the solubilization of the polyuronides during ripening is the alteration of the neutral sugars. The means by which the

turnover of neutral sugar-containing wall constituents is accomplished are not nearly as clear as the mechanism of pectin solubilization (Labavitch, 1981). Higher contents of galactan are associated with firmer strawberry cultivars, while the neutral sugar composition is fairly constant during ripening (Wade, 1964; Neal, 1965). Soluble sugars increase steadily during development and ripening, and are maintained throughout senescence (Woodward, 1972). Total sugars increases logarithmically as a cell basis after 35 days petal fall (Knee et al., 1977). Galactose and arabinose of polyuronide origin increased during ripening though rhamnose decreased (Huber, 1984). Neutral sugar composition of ion-exchange chromatographic peaks of water soluble polyuronide and chelator soluble polyuronide were intensively examined by Wesche-E. et al., (1986). Arabinose and galactose were detected as major sugars followed by xylose and glucose.

Neutral sugar side chains attached to the polyuronides may be involved in cross-linking to cellulose microfibrils (Keegstra et al., 1973; Knee et al., 1975). Strawberry cell wall contains lower levels of arabinose, galactose and xylose than apple cell wall and this may indicate that there are fewer side chains available for cross-linking of polyuronide to microfibrils (Knee et al., 1977). The increase in xylose, mannose and glucose residues during ripening suggests that hemicellulose was either being

degraded or released from interpolymeric bonds. This suggestion may provide some clues for changes of the elution pattern of hemicellulose from Ultrogel AcA 34 (Huber, 1984).

While not amongst the predominant sugars in strawberry, rhamnose exhibited the most dramatic increase (five fold), resulting in an overall decrease in the molar ratio of galacturonic acid to rhamnose with ripening (Huber, 1984). This increase of rhamnose is perhaps related to the increase observed for arabinose and galactose since they are apparently linked to polyuronides via rhamnosyl moieties (McNeil et al., 1982). Wesche-E. et al., (1986) also reported that changes in rhamnose levels may be related to the texture of the strawberry fruit, i.e. higher at the ripe and overripe stages in the softer cultivar and lower in the firmer culticar. Analyses have revealed that rhamnose insertions resulted in a marked kinking of the parent polymer, minimizing the frequency of interaction of any two adjacent chains (Grant et al., 1973; Morris et al., 1977).

Analytical methods

Extraction and fractionation of cell walls

Extraction of cell wall polysaccharides:

In preparing plant materials for isolation of individual compounds, the most important precaution is the avoidance of artifacts. Oxidation and hydrolysis are the most common degradative process; and if constituents are sought that are subject to those reaction, care must be taken to reduce such effects (Robinson, 1983). Probably the safest general method for all eventualities is immersion in liquid nitrogen followed by freeze-drying, and extraction of dried material with a solvent that does not permit degradative changes to occur. It is well known that strawberry achenes contain relatively large amount of phenolics that could affect the isolation of pectic substances during the extraction procedure. Therefore, achene removal in the early stage of the extraction is practiced by some workers (Wade, 1964; Wesche-E. et al., 1986). With the use of cold 0.1% saline, Wade (1964) was able to isolate cell wall material from strawberry fruit at different stages of ripeness as well as from preserves. The use of saline rather the distilled water was found to

be necessary to avoid excessive swelling of the tissue and the consequent difficulty in centrifugation as washing proceeded. The tissue was finally washed three times with acetone and air dried. Neal (1965) obtained ethanol insoluble residues by blending one volume of tissue with three volumes of 95% ethanol and suspended successively in ethanol and acetone. Sistrunk and Moore (1967) extracted cell wall material by mixing tissues with acidified 95% ethanol (0.1% HCl). Alcohol-insoluble residues were washed with large volumes of ethanol to remove any residual C-15 glycosides and refluxed for 30 min in ethanol (Woodward, 1972). The preparation was then washed with ethanol and ether shortly before being air dried. The purified cell wall was obtained by macerating tissues in Tris-DIECA with successively washes and then freeze-dried (Knee et al., 1977). Cell wall homogenates were prepared by homogenizing one volume of tissue in four volumes of ethanol containing 20 mM EDTA (Huber, 1984). The homogenate was then heated in a boiling water bath, washed with 1 L each of 100 mM NaCl and acetone followed by 0.5 L chloroform-methanol (2:1, v/v). Wesche-E. et al., (1986) developed a procedure based on an adaptation of methods used by O'Berine (1980), Knee (1973) and Tetley (1984) for isolating strawberry cell wall polysaccharides. Cell wall material was extracted with 80% acetone with repeated washes of 80% and 100% acetone, then freeze-dried for further fractionation.

Commonly, strawberry tissue is pre-extracted with 80% ethanol or acetone solutions before the pectic substances are obtained; however, there is some uncertainty as to whether contact with ethanol alters the degree of esterification of pectic substances under these circumstances (Joslyn and Duel, 1963). Knee (1973a) reported that when comparable samples of apples tissue wereextracted with cold acetone and with ethanol under flux, more insoluble material was recovered with acetone. The preliminary extraction of pectic substances with 80% acetone or ethanol removes sugars and other low molecular weight compounds, inactivates enzymes and provides a convenient starting material for further analysis. By assaying for the amount of methanol and reducing sugars released from the acetone insoluble solid suspension, O'Berine (1980) developed a procedure for enzyme inactivation prior to the extraction of pectic substances. A 10 minute reflux in 80% acetone was considered adequate for the inactivation of pectin-methylesterase and other reducing sugar releasing enzymes.

Fractionation of cell wall polysaccharides:

Fractionation of cell wall materials is less consistent among the researchers than the extraction of cell wall polysaccharides. Wade (1964) separated cell wall

polysaccharides into the soluble and the insoluble parts and studied only the insoluble isolate. Neal (1965) extracted the cell wall fractions, prepared from fruits at three stages of maturity, with distilled water, EDTA in Tris-HCl buffer and pectasin enzyme. The EDTA treated cell wall does not lead to a complete removal of the pectin from the separated cell surfaces. This contrasts with the separation of the pectasin that generally results in a complete loss of the pectin from the cell walls. Cell wall materials extracted with acidified ethanol were further treated with water, 0.05% Calgon, 0.05N NaOH, 1N NaOH and 70% H_2SO_4 (Sistrunk, 1967). The 1N NaOH-soluble fraction was determined to be as hemicellulose, and the 70% H_2SO4-soluble fraction to be as cellulose by the phenol method. Since the Calgon, 0.05N NaOH, and 1N NaOH fractions were small and positively correlated, they were combined into one fraction. Woodward (1972) extracted soluble pectic polysaccharides by suspending acetone-insoluble residues in 0.05 M EDTA at 20°C for 24 hr. The residue obtained from the extraction contained the insoluble pectic polysaccharides, which were then extracted with a pectinase. All filtrates were analyzed for polyuronide and neutral polysaccharides. The amount of polyuronides in the pectinase-treated filtrates as well as the soluble polyuronides were taken to represent the total pectin content of the cell walls. Knee et al., (1977)

fractionated Tris-DIECA (pH 8) pretreated cell wall tissues into a Tris base (pH 10) soluble fraction and a pure cell wall fraction. The Tris-DIECA soluble fraction was considered to be the low molecular weight fraction (mol wt. < 1,000). The Tris base-soluble fraction represented the ionically bound high molecular weight material. Huber (1984) obtained soluble polyuronides by suspending ethanol powder in Na-acetate buffer containing 20 mM EDTA . The suspensions were filtered and washed with extraction buffer. The combined filtrate and washing were either used directly or dialyzed. Hemicellulose fractions were extracted from the cell wall powder instead of the residues after the extraction of the soluble polyuronides. Briefly, 250 mg of cell wall were placed in 7 mL 4N NaOH containing 9 mg mL^{-1} $NaBH_4$. After two successive 8 hr extractions, the combined filtrates were neutralized with acetic acid and dialyzed successively against tap water, 10% methanol and distilled water. This preparation was used directly or stored at -20°C. Wesche-E. et al., (1986) sequentially fractionated acetone insoluble powder into four different fractions according to their solubility in different extraction media. Water-soluble fraction was obtained from Tris-HCl buffer, pH 7.5. Chelator-soluble fraction was obtained by refluxing EDTA buffer at 95°C for 4 hr. Base-soluble fraction (BSP) was obtained by 4N NaOH extraction for 4 hr. The residues remained after the

separation of BSP was considered to be cellulose-like material.

Separation of pectic substances

Ion exchange chromatography is particular valuable in separating the acidic polysaccharides from the neutral polysaccharides and is also useful in separating those polysaccharides that contain differing amounts of acidic residues (Darvill et al., 1980). The selection of the correct ion exchange functional group is determined by the effective charge of the biopolymer and its pH range of stability. It is the charge that determines whether the biopolymer will bind to the ion exchanger or not. The charge carried by the Diethylaminoethyl Cellulose (DEAE cellulose) varies with the pH value of the system. It is the charged or protonated form of the DEAE group that is available for the ion exchange mechanism. The anion which acting as a counter ion may be acetate, chloride, citrate, phosphate, etc. and these would be exchangeable (Whatman Chemical Separation Inc., 1981).

Neukom et al., (1960) first reported the separation of pectic substances on DEAE cellulose column (Knee, 1970). These authors found that neutral polysaccharides were eluted only when a gradient of sodium hydroxide

concentrations was applied. Heri et al., (1961) later reported that pectic polymers were more strongly bound to DEAE cellulose as their degree of polymerization increased or their esterification and content of neutral sugar side chains decreased. Jermyn (1962) criticized the procedure of Neukom et al., (1960) in that the methyl-esterified polygalacturonides would be undergoing de-esterification in alkaline conditions. The effect of the number of free carboxyl groups on the separation of pectic substances on DEAE chromatography is demonstrated by the gradual decrease in methoxy content and degree of esterification with the increase of fraction numbers (Smit and Bryant, 1967). By using viscosity measurement in 1% Calgon, no regular changes in molecular weight were observed and none of the fractions had a molecular weight as high as the original pectin. As the pectic substances were separated, the first concern was to examine the materials as closely as possible to the molecular state in the intact cell wall. Systems showing the least amount of interference were chosen for chromatography (Knee, 1970). The first chromatographic system to be tried involved the use of sodium chloride gradients together with a constant concentration of Tris-HCl buffers including 0.001 M EDTA at pH 8.0 and 8.9. The pectic substances were almost unretarded in these systems. Subsequently, a more satisfactory result was obtained from columns equilibrated with phosphate buffer at

pH 6,5 including 0.001 M EDTA. In order to separate neutral and acidic pectic components the initial phosphate concentration had to be 0.005 M. The inclusion of EDTA in all buffers used for chromatography might have contributed to the success of this system, as EDTA might eliminate the competition between calcium ions and cellulose exchangers to bind with polyuronides (Knee, 1970).

For extraction at an elevated temperature, pH 4 would seem to be the most satisfactory. The use of lower pH values would entail more extensive hydrolysis of neutral polysaccharides, while at higher pH values de-esterification and transelimination would become significant reactions. Thus there is a need for an extraction medium which can be used at low temperature (i.e. cyclohexanediamine tetraacetic acid, CDTA) to provide a relatively undegraded fraction representative of the bulk of the pectin in a plant tissue (Jarvis et al., 1981). If such material could be isolated, DEAE cellulose chromatography would be a very useful means of separating its component polymers. This would in turn lead to a new understanding of some of the molecular changes which are involved in the softening of fruits during ripening.

Huber (1984) demonstrated an ion-exchange chromatography of polyuronides on a bed (15 cm high, 1.3 cm wide) of DEAE Sephadex which had been equilibrated and packed in Na-phosphate buffer (15 mM, pH 6.8) containing 20

mM NaCl and 5 mM Na_2EDTA. Polyuronides (8 mg), after dialysis against the equilibration buffer, were applied to the column and eluted with the phosphate buffer. After 60 mL were collected, the column was washed with a NaCl gradient generated from 150 mL phosphate buffer and 150 mL buffer containing 800 mM NaCl. Fractions of 5 mL were collected at a flow rate of 10 mL . cm^{-2} . hr. Wesche-E. et al., (1986) carried out an ion-exchage chromatography of water-soluble polyuronides and chelator-soluble polyuronides on a bed (40-50 cm high, 0.9 cm wide) of microgranular DEAE-cellulose. Dry polyuronides (75 mg) were applied to the column and the bound polyuronides were eluted with a linear gradient of phosphate buffers. Three major peaks were eluted in this chromatographic system. The first peak was eluted without the gradient and was rich in neutral sugars. The second peak was eluted in the early stage of the gradient and was rich in neutral sugars. The third peak was also eluted in the gradient area but was mainly consisted of uronic acids.

Quantitation of uronic acid and neutral sugars

Uronic acid:

In study of strawberry cell wall polysaccharides, Wade (1964) and Neal (1965) determined the anhydrouronic acid by using the carbazole method of McComb and McCready (1957). Woodward (1972) reported the polyuronide content of strawberry fruits as the equivalents of anhydrouronic acid by using an automated carbazole system modified by Bitter and Muir (1962). Knee et al., (1977) estimated the content of polygalacturonate in cell wall preparations by using the method of Bitter and Muir (1962). Huber (1984) determined the equivalents of galacturonic acid by using the m-hydroxydiphenyl procedure (Blumenkrantz and Asboe-Hanson, 1973). Wesche-E. et al., (1986) analyzed the total uronic acid contents by using the m-hydroxydiphenyl method (A_{520}, Blumenkrantz and Asboe-Hanson, 1973).

Thibault (1979) compared the carbazole method and m-hydroxydiphenyl method in relation to the reduction of interferences of different neutral sugars of plant cell wall origin. The author reported that m-hydroxydiphenyl method was approximately ten times more specific than the carbazole method for galacturonic acid, and automation reduced even more interferences of neutral sugars.

Neutral sugars:

Neutral polysaccharides of the cell wall were determined by paper chromatography of the monnomeric sugars (Wade, 1964). Results from duplicate hydrolysates agreed within ± 5-10% of the mean value. No correction was made for possible destruction of sugars during hydrolysis. Neal (1965) estimated neutral sugar contents by the same method of Wade (1964). Woodward (1972) determined neutral polysaccharides as anhydroglucose units by using the automated sulphonated alpha-naphthol system (Devor, 1950). Knee et al., (1977) estimated the total sugar contents by reaction with sulphonated alpha-naphthol against a sucrose standard (Devor, 1950). For estimation of neutral monosacchride residues, cell walls of strawberry fruits were hydrolyzed and derivatized to alditol acetates followed by separation of gas chromatography on columns of 3% ECNNS-M on Gas Chrom Q or 3% OV-275 on Chromosorb W. Huber (1984) estimated the pentose content of chromatographic fractions of polyuronides by reaction with orcinol (Dische, 1953). Neutral sugar composition of polyuronides and hemicelluloses were estimated by a gas chromatography column packed with SP 2340 (Supelco, Inc, Bellefonte, PA). Wesche-E. et al., (1986) determined total neutral sugars by using the alpha-naphthol method (Devor, 1950). No correction was made for the possible formation

of the chromophore by uronic acids. Neutral sugar composition of cell wall polysaccharides were estimated by the method of Mankarios et al., (1979) on a gas chromatography column packed with 0.3% Reoplex-400 and 0.6% OV-275 coated on acid washed Chromosorb W, 80-100 mesh.

Material and Methods

Source and treatment of strawberry fruits

Individually quick frozen (IQF) Selva strawberries (Fragaria x ananassa, Duch) were provided by General Foods Corporation in a sealed container from 1984 harvest in California. Fresh Selva strawberries were handpicked in late August, 1984, from the North Willamette Experimental Station, Aurora, OR. The fruits were capped, washed, halved and either used in study of the work of compression or frozen in an air blast freezer at -40°C. IQF Selva halves were placed in polyethylene bags, sealed in plastic containers, and stored at -10°C.

Work of compression and drip loss

Fresh Selva halves (150 g) were loaded into the shear cell of an Allo-Kramer shear press, model 52 HE, to obtain the work of compression. The range was set at 5% with a 5000 lb proving ring to give a full scale (5 inches) reading of 250 lbs force. Force rate is the force represented by 1 inch of height on chart (i.e. 1/5 x full scale force value). The shear curve was estimated by use

of a planimeter. Work of compression was obtained from area of the curve (square inches) multiplying by the force rate (Lbs. per inch).

Drip loss was determined on 150 g frozen strawberry halves which had been warmed to -7°C. The halves were arranged between two 9 cm diameter glass funnels to form a single layer, and were allow to thaw at 22°C controlled room temperature. A strip of "Kimwipe" (cellulose tissue) was wrapped at the base of the funnel to absorb condensation from the glass funnel. The volume of the drip loss was measured at the end of 2 hr, and the residual halves were used in the study of work of compression. After the press the berries were used in the determination of total solids, soluble solids, total acid and pH.

Total solids, soluble solids, total acid, and pH

Total solids:

Weigh a 10 g aliquot of strawberry puree in a dried aluminum foil dish. Dry under 27-28 inch vacuum at 70°C for 24 hr. Cool in a desiccator and weigh. Total solids were calculated as dried weight over original weight times 100.

Soluble solids:

Use 10 g of puree, squeeze 1 to 2 drops through two sheets of Kimwipe and place on an Abbe refractometer calibrated in ^{o}Brix (% Sucrose) or Refractive Index (R.I.). Run temperature-controlled water through refractometer to obtain a constant 20^{o}C. Resulted are reported as % Sucrose.

Total titratable acidity (as citric acid):

Dilute 10 g of puree with 100 ml distilled water and titrate the solution with 0.1 N NaOH to pH 8.1.

$$\% \text{ acid} = \frac{\text{ml NaOH x Normality x 0.064 x 100}}{\text{weight of samples}}$$

pH:

Measure pH directly on puree with Corning pH meter model 125.

Preparation of the acetone insoluble solids (AIS)

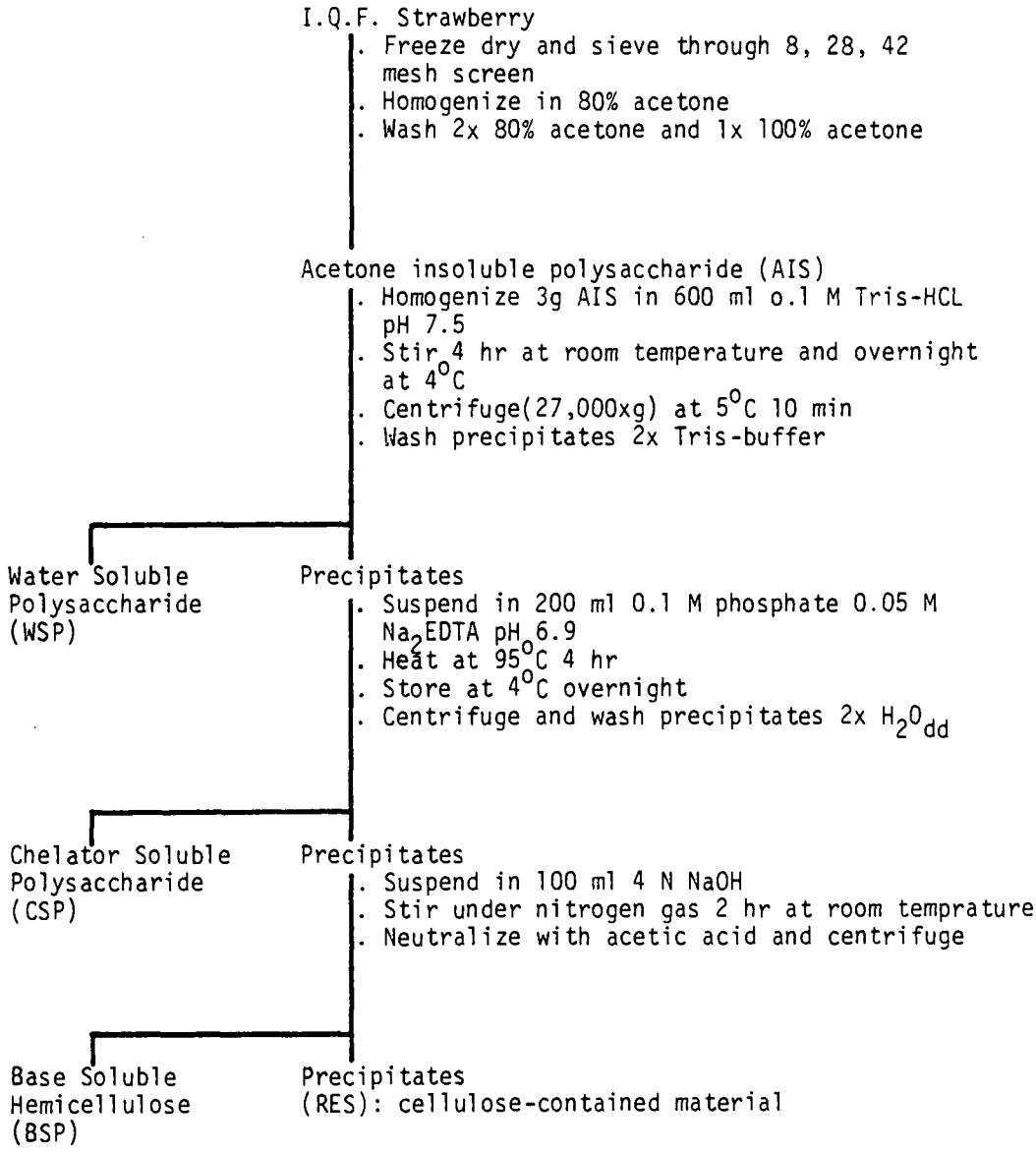
The fractionation procedure used in this study was developed by Wesche-E. et al., (1986). The overall procedure is shown in Figure 1. The freeze-dried strawberries were passed successively through 8, 28, 42 mesh stainless steel screens to remove achenes. The powder obtained was mixed with four volumes of acetone and stirred; the supernatant was removed after the precipitate had settled. Eighty percent acetone was added to restore the original volume and the mixture was stirred overnight at room temperature. The acetone was removed by filtration through Whatman No. 541 paper. The AIS were then washed with one volume each of 80% and 100% acetone and dried. The AIS were kept in sealed containers until used.

Water soluble polyuronides (WSP)

The AIS was suspended in 4 volumes of acetone and refluxed for 10 min to inactivate enzymes. To 3 g of refluxed AIS 600 ml of 0.1 M Tris-Hcl pH 7.5 elution buffer (EB) were added. After homogenization for 60 sec at 80 rpm with a Virtis Homogenizer the mixture was stirred at room temperature for four hr and allowed to stand at 4^{o}C overnight. The insoluble material was separated by

Figure 1. Preparation and fractionation of strawberry cell wall polysaccharides.

Figure 1.


I.Q.F. Strawberry
. Freeze dry and sieve through 8, 28, 42 mesh screen
. Homogenize in 80% acetone
. Wash 2x 80% acetone and 1x 100% acetone
Acetone insoluble polysaccharide (AIS)
. Homogenize 3g AIS in 600 ml o.1 M Tris-HCL pH 7.5
. Stir 4 hr at room temperature and overnight at 4°C
. Centrifuge(27,000xg) at 5°C 10 min
. Wash precipitates 2x Tris-buffer
Water Soluble Polysaccharide (WSP)
Precipitates
. Suspend in 200 ml 0.1 M phosphate 0.05 M Na2EDTA pH 6.9
. Heat at 95°C 4 hr
. Store at 4°C overnight
. Centrifuge and wash precipitates 2x H2Odd
Chelator Soluble Polysaccharide (CSP)
Precipitates
. Suspend in 100 ml 4 N NaOH
. Stir under nitrogen gas 2 hr at room temprature
. Neutralize with acetic acid and centrifuge
Base Soluble Hemicellulose (BSP)
Precipitates (RES): cellulose-contained material

centrifugation at 27,000x g at 5°C for 10 min. The precipitate was washed twice with EB and after repeating the centrifugation steps the precipitate was stored at 4°C for use in further fractionation. Four volumes of acetone were added to the combined supernatants and the mixture was left overnight at room temperature. The precipitate formed was separated by filtration through Whatman No. 541 filter paper. The precipitate was washed with 100% acetone, resolubilized in deionized distilled water, and freeze-dried. The "cotton-like" material was stored in a sealed container.

Chelator-soluble polyuronides (CSP)

The precipitate obtained after centrifugation in the step above was suspended in 200 ml of 0.1 M Na-Phosphate 0.05 M EDTA-Na_2 pH 6.9 buffer. The mixture was homogenized in a virtis homogenizer for 60 sec at 80 rpm and stirred for four hr in a water bath at 95°C. The precipitate was separated as above by centrifugation, washed twice with distilled water and stored at 4°C. The combined supernatants were filtered through Whatman No. 541 filter paper, placed in Millipore No.1 dialysis tubing and dialyzed for 48 hr against several changes of distilled water. After dialysis the sample was frozen and

freeze-dried. This resulted in a "cotton-like" preparation that dissolved readily in water.

Base-soluble polysaccharides (BSP)

The precipitate obtained after separation of the CSP was suspended in 100 ml of 4 N NaOH and stirred for two hr under nitrogen at room temperature. After neutralizing with acetic acid the precipitate was separated as above by centrifugation. The precipitate was washed twice with deionized distilled water and stored at 4°C. The combined supernatants were dialyzed for 48 hr against several changes of distilled water. The sample was freeze-dried and stored in a sealed container.

Glucan residue (RES)

The precipitate obtained after separation of the BSP fraction was washed twice with 80% acetone and 100% acetone. It was then frozen in liquid nitrogen, freeze-dried, and stored for later use.

Ion-exchange chromatography of WSP and CSP

Ion-exchage chromatography of WSP and CSP was performed on a bed (40-50 cm high, 0.9 cm wide) of microgranular DEAE-cellulose (Sigma chemical Co., St. Louis, MO) which had been equilibrated and packed in Na-Phosphate buffer (5 mM pH 6.9, EqB) containing 1 mM EDTA-Na_2. Freeze-dried polyuronides (60 mg) dissolved in 100 ml of deionized distilled water were applied to the column and eluted with 75 ml EqB. The bound polyuronides were eluted with a linear gradient generated from 120 ml EqB and 120 ml 500 mM Na-Phosphate buffer containing 1 mM EDTA-Na_2 pH 6.9. The gradient elution was followed by sequential washes with 60 ml each of 500 mM and 1000 mM Na-Phosphate (pH 6.9) containing 1 mM EDTA-Na_2. The flow rate was 1.13 ml/min and timed 5 min fractions (5.65 ml) were collected. Uronic acid content (A_{520}) was measured using an adaptation of the automated version (Thibault, 1979) of the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). Total sugars, pentoses and hexoses (A_{560}), were measured using an adaptation of the automated version (Fuller, 1967) of the alpha-naphthol method (Devor, 1950).

Colorimetric methods

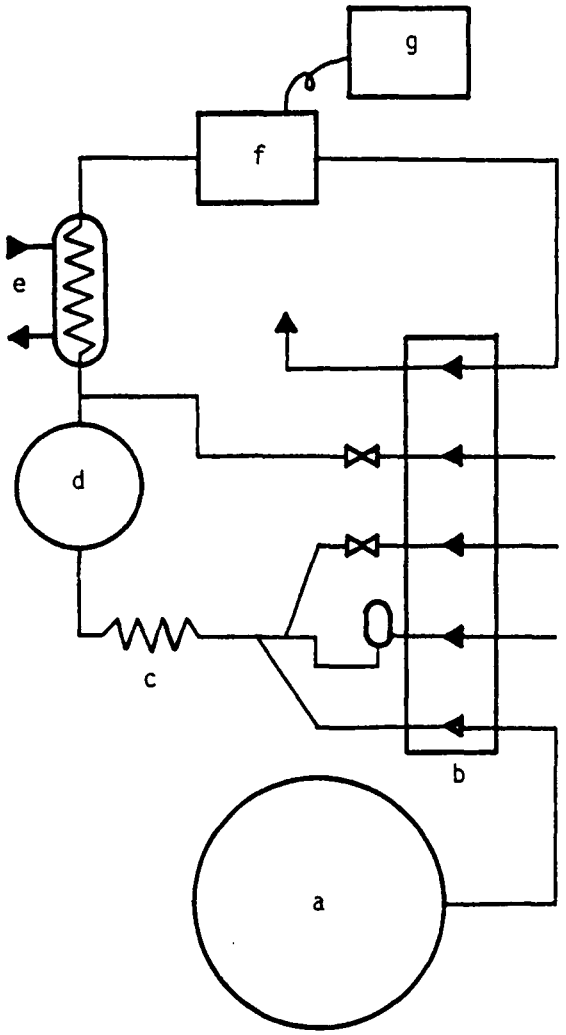
Total uronic acid content was determined using the m-hydroxydiphenyl method (A_{520}, Blumenkrantz and Asboe-Hansen, 1973). Automation of measurements was similar to the flow pattern described by Thibault (1979). The reagents, their corresponding pump tube (internal diameter) and nominal flow rate were as follow: 0.4% alpha-naphthol in 96% sulfuric acid, 2.29 mm acidflex, 2.23 mL/min; air, 1.85 mm clear standard, 2.00 mL/min; 0.23 mL/min samples of acidic sugar, 0.76 mm clear standard; 0.23 mL/min of 0.04% m-hydroxydiphenyl, 0.64 mm solvaflex; colorimeter return, 2.54 mm acidflex, 2.11 mL/min. In analysis of discrete samples, a rate of 20 samples per hr was used. The automated scheme is described in Figure 2. Neutral sugars were determined using the sulphonated alpha-napthol method (A_{560}, Devor, 1950) and were not corrected for the chromophore formed by uronic acids.

Gas chromatography of cell wall polysaccharides

All of the fractions obtained from DEAE-column were dialyzed and freeze-dried and 10 to 20 mg aliquots were subsequently hydrolyzed and acetylated as described by Mankarios et al., (1979). The overall procedure employed

Figure 2. Automated scheme (taken from Thibault, 1979) of the measurement of uronic acid in the pectic fractions by the method of metahydroxydiphenyl (MHDP).

Figure 2.

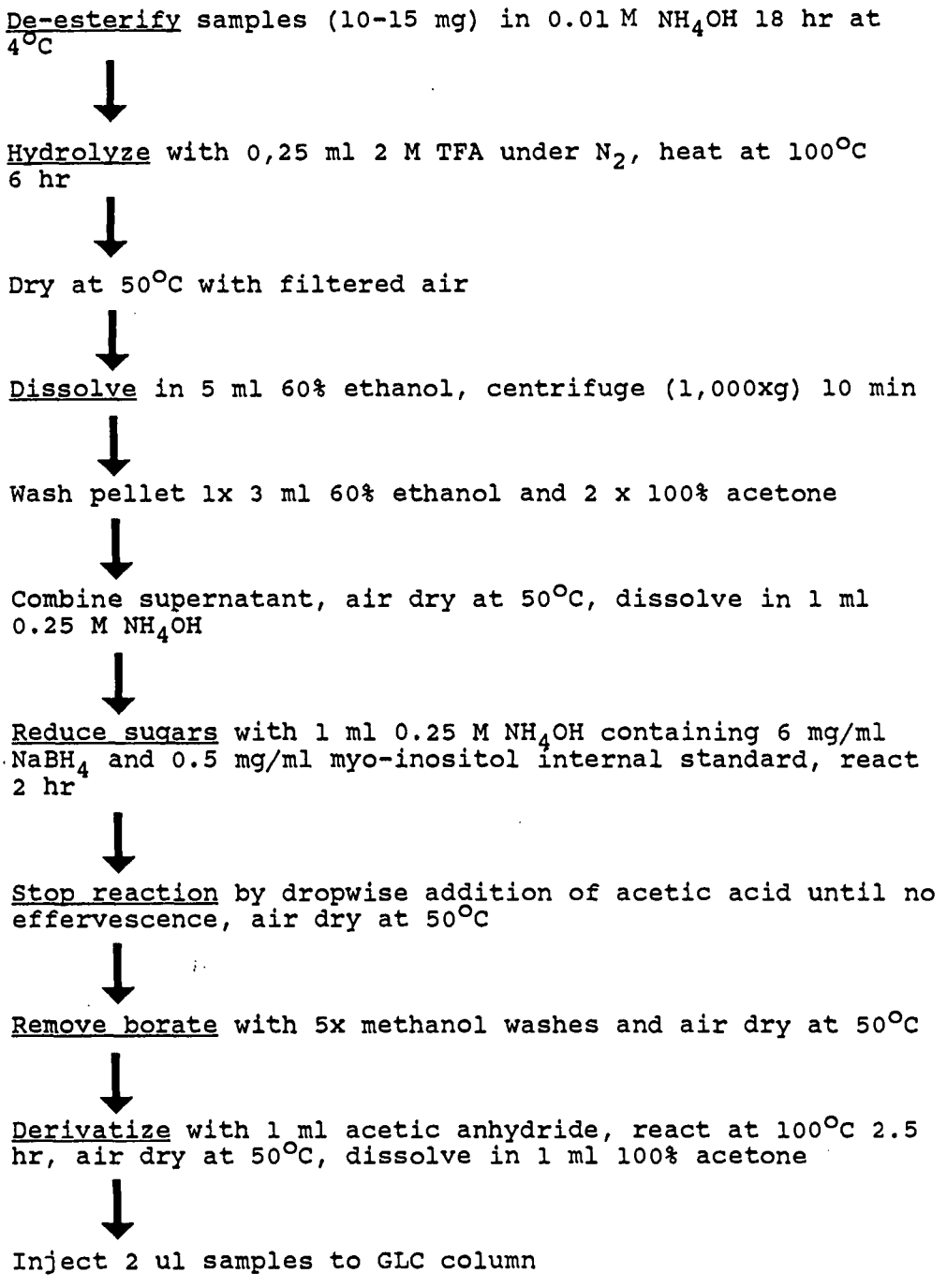


tube	delivery (ml/min)
acidflex	2.11
CS	0.23
CS	2.00
acidflex	2.23
CS	0.23

a: sample distributor
b: proportioning pump
c: mixing coil
d: water bath
e: cooling coil
f: spectrophotometer
g: recorder

Figure 3. Steps in the hydrolysis and preparation of alditol acetate of neutral sugars from pectic fractions for GLC analysis.

Figure 3.


De-esterify samples (10-15 mg) in 0.01 M NH_4OH 18 hr at 4°C
Hydrolyze with 0,25 ml 2 M TFA under N_2, heat at 100°C 6 hr
Dry at 50°C with filtered air
Dissolve in 5 ml 60% ethanol, centrifuge (1,000xg) 10 min
Wash pellet 1x 3 ml 60% ethanol and 2 x 100% acetone
Combine supernatant, air dry at 50°C, dissolve in 1 ml 0.25 M NH_4OH
Reduce sugars with 1 ml 0.25 M NH_4OH containing 6 mg/ml $NaBH_4$ and 0.5 mg/ml myo-inositol internal standard, react 2 hr
Stop reaction by dropwise addition of acetic acid until no effervescence, air dry at 50°C
Remove borate with 5x methanol washes and air dry at 50°C
Derivatize with 1 ml acetic anhydride, react at 100°C 2.5 hr, air dry at 50°C, dissolve in 1 ml 100% acetone
Inject 2 ul samples to GLC column

is outlined in Figure 3. A FID instrument (Varian Aerograph Series 1400) and all-glass column was employed. The column and separation conditions used were as follows: 9 ft x 2 mm column packed with 0.3 % Reoplex-400 and 0.6 % OV-275 coated on acid washed chromosorb W, 80-100 mesh. Flow rate was 10 ml N_2/min. Separations were programmed from 140°C to 180°C at 1°C/min and held isothermally at 180°C for the remainder of the separation.

Results and Discussion

Chemical and physical characteristics

Table 1 compares the three strawberry cultivars for their general chemical and physical characteristics in their commercial ripe stage. Selva is desirable as an exceptionally firm cultivar from California (Bringhurst and Voth, 1982). Totem and Benton are cultivars commercially grown in Oregon. Totem is considered a firm variety , whereas Benton is considered a tender variety (Northwest Cold Pack Company, 1983). Measurements of Selva were repeated with two replications. The data for the Totem and Benton samples were obtained from the 1983 harvest (Varsveld, 1984). The purpose of comparison is to determine the compositional factors which account for the unusual textural qualities of the Selva strawberry.

The most prominent differences among the three strawberry cultivars are the work of compression and the drip loss. Fresh Selva halves shows three to four fold the shear resistance as contrasted to fresh Totem halves and fresh Benton halves. Thawed Selva halves show two to four fold the shear resistance compared to thawed Totem halves and Benton halves. Totem shows more shear resistance than Benton for both fresh and thawed strawberry halves. The

Table 1. Chemical and physical characteristics of Selva, Totem, and Benton strawberry.

	Selva[a]	Totem[b]	Benton[b]
% Total solids	11.5 ± 0.10	9.2	11
% Soluble solids	10.4 ± 0.30	8.5	9.5
% Total acid	0.86 ± 0.01	1.0	1.1
Ratio of soluble solids to total solids	12.1 ± 0.40	8.5	8.6
pH	3.68 ± 0.07	3.4	3.3
Work of compression (inch-lbs/150g)			
Fresh	60.0 ± 6.00	27	18
Thawed	27.0 ± 2.70	12	6
Drip loss	7.75 ± 2.50	19	42

a: with two replicates
b: with one analysis (Varsveld, 1984)
c: determined using Allo-kramer shear press
d: juice released from 150g frozen berry halves thawed at 22°C for 2 hr

unusual firm characteristic of Selva is indicated by the equivalent work of compression of thawed Selva and fresh Totem. The thawed Selva is even firmer than fresh Benton as expressed from the readings of shear press.

Difference in drip loss is inversely proportion to the difference of the work of compression among the three strawberry cultivars. Drip loss of Selva is about one fourth of Benton and one third of Totem. In general, drip loss values below 20 ml/150 g sample were associated with strawberries which didn't shrink or become flaccid when completely defrosted (Varsveld, 1984). Drip loss of Selva and Totem fall within this range.

Total solids, soluble solids and pH are higher in Selva than in Totem and Benton. Total acid is the lowest in Selva. When frozen strawberries were cut into halves, Selva showed the least pigmentation around microtubules as compared to Totem and Benton. The ratio of soluble solids to total acid is considerably higher in Selva.

The large work of compression and small drip loss for Selva fruit suggests that the cell wall wholeness is better maintained throughout the freezing period as compared to Totem and Benton. The study of the work of compression agrees well with Bringhurst and Voth's (1983) report that Selva is an exceptionally firm cultivar. The lower work of compression and the higher drip loss of Benton as compared to Totem agree well with the commercial findings that

Benton is a tender cultivar (Northwest Cold Pack Company, 1983).

In the assessment of strawberry quality for fresh and frozen fruits, Sistrunk and Moore (1967) found that textural measurements by shear press and viscometer were related directly to drained weight, water soluble pectin, hemicellulose and cellulose; the investigation included three cultivars for two harvests and two maturities. The present study of the work of compression and drip loss are in good agreement with the findings of Sistrunk and Moore (1967) and Sistrunk et al., (1983).

Fractionation of acetone insoluble solids (AIS)

Table 2 shows the quantities of the four fractions obtained from extraction of acetone insoluble solids (AIS) of strawberry fruits. Also included are values normalized to 100 percent recovery which facilitates comparison of the proportions of the different fractions. There was reasonably good agreement between replications for the quantities of the four fractions obtained in extraction of Selva AIS. The total AIS obtained from the freeze-dried powders is also included in table 2. The quantity of AIS was high for Selva and Benton, and low for Totem. Recovery of the sum of the four fractions from AIS was low for Selva as compared to Totem and Benton.

The most striking result is that Selva contained relatively low quantities of hemicellulose or base-soluble polysaccharides, BSP. The BSP fraction was obtained by treating the residue remaining after the extraction of the CSP with 4 N NaOH. Wesche-E. et al., (1986) reported that both Totem and Benton strawberry showed a marked decrease in BSP with ripening. Firm underripe fruit contained higher amounts of BSP.

Solubilization of polyuronides (pectin) was done in two steps. Water soluble polyuronides (WSP) were first obtained by buffer extraction of the AIS without heat. This fraction represents polyuronides not bound covalently

Table 2. Fractionation of acetone insoluble solids (AIS) obtained from three strawberry cultivars at commercial ripe stage. Results reported as g per 100 g AIS.

FRACTION	SELVA		TOTEM		BENTON	
	Abs	Nor	Abs	Nor	Abs	Nor
AIS/FDP	17.8 ± 0.2		12.0		16.0	
WSP	28.3/29.3	38 ± 2	29	31	27	31
CSP	21.9/22.0	29 ± 2	26	28	19	22
(WSP+CSP)	50.2/51.3	67 ± 4	57	59	46	55
BSP	1.70/3.30	3 ± 1	10	10	10	12
RESIDUE	19.9/25.1	30 ± 3	29	31	26	35
RECOVERY	71.8/79.7	100	84	100	82	100

Abs: absolute quantities;
Nor: normalized values were the sum of the 4 fractions = 100%;
FDP: freeze-dried powders;
WSP: water-soluble polysaccharides;
CSP: chelator-soluble polysaccharides.

to other cell wall polysaccharides (O'Beirne, 1980). Chelator soluble polyuronides (CSP) was then obtained by heating the residue remaining after the extraction of the WSP in the buffer containing EDTA. The CSP represents the heat and chelator soluble polyuronides fraction. The absolute quantities of WSP were fairly constant among the three strawberry cultivars. Selva possessed the largest quantity of normalized WSP. Totem contained the largest absolute quantity of CSP but Selva had the largest normalized value of CSP, suggesting that these two samples contain higher quantities of polyuronides bound to the cell wall matrix by calcium ions. The magnitude of total soluble polyuronides (WSP+CSP) are similar to that of CSP for both absolute quantities and normalized values. Selva contains the largest normalized quantity of total soluble polyuronides, due to its low quantity of BSP.

The last cell wall fraction consists of the residue remaining after extraction of the other fractions. Selva contained the lowest amounts of this fraction. The role of this fraction in fruit softening has received less attention than other cell wall fractions in textural investigations (Dilley, 1970). However, Barnes and Patchett (1976) reported that some cellulase type enzymes were involved in the ripening process. One would expect such enzymic activity to reduce the quantity of this fraction.

Total soluble polyuronides of strawberry cell wall reported by Huber (1984) did not distinguish WSP from CSP. An increase in WSP with ripening for Totem and Benton was reported by Wesche-E. et al., (1986). The high content of normalized WSP in Selva was contrary to the implied association of softer texture with increased level of WSP. A decrease of CSP polyuronides was observed during ripening of Totem and Benton strawberry fruit (Wesche-E. et al., 1986). Underripe fruits seems to be associated with higher quantities of CSP polysaccharide. The relatively high quantities of the CSP of Selva and Totem may relate to the firmer characteristic of underripe fruits. The exceptionally firm characteristic of Selva may be due to the high content of total polyuronides which is considered to be the cementing materials between plant cells (Doesburg, 1965). The study of Wesche-E. et al., (1986) also showed that Totem solubilized more total polyuronides than Benton throughout the fruit development.

Elution profiles of WSP and CSP

Figure 4 and Figure 5 show the separation of WSP and CSP of three commercial ripe strawberry cultivars on the DEAE-cellulose anion exchange column. Chromatograms of Totem and Benton were obtained from the study of Wesche-E. et al., (1986). Different scale of ordinate was used among the three strawberry cultivars in Figure 4 and Figure 5. Sample loading of Totem and Benton were corrected to allow for direct comparison to Selva. Peak areas are shown on Figure 6 and Figure 7 to facilitate comparison of the proportions among the three strawberry cultivars. All fractions were monitored for both neutral sugar and anhydrouronic acid content. The intention of this procedure is to separate the neutral polysaccharide chains that are not structural components of the polyuronides (Wesche-E. et al., 1986). The peak area of neutral polysaccharides that are eluted in the void volume (W1) were greatest for Totem, followed by Benton and then Selva (Fig 4 and Fig 6A). Selva contained about one third the quantity of neutral sugars in W1 as Totem. Two major peaks (W2 and WP) were eluted with the phosphate gradient. In general, W2 were eluted below 250 mM gradient and were high in neutral sugars (Fig 4 and Fig 6B). The peak area of W2 in Selva was much smaller than Totem and Benton (Fig 6B).

Figure 4. Elution profiles of water-soluble polyuronides of Selva, Totem and Benton strawberry from DEAE chromatography. Detector response for both neutral sugars and uronic acids are given. Na-phosphate buffer (5 mM, pH 6.9) with a linear gradient (500 mM, pH 6.9, 1 mM EDTA-Na_2) was utilized. Sample loading = 60 mg; flow rate = 1.13 ml/min. W1, W2, and WP are peaks from DEAE chromatography.

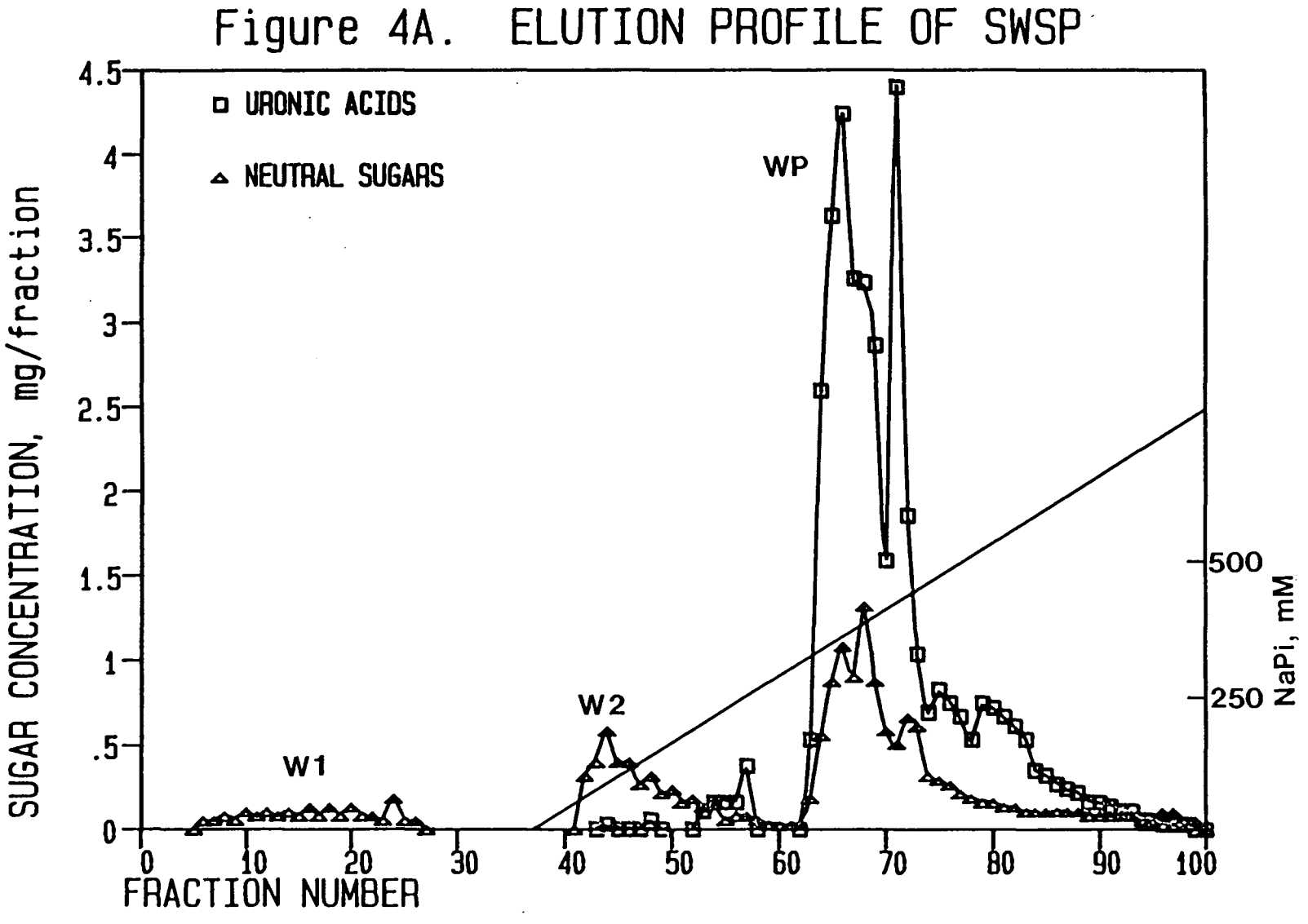


Figure 4A. ELUTION PROFILE OF SWSP
URONIC ACIDS
NEUTRAL SUGARS
WP
W2
W1
SUGAR CONCENTRATION, mg/fraction
FRACTION NUMBER
NaPi, mM
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
0
10
20
30
40
50
60
70
80
90
100
500
250

Figure 4.

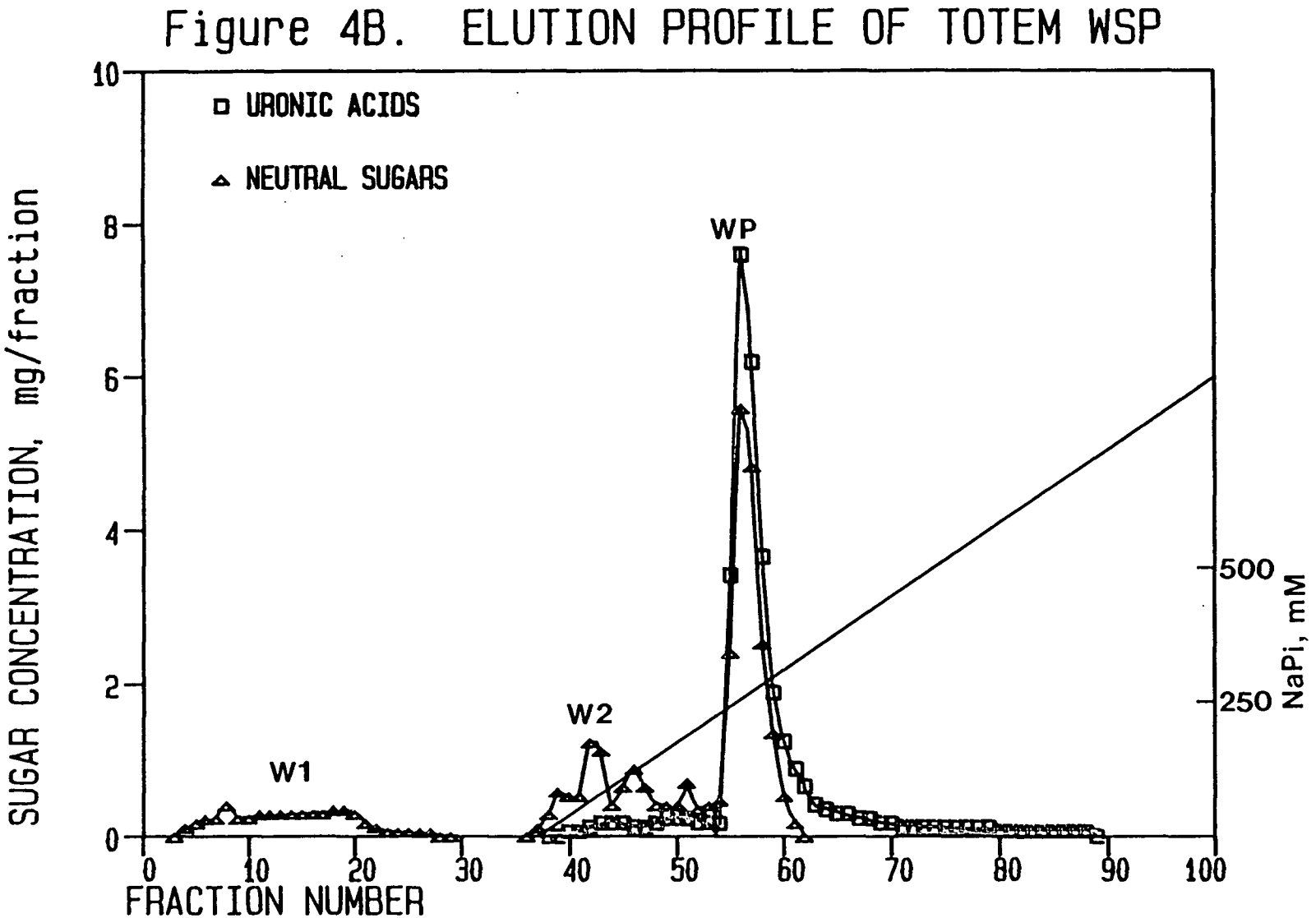


Figure 4. Continued

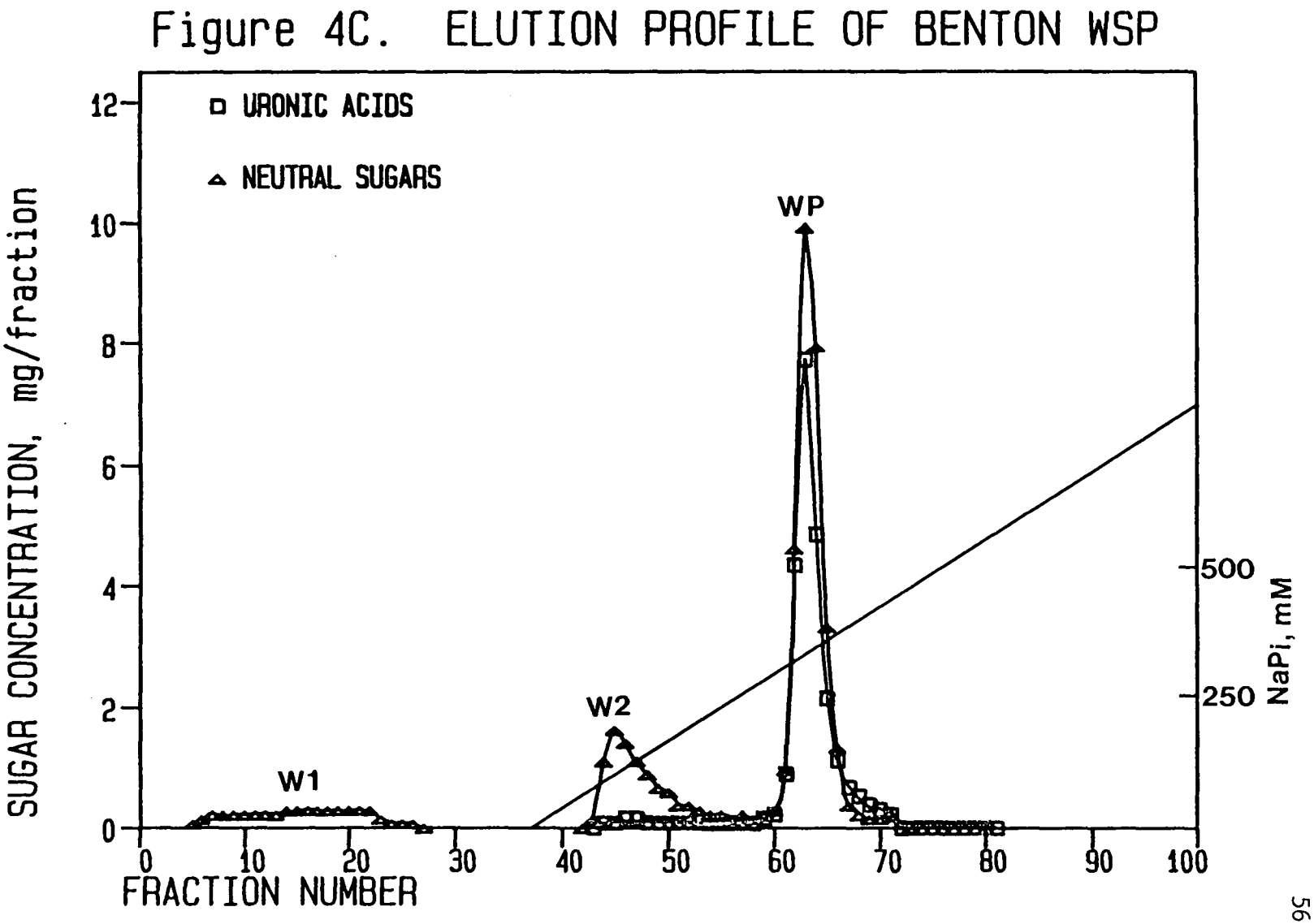


Figure 4. Continued

Figure 5. Elution profiles of chelator-soluble polyuronides of Selva, Totem and Benton strawberry from DEAE chromatography. Detector response for neutral sugars and uronic acids are given. Na-phosphate buffer (5 mM, pH 6.9) with a linear gradient (500 mM, pH 6.9, 1 mM EDTA-Na_2) was utilized. Sample loading = 60 mg; flow rate = 1.13 ml/min. C1, C2 and CP are peaks from DEAE chromatography.

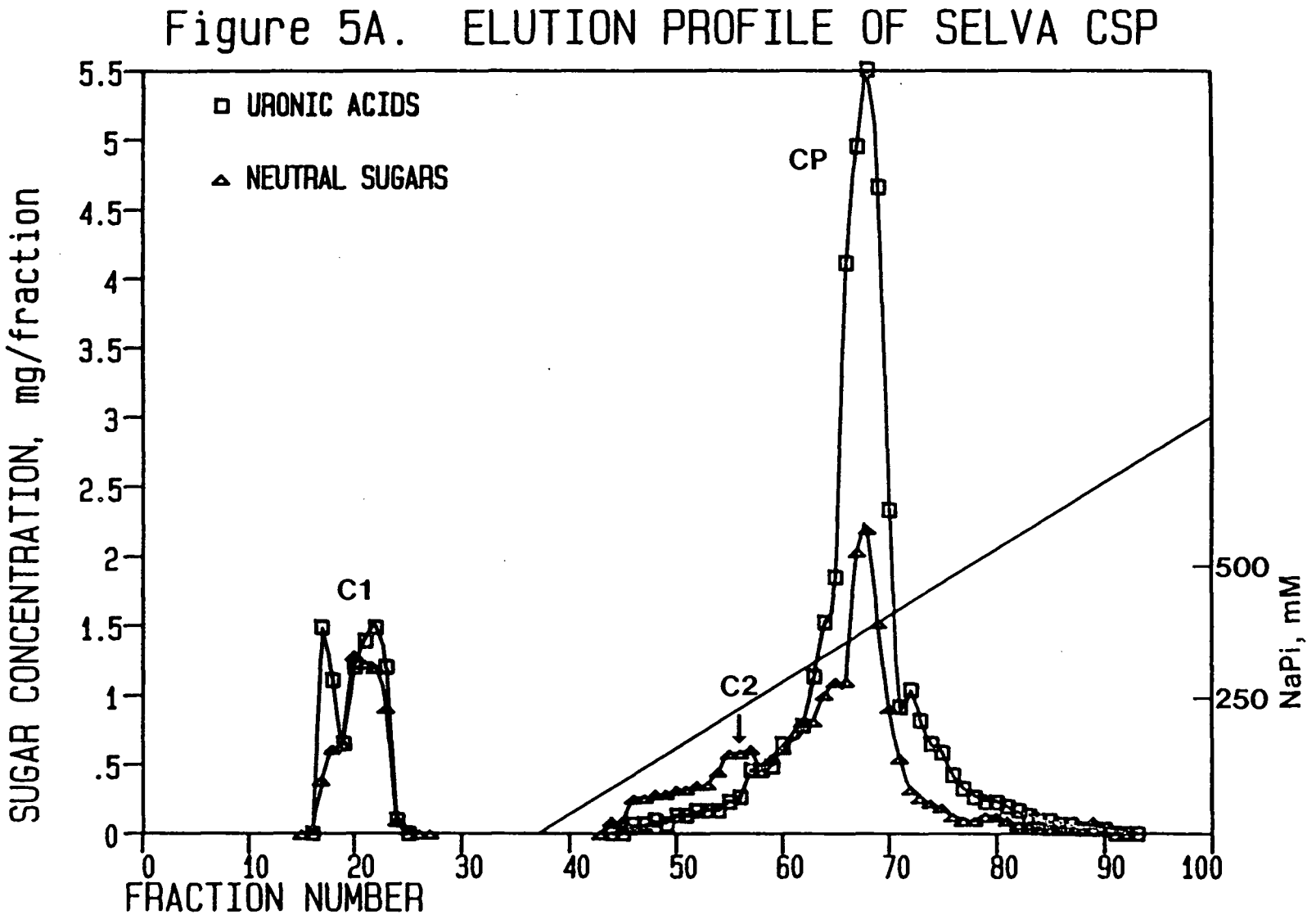


Figure 5A. ELUTION PROFILE OF SELVA CSP
URONIC ACIDS
NEUTRAL SUGARS
CP
C1
C2
SUGAR CONCENTRATION, mg/fraction
FRACTION NUMBER
NaPi, mM
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
0
10
20
30
40
50
60
70
80
90
100
500
250

Figure 5.

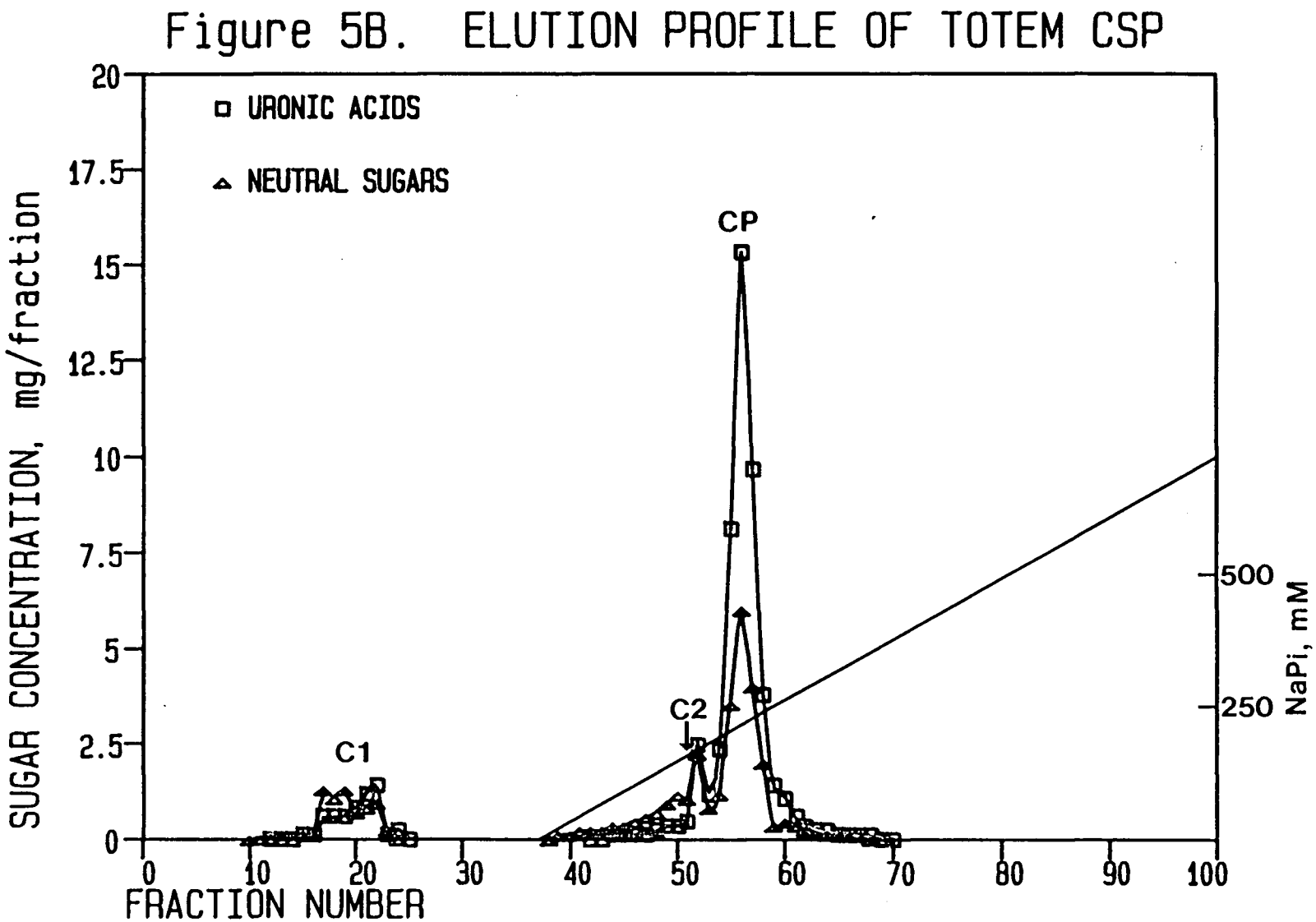


Figure 5. Continued

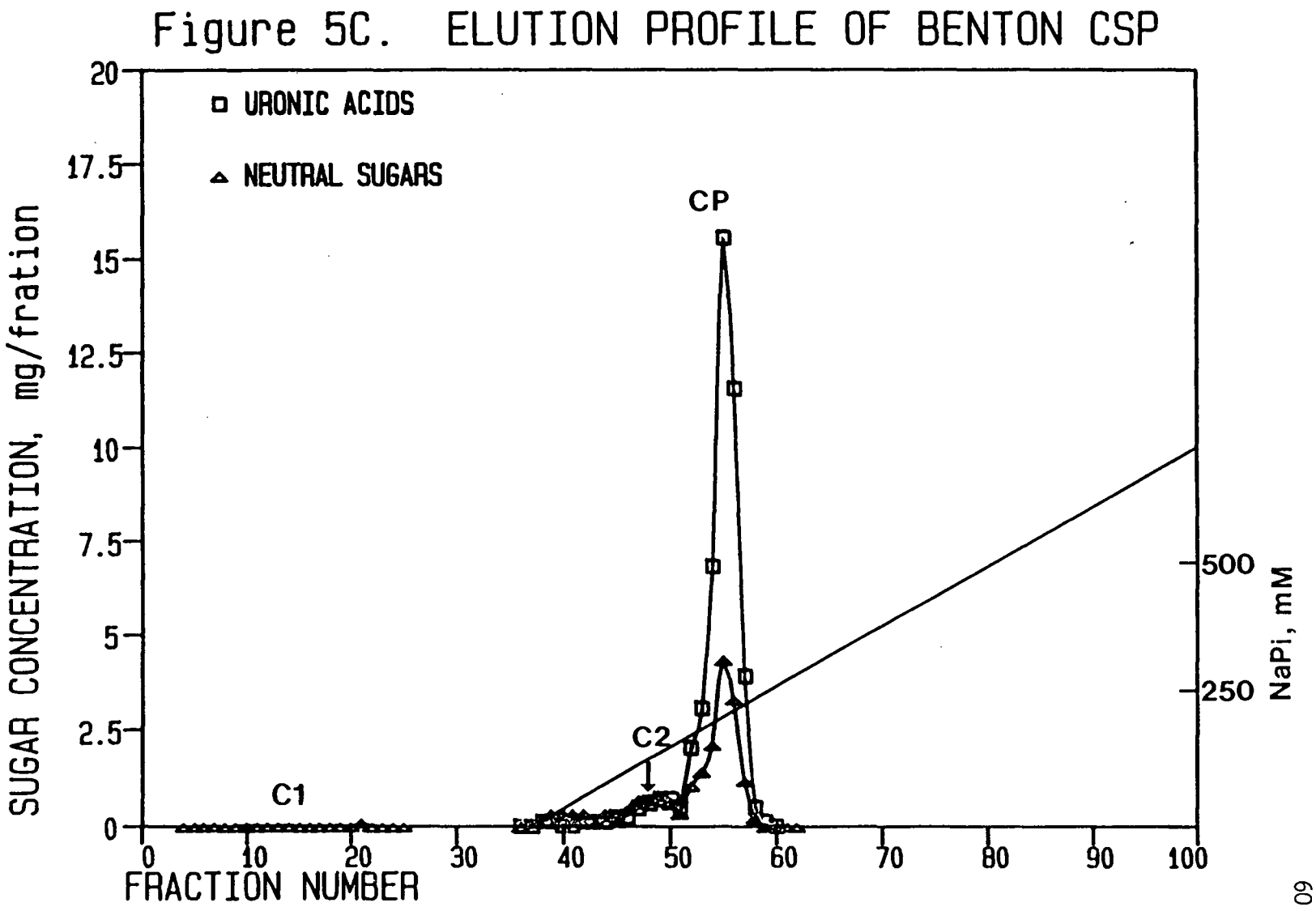


Figure 5. Continued

Figure 6. Peak areas of water-soluble polyuronides of Selva, Totem and Benton strawberry from DEAE chromatography. W1, W2 and WP are peaks from elution profiles of water-soluble polyuronides.

Figure 6A. WSP-W1

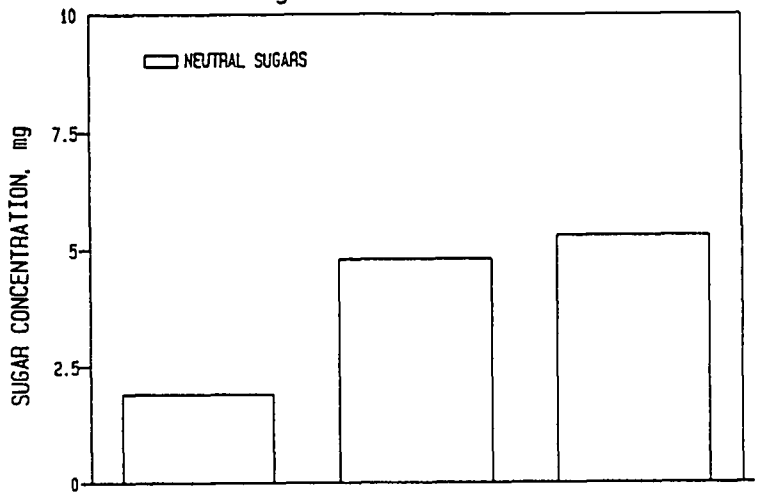


Figure 6B. WSP-W2

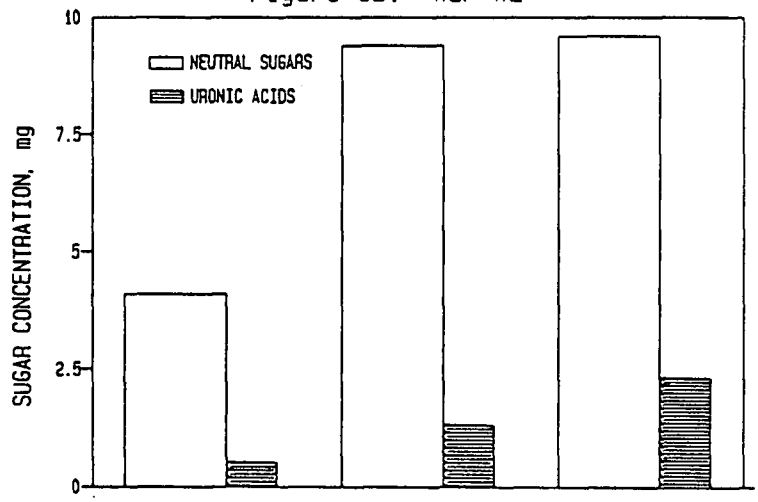


Figure 6C. WSP-WP

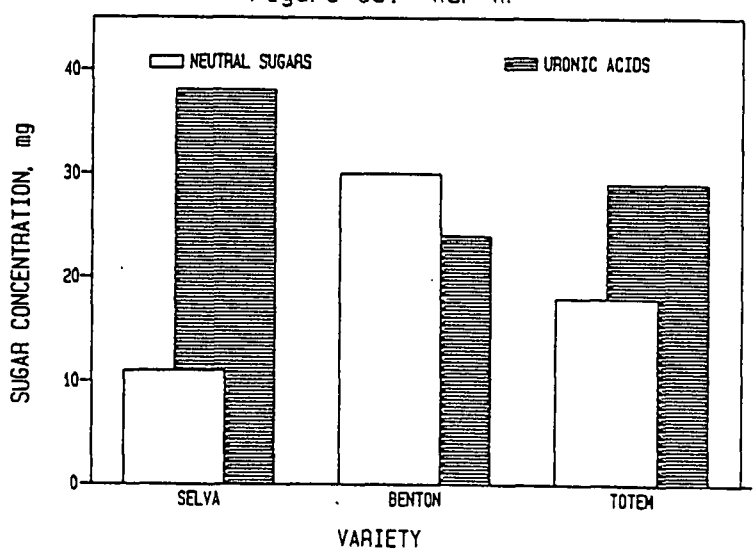


Figure 6.

Figure 7. Peak areas of chelator-soluble polyuronides of Selva, Totem and Benton strawberry from DEAE chromatography. C1, C2 and CP are peaks from elution profiles of chelator-soluble polyuronides.

Figure 7A. CSP-C1

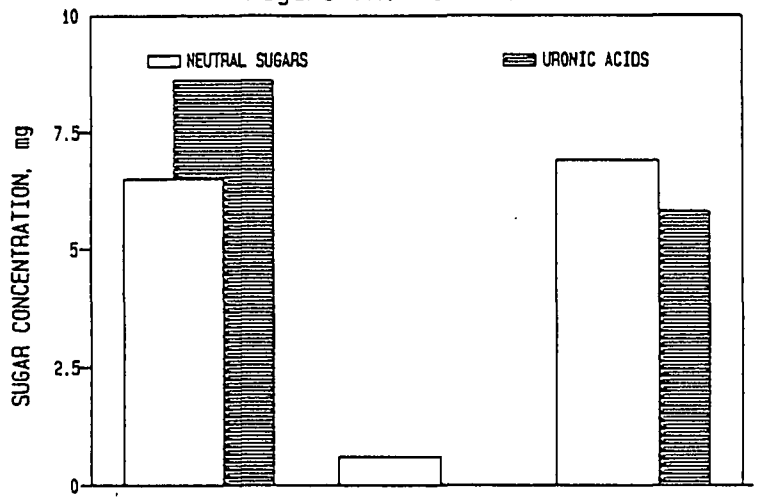


Figure 7B. CSP-C2

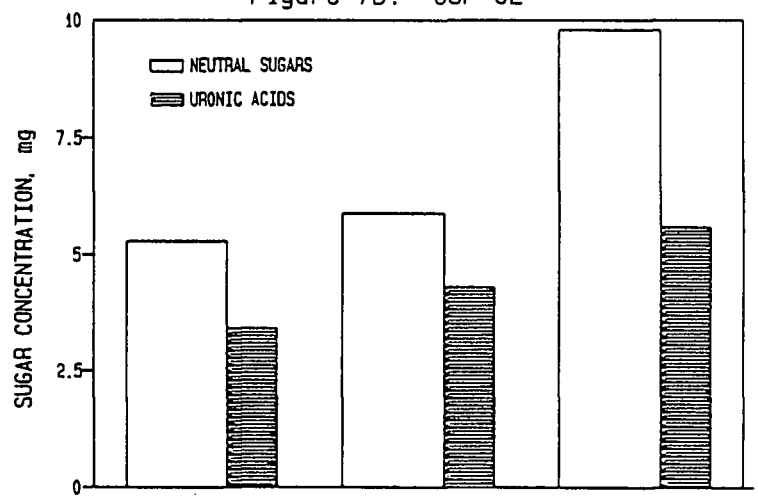


Figure 7C. CSP-CP

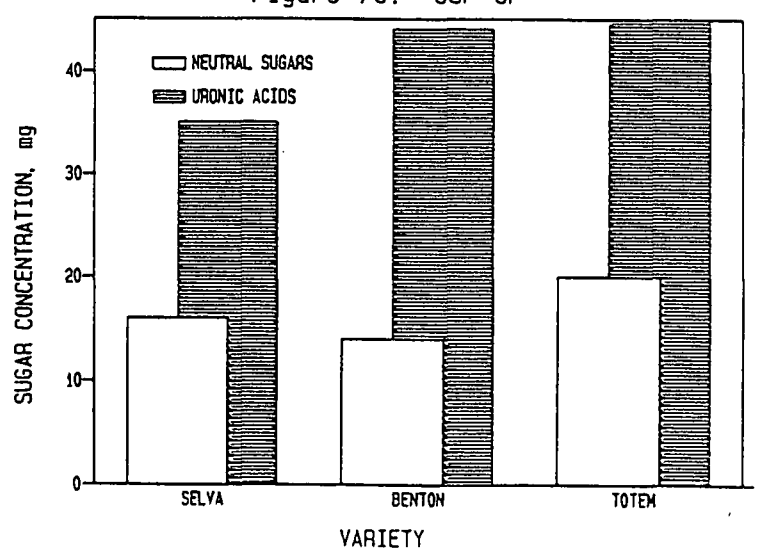


Figure 7.

W2 was eluted in the gradient area which indicated some charges could be attached to the polymer (Fig 4). On account of its predominant neutral sugar characteristics but few uronic acid charges, it is possible that W2 is part of the neutral sugar side chains of the polyuronides.

Selva WP had the longest retention time and the widest peak distribution (Fig 4). In general, the WP peaks were high in uronic acid (Fig 6C). Selva contained the largest quantity of uronic acid (Fig 6C) and the highest ratio of uronic acid: neutral sugars (Table 3). Benton contained the largest quantity of neutral sugars which even exceeded the uronic acid content (Fig 6C). The excessive neutral sugars may partly be from the side chains that are attached to polyuronides or partly be from polymers such as xylan, arabinan, or galactan that are associated with pectin macromolecules. Two distinct peaks were found in Selva WP. One is of 3.5 ratio of uronic acid: neutral sugars (fraction 63 to fraction 74), the other is of 2.6 ratio of uronic acid: neutral sugars (fraction 74 to fraction 96). Ratio of 3.5 peak possessed most of the WP area; but ratio of 2.6 peak represented the one that must be replaced with gradient larger than 500 mM ionic strength. A comparable peak was not found in Totem and Benton.

Table 3. Ratio of anhydrouronic acid to neutral sugars for DEAE chromatographic peaks of three strawberry cultivars.

Fraction	Peak	Selva	Totem	Benton
WSP	W-1	-	-	-
	W-2	0.12	0.24	0.14
	WP	3.33	1.64	0.79
CSP	C-1	1.33	0.84	-
	C-2	0.46	0.57	0.73
	CP	2.19	2.27	3.16

WSP: water soluble polysaccharides;
CSP: chelator soluble polysaccharides;
W1, W2, WP, peaks from DEAE chromatography of WSP
C1, C2, CP, peaks from DEAE chromatography of CSP

Totem WP peak contained a tailing of the uronic acid under which no neutral sugars were detected (Fig 4B). This may indicate a possible existence of a homogalacturonan. Most of the Totem WP peak was eluted before fraction 60 while the Benton WP peak just started to be eluted after this fraction. A considerable quantity of the Selva WP was still eluted after the fraction 70 which was not found in Totem and Benton.

The peak areas show that the CSP fractions are proportionately higher in uronic acid than the WSP fractions (Fig 6 and Fig 7). In the void volume , a small peak (C1) was eluted, containing both uronic acid and neutral sugars except that uronic acids were absent in Benton (Fig 5C and Fig 7A). Due to its lessened tendency to remain in the gradient and its ability to react with metahydroxyldiphenyl (MHDP) chromogen, it is possible that C1 contained a relatively high proportion of esterified residues (Aspinall, 1980). Selva C1 contained both higher uronic acid (Fig 7A) and the ratio of uronic acid: neutral sugars (Table 3). A large peak was eluted when the gradient was applied. This major peak (CP) contained a leading shoulder, C2, which was high in neutral sugars (Fig 7B). The retention time of Selva C2 was close to Totem CP and Benton CP. As in the case of W2, C2 has the characteristics of neutral sugars but with fewer charges

than CP, it is likely that they are structural component of the polyuronides.

Selva CP has a longer retention time and wider peak distribution than Totem CP and Benton CP (Fig 5). Essentially all materials eluted for Benton by fraction 60 and for Totem by fraction 70. The CP peak was high in uronic acid and contained a similar quantity of neutral sugars (Fig 7C).

Wesche-E. et al., (1986) found that underripe strawberry fruit contained broader peaks of longer retention time due to higher molecular weight and/or charge. The elution profile of the ripe Selva is rather similar to that of underripe Totem and Benton. The longer retention time and the wider peak distribution suggested that Selva pectin species may have larger charges and molecular aggregates. The wider peak width also indicates that Selva pectin species are of various charges and sizes. The peaks in Totem and Benton are sharper with a shorter retention time, suggesting that its pectin species are of more equal sizes and lower charges. Both WP in WSP and CP in CSP of Selva were retained in the DEAE-cellulose column longer than that of Totem and Benton. This greater binding feature of Selva may partly explain the exceptional firm characteristics of Selva strawberry fruit.

Neutral sugar composition of AIS fractions

The neutral sugar composition in the different fractions of strawberry AIS are listed in Table 4. In the WSP fraction, galactose is the major sugar followed by arabinose and xylose. Smaller proportion of rhamnose was present. Selva contained lower xylose and higher glucose than Totem and Benton. The uronic acid: rhamnose ratios were higher in Selva and Totem than in Benton. Rhamnose is believed to be the kinking points along the rhamnoglacturonan backbone (Rees and Wight, 1971).

Galactose and arabinose are the major sugars in the CSP fraction. Galactan and arabinan are known to frequently be associated with polyuronides (Darvill et al., 1980). Smaller quantities of glucose, xylose and rhamnose are also present. Xylose is still relatively low in Selva. Totem and Selva contains less rhamnose and more glucose than Benton. The uronic acid: rhamnose ratios is higher in Selva than in Totem and Benton. Rhamnose quantities are higher in CSP than in WSP. However, the WSP shows higher uronic acid: rhamnose ratios than the CSP.

Xylose and glucose are the major sugars in the BSP fraction. Considerable amounts of galactose are also present and amounts are higher in Selva and Totem than in Benton. Selva contains proportionately more arabinose and rhamnose than Totem and Benton but less glucose.

Table 4. Neutral sugar composition of cell wall fractions obtained from Selva, Totem and Benton strawberry.

Fraction	Cultivar	Neutral sugars					
		RHA	ARA	CAL	XYL	GLU	AUA/RHA
WSP	Selva	2	12	64	5	18	3.1
	Totem	2	9	69	17	0	3.5
	Benton	4	16	69	10	0	1.2
CSP	Selva	5	34	44	2	15	1.0
	Totem	8	18	34	19	17	0.5
	Benton	11	23	52	6	1	0.4
BSP	Selva	3	19	27	27	27	
	Totem	1	0	23	26	56	
	Benton	0	4	12	37	37	
RESIDUE	Selva	5	5	24	15	50	
	Totem	1	3	13	14	63	
	Benton	3	4	9	22	57	

WSP: water soluble polysaccharides
CSP: chelator soluble polysaccharides
BSP: base-soluble polysaccharides
RHA: rhamnose, ARA: arabinose, GAL: galactose, XYL: xylose, GLU: glucose, AUA, anhydrouronic acid determined colorimetrically.

Glucose is the major sugar in the residue followed by xylose and galactose. Smaller quantities of arabinose and rhamnose are also detected. Selva contains relatively high proportions of rhamnose and galactose.

Rhamnose is a particularly significant component of the polyuronides. It is considered to be a site of attachment of sidechains and to be responsible for causing the polyuronide chain to kink thus decreasing the possibilty of interaction between chains (Morris et al., 1977, Huber, 1984). The extent of interpolymeric association is determined by the relative content of rhamnose along the rhamnogalacturonan backbone. Decreased levels of rhamnose or high ratios of uronic acid: rhamnose may then result in a strengthening of the cell wall (Wesche-E. et al., 1986). The exceptionally firm characteristic of Selva strawberry fruit may be partially explained by the role of rhamnose on the textural profile. In Huber's (1984) study, an association of the high ratio of uronic acid: rhamnose with underdeveloped strawberry fruit which is firmer in texture was reported.

A good separation of the polyuronide fractions, WSP and CSP, from the hemicellulose fraction, BSP, is indicated by the low content of xylose in both WSP fraction and CSP fraction. Total xylose content of Selva in the four fractions is much lower than that of Totem and Benton. This is in good agreement with the results of Table 2

which shows that Selva contains low quantities of hemicelluloses (BSP).

The relative high content of arabinose and galactose of Selva BSP may indicate an arabinogalacatan included in the hemicellulose fraction. Bauer et al., (1973) reported that significant and reproducible amounts of arabinose and galactose were found in the sycamore extracellular polysaccharides material bound to cellulose which can be partially eluted from cellulose with 8 M urea or 1 N NaOH. In a study of the cell wall structure of bean epicotyl, Nishitani and Masuda (1981) also found that the hemicellulose consisted of xyloglucans, arabinoglactans and polysaccharides composed of xyloses and/or mannoses. This arabinogalactan may have an important role in connecting the rhamnogalacturonan to the glycoprotein in the cell wall (Albersheim, 1976). The presence of a terminal rhamnose in the arabinogalactan polymer was also reported by Keegstra et al., (1973). This may account for the detection of rhamnoses in the hemicellulose fraction of strawberry cell wall.

Conclusions

1. Fresh Selva halves showed two to three fold the shear resistance as compared to fresh Totem halves and Benton halves. Thawed Selva halves had the same shear resistance as fresh Totem halves and even higher shear resistance than fresh Benton halves. Drip loss was the lowest for thawed Selva halves and the highest for thawed Benton halves.

2. The quantity of acetone-insoluble solids obtained from freeze-dried powders of strawberry fruits was high for Selva and Benton, and lower for Totem. Selva contained higher amounts of total soluble polyuronides and the lowest quantities of hemicellulose fraction as compared to Totem and Benton.

3. DEAE cellulose chromatography of water-soluble polyuronides and chelator-soluble polyuronides showed that Selva had a longer retention and wider peak distribution, suggesting that its pectin species contained higher molecular weight and/or charge. The wider peak width also indicated that its pectins were of various charges and sizes. The elution profile of ripe Selva is similar to that of underripe Totem and Benton.

4. Ratios of uronic acid to rhamnose, which indicates the extent of the interpolymeric association among the pectin chains, were higher in both Totem and Selva than Benton for the water-soluble fraction; the ratio was higher in Selva than in Totem and Benton for the chelator-soluble fraction. The higher ratio of uronic acid to rhamnose is able to expose longer length of junction zones between neighboring pectin chains and is indirectly related to firmer texture of strawberry fruits.

5. The relative high content of arabinose and galactose of Selva BSP may indicate that an arabinogalactan is included in the hemicellulose fraction. This arabinogalactan may have an important role in connecting the rhamnogalactan to the glycoprotein in the cell wall due to the relative high amount of rhamnose detected in Selva BSP.

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