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Title: Strawberry Cell Wall Polysaccharides: an Intervarietal Comparison of Compositional, Physical, and Textural Properties

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Maintaining the structural integrity of cell walls largely determines the texture of fruit products during freezing processes. Strawberries undergo major textural changes when frozen, and varietal differences in quality after thawing are not readily predicted from mechanical and sensory testing of the fresh fruit. The objectives of this study were to develop a quantitative sensory texture profile of three strawberry varieties, individually quick frozen (IQF), to determine differences in cell wall composition and structure, and to relate the sensory and compositional profiles to differences in instrumental values of firmness.

Intervarietal differences in IQF strawberries of Benton, Totem, and Selva varieties were examined and a summary of major findings follows.

Drip-loss measurements, and work-of-compression on thawed berries were correlated to sensory ratings. Puree viscosity was highest in Selva samples, lowest in Bentons and shows potential as a screening test for strawberry cultivars. Firmness of thawed, whole Selva berries was rated 4x and 2x as compared to Bentons and Totems, respectively, via sensory profile evaluation with magnitude estimation scaling. Fractionation of fruit cell walls showed Selva had the highest percentages of acetone-insoluble solids. Total pectin content and absolute weight percentages of uronic acids and neutral sugars did not show major differences. Ratios of: 1) uronic acids to neutral sugars, 2) uronic acids to rhamnose, and 3) neutral sugars to rhamnose followed trends of increasing with varietal firmness in water-soluble polysaccharides (WSP) and decreasing in chelator-soluble polysaccharides (CSP). Ratios of galactose to arabinose decreased with increasing firmness scores in WSP and increased in CSP. Selva had the highest amount of high molecular-weight polymers in water-soluble and chelator-soluble polysaccharides.

Strawberry Cell Wall Polysaccharides:
An Intervarietal Comparison of Compositional,
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INTRODUCTION

Strawberries excel in popularity among fresh and processed fruits because of their delicate flavor, succulent texture, and attractive color. Textural firmness is emphasized as a major characteristic for both uses. "Berry wholeness" is a major factor determining freezing and jamming quality, as thawed strawberries become mushy and exude large quantities of juice. For transport to fresh markets berry flesh and skin must be resistant to injury and bruising (Lawrence, 1985).

Strawberries, ranking 25th of the economically important crops in Oregon, were valued at $16.3 million in 1985. The majority of the crop goes to processing as IQF, sliced, or whole sugar packs (4+1) (Miles, 1986). Oregon strawberries are recognized for their distinctive flavors and color intensity, but texture of slices and berries after thawing is poor in many flavorful varieties. With competition increasing from California and Mexico berries also going to processing, prices to growers are lower than ever before. Improvement of existing strawberry cultivars and development of new ones with increased firmness qualities are strategies for competing in both the fresh and frozen markets.

Screening tests for strawberry cultivars include chemical characteristics of pH, soluble solids (°Brix), titratable acidity, and total solids. Workers have not demonstrated correlations between these indices and varietal texture quality (Robinson et al, 1947, Sistrunk and Morris, 1967; Simpson, 1980). Penetrometer and shear press measurements correlate well with sensory firmness intensity ratings when performed on strawberries at the same stage of processing, fresh or frozen (Wolford, 1961). However, fresh berries with good texture quality can not be assumed to retain their high texture scores after freezing and thawing (Sistrunk et al, 1960).

Mechanisms involved in strawberry softening are not yet well understood, but are mainly attributed to changes in the intricately constructed cell wall matrix. From microscopic observations, strawberries are characterized by large cells with thin walls (Neal, 1965). After freezing processes, rupture of parenchyma cells in cortical tissue was closely associated with soft texture and juice release (Armbruster, 1967; Szczesniak and Smith, 1969). Changes in sensory textural properties of wholeness and firmness were also examined using category scaling and qualitative descriptions obtained from a trained texture profile panel (Armbruster, 1967; Szczesniak and Smith, 1969).

Several studies have followed changes in cell wall polysaccharides with ripening. Wade (1964) found no differences in cell wall composition to account for textural changes. Total soluble pectic polysaccharides were observed to increase with ripening (Woodward, 1972; Knee et al, 1977; Huber, 1984). Wesche-Ebling et al. (1985) reported that water-soluble polysaccharides increased concurrently with decreases in chelator-soluble polysaccharides. Rhamnose-induced kinking in pectic chains appears to strongly associated with strawberry softening. Rhamnose levels in pectic fractions increased in relation to uronic acids with maturity (Huber, 1984; Wesche-Ebling et al, 1985).

A role for enzymatic softening in strawberries by endo-polymethylgalacturonase and polygalacturonase proposed by Gizis (1963) and suggested by Woodward (1972) has not been supported by recent findings. Barnes and Patchett (1976) observed pectinmethylesterase and a "C_x" cellulase activities in strawberries and suggested that the cellulase aided the solubilization of xyloglucans by hydrolyzing glycosidic linkages to cellulose. Results from gel permeation and ion exchange chromatography (Huber, 1984; Wesche-Ebling et al, 1985) provided no evidence for hydrolytic breakdown for the rhamnogalacturonide backbone. Increases in proportions of neutral sugars with the pectin chains were observed with maturity.

Little work has been directed to examining varietal differences in the strawberry cell wall. It is not yet clear whether varietal differences in cell wall pectic fractions follow the same trends as changes related to maturity and softening. Lin (1986) compared pectic polysaccharides of red-ripe Benton, Totem, and Selva strawberries. These varieties were chosen to represent extremes of textural properties in strawberries. Bentons are soft berries produced in the Pacific Northwest primarily for processing, but also marketed fresh to a lesser extent. Totems are heavily pigmented fruit produced for processing and for fresh market. The Selva berry is a California cultivar of extreme firmness under development for production in Oregon and Washington (Daubeny, 1985). Elution patterns of pectic fractions from Selva berries from ion exchange chromatography showed more highly charged and/or larger rhamnogalacturonide polymers than from the other varieties. This pattern was similar to profiles from under-ripe Benton and Totem fruit.

The objectives of this study were: 1) to extend the compositional data base on cell wall polysaccharides in Benton, Totem, and Selva varieties, 2) to develop a quantitative sensory profile of textural properties in thawed IQF berries using magnitude estimation scaling, 3) to relate these sensory textural values to instrumental measures of texture, and 4) to determine physical or chemical characteristics that could provide good screening indices for textural quality after freezing.

LITERATURE REVIEW

Effects of freezing on strawberries

Freezing effects on cellular structure:

Textural changes in frozen strawberries are determined primarily by the extent of structural damage in parenchymal cells of the berry cortex. Armbruster (1967) found that epidermal and xylem cells were not changed, but that parenchymal cells rupture without cell separation. Also, smaller cells in the cortex suffered less rupture damage than large ones. Mohr (1971) noted that the role of protoplasmic structures was insignificant in influencing the texture of high moisture, edible plant tissues. Szczesniak and Smith (1969) described cellular damage in syrup-packed frozen strawberry halves as cytoplasmic disorganization, cell plasmolysis, and folding of the cell wall with some rupture. Large and thin-walled cells ruptured most easily, and they suggested that freezing caused changes in the cellular structure that decrease the water holding capacity of structural polysaccharides. Cellular damage caused by freezing was also correlated to changes in texture and tissue resistance, measured by the General Foods Texturometer, in green beans (Brown, 1967) and apple tissue (Sterling, 1968).

Freezing effects on chemical composition:

Frozen strawberries increase in percent soluble solids, pH and titratable acidity and decrease in total solids and alcohol-insoluble solids (AIS) (Rao, 1966). Small berries and those frozen quickly, blast-frozen at -20°F, changed the least (Woodroof, 1938; McArthur, 1945; Rao, 1966; Armbruster, 1967), although Sistrunk et al.(1960) observed increased shear press values and a decreased syrup viscosity in thawed berries from a slow freezing rate. Weight loss with thawing was inversely related to total solids, AIS, pectic substances, cellulose, ash in berry flesh, and minerals. Syrup concentrations of sugars, ash, titratable acids, pectins and minerals were directly related to weight loss. Wade (1964) detected no changes in methoxylation or acetylation or in the proportions of cell wall polysaccharides. Reid et al.(1985) found reduced amounts of total pectin, especially in the water-soluble fraction, in a survey of many frozen fruits and vegetables.

Effects of processing factors on texture

Processing factors from harvest through transport and storage affect strawberry texture quality. Removal of field heat retards breakdown. Firmness of berries cooled from 72° to 36°F measured by plunger-testing increased, but rupture testing revealed no major changes, indicating that the epidermis of the fruit was most toughened by cooling (Rose et al, 1934). Sistrunk et al.(1960) found that date of harvest, variety and length of time of mixing berries with sugar had the most impact on the character of frozen sliced strawberries. Incorporating sugars and hydrocolloids with fruit improved the texture of frozen thawed strawberries (Armbruster, 1967; Aref et al, 1956; Wegener et al, 1951; Hoover and Dennison, 1955). Simpson (1980) found that frozen sugared berries, especially whole fruit, were firmer than the fresh product. He considered it an effect of changes in the skin rather than texture improvement. The type of equipment for mixing the fruit and sugar also influenced fruit wholeness (Talburt et al, 1955).

Holding strawberries up to 18 hr at room temperature before freezing did not decrease the firmness of frozen sliced strawberries (Sistrunk et al, 1960), and fresh berries increased in firmness and mechanical elasticity when stored up to 7 days (Ahmed and Dennison, 1972). Even when protected from moisture loss, berries stored at 4°C for 10 days became tough, rubbery, and less turgid (Johnson et al, 1965).

Testing methods for screening strawberry varieties for processing

Chemical tests:

Much of the study on varietal differences in chemistry, mechanical properties and effects of processing is oriented to developing adequate screening tests for cultivar development. Relating objective mechanical and chemical results to acceptable fruit quality is important to identify desirable and undesirable characteristics in new crosses and selections. Soluble solids, dry weight, and total acidity are routine tests on fruits that broadly define quality in breeding lines (Sistrunk and Moore, 1967). With respect to strawberry texture, Robinson et al.(1947) found no relation of varietal freezing adaptability with pH, ascorbic acid content, color, soluble solids, and free acids. Seasonal variation in these characteristics and in alcohol-insoluble solids precludes their use as predictive indices for texture quality (Simpson, 1980). Jewell (1973) recommended achene density, the number of achenes per centimeter square of berry surface area, as a criterium for selecting cultivars resistant to breakdown during processing.

Mechanical assessments of strawberry texture:

Objective instrumental measurements of strawberry texture have assessed the mechanical parameters: work of compression, maximum rupture force, penetration force, elasticity, and viscosity of whole fruit or syrup. The Kramer shear press is often used on fresh and frozen strawberries to measure firmness as work of compression and maximum force to rupture, although correlations vary between its values and sensory or chemical characteristics. Sistrunk et al.(1960) noted a relationship between shear-press firmness and viscosity of syrup from thawed slices. Harvest date and maturity influenced correlations of shear-press with drained weight, water-soluble pectin and cellulose in frozen strawberries (Sistrunk and Moore, 1967), but shear values were still considered a major index defining differences in character and wholeness. Sensory wholeness scores for frozen, sugared, mechanically-harvested berries did not follow the same trend as puree viscosity and shear press measurements on the raw product (Morris et al, 1979). Changes in sensory hardness were not detected in forces required to shear fresh strawberries stored 7 days or irradiated with a 150 Krad dose (Ahmed and Dennison, 1972), or frozen sugared berries from 10 cultivars (Simpson, 1980). A specially designed penetration instrument used to assess the effects of prefreezing treatments on strawberry texture had the sensitivity to measure forces to penetrate both epidermal and cortical tissues (Armbruster, 1965). Multiple measuring instruments more completely simulate mastication properties than does the shear press or penetrometer. Penetration, compression and hysteresis characteristics in stored and irradiated strawberries was examined using an Instron Universal Testing Instrument Model TM (Ahmed and Dennison, 1972). Szczesniak and Smith (1969) selected the General Foods Texturometer to profile a textural differences between fresh strawberries at three stages of maturity, frozen-thawed berries, and rehydrated, freeze-dried berries because several characteristics could be measured in each sitting.

Rheological properties of pureed strawberries

Fruit purees typically exhibit non-Newtonian flow behavior which is greatly influenced by pectin content. Pectins may contribute to time dependent thixotropic and rheopectic properties, depending on whether the shear stress decreases or increases with time. However, purees normally fall into one of two classes of time independent, non-Newtonian behavior: 1) plastic (Bingham) where a minimum yield stress must be exceeded before flow begins; and 2) pseudoplastic in which the curve begins at the origin, but a proportional increase in shear rate gives a less than proportional shear stress (Bourne, 1982). A simple empirical relationship, termed the power law from logarithmic plots of shear stress versus shear rate over a wide range of shear rates, is often evident for pseudoplastic foods. This equation is written as:

$$\log \sigma = n \log y + \log K$$

or

$$t = K y^n.$$

The equation for plastic fluids is : $\sigma = \sigma_o + K y^n$, where σ_o is the yield stress. K is a consistency factor and n is a flow index relating to the extent of deviation from non-Newtonian behavior.

Temperature markedly decreases the viscosity of fruit juices, but the effect decreases with increasing solids content. Saravacos (1970) reported that increased temperatures caused only slight decreases in "K" and no changes in "n" in purees of 10-16 ^{o}Brix. The well established dependency of flow characteristics on concentration, size and shape of suspended particles accounted for the neglible effect of temperature on the apparent viscosity of apple sauce (11 ^{o}Brix). An activation energy for viscous flow in a puree can be calculated from a modification of the Arrhenius equation:

$$\mu_a = \mu_o \, e^{\Delta E/RT},$$

where T is absolute temperature (^{o}K). μ_a is apparent viscosity, μ_o is an intrinsic property of the liquid, and ΔE is the activation energy obtained from a logarithmic plot of log vs. $1/_T$ according to (Holdsworth, 1971):

$$\log \mu_a = \log \mu_o + (\Delta E/R)(1/T).$$

This activation energy is inversely related to the concentration of suspended solids (Saravacos, 1970).

Viscosity of pureed strawberries and syrup from thawed fruit, recorded with a Stormer Viscometer using different sized falling weights, was related to shear-press values, drained weights, water soluble pectin, and the inclusion of underripe berries (Sistrunk and Moore, 1967; Spayed and Morris, 1981; Sistrunk et al, 1983). The viscosity of syrup from frozen strawberries, determined with a 300 Oswald pipette, decreased with harvest date (Sistrunk et al, 1960). Woodward (1972) estimated intrinsic viscosity of soluble pectic polysaccharides with a "U" tube viscometer.

Shaller et al.(1970) used a Contraves Rheomat 15, a coaxial cylinder rheometer, to characterize the rheological properties of strawberry purees, which an earlier report had called pseudoplastic (Oktabec and Ambros, 1965). Frozen strawberry puree, after thawing and cooling, showed non-Newtonian, almost Bingham plastic flow behavior for the range of shear rates used (50 - 200 sec^{-1}). Logarithmic plots of temperature vs logarithm of plastic viscosity proved to be linear. Enzymatic degradation of pectin did not change the type of flow behavior, but decreased the puree viscosity and yield stress. Saravacos and Moyer (1967) determined rheological constants of fruit purees at two temperatures using a Brookfield Viscometer (Model RVT) which is commonly used in the food industry. For non-Newtonian liquids, the geometry of this rotational viscometer is not amenable to rigorous mathematical analysis, but the instrument can be used to measure time dependency and hysteresis behavior since it is not affected by suspended particles (Bourne, 1982).

Pureed strawberries decreased in viscosity from immature green fruit to the color inception stage, then stabilized in ripe fruit with the apparent decomposition of cellulose and increase in water-soluble pectin (Spayed and Morris, 1981). Combinations of pureed green and ripe strawberries mixed with ripe sliced fruit resulted in higher viscosity than 100% sliced ripe fruit. Mixtures containing up to 40% puree were rated acceptable by a sensory panel (Sistrunk et al, 1983).

Sensory evaluation of strawberry texture

Texture evaluations of strawberries:

Most workers involved with cultivar screening tests are concerned with relationships between chemical composition or mechanical resistance to breakdown and sensory scores of the firmness component of "texture." In many sensory assessments of strawberry texture performed for this purpose, "texture" by mouth and "berry wholeness," or "appearance" are scaled as quality factors, along with color and flavor, using fixed point category scales and various panel sizes (Wolford, 1961). Sensory tests using category scales have worked with six categories and six judges examining 300 cultivars (Robinson, 1947), ten samples scaled on nine levels (Simpson, 1980), and eleven point scales and eight panelists judging 20 samples (Sistrunk and Moore, 1967). Processing and storage effects on mixtures of sliced fruit with purees of ripe and green fruit were evaluated by 12-15 panelists using a ten point category scale (Sistrunk et al, 1983). Ahmed and Dennison (1971) used ten "semi-trained" panelists and a six point scale to examine storage and irradiation treatments of fresh strawberries. A berry "wholeness factor" calculated from percentages of hand-sorted strawberries in different stages of brokenness was proposed as a varietal screening index (Jewell et al, 1973).

Few reports in the literature concerning strawberries relate sensory textural attributes to chemical and physical characteristics and fruit cell wall structure. Armbruster (1967) observed strawberry cell walls microscopically and conducted paired difference tests for firmness on three berry varieties. Szczesniak and Smith (1969) obtained qualitative verbal descriptions to differentiate among sample types of fresh strawberries at three stages of ripeness; frozen and freeze-dried berries were also examined. Softening, loss of crispness, and easy juice release accompanied cellular changes caused by senescence and processing. The texture profiling technique was followed, but quantitative differences among sample types were not detected.

Sensory texture profile techniques:

Descriptive profiling techniques identify, describe and quantitate sensory (visual, textural, auditory, and gustatory) qualities of a given product (Gillette, 1984). Texture profiling is one of several methods to describe quality differences in foods and complies with the criteria for objectivity (Bourne, 1982): 1) Freedom from personal bias. Panelists are trained to rate intensity, not acceptability. 2) Repeatability. Results from two panels or the same panel over time have been reproducible, even 16 months after the first test (Szczesniak et al, 1975). Descriptive profiles aid the monitoring of changes in a food product over time, effects of ingredient substitutions, or differences between similar products.

Szczesniak (1963) pioneered the classification of textural characteristics of foods based on mechanical and geometrical properties, order of appearance, and moisture content. Standard rating scales which correlated sensory analyses to mechanical measurements of texture were then developed (Szczesniak et al, 1963a). Brandt et al.(1963) presented the general methodology for developing a texture profile on which Civille and Szczesniak (1973) expanded with guidelines for training an expert texture profile panel. Civille and Liska (1975) described modifications of this General Foods Sensory Texture Profile technique.

Panel training involves selecting motivated panelists with time available to participate and thoroughly familiarizing them with texture properties, rating scales, testing techniques, and the products for evaluation. A list of descriptive terms is compiled by the panelists and used to develop a comparative ballot. This ballot is used to identify and quantify small-differences in textural properties. One sample is sometimes picked as the ideal to be reproduced and samples are scaled as more or less than the reference for each attribute.

Cardello (1982) reported that psychophysical exponents were greater for ratings from trained panelists than from consumer judges. Trained texture panelists showed less variance in their scoring than consumer panelists, but mean scores from trained and consumer panels were not significantly different. Large numbers of descriptors are often listed on ballots which may denote interdependent textural properties. Toda et al.(1971) and Syarief, et al.(1985) proposed that reducing the number of character notes through principle component analysis would increase reliability and evaluation validity, save evaluation time and cost, and reduce panel fatigue.

Factors to consider in texture analysis are the type of test, temperature, sample geometry, and having a representative subsample. Textural tests may be non-oral, such as cutting fruits, squeezing bread loaves, fracturing crackers, stirring semi-solids or liquids, or observing shape retention (Bourne, 1982). Most oral mastication testing is described by Szczesniak (1963). Temperature control is especially important when testing berries since refrigeration has been shown to increase firmness. Geometric properties influence compression and rupture forces, and size affects chewiness (Brandt et al, 1963).

Category scales or line scales are the usual means for quantitating texture properties (Szczesniak, et al, 1963; Sistrunk and Moore, 1967; Stone, 1974; Cardello et al, 1982). Since correlating sensory results with values from mechanical measures of texture is the objective of many testing programs, magnitude estimation scaling offers several advantages over other scaling techniques: 1) no arbitrary limits on scaling inherent in interval or ordinal scaling. 2) increased sensitivity for detecting differences. 3) less biased technique for finding correlations and mathematical functions between sensory and instrumental values. 4) increased use by psychophysicists to describe sensory functions (Moskowitz, 1982). Magnitude estimates must be transformed before they can be compared, because high variances in the raw ratio values obscure true differences between samples. Procedures for geometric normalization of magnitude estimates have been described by Lane et al.(1961), Moskowitz (1971, 1977), and McDaniel (1974). Arithmetic means and geometric means of normalized values are open to the full range of statistical testing.

Strawberry softening with maturity

Changes in strawberry cell wall polysaccharides during ripening and senescence:

Examinations of differences in cell wall polysaccharides related to textural differences have dealt primarily with changes occurring in the fruit during ripening. With increasing maturity, the texture of strawberries softens considerably while the color is developed. The strawberry is generally considered a non-climacteric fruit, not showing increased synthesis of ethylene during ripening, but it does enter a period of rapid ripening shortly before harvest (Woodward, 1972). Cells enlarge considerably and cell walls of adjacent cells separate from each other (Neal, 1965). Total sugars increase logarithmically for 35 days after petal fall (Knee et al, 1977). The greatest changes in texture occur between the first appearance of red coloration and the development of a uniform redness.

Wade (1964) detected no major differences in the proportions of component anhydro-sugars or ester groupings of the insoluble cell wall polysaccharides and concluded that differences occurred in the nature of associations between the insoluble pectic substances. Increases in the proportions of water-soluble polysaccharides have been related to ripening (Neal, 1965; Woodward, 1972; Knee et al, 1977; Huber, 1984; Wesche-E. et al, 1986), with corresponding decreases in fractions extracted using cation-chelating agent EDTA (Wesche-E. et al, 1986). Knee et al.(1977) traced the solubilization of soluble high molecular weight neutral and uronic acid polymers from the cell wall with C^{14}-labelled sugar assimilates. Net synthesis of total polyuronides, but not neutral polysaccharides, occurred before color development began (Woodward, 1972).

Enzymic role in softening of strawberries:

In many fruit types, the softening that accompanies ripening involves enzymatic activity that causes a loss of cell wall material by dissolution or degradation of polyuronides. The nature of this polyuronide solubilization in strawberry cell walls is still unknown, but the role of enzymatic depolymerization, an activity observed during tomato and avocado softening (Pressey, 1977), has been examined. Polygalacturonases which are primarily endo-hydrolases of pectic acid, are found in many fruits and are associated with loss of firmness in peaches (Pressey et al, 1971) and tomato fruit (Huber, 1983). There is confusion in some reports as to whether hydrolytic activity may be of microbial origin (Pilnik and Voragen, 1970). Pectinmethylesterase is more widespread, but its role in ripening is still undefined, although it is thought to prepare pectic chains for cleavage by hydrolyzing methoxyl esters adjacent to free carboxyl groups.

Enzymatic activity responsible for changes in cell wall polysaccharides that effect changes in texture with maturity is partially characterized. The involvement of an endo-polymethylgalacturonase in hydrolyzing pectic polymers in strawberries was reported (Gizis, 1963) and its role was suggested in the decrease in specific viscosity of soluble pectic polysaccharides with ripening (Woodward, 1972). However, this activity has not been confirmed by other workers (Neal, 1965; Barnes and Patchett, 1976).

A key mechanism proposed for maintaining fruit firmness in strawberries is the formation of calcium-stabilized 'ionic' bonds crosslinking carboxyl groups of adjacent polyuronide chains (Neal, 1965). Separation of strawberry parenchyma cells was suggested to result from increased methylation of carboxyl groups on polyuronides in the middle lamella. A reported decrease in pectin-methylesterase activity during sensescence supports Neal's suggested mechanism, since a reduction in demethylating versus methylating activity would be expected to destroy crosslinks with divalent cations (Barnes and Patchett, 1976).

Barnes and Patchett (1976) observed a C_x- type cellulolytic activity in strawberries that increased from the ripe to the overripe stage. This glucanase activity was suggested to act on glycans similar to the xyloglucans isolated from sycamore callus cells (Darvill et al, 1980) to allow for sliding growth expansion with the eventual release of polyuronic acids during ripening. Hemicellulose polymers decreased in molecular size during ripening, while the only compositional changes were consistent decreases in arabinose and galactose (Huber, 1984). However, no hemicellulase activity similar to that indicated in tomato fruit was apparent (Huber, 1983).

Current model of pectin structure in primary cell walls

Pectins are one class of complex polysaccharides in the primary cell wall of dicotyledonous plants which are responsible for maintaining intercellular adhesion. Their location and possible molecular associations with cellulose and hemicellulosic polysaccharides in the cell wall was first modeled by Keegstra et al.(1973) from studies of suspension-cultured sycamore cell. Pectins are block co-polymers containing branched and unbranched blocks of galacturonate chains with rhamnose insertions to which side chains of neutral sugars may be linked. Side chains include mostly galactose, and arabinose, with minor quantities of fucose, xylose, and apiose, in a few cases. Galactan, arabinan and galactoarabinan side chains predominate and may have several levels of branching (Jarvis, 1984). Fry (1982, 1983) suggested that ferulic acid esters that terminate (1,4')-D-galactan and -(1,5')-L-arabinan side chains dimerize to alkali-labile diferulate cross bridges between adjacent pectin molecules.

Pectins are held in the cell wall matrix by their ability to form non-covalent gels and can be extracted by water or strong chelating agents (Jarvis, 1982). Galacturonate residues are fully methyl esterified during synthesis and are deesterified as required at the cell wall location. Randomly distributed acetyl esters may occur at free -OH groups at C-2 or C-3. In the normal gel type in cell walls, co-ordinated calcium ions bind non-esterified galacturonic acid blocks through salt linkages to form junction zones. The presence of rhamnose-linked side chains and methoxyl or acetyl ester groups on the galacturonate backbone prevents aggregation and terminates a binding segment (Lau et al, 1983). Xyloglucan polymers, part of the hemicellulosic polysaccharides, appear to be non-covalently bound to cellulose and possibly glycosidically linked to pectin chains (McNeil et al, 1984). For strawberries, Barnes and Patchett's (1976) suggestion that these polymers released from the insoluble fraction of the cell wall influence softening is supported by observations of decreases in xylose and glucose with ripening (Wesche-E., et al, 1986).

Analytical procedures

Fractionation of strawberry cell wall polysaccharides:

Strawberry cell wall polysaccharides have been extracted and fractionated on the basis of selective solubility properties in water and calcium-chelating buffers, ion exchange properties, and molecular size. Wade (1964) isolated a cell wall preparation from which water-soluble polysaccharides appear to have been discarded. Fresh strawberry cortical tissue was blended and washed with cold saline, that was later discarded, before treatment with acetone. Neutral sugar composition of this preparation was determined by hydrolysis and paper chromatography. Neal (1965) performed extractions with water, Tris-HCl containing 50 mM EDTA (pH 9.0), and Pectasin on different samples of alcohol-insoluble substances. Purees of frozen-thawed berries were extracted with 95 % ethanol (Sistrunk and Moore, 1967), then sequentially extracted this acetone-insoluble material with water, 0.5% Calgon, 0.05 N NaOH, and 1 N NaOH. The 1 N NaOH extract was initially considered hemicelluloses and the residue cellulose, but Calgon, 0.5N NaOH, and 1N NaOH extracts were combined because their contents of uronic acids (carbazole method, Dietz and Rouse, 1953) were small and positively correlated.

Woodward (1972) prepared alcohol-insoluble residue from fresh berries macerated in HCl-MeOH for anthocyanin extraction. Soluble pectic polysaccharides were extracted from dry AIS with 0.1 M sodium phosphate buffer (pH 6.9) containing 50 mM EDTA and a few drops of toluene. Insoluble pectic polysaccharides in the residue were further released with a pectinase enzyme. Total pectin content was considered the sum of soluble and insoluble pectic polysaccharides. A number of average molecular weights of polysaccharide extracts were estimated by determinations of intrinsic viscosities. A strawberry cell residue was prepared for study of enzymatic degradation from a slurry of fresh tissue homogenized with salt, EDTA, and PVP (pH 8.0) at 3°C (Barnes and Patchett, 1976). After centrifugation, the supernatant was made to 72% acetone and allowed to stand at -24°C to precipitate polysaccharides. Enzymatic hydrolysis of polysaccharides were followed by viscosity values measurements of a cell wall slurry. Knee et al (1977) macerated small quantities of tissue (wt < 1 g) in Tris-DIECA (0.2M Tris with 0.01M sodium diethylthiocarbamate, pH 8.0) to obtain soluble pectic polysaccharides. Ionically bound material was extracted in Tris base (pH 10), and the remaining residue was considered purified cell wall. Gel-filtration through Sephadex G-25 separated free sugars from polysaccharides and molecular weight fractions were passed over an ion-exchange resin (DEAE) to separate neutral sugars from uronic acid polymers. Collected fractions of each were combined for spectrophotometric quantitation of neutral sugars and anhydro uronic acids.

Huber (1984) obtained an ethanol powder from fresh strawberries by homogenizing fruit in 100% ethanol, freezing overnight at -20°, and filtering the precipitate. Total soluble polyuronides were extracted from this powder using a Na-acetate buffer with EDTA. Hemicelluloses were extracted from a cell wall preparation, previously heat-treated with a solution of sodium chloride with EDTA, by 4 N NaOH with $NaBH_4$. Gel-filtration of the polyuronide and hemicellulose solutions were performed on Ultrogel AcA 24 and 34, respectively. Polyuronides were eluted with sodium acetate-acetic acid buffer, pH 5.0, containing EDTA and 50 mM NaCl. Hemicelluloses were eluted with a sodium citrate-phosphate buffer containing 100 mM NaCl.

Wesche-E. et al.(1986) developed a technique for isolating fractions of strawberry cell walls based on those by O'Beirne (1980), Knee (1973), and Tetley (1984). Achenes were removed from freeze-dried berries as they were powdered. Cell wall substances were isolated as insoluble solids in 80 % acetone (AIS), then freeze-dried for further extractions. The AIS powder was sequentially fractionated with distinctions made between water-soluble and chelator-soluble polyuronides: 1) water-soluble polysaccharides (WSP), Tris-HCL buffer, pH 7.5. 2) chelator-soluble polysaccharides (CSP), Na-phosphate, pH 6.9, heating under reflux in 95°C water-bath 4 hr. Weight percentages of the fractions were determined from lyophilized solutions. Aliquots of solubilized WSP or CSP were stored at -20°C, then applied to an ion exchange column to examine neutral and uronic acid polymers.

Analysis of molecular size distributions of pectic polysaccharídes has been aided by recently developed size-exclusion columns for high-performance liquid chromatography (HPLC) equipment to analyze polar polymers. Fishman et al (1984), using a Waters µBondagel E-Linear column, took advantage of the speed of this analysis to demonstrate effects of mobile phases, purification method, chemical modification and methoxyl content on pectin solution behavior. Barth (1980) reported a dependence of peak maximum on sample concentration.

Characteristics of Benton, Totem and Selva strawberries.

Fresh Bentons have been described as being tender - lacking firmness, but medium in firmness and toughness when thawed (Northwest Cold Pack Co., 1983). They are medium to light red berries and are generally high in sugar and acid, averaging 10.2^{o} Brix and 1.10 % titratable acidity. The flavor is clean, distinctive and in great demand with food processors. A high rate of malformation was noted in this variety (Gilbert and Breen, 1985), but they composed 35-40 % of the strawberry acreage in Oregon in 1981, due to high crop yields and their viral resistance. Their soft texture has caused processing problems.

Totems have the darkest pigmentation of commercial cultivars grown in Oregon, almost black when over-ripe, and composed 10% of the strawberry acreage in 1981. Totems are medium in sugar content and low in acid (averages of 9.9^{o}Brix and 0.85 % titratable acidity). They are firmer than Bentons maintaining superior fruit identity in processed products and considered adaptable for fresh marketing.

Selva is a day-neutral cultivar developed in California with extreme firmness and a long shelf life. These berries, currently used in processing and for the fresh market, are being tested for production in Oregon and Washington (Daubeny, 1985). The flavor of Selva strawberries is bland and sometimes described as grassy. Thawed berries from the 1984 season had shearpress values as high as readings for fresh Bentons (Lin, B.L., unpublished data).

MATERIALS AND METHODS

Samples

During the 1985 season, red-ripe strawberries (Fragaria x ananassa), Benton and Totem varieties, were handpicked from two commercial fields and from a field plot at the OSU Lewis Brown Farm, Corvallis, over three non-consecutive days of high temperatures above 100°F. Selva variety strawberries were received as two 50 lb shipments (four unrefrigerated flats) in late July and mid-September from the Shinta Kawahara Co. in Watsonville, CA via overnight Air Federal Express shipment. The samples were designated Selva-A and Selva-B, respectively. All berries were inspected for mold and bruises, rinsed under tap water, and capped. A portion of each lot was halved, and all fruit was individually quick frozen at -40°C, packed in two layers of polyethylene bags, and stored at the same temperature.

Reagents

M-hydroxydiphenyl colorimetric reagent came from Eastman Kodak Co, Rochester, N.Y. Dichloromethane, dimethyl sulphoxide, 1-methylimidazole, alpha-naphthol and dextran standards in the following average molecular weights: 2×10^6, 5.31×10^5, 7.12×10^4, 4.06×10^4, 9.00×10^3 were obtained from Sigma Chemical Co., St. Louis, MO. Reagent grade anhydrous sugar standards glucose, galactose, mannose, xylose, arabinose, ribose, and myo-inositol came from Mallinckrodt Chemical Works, St. Louis, MO, and were stored in a dessicator at room temperature. Apiose was obtained by hydrolysis of the dimer apiose di-O-isopropylidene, purchased from Sigma Chemical company, according to a procedure by Bell (1962). Mono- and di-basic sodium phosphate and Tris buffer salts, disodium EDTA reagents, and mineral acids and bases were of analytical grade. EDTA buffer was a 0.1 M phosphate buffer at pH 6.9 with 50 mM EDTA.

Physical properties

Drip loss:

Frozen berry halves (150 g) were placed in 9 cm diameter glass funnels, fitted with wire mesh screens, and resting in 100 ml graduated cylinders. Thermometers inserted into the center of berries present in the middle of the funnel showed an average temperature change of $-12^{o}C$ to $4^{o}C$ in three hours, in a controlled temperature room at $22^{o}C$.

Resistance to shear:

Resistance to shear was determined when the berries had reached an internal temperature of $65^{o}F$. Samples were placed in the cell of a modified Kramer shear-press, using the 500 lb capacity proving ring, to give a full scale reading of 100 lbs force. Work of compression curves were measured using a planimeter and square inches were multiplied by the factor 20 to obtain inch-lb units.

Viscosity of purees from frozen strawberries:

Approximately 200 g samples of frozen strawberry halves were thawed 18 hr at 4^{o}C in 600 ml beakers covered with parafilm. Thawed berries and juice were blended in a Waring blender, Variac setting 80, for 1.0 min and poured into a 250 ml beaker. Viscosity was measured with a rotational viscometer, Brookfield Viscolectric (Model RV). Spindle 3 and a shear rate of 20 rpm were selected for full scale readings during preliminary measurements of heating effects on fresh strawberries. The temperature of puree samples was increased by placing the beaker in a water bath heated to 75^{o}C and gently stirring the puree with a teaspoon. To examine the type of fluid behavior in purees of Totems and Selva-B berries, the rotational speed of the spindle was changed stepwise from 2.5 - 50 rpm, at 30^{o}C.

Instrumental readings were converted to apparent viscosity (centipoises) using tables supplied by the manufacturer, and to shear stress by multiplying the apparent viscosities by the shear rate ($^{rpm}/_{60}$), according to the simple Newtonian rheological model:

N x (shear rate) = shear stress (Charm, 1960).

<u>Sensory texture profiling of frozen strawberries</u>

Materials.

Samples for sensory texture profiling were whole IQF frozen strawberries of the Benton, Totem, and Selva varieties pooled from each harvest replication. Appropriate reference standards for each descriptor are presented in Table 1.

Training of panelists.

Eight panelists with prior sensory panel experience were selected on the basis of their interest and time availability. Training periods consisted of six sessions of 45-60 min each. Panelists were familiarized with texture definitions and the range of textural differences in frozen strawberries. A list of texture descriptors was developed for frozen strawberries, and those terms best fitting sensory texture characteristics classified by Szczesniak (1963) and Szczesniak, et al (1963) were chosen for evaluation.

For each descriptor, techniques were developed with the panelists to ensure that equivalent portions of each berry and equal-sized samples were evaluated. Panelists received a paring knife and a six-inch ruler for slicing berries in half longitudinally. Excess width and length of the berry halves were then trimmed off at 1.5 cm from the top and 1.5 cm from the side, measured at the widest point. Panelists also received plastic spoons and a styrofoam cup for expectoration.

During training, panelists practiced slicing and testing methods with discussion of their individual ratings of practice sample sets for each attribute. The final texture definitions and a sample ballot are given in the appendix.

Table 1. Sensory - Texture descriptors and reference standards for frozen strawberries.

Final descriptors	Reference
firmness	1/2 in cube Kraft Velveeta cheese
fibrousness	slice of Del Monte 'Lite' sliced peaches, 1/2 in long.
juice release	1/2 in cube "My-T-Fine"canned mushrooms
total mouth moisture	" "
chewiness	No. of chews required before swallowing 1/2 in cube of strawberry.

Testing conditions.

All samples were evaluated at room temperature (25^{o}C), under red lighting, in individual testing booths. Two replications of tests for each attribute were completed over two successive days. Panelists received one tray per attribute containing three coded sample cups of two strawberries each and one cup of the reference food. They were instructed to evaluate one half of each berry, to increase the number of judgements per sample. For all descriptors, except chewiness, samples were scaled using magnitude estimation with the reference valued at 50. Chewiness was scored as counting number.

Panelists also rated visual texture characteristics of strawberry samples. Ten berries of each variety were thawed in coded white bowls and evaluated under a Macbeth Color Identification Lamp (Executive model light box) on the daylight setting. Magnitude estimates of each attribute were assigned in comparison to Totem berries as the reference, with a value of 50.

Data Analysis.

For each attribute per testing session, the 16 magnitude estimates which the panelists assigned were normalized by the procedure of Cardello (1977). The following summarizes this proceedure: 1) geometric means from all ratings of each panelist per sample per session were calculated; 2) grand geometric means from each sample mean per judge per session were calculated; 3) each panelists estimate was divided by the ratio

$$\frac{\text{Sample Geometric Mean}}{\text{Grand Geometric Mean}}$$

per subject per sample. The normalization calculations were performed on an IBM-PC using Lotus 1-2-3 software. Three-way analysis of variance on each texture descriptor was performed on the normalized estimates using statistical software developed by David Lundahl (1986). Correlations between descriptors were obtained through use of the Number Cruncher Statistical System (vers. 4.1).

Chemical Analysis

Screening tests:

A sub-set of strawberry samples was pureed in a Waring blender, Variac setting 80, for total solids, pH, °Brix, and titratable acidity measurements. Total solids were determined on 10 g samples of puree through vacuum oven drying (-29 psi) at 60°C for 24 hrs. pH measurements were made with a Corning pH Meter (Model 125) using a combination gel-filled electrode. °Brix values were read from a Bousch and Lombe temperature-regulated refractometer at 20°C. Titratable acidity, calculated as % citric acid, was determined on 10.0 g samples diluted to 100 ml with distilled water and titrated to an end point of pH 8.1, with 0.1 N NaOH.

Isolation and fractionation of cell wall polysaccharides:

Frozen berry halves were lyophilized for a minimum of 4 days in 1200 ml Virtis glass flasks with adapters for a Labconco Deluxe Bench Top Freeze Dryer. To remove achenes, dried fruit was crushed and the powder sieved through 8, 28, and 45 mesh screens. The resulting powder was stored in a dessicator until needed for the extraction of cell wall polysaccharides. Extraction procedures developed by Wesche-E. et al.(1985) were applied to the dry strawberry powders to obtain acetone-insoluble solids (AIS) and the subsequent fractions of water-soluble pectins (WSP), chelator-soluble pectins (CSP), base-soluble polysaccharides (BSP), and insoluble residue (RES). Figure 1 presents this extraction scheme.

Two replications of AIS extractions were performed for each variety. From each AIS powder obtained, separate extraction sequences were followed for Benton, Totem, and Selva-A samples. For Selva-B samples, the resulting off-white AIS powders from replicate extractions were pooled and used to obtain two sets of polysaccharide fractions. All cell wall fractions were stored in a dessicator at room temperature.

Procedure developed by Dr. Pedro Wesche-E.

IQF strawberry halves

Freeze Dry
Remove achenes - Sieve through 8, 28, 42 mesh screens.

80% acetone extraction

Acetone soluble sugars, pigments, phenolics

Cell Wall Prep (AIS)

0.1 M Tris-HCl pH 7.5

acetone ppt., lyophilize
Water soluble pectin (WSP)

ppt.

0.1 M NaPi, 0.05 M EDTA, pH 6.9

dialyze, lyophilize
Chelator soluble pectin (CSP)

ppt.

4 N NaOH

dialyze, lyophilize
Base-soluble polysaccharides (BSP)

Residue

Figure 1 Isolation of strawberry polysaccharide fractions

Analysis of uronic acid and neutral sugars:

Total uronic acid content and neutral sugars were determined on solutions of lyophilized WSP, CSP, and BSP extracts. CSP and BSP powders of all varieties dissolved immediately at 1 mg/ml concentrations. WSP samples, however, were slow to wet and required extensive agitation before it completely dissolved, after 4 hrs at room temperature. Anhydro-galacturonic acid equivalents (A_{520}) were measured on an Autochrome autoanalyzer system (Thibault, 1979), a modification of the m-hydroxydiphenyl method (Blumenkrantz and Asboe-Hanson (1973). Neutral sugars (A_{560}), reported as anhydroglucose units, were also measured automatically using sulphonated alpha-naphthol (Devor, 1950) and were not corrected for the absorbance of the uronic acid chromophores.

Neutral sugar composition:

Samples.

Lyophilized cell walls fractions of AIS, WSP, and CSP from the four varietal sample groups were analyzed for neutral sugar composition.

Hydrolysis of plant cell walls.

Ten mg of polysaccharide fractions, weighed into Kimex screw-top test tubes, were treated with 72% (w/w) H_2SO_4 (250 ul) under nitrogen at room temperature. Agitation using a vortex stirrer was used to aid dissolution. After 45 min, the acid was diluted with EDTA buffer (2.0 ml) to give 1.35 M sulfuric acid, and the tubes were heated 6 hrat 105°C under nitrogen. Tubes were cooled and neutralized with 15 M ammonia (0.64 ml) to give 1.35 M ammonia. Myo-inositol (0.05 mL of a 20 mg/mL solution) was added as an internal standard. Aliquots of 0.1 mL were reduced and acetylated as described by Blakeney et al (1983).

Preparation of standard sugar solutions.

Three concentrations of sugar mixtures at approximately 5, 7.5, and 11 mg of each sugar were reduced and derivatized as described above.

Gas-liquid chromatography.

The alditol-acetates were separated on a FSOT glass-capillary column (15 m x 0.53 mm id, DB-225, J & W Co., California) fitted to a Varian Aerograph series 1400 equipped with a flame-ionization detector. High-purity helium was used as the carrier gas with a flow rate of 165 cm/sec (determined by injecting dichloromethane). Samples of 1-uL were injected directly onto the column. The oven temperature was kept for 4 min at 198°C following injection and then raised at 4°C/min to 225°, where it was held for 7 min. The injection port and detector temperatures were 250°and 270°C, respectively.

Molecular weight distribution of polysaccharides in frozen strawberry cell wall extracts and free run juice:

Materials.

Polysaccharide samples were lyophilized WSP, CSP, and BSP extracts from Benton, Totem, Selva 1, and Selva 2 strawberries. For free-run strawberry juice, composite samples of frozen berries (approximately 150 g) from each varietal group were thawed in 600 ml beakers at 25°C for 6 hr.

High Performance Size Exclusion Chromatography (HPSEC) Equipment.

The running buffer was of 50 mM sodium phosphate, pH 6.5, pumped by a Varian 5000 Liquid Chromatograph System, Varian Associates, Inc., Walnut Creek, CA. Two Waters Associates, Bondagel columns in series, E-1000 and E-Linear (30 x 0.39 cm each), performed the high-performance size exclusion chromatography. Sample peaks, detected by a Varian Aerograph Refractive Index Detector, Palo Alto, CA, were recorded by an Hewlett-Packard 3380 A Integrator, which listed retention times, peak areas, and percentages of total peak area.

HPSEC Sample preparation.

Polysaccharide extracts: Typically, 3-5 mg of a lyophilized cell wall extract was weighed into a tared glass test tube, and the volume of mobile phase required to obtain the desired sample concentration was added. With concentrations of 2 mg/ml, CSP and BSP samples dissolved immediately; WSP samples required about 1 hr at room temperature and frequent agitation before completely dissolving. Free-run juice from each sample described above was drained off and 20 ml aliquots were centrifuged 30 min in Kimex test tubes using a bench-top centrifuge. The upper 10 ml were removed from the test tubes for testing. From this sample, 1 ml aliquots were diluted to 25% of single strength for injection onto the column.

RESULTS

Chemical Characterization

Table 2 lists the chemical and physical properties of the four strawberry samples. Benton fruit was highest in total solids, ^{o}Brix, and pH. Titratable acidity and ^{o}Brix were lowest in Selva fruit, and Totems had the highest pH.

Physical properties

Work of compression and drip loss:

Thawed Selva berries of both sample sets gave work of compression values (Table 2) much higher than those for Totems and Bentons. Values for Totems were twice as large as those for Bentons. Drip loss volumes were highest in Bentons and very low in Selva fruit. These values showed an inverse trend with work of compression. Selva-A required more work of compression and lost less juice than Selva-B berries. Differences in chemical characteristics did not correspond to the trends in firmness or drip loss, but measurements on Bentons and Totems were similar to the range of values obtained previously for these varieties (Varseveld, unpublished data).

Table 2. Physical and chemical characteristics of thawed strawberries.

	Benton	Totem	Selva-A	Selva-B
A. Physical				
Work of compression[a] (in.*lb.)	7.0	13.2	20.0	18.9
drip loss (ml)[b]	28	20.5	3.5	4.5
B. Chemical				
pH	3.52	3.39	3.42	3.49
Brix	10.1	8.0	8.6	9.4
T.A.	0.96	0.97	0.91	n/a
Total solids (%)	11.3	10.6	10.8	9.8

[a]determined using Kramer Sheer Press.
[b] Juice released from 150 g frozen berry halves thawed for 2 hr.,
internal temperature change: 20 to 45°F.

Viscosity of pureed thawed strawberries:

In Figure 2, the logarithm of apparent viscosity of purees of thawed strawberries are plotted against the inverse of absolute temperature over the range of 10-60°C, for two sample replications. Purees from Selva-A berries were approximately twice as viscous at all temperatures as were Benton samples. Viscosity ranges for Selva-B and Totem samples overlapped, but Selva-B had higher mean viscosities over all temperatures.

Linear relationships obtained from these plots of apparent viscosity (log N) versus the reciprocal of absolute temperature (T^{-1} where T is $^{\circ}$K) can be described by the Arrhenius equation, $\log \eta = {}^{(\wedge E)}/_{(2.3RT)} + B$. ^E is the activation energy for viscous flow, R is the gas constant and B is a constant (Saravacos, 1970). Table 3 gives the coefficients describing these linear relationships. The activation energies calculated for strawberry purees agree closely with those Saravacos reported for peach and pear purees (1.7 and 1.9, respectively). As a comparison, the activation energy for water, between 20°and 70°C is 3.56 kcal/g mole (van Wazer et al., 1963). In these varieties, pureed Benton berries had the highest ^E values and Selvas had the lowest, with a slight decrease in ^E from Selva-A to Selva-B. Replications of each sample set were not significantly different.

Figure 3 shows the viscosity decreasing with increasing sheer rate for Totem and Selva-B berries. This plot indicates that these purees were non-Newtonian fluids possessing pseudoplastic properties with a yield stress at the shear rates used. Schaller et al (1970) reported that pseudoplastic behavior was improbable in thawed and heated strawberry purees, but their range of sheer rates was much higher than sheer rates used in this study. They also noted that the type of flow behavior did not change over increases in temperature from $20^{o}$to $60^{o}C$.

Figure 2. The effect of temperature on the viscosity of strawberry purees. IQF strawberry halves were thawed overnight at $4^{o}C$ and homogenized 1 min in a Waring Blender, Variac setting 80.

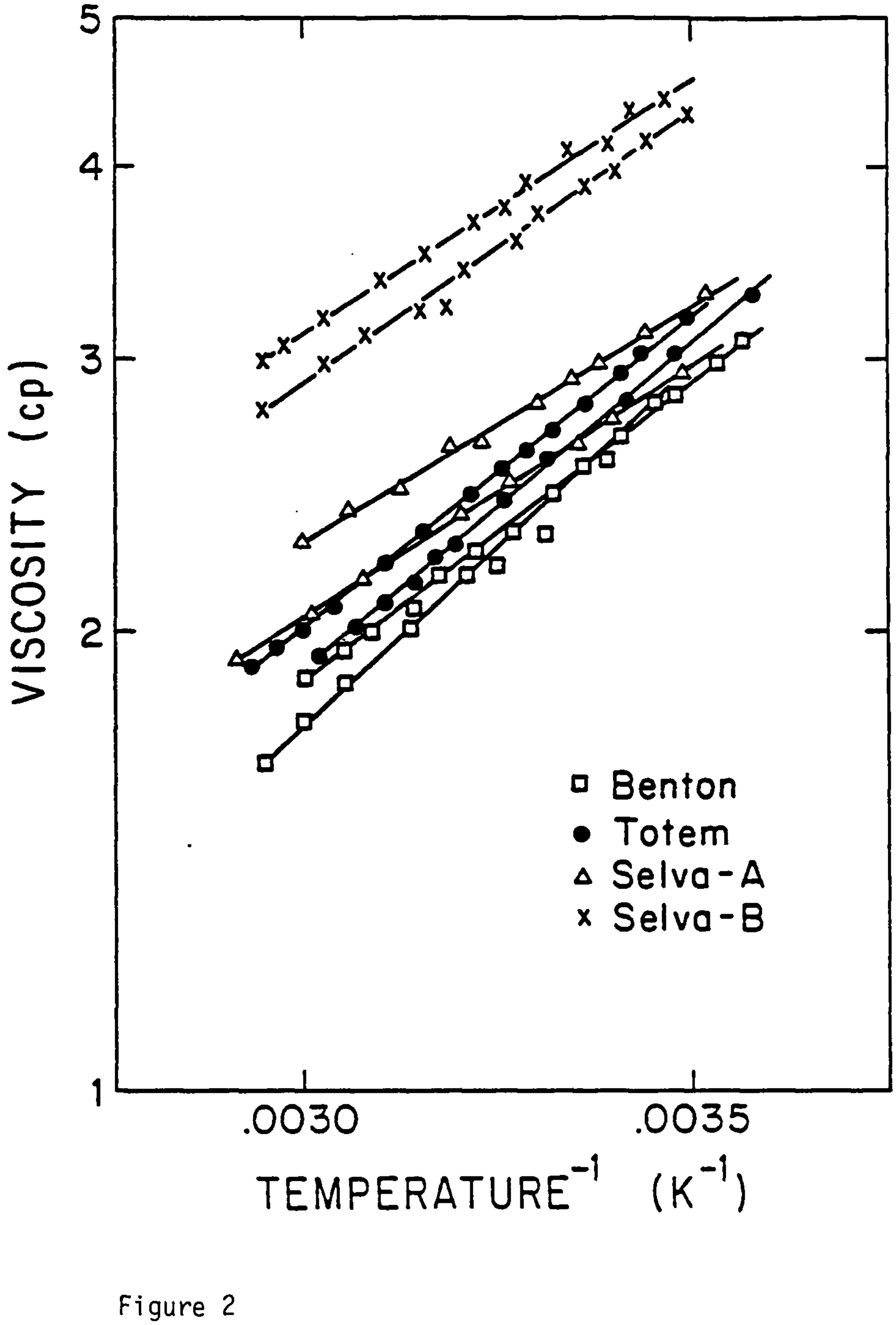

5
4
3
2
1
VISCOSITY (cp)
.0030
.0035
TEMPERATURE^{-1} (K^{-1})
Benton
Totem
Selva-A
Selva-B

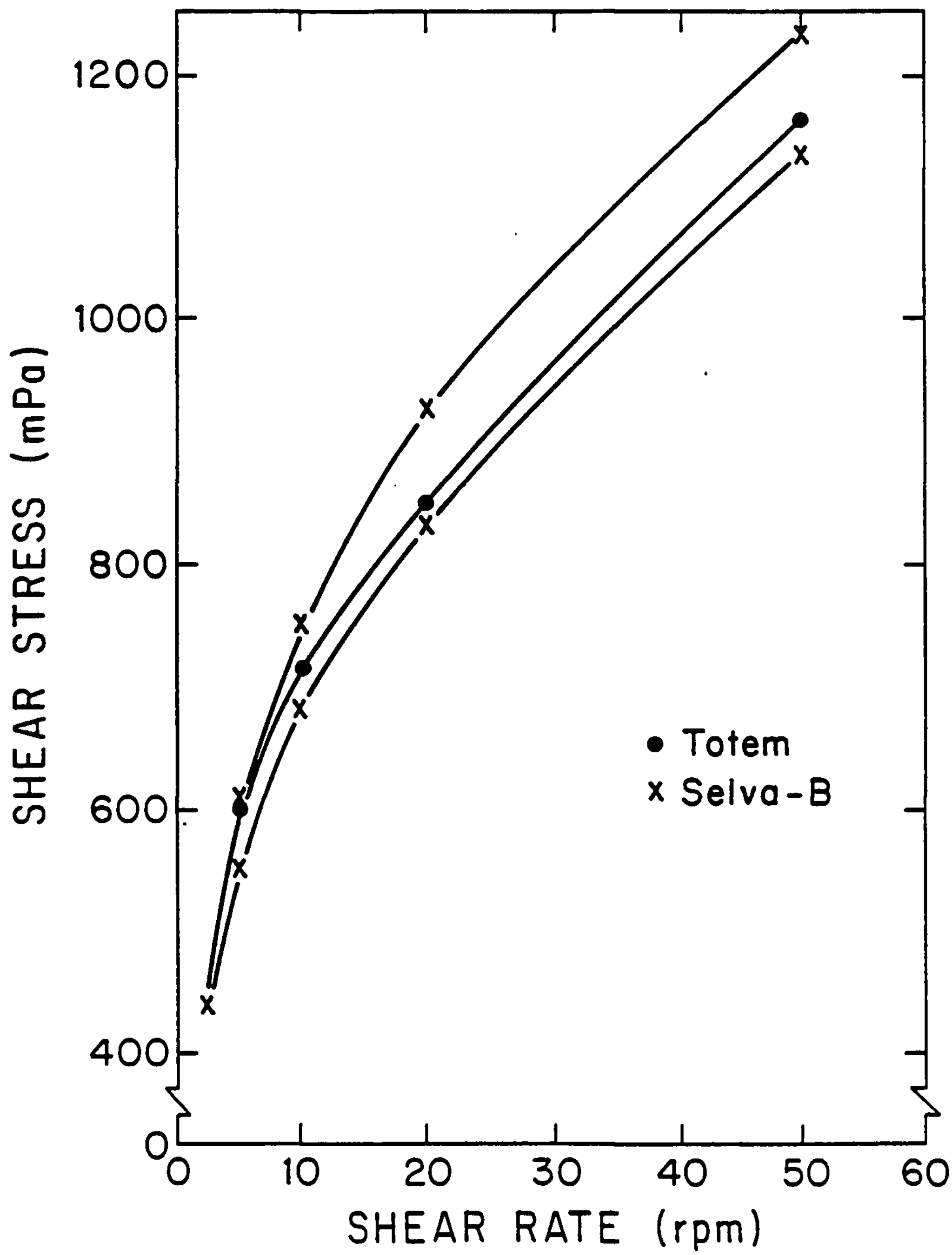
Figure 2

Table 3. Effect of temperature on viscosity of strawberry purees: Arrhenius constants, activation energies, and regression coefficients.

Sample	Slope (log cp[a])*K	Intercept log cp[a]	Activation energy E, kcal/g mole	Regression coef., r
Benton	380	2.13	1.74	0.994
	463	1.85	2.12	0.995
Totem	413	2.06	1.89	0.996
	420	2.02	1.92	0.992
Selva-A	340	2.44	1.58	0.987
	323	2.53	1.48	0.961
Selva-B	326	2.33	1.49	0.983
	303	2.45	1.39	0.993

[a]Viscosity in centipoises.

Figure 3. Effect of shear rate on shear stress in strawberry puree. Totem and Selva-B samples, 30°C.



1200
1000
800
600
400
0
SHEAR STRESS (mPa)
0
10
20
30
40
50
60
SHEAR RATE (rpm)
● Totem
x Selva-B

Figure 3

Sensory Texture Profile

Figure 4-A shows the sensory profile of oral texture properties measured on Benton, Totem, and Selva varieties of thawed IQF strawberries. Selva berries were significantly different from Bentons and Totems in four of five texture characteristics. Selva fruit rated four times greater than Bentons in firmness and twice as firm as thawed Totem berries. Fibrousness was two times higher in Selvas and about 1.5 times higher in Totems than in Bentons. In the "juiciness" properties, Benton berries were intermediate, and not significantly different than either Totems or Selvas. Benton strawberries were approximately half as "chewy" as Selvas and two-thirds as chewy as Totems. Benton and Totem fruit are qualitatively similar, but Benton berries rated lower than Totems in structural descriptors.

Table 4-A shows means of the normalized values for each descriptor and Table 5 gives the F ratios from three-way analysis of variance on each attribute. The treatment effect was highly significant ($p \leq 0.01$) in every descriptor except total mouth moisture, which was also significant ($p \leq 0.05$). Firmness, fibrousness, and chewiness descriptors had F values for treatment effect that were 2X to 9X larger than the next most significant source of error. Chewiness also showed the greatest panelist effect. This is not unusual since these counting values were not normalized, and judges often vary in their chewing rate and manipulation of the sample in their mouths, despite their training (Bourne, 1983). Juiciness ratings were heavily influenced by the day of testing, and the panelist effect was significant or highly significant

in all tests. Panelist x treatment effects were also highly significant in all tests and were greater than the treatment effect for the total mouth moisture descriptor.

Figure 4. Sensory texture profile of thawed IQF strawberries: Benton, Totem, Selva varieties.

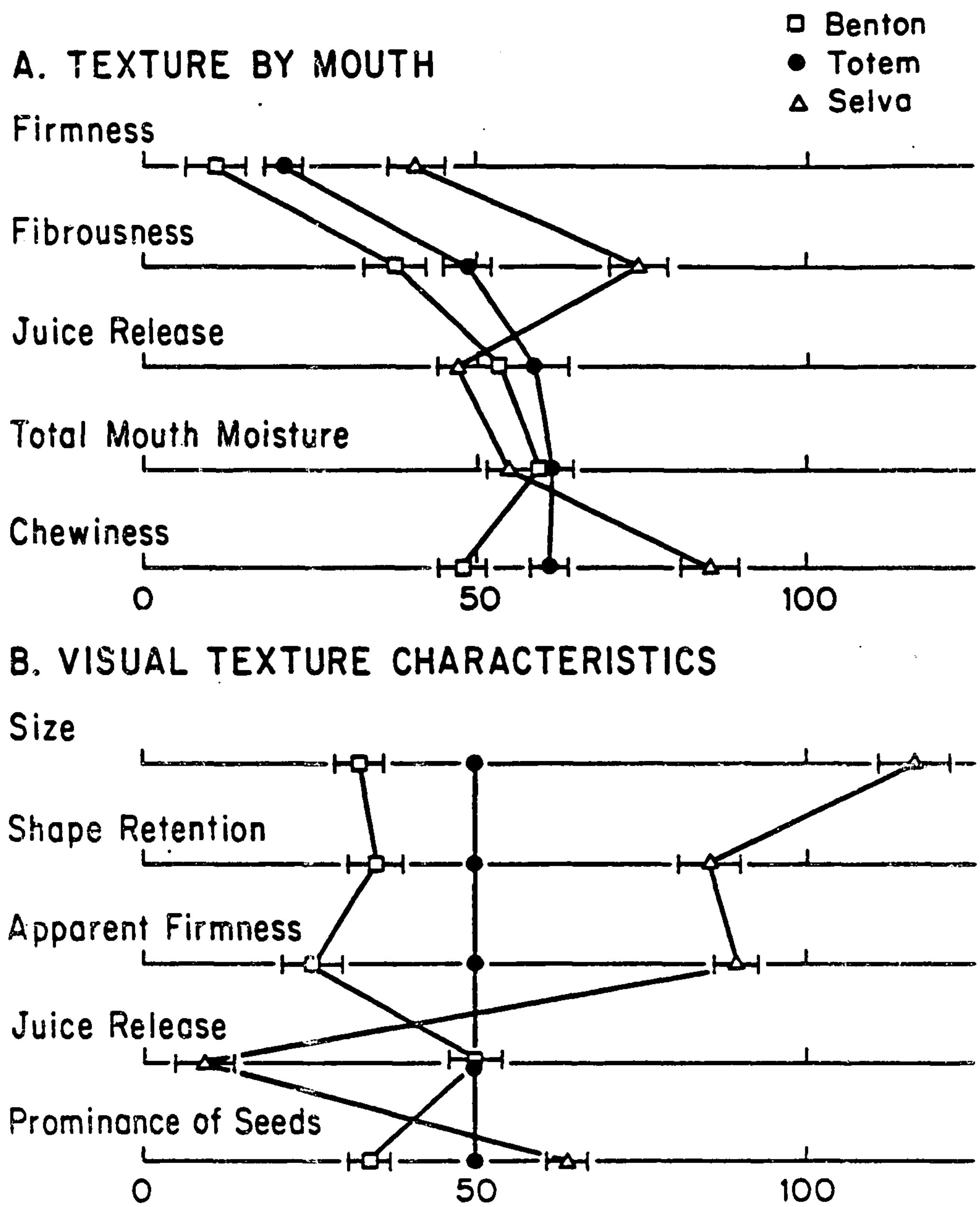

Benton
Totem
Selva
A. TEXTURE BY MOUTH
Firmness
Fibrousness
Juice Release
Total Mouth Moisture
Chewiness
0
50
100
B. VISUAL TEXTURE CHARACTERISTICS
Size
Shape Retention
Apparent Firmness
Juice Release
Prominance of Seeds
0
50
100

Figure 4

Table 4. Sensory texture profile of thawed IQF strawberries using magnitude estimation scaling.

A. Texture by mouth.

Attribute	Benton	Totem	Selva	lsd. (P<0.05)
Firmness	10.8[a]	20.1[b]	39.3[c]	8.6
Fibrousness	37.5[a]	47.9[b]	73.9[c]	8.9
Juice release	52.8[a,b]	58.4[b]	46.84[a]	10.4
Total mouth moisture	59.0	62.2	55.4	10.4
Chewiness	4.7[a]	6.09[b]	8.50[c]	.77

B. Visual texture characteristics.

Attribute	Benton	Totem	Selva	lsd (P<0.05)
Size	32.2[a]	50[b]	117.0[c]	11.4
Shape Rentention	35.1[a]	50[b]	84.9[c]	9.4
Apparent Firmness	31.3[a]	50[b]	88.6[c]	9.0
Free Run Juice	51.1[a]	50[a]	8.4[b]	6.8
Prominence of Seeds	34.4[a]	50[b]	62.9[c]	5.5

Values with the same letter in the same row are not significantly different.

Table 5. Sensory texture profile of thawed IQF strawberries: F ratios from three-way ANOVA.

Source of Error	df	Firm	Fiber	Juice Release	Total Mouth Moisture	Chewy
Panelist	7	2.63*	8.23**	4.18**	4.44**	48.64**
Day	1	7.10*	3.37	119.54**	191.93**	0.37
Pan*Day	7	0.44	5.80**	2.83*	3.68**	0.97
Treatment	2	245.98**	196.89**	11.96**	3.68*	87.17**
Pan*Trt	14	9.54**	22.85**	4.51**	4.58**	4.57**
Day*Trt	2	1.32	19.37**	1.74	1.25	2.53
Pan*Day*Trt	14	3.05**	12.35**	4.40**	0.99	2.07*
Error	48					

*F ratios are significant at $P \leq 0.05$.

**F ratios are significant at $P \leq 0.01$

Table 6. Sensory texture descriptions of three strawberry varieties.

Variety	Visual Features	Texture by Mouth
Benton:	Soft, slimy, High juice loss Greatly wrinkled skin "Grainy" flesh Seeds are deeply sunken.	Mushy, flesh disintegrates No sudden juice release Skin tough, rubbery Obvious tough, stringy fibers with little surrounding flesh.
Totem:	Spongy, mushy Sticky liquid covers skin. No air spaces in pith region, but center core remains hollow.	Skin peels away from flesh Juice release constant when chewed. Individual fibers less distinct, more chunky.
Selva-A:	Seeds not sunken into skin. Skin wet and slippery. Cells in pith region appear intact.	Fibers bulbous and lumpy Large fleshy sections separate. Skin resistance not noticeable.

Figure 4-B shows profiles of visual texture characteristics of the three strawberry varieties. Table 4-B lists the mean values for each attribute. The relative rankings on these descriptors corresponded well to oral texture ratings. Besides the size differences noted above, Selva fruit appeared firmer by retaining their original shape better than the other varieties. Their seeds remained prominent on the surface of the berry skin, in contrast to Benton and Totem berries whose achenes receded into pockets. Benton and Totem berries produced similar juice release and rated approximately four times greater than Selva.

Table 6 lists panelists descriptions of thawed berries from each variety. Benton strawberries were described as "sacs of juice, enclosed in a tough skin, and held together with stringy fibers." This juice was noted to be more transparent in Benton samples than in Totem or Selva fruit. Also, these berries retained no visible air spaces within the pith region. From histological studies of freezing treatments with strawberries, Armbruster (1967) reported these "stringy fibers" to be vascular microtubules that connect the pith to the achenes. In contrast, the flesh of thawed Selva fruit was considered "spongy, sections separated easily when bitten, and fibers were bulbous and pulpy." Panelists also noted that the juice of thawed Selva berries was less transparent than juices from Totem and Benton samples.

Fractionation of cell wall polysaccharides

Weight percentages of extracted cell wall fractions:

Table 7 lists the weight percentages of extracted cell wall polysaccharides from each strawberry variety. AIS,which consists almost entirely of acetone-insoluble polysaccharides (Wade, 1964), was 27-33% higher in both groups of Selva fruit than in the Benton and Totem solids. These levels in Selva are slightly higher than results obtained from the 1984 season showing AIS at 17 % of dry weight (Lin, 1986). Variability between replications was low when values were expressed as weight percentages of AIS. Replicate values of CSP, BSP, and RES showed large differences between extracts from different AIS preparations. This variability was highest in Benton samples. In comparison with results from 1984 season, AIS percentages in Benton and Totem samples were reported as 16 % and 12 %, respectively (Wesche-E. et al, 1985). In our investigation, weight percentages of AIS were lower in Benton samples and higher in Totems than those previously found.

Weight percentages of the cell wall fractions were significantly different between varieties due to low values for Selva-B extractions in every category, especially for CSP. This sample showed the greatest replication precision in weight percentages, possibly since these extractions were performed on a pooled AIS sample rather than separate AIS samples. Table 8 gives proportions of soluble and insoluble polysaccharides when values were normalized to 100% of AIS weight recovered. These values showed little varietal differences. Levels of water-soluble pectins were higher in Selva-B than in Selva-A.

CSP levels were lowest in Benton extracts and compared well with percentages found in this variety from the 1984 season (Lin, B.L., unpublished data). In all varieties, CSP proportions were approximately one and a half times greater than WSP levels. Results reported for 1984 season strawberries showed WSP higher than CSP by the same proportions. Major differences in the total pectic fractions (WSP+CSP) were evident only in the Benton variety for which a very low CSP level was obtained. Mean BSP levels were highest in Selva-A and lowest in Selva-B fruit. Benton samples had the highest levels of insoluble glucan residue, which was higher in Selva-B than in Selva-A.

Table 7. Fractionation of cell wall polysaccharides from freeze-dried strawberries.[a]

		(% D.W.)	Wt % of AIS			Recovery
	AIS	WSP	CSP	BSP	RES	(% AIS)
Benton	14.7	26.5	33.7	8.7	31.1	100.0
	14.5	20.1	14.3	12.6	39.2	86.2
mean	14.6	23.3	24.0	10.6	35.2	93.1
Totem	13.6	25.7	42.1	9.3	26.3	103.3
	14.0	19.3	43.6	9.8	23.3	95.9
mean	13.8	22.5	42.8	9.5	24.8	99.6
Selva-A	19.7	23.5	37.8	12.2	26.5	100.0
	16.2	22.5	44.2	13.4	22.3	103.0
	18.0	23.0	41.0	13.3	24.4	101.5
Selva-B	19.4	20.5	29.9	4.7	19.6	74.6
	19.0	20.4	29.6	7.2	27.6	84.9
mean	19.2	20.5	29.7	6.0	23.6	79.8

[a] Fractions are: AIS, acetone-insoluble solids,
WSP, water-soluble polysaccharides,
CSP, chelator-soluble polysaccharides,
BSP, base-soluble polysaccharides,
RES, insoluble residue.

Table 8. Fractionation of cell wall polysaccharides from freeze-dried strawberries.[a]

	AIS (% D.W.)	WSP	CSP	BSP	RES	(WSP+CSP)
		Normalized wt % of AIS				
Benton	14.7	26.5	33.7	8.7	31.1	(60.2)
	14.5	23.3	18.6	14.6	45.5	(39.9)
mean	14.6	25.0	25.8	11.4	37.8	(50.8)
Totem	13.6	24.9	40.7	9.0	25.4	(65.6)
	14.0	20.1	45.5	10.2	24.2	(65.6)
mean	13.8	22.6	43.0	9.5	24.9	(65.6)
Selva-A	19.7	23.5	37.8	12.2	26.5	(61.2)
	16.2	21.7	42.8	13.9	21.6	(64.5)
mean	18.0	22.6	40.3	13.1	24.0	(62.9)
Selva-B	19.4	27.4	40.0	6.3	26.3	(67.4)
	19.0	24.1	34.9	8.5	32.5	(59.0)
mean	19.2	25.6	37.3	7.5	29.6	(62.9)

[a] Fractions are: AIS, acetone-insoluble solids,
WSP, water-soluble polysaccharides,
CSP, chelator-soluble polysaccharides,
BSP, base-soluble polysaccharides,
RES, insoluble residue.

Uronic acid and neutral sugars in soluble polysaccharide fractions:

Table 9 gives the results of colorimetric analyses for total uronic acids and neutral sugars. Polyuronides in the soluble pectic fractions constituted 32-68 % of the water-soluble pectins, 40-68 % of the chelator-soluble pectins and 2.5-9.0 % of the hemicellulosic fractions (Table 7 and 8). Figure 5-A shows the ratios of uronic to neutral sugars in the WSP and CSP fractions. In WSP fractions, this ratio tended to increase with increasing varietal firmness (Figure 2-A). The mean ratio for Selva-A WSP was twice that for Benton WSP, and values from Totem and Selva-B fractions fell between the extremes. This trend was reversed in CSP fractions, as uronic to neutral sugar ratios decreased from Benton to Selva-A samples.

Figure 5-B illustrates the relationships between galacturonic acid and rhamnose which exhibited a similar, but stronger, trend of increasing with firmness in WSP and decreasing in CSP extracts. The anhydrouronic acid/rhamnose ratio in Selva-A is twice that of Benton in WSP and half the value in CSP. The ratio between total neutral sugars and rhamnose showed this same trend with high precision between replications (Figure 5-C). This value was again two times higher in WSP fractions from Selva fruit than from Totems or Bentons.

Table 9. Proportions of uronic acids (U.A.) and neutral sugars (N.S.) in polysaccharide extracts from strawberry cell walls. (ug/mg dry fraction)

Sample	Uronic Acids	Neutral Sugars	U.A./N.S	U.A./rh	N.S./rh
Benton:					
WSP-1[a]	528	516	1.023	7.13	6.97
WSP-2[b]	325	478	0.680	4.03	5.93
mean	427	497	0.652	5.58	6.45
CSP[c]	557	268	2.076	14.10	6.78
BSP[d]	57	690	0.083		
Totem:					
WSP-1	643	631	1.019	7.10	6.96
WSP-2	680	651	1.045	7.85	7.51
mean	662	641	1.032	7.47	7.23
CSP-1	669	383	1.747	9.63	5.62
CSP-2	602	612	0.094	5.53	5.62
mean	636	498	1.365	7.66	5.62
BSP-1	35	370	0.095		
BSP-2	30	479	0.063		
[e]Selva-B:					
WSP-1	512	749	0.684	8.36	12.25
WSP-2	494	789	0.642	14.37	22.45
mean	503	759	0.663	11.37	17.35
CSP-1	410	505	0.612	6.29	7.75
CSP-2	400	455	0.879	7.10	8.07
mean	405	480	0.846	6.69	7.90
BSP-1	89	926	0.096		
BSP-2	59	600	0.098		
mean	74	763	0.097		
Selva-A:					
WSP-1	651	530	1.228	14.64	12.08
WSP-2	522	264	1.977	23.83	12.05
mean	587	397	1.603	19.34	12.07
CSP-1	675	693	0.974	7.47	7.67
CSP-2	407	373	1.091	4.17	3.82
mean	541	533	1.033	5.82	5.75
BSP-1	34	280	0.121		
BSP-2	25	294	0.085		
mean	30	287	0.103		

[a]Water-soluble polysaccharides, 1st replication.
[b] " " " , 2nd replication.
[c]Chelator-soluble polysaccharides.
[d]Base-soluble polysaccharides.
[e]Selva-B fractiona were extracted from pooled AIS; other varieties were extracted form separate AIS.

Figure 5. Ratios of uronic acids, neutral sugars, and rhamnose in polysaccharide fractions of strawberry cell walls: Benton, Totem, Selva-B, Selva-A.

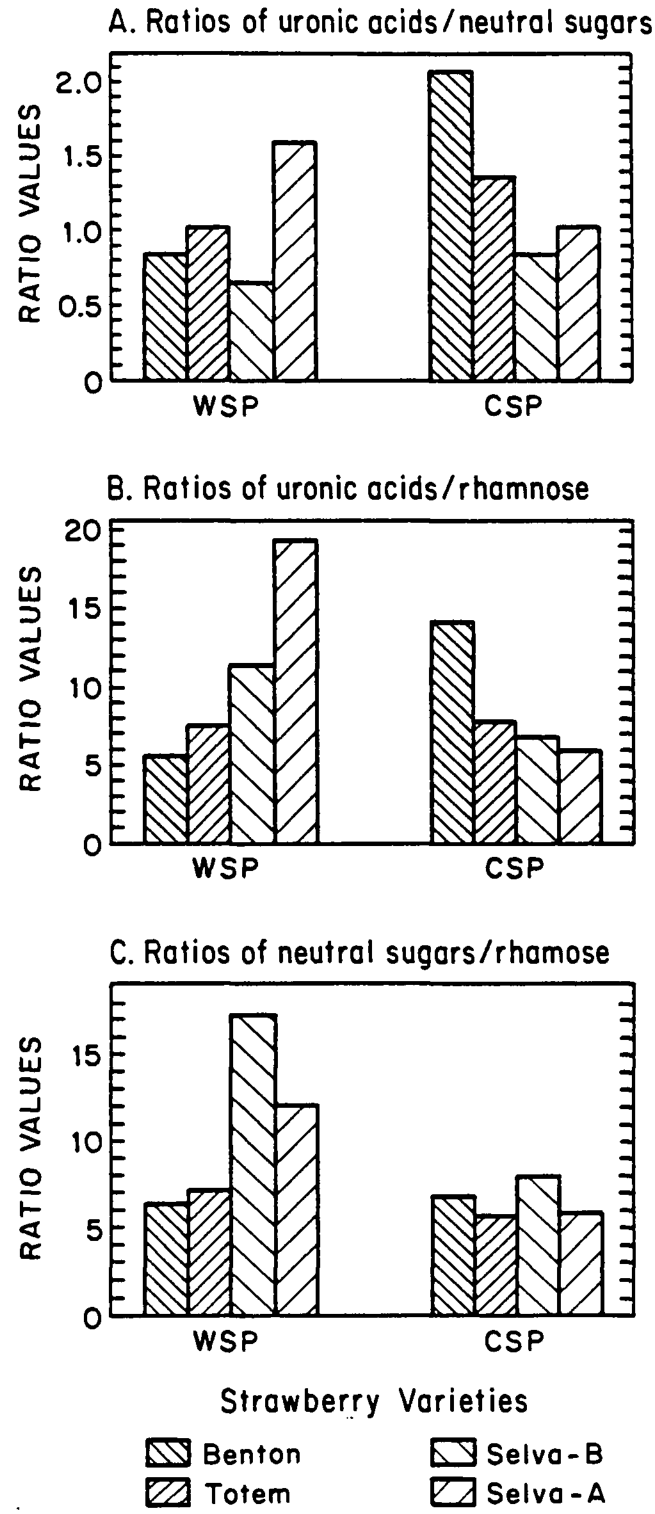

A. Ratios of uronic acids/neutral sugars
RATIO VALUES
2.0
1.5
1.0
0.5
0
WSP
CSP
B. Ratios of uronic acids/rhamnose
RATIO VALUES
20
15
10
5
0
WSP
CSP
C. Ratios of neutral sugars/rhamose
RATIO VALUES
15
10
5
0
WSP
CSP
Strawberry Varieties
Benton
Selva-B
Totem
Selva-A

Figure 5

Composition of neutral sugars in cell wall extracts:

Table 10 gives the molar percentages of neutral sugars present in cell wall fractions. Glucose, predominantly from cellulose, comprise 34-42 % of the AIS cell wall polysaccharides. Totem had the lowest glucose levels in this fraction. Rhamnose, arabinose and galactose are predominant in the WSP and CSP fractions. Xylose and glucose are present in smaller, almost equivalent proportions in WSP, but little xylose and no mannose were found in CSP extracts.

Although other neutral sugar proportions in the AIS and pectic extracts did not significantly relate to textural differences, ratios of galactose to arabinose in WSP were inversely related to fruit firmness. The molar ratios in Benton samples were twice as large as those in Selva samples. In CSP, however, the trend was reversed with Selva-A having the highest ratio of galactose to arabinose. It was not possible to quantitate apiose, but it was detected in trace amounts only in Selva WSP and CSP extracts, eluting after xylose with a retention time of 5.4. Figure 6 is an example of a gas chromatogram of the sugar acetate derivatives and shows the trace apiose peak. An unidentified peak eluting just before mannose (retention time of 7.0) was a significant feature in chromatograms of WSP sugars from all four berry samples. In WSP extracts, it produced quantifiable peak areas approximately equal to those of galactose, but occurred in only trace amounts in CSP fractions. Barbier and Thibault (1982) reported finding 2-desoxyglucose, a rare sugar, in pectic fractions isolated from cherry fruits. It eluted in the same position relative to mannose as this unknown peak, but was present in much smaller proportions than the peak area this analysis suggests.

Table 10. Neutral sugar composition of strawberry cell wall extracts. (normalized to molar % basis)

Sample	Rham	Fuc	Arab	Xyl	Man	Gal	Glu
Benton:							
[a]AIS-1	5.4	2.0	10.3	14.6	7.6	21.6	36.5
AIS-2	4.2	0.3	10.6	15.5	6.4	20.8	42.2
mean	4.6	1.1	10.5	15.1	7.0	21.2	40.4
[b]WSP-1	11.2	5.6	16.9	10.6	0.2	36.1	17.2
WSP-2	13.1	3.8	20.4	10.5	0.9	37.3	14.0
mean	12.1	4.7	16.7	10.6	0.6	37.7	15.6
[c]CSP-1	11.1	4.0	26.1	4.1	0.0	39.2	13.5
CSP-2	10.7	3.6	26.0	2.6	0.0	45.9	9.1
mean	10.9	3.6	26.0	3.5	0.0	42.5	11.3
Totem:							
AIS-1	4.2	2.6	14.0	17.4	7.6	16.6	35.6
AIS-2	10.6	0.0	10.6	19.3	6.4	20.0	32.9
mean	7.5	1.3	12.3	16.3	7.0	19.3	34.2
WSP-1	11.0	6.6	22.9	14.4	0.2	22.1	20.6
WSP-2	10.5	7.6	21.6	15.9	1.7	24.2	16.5
mean	10.6	6.2	22.3	15.2	0.9	23.1	19.6
[e]CSP-1	0.0	0.0	0.0	0.0	0.0	66.2	33.6
CSP-2	13.6	11.5	25.0	4.1	0.0	28.5	17.1
mean	12.7	10.6	23.0	3.6	0.0	32.2	17.6
Selva-A:							
AIS-1	9.6	0.0	13.6	10.6	4.1	17.8	44.4
AIS-2	2.6	4.0	15.6	13.2	5.3	20.6	36.2
mean	6.2	2.0	14.6	11.9	4.7	19.3	41.3
WSP-1	23.1	0.0	24.2	11.0	0.6	26.4	14.7
WSP-2	6.3	5.5	37.9	9.7	0.7	27.3	12.6
mean	14.6	2.6	31.1	10.3	0.6	26.9	13.6
CSP-1	10.2	3.4	26.4	2.0	0.6	51.5	3.9
CSP-2	15.3	5.1	19.5	2.5	0.0	45.6	12.0
mean	12.6	4.3	23.9	2.2	0.3	46.5	6.0
[d]Selva-B:							
AIS-1	5.7	1.9	11.5	12.0	5.6	17.9	45.1
AIS-2	7.0	1.7	13.1	13.4	5.9	20.0	39.1
mean	6.4	1.6	12.3	12.7	5.6	16.9	42.1
WSP-1	6.2	5.5	30.9	13.1	1.2	30.1	13.0
WSP-2	3.4	10.6	31.7	13.8	1.0	25.6	13.7
mean	4.6	6.1	31.3	13.5	1.1	26.0	13.4
CSP-1	10.1	4.5	26.6	1.9	0.0	46.0	6.6
CSP-2	9.7	7.3	27.2	0.3	0.0	45.9	9.6
mean	9.9	5.9	27.9	1.1	0.0	47.0	6.2

[a]Acetone-insoluble polysaccharides. 1st replication.
[b]Water-soluble polysaccharides. 1st replication.
[c]Chelator-soluble polysaccharides.
[d]Selva-B fractions were extracted from pooled AIS; other varieties were extracted form separate AIS.
[e]Poor hydrolysis ocurred in this sample.

Figure 6. Profile of alditol acetates in Selva-A WSP fraction by gas chromatography. Saples were analyzed on a J & W Macrobore capillary comumn, DB 225, id 0.53 mm, 15 m long with helium carrier gas at 165 cm/sec; FID detection. Myo-inositol is present as an internal standard.

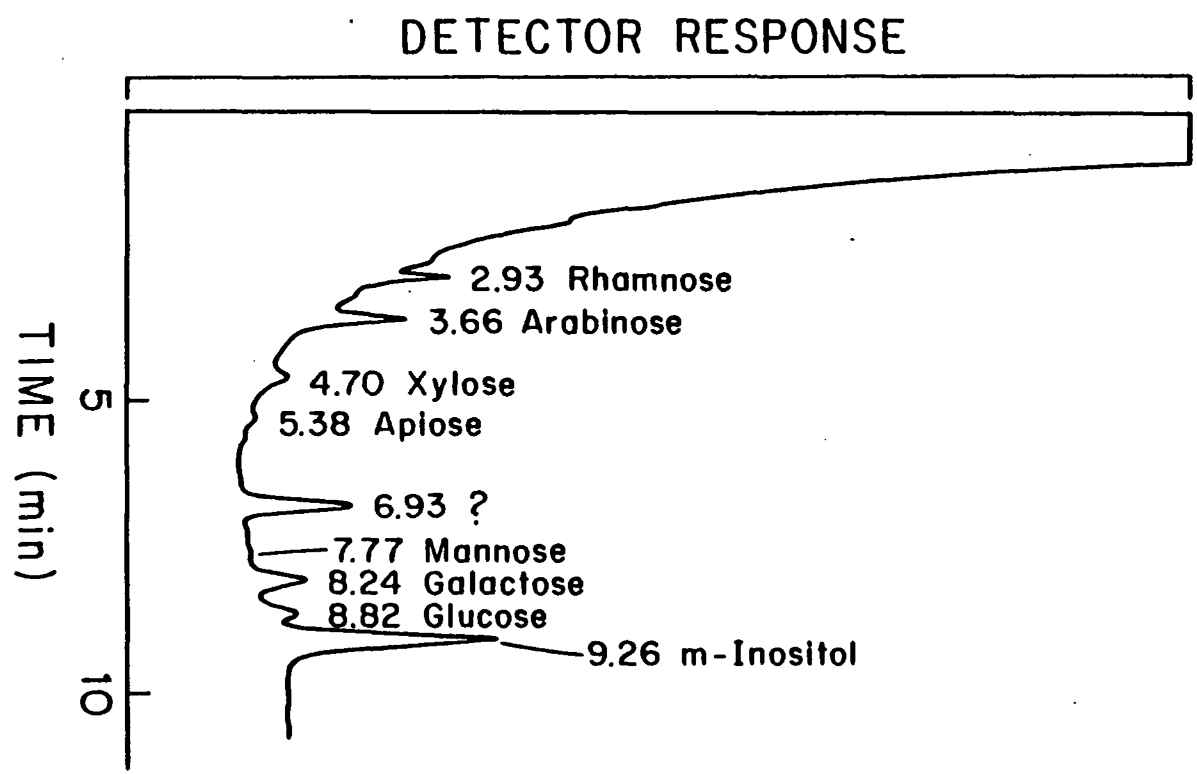

DETECTOR RESPONSE
TIME (min)
5
10
2.93 Rhamnose
3.66 Arabinose
4.70 Xylose
5.38 Apiose
6.93 ?
7.77 Mannose
8.24 Galactose
8.82 Glucose
9.26 m-Inositol

Figure 6

Molecular weight distributions of cell wall polysaccharides:

Figure 7 shows the molecular weight distributions of substances in free run juice of thawed strawberries. These profiles are similar for all samples, except for the broad distribution of high molecular polymers in Selva-A samples. Figure 8 shows the molecular weight distributions of polymers within the water-soluble cell wall extracts. These profiles were similar to those of the free run juices for samples from Totem and Selva variety fruit. Both the juices and the WSP from these varieties showed a high-molecular weight peak in the excluded volume and a large peak at the total elution volume. Retention times for this high M_w peak were slightly higher in both Selva samples, and Selva-A had a broader distribution of these polymers which almost resolved into two peaks. In free run juice, another peak of polymeric material showing an average molecular weight of approximately 6000 D was present in substantial proportions in all samples except Selva-A. This peak was not present in chromatograms of WSP from any sample but did appear in all CSP fractions.

Profiles of Benton WSP extracts showed the most significant differences between the other three samples. Benton WSP fractions did not have the high molecular weight peak maxima present in free-run juice from thawed Benton strawberries. That this high molecular weight peak was present in juice from thawed berries and absent in the water-soluble extract indicates that high-molecular weight species or aggregates were still intact after the rapid freezing process, and eluted from the cell wall into the juice. This suggests that the polymers in Benton WSP were more easily broken down or broken apart in the extraction process than were polymers from the other two varieties.

Totem WSP extracts #1, 2, 3, however, showed maxima at retention times (R.T.) of 5.18, 5.30, 5.42, respectively, which corresponded to average molecular weights of approximately 700,000 - 1,000,000 D. Profiles of Totem and Selva WSP extracts were very similar, except for the early eluting peak in Selva-A extracts whose mean R.T. was used as the V_o in calculating K values. This Selva-A sample indicated the presence of higher molecular-weight polymeric material than appeared in Selva-B or Totem extracts. In CSP extracts (Figure 9), the proportionate amounts of large polymeric substances increased with firmness scores. Sharp Selva-A peaks demonstrated the largest quantity of high molecular weight compounds and a small peak of approximately 6000 D. The main peak in Selva-B eluted slightly ahead of those in Selva-A and had more small polymers. Totems had a lower, slightly broad distribution of large polymers, and Benton CSP is even broader in the high molecular weight region, from approximately 100,000 to 500,000 D with no clear peak maximum.

Huber (1984) also noted that using gels with different exclusion limits showed little differences in profiles of soluble polyuronides from strawberries, since the same peak of polymers always eluted in the void volume. The elution behavior of Selva polyuronides suggests that these polymers may have a greater affinity for aggregation rather than a higher molecular weight. This aggregation behavior may also be resposible for the difficulties in rehydrating the freeze-dried water-soluble extracts for analysis. Szczesniak and Smith (1969) and Kao (1967) suggested that complete removal of water bound by cell wall polysaccharides causes structural, or conformational changes that reduce the polymers' water binding capacity.

Figure 7. HPLC-size exclusion chromatography of juice from thawed IQF strawberries. Samples were diluted to 25 % of full strength. Profiles represent soluble polymeric substances from: A) Benton, B) Totem, C) Selva-A, D) Selva-B strawberry samples.

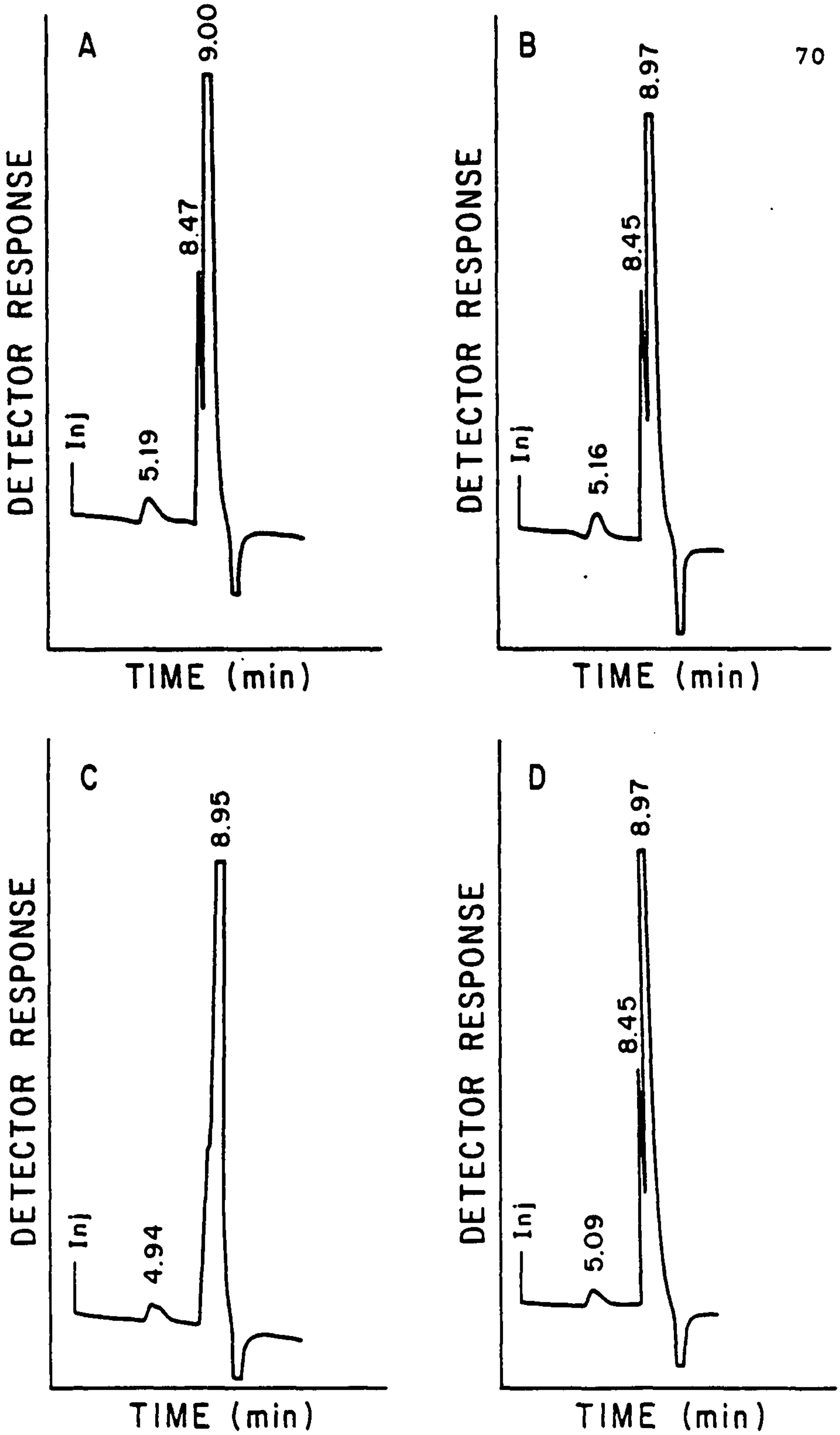

A
9.00
8.47
5.19
Inj
B
8.97
8.45
5.16
Inj
C
8.95
4.94
Inj
D
8.97
8.45
5.09
Inj
DETECTOR RESPONSE
TIME (min)

Figure 7

Figure 8. HPLC-size exclusion chromatography of water-soluble polysaccharides from strawberry cell walls. Profiles represent soluble polymeric substances from: A) Benton, B) Totem, C) Selva-A, D) Selva-B strawberry samples.

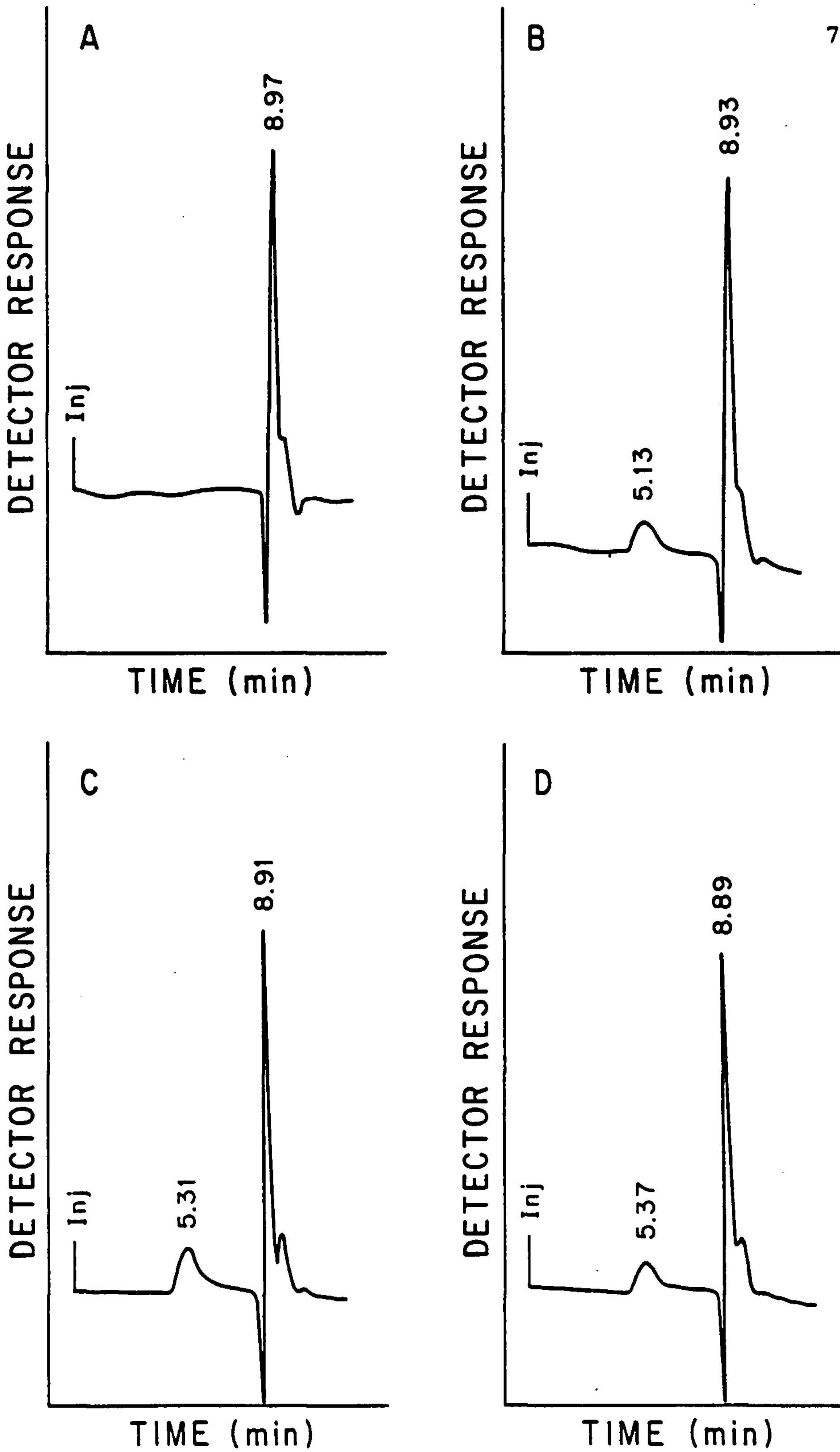

A
8.97
Inj
DETECTOR RESPONSE
TIME (min)
B
8.93
5.13
Inj
DETECTOR RESPONSE
TIME (min)
C
8.91
5.31
Inj
DETECTOR RESPONSE
TIME (min)
D
8.89
5.37
Inj
DETECTOR RESPONSE
TIME (min)

Figure 8

Figure 9. HPLC-size exclusion chromatography of chelator-soluble polysaccharides from strawberry cell walls. Profiles represent soluble polymeric substances from: A) Benton, B) Totem, C) Selva-A, D) Selva-B.

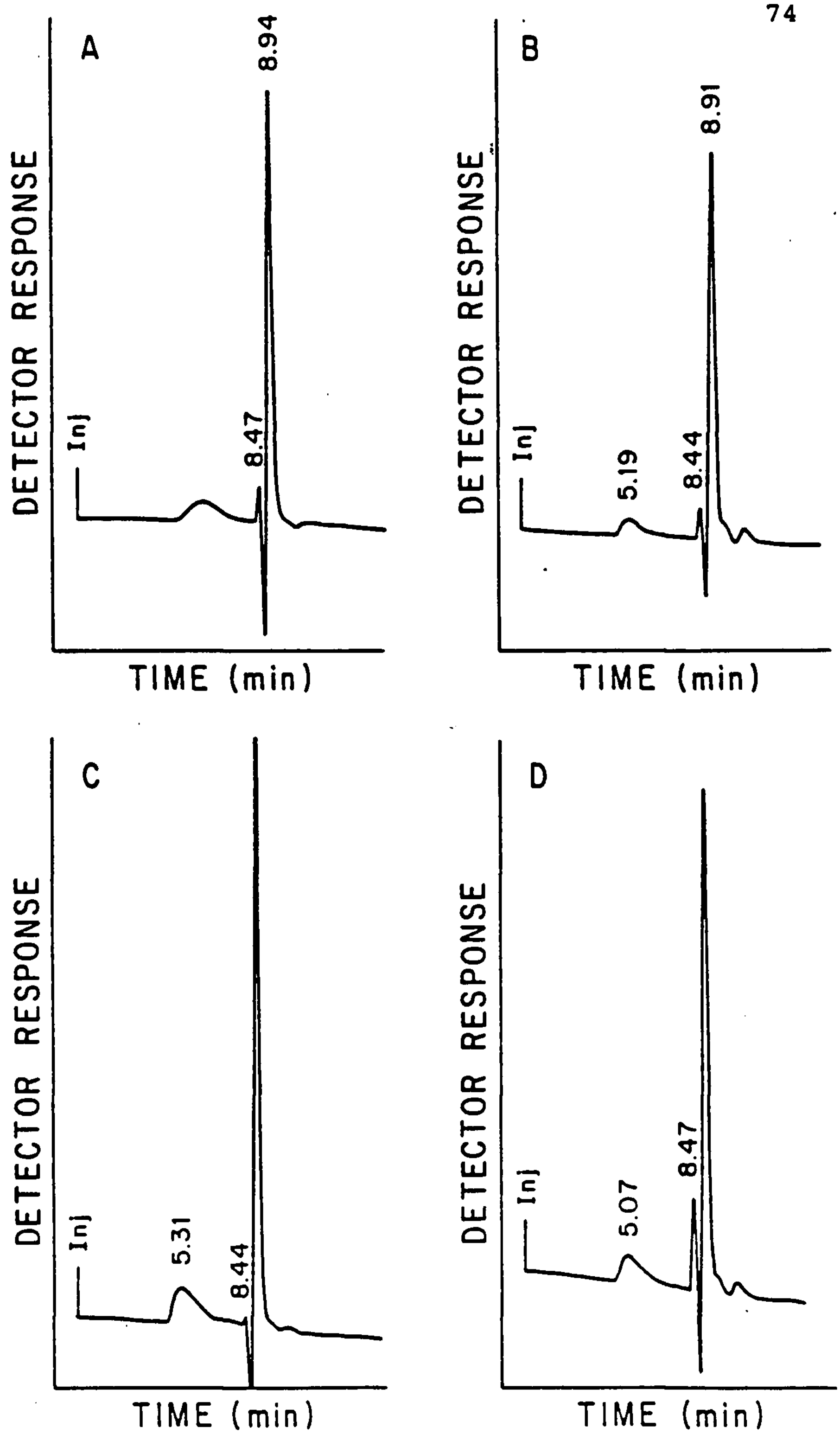

A
8.94
8.47
Inj
DETECTOR RESPONSE
TIME (min)
B
8.91
8.44
5.19
Inj
DETECTOR RESPONSE
TIME (min)
C
5.31
8.44
Inj
DETECTOR RESPONSE
TIME (min)
D
8.47
5.07
Inj
DETECTOR RESPONSE
TIME (min)

Figure 9

For calibrating the size exclusion chromatography, dextran standards demonstrated a similar linear relationship between log M_w and the partition coeficient, K_{av}, as reported by Fishman, et al (1984). The partition coefficient K^{av} was calculated from:

$$K = \frac{V_e - V_o}{V_t - V_o}.$$

Here, the void volume V_o was determined from the maximum of the first pectin peak, Selva-A WSP, detected in a sample extract. This peak eluted 20 seconds before the highest M_w dextran of 2,000,000 D. For estimating molecular weights of pectic substances, partition coefficients for each chromatographic peak were inserted into the regression equation,

$$\log M_w = b + m K_{av},$$

determined from the dextrans over the range of 9000-2,000,000 D (Fishman, et al, 1984).

DISCUSSION

The strawberry varieties were chosen for this study to represent the range of textural properties encountered in commercial strawberry cultivars. Bentons, a soft Pacific Northwest variety, produces high yields but its high berry malformation rate and poor texture causes processing quality problems (Sjulin, 1985). Totems are a firmer fruit, with good potential for fresh and processing markets and resistance to viral infection (Lawrence, 1985). They have good keeping quality and retain their fruit identity in jams and other processes. Selvas are a very firm, day-neutral, California variety under development for production in the Northwest. Strawberry breeders, growers, and food processors are interested in reasons for these differences in textural properties and objective testing methods to speed the selection process for cultivars with the best fruit character and plant heartiness.

Physical properties

The apparent viscosity of strawberry purees from frozen-thawed Benton, Totem, and Selva berries corresponds well to both objective and sensory firmness ratings. The greater effect of temperature on viscosity in Benton strawberry purees possibly reflects the break down of internal flesh macrostructures noted in sensory texturedescriptions. As a result, the average size of particles distributed in the suspension is smaller and the activation energy for viscous flow increased. Totem berry purees were intermediate in viscosity and showed intermediate ^E values; they equaled Selva-B samples in viscosity values. Selva-B purees were less affected by temperature than the Selva-A samples. Different relative contributions from serum versus that from suspended particles may account for Selva-B's similarity to Totem. Apparent viscosity of Selva-A puree greatly exceeded that of all other samples and reflected this fruit's extreme resistance to cortex cell separation and its sensory firmness scores.

Saravacos (1970) compared flow properties of heated juices and purees. He noted that the concentration, size, and shape of suspended particles primarily determines viscosity in the purees and pulps. As temperature only slightly affects the flow properties of suspended solids, it has a small over-all effect on puree viscosity, unless sugar is added or the puree is concentrated. Duran and Costell (1982) attributed the initial flow resistance of a fruit puree to particle size and interaction between particles. Sistrunk and Moore (1967) also directly correlated the viscosity of serum from frozen strawberries with shear press measurements.

Although detailed structural information can not be determined from this proceedure, a strong relationship between the viscosity of pureed strawberries and varietal differences in firmness may find application as a quality control or screening test for cultivar breeding.

<u>Sensory texture profile</u>

Texture profiles developed for the three strawberry samples are quantitative descriptions of texture perceptions imparted by the integrated physical andchemical properties of the fruit. Totem berries were more intense in most textural characteristics than Bentons, and the extent of difference between these varieties was fairly constant. In contrast, Selva fruit was extremely higher in firmness, fibrousness, and chewiness and extremly low in juiciness properties. The firmness ratios of 1:2:4 for Benton, Totem, and Selva, respectively, follow the same strong trend with the mechanical measure of work of compression, the drip-loss volume, and viscosity measurements made on purees from this sample.

Other assessments of frozen strawberry texture in the literature report this inverse relationship of firmness to juice release (Szczesniak and Smith, 1969; Sistrunk and Moore, 1967; Rao, 1966; Sistrunk et al, 1960). This trend was also noted in preliminary textural measurements made on fresh berries of these varieties. The freezing process produces disoriented cell organization, plasmolysis and folded cell walls with some rupture in cortical cells (Szczesniak and Smith, 1969). Freezing decreases the water-holding capacity of parenchyma cell walls and xylem fibers. Varietal differences in the structure of these fibers could determine the retention of cellular and intercellular water on thawing.

Panelists reported that the sensation of juice release was difficult to quantitate in Benton berries, which may account for the relative ranking of mean juiciness ratings among the varieties. Benton fruit had lost much of its free-run juice during thawing and the berry flesh seemed to disintegrate, or dissolve, in the mouth, whereas cortex cells in Totem and Selva fruit had retained more moisture that could be released suddenly when compressed and bitten. In addition, berry skins in all three varieties gave a "slimy," moist sensation that may have interferred with perceptions of a sudden juice release. The skin of Selva berries had the most noticable "sliminess." Askingpanelists to distinguish between juice release and total mouth moisture seemed to aid them in concentrating on the juice directly coming from the berries and minimized confusion from salivation.

Sensory texture analysis of fruits is influenced by the variability within samples as much as by differences between treatment groups and panelists. Interactions observed between panelists and strawberry variety (Table 4) occur when some panelists score the varieties in an opposite direction from the rest of the panelists (McDaniel, 1981). These interactions could be due to panelist confusion of the attribute scored or intrasample variability. Since each attribute was judged on different berries, panelists received non-uniform samples and ranking differences by different panelists was likely to occur. However, highly significant quantitative differences were obtained for four out of five oral texture characteristics of Benton, Totem, and Selva strawberries.

This inherent variability within sampling lots of strawberries was minimized where possible by carefully sorting the pooled samples to present berries of the same maturity, relative size and color. The intensely dark red color in Totem fruit and the size of Selva berries were possible influences on textural perceptions. Selva strawberries are approximately 2 - 3 times larger than the other samples, as the visual profile shows (Figure 2-B). Some judges continued to use larger ranges when assigning magnitude estimates than others did, which increased data variability even in the normalized values.

The statistical independence of texture quality descriptors was examined through correlation analysis. Table 9 shows that the correlations among descriptors were fairly low, but highly significant, ($P<0.01$), between firmness, fibrousness, and chewiness scores (Table 11). Measurements on these terms represent mechanical parameters

Table 11. Correlation-variance matrix of sensory profile descriptors for frozen strawberries.

	Firm	Fiber	Juice Release	Total Moisture	Chewy
Firm	215	0.52**	-0.06	0.01	0.35**
Fiber		615	0.02	-0.02	0.26**
Juice Release			347	0.64**	0.07
Total Moisture				404	-0.13
Chewy					9.6

**Values are significant at the $P \leq 0.01$ level.

influenced by structural solids (Syarief, 1985). Firmness and fibrousness were the most strongly correlated characteristics. Chewiness was significantly related to both of these, even with a correlation coefficient of only 0.26 with fibrousness. Structural fibers having a strong influence on berry integrity may also be expected to require more mastication to break down. Juice release and total mouth moisture scores were completely independent of firmness, fibrousness and chewiness, but were highly correlated to each other. Chewiness and total mouth moisture also showed an insignificant negative correlation. The foods chosen as reference standards for oral texture descriptors appear to be appropriate, except for firmness. Means of judgements across all panelists and replications for fibrousness and juiciness characteristics were only slightly higher than the reference value of 50. For firmness, a softer reference than Velveeta cheese would have been more appropriate since the overall mean sample score was only 25. However, panelists performed consistently with this standard, and the firmness descriptor had the lowest standard error. The firmness of Philadelphia cream cheese (standard #1 in the Szczesniak firmness scale) (Szczesniak, 1963) was more similar to frozen strawberries than Velveeta cheese, but also more temperature sensitive and difficult to handle.

Some modifications in these procedures for profiling strawberry texture may increase the sensitivity of describing varietal differences. The independence of sample judgements could be increased by using one berry per coded cup and increasing the number of sample cups per tray. Introducing notation for recording a covariant, such as size, may account for more of the variability within samples. A more appropriate standard could be sought for comparing "firmness" in fibrous products, since cellular fruit products behave differently under compression andrupture forces than the Velveeta cheese does (Peleg, 1983). Increasing panelists' training to include more strawberry attributes would broaden the profile.

Magnitude estimation scaling was chosen to look at the potential for applying power funcitons to fit a relational function between sensory ratings and rheological properties. The generalized form of a power function is

$$S = kI^n$$

where S is the sensory response, k is a proportionality constant, and I is the objective measurement of the intensity of a physical characteristic (Kapsalis and Moskowitz, 1977). Lin and Rao (1981) recommended the use of power functions to better fit rheological and sensory ratings of canned peaches. They found significant positive correlations between AIS levels and sensory scores for hardness and elasticity, and negative correlations for graininess, defined equivalently to the "fibrousness" term used here. Scatter plots of the means of firmness scores vs. viscosity at 25°C, or vs. drip loss volume indicates that a power function may be appropriate to describe these relationships.

Chemical analysis

The chemical analysis of the strawberry cell walls support the suggestions of Huber (1984) and Wade (1965) that varietal differences in firmness result from differences in the structural detail and forces of polymer association rather than with large compositional differences. Levels of acetone-insoluble solids are consistant with relative textural differences in the fruit after freezing. Selva fruit has the largest quantity of AIS available on a dry weight basis to produce the structural matrix, and Bentons have the least.

Woodward (1972) found that strawberry cell wall alcohol-insoluble solids as a percentage of fresh weight decreased with fruit development and ripening, but increased on a per fruit basis. He reported that the cell wall expansion exceeded total polysaccharide synthesis. However, Knee and co-workers (1977) found that cell wall polysaccharide synthesis ceases in ripe strawberries. Wesche-E. et al (1986) found that AIS levels in Bentons decreased greatly from the under-ripe to ripe stages, then increased slightly in over-ripe berries. AIS in Totem fruit changed little until the over-ripe stage, when this fraction increased substantially. Lin and Rao (1981) also found strong correlations between alcohol-insoluble solids, sensory hardness ratings and elasticity constants from Instron measurements.

The agreement of these results with those from the 1984 season showing AIS at 17 % of dry weight (Lin, 1986), reinforces this measurement as a varietal trait. Seasonal variation was evident in AIS values of Benton and Totem strawberries, as values from the 1984 season fruit were 16% and 12%, respectively, for these varieties. Increased WSP values from Selva-A to Selva-B may reflect increased WSP with softening similar to that reported by Woodward

(1972) and Wesch-Ebling (1986). This is supported by lower shearpress values, higher drip loss, and lower puree viscosities in Selva-B than in Selva-A. Percentages of base-soluble polysaccharides and insoluble residue agreed with those reported by Wesche-E., et al (1986), with Totem residues being somewhat lower.

The presence of branches, typically composed of neutral sugars have been recognized to influence noncovalent interpolymeric associations of polyuronides (Jarvis, 1984). Water-soluble polymers in Selva-A fruit showed a greater amount of uronic acid units to neutral sugars than Bentons, Totems and even Selva-B berries did. Differencesn molar ratios of anhydrouronic acids per rhamnose in WSP extracts strongly supports the hypothesized role of rhamnose in the polyuronide backbone. If interpretation of compositional analysis follows the structural model of a primary cell wall proposed by Rees and Wight (1971), rhamnose introduces kinks in the polyuronide chains. These kinks increase steric hinderance between chains and decrease side by side linear associations of polymers in the cell wall matrix. Huber (1984) also noted a dramatic decrease in molar ratios of galacturonic acid to rhamnose with ripening in soluble polyuronides, to one-fifth of that in the green fruit. The molar ratio he reported for the ripe stage (20 AUA units per rhamnose) equals that found in the WSP of ripe Selva-A berries. This finding may indicate that water-soluble pectic chains from Selva berries may have longer straight, "smooth" homogalacturonan regions and less frequent neutral sugar branches.

The distribution of these side chains may prove very important. Voragen et al.(1985) found that side chains are present in "hairy blocks" in apple pectins, rather than evenly spaced. Rombouts (1985) also reported the presence of the "hairy" regions of neutral sugar side chains attached to the rhamnogalacturonan backbone of sugar beet pectins. However, the possible differences in branching or kinking in the polyuronides caused no differences in the normalized proportions of WSP extracted from AIS in Benton, Totem, and Selva strawberries in this study.

The WSP of Selva-A also had the most neutral sugars per rhamnose. Structural analysis of rhamnogalacturonan I isolated from suspension-cultured sycamore cells revealed the presence of up to 30 different glycosidic side chains attached to O-4 of the 2,4-linked L rhamnosyl residues (McNeil et al, 1982). The neutral sugars in the Selva-A could indicate that side chains linked to rhamnose are longest in this variety or, more likely, that these neutralsugars compose polymeric chains unattached to the polygalacturonides (Wesche-E. et al, 1986; Huber, 1984).

Relationships between galactose and arabinose also changed with varietal firmness. In Benton WSP, galactose is the major neutral sugar. The galactose:arabinose ratio decreases in Totem and in Selva arabinose predominates. The primary cell walls of many dicots contain various polymers that appear to be pure -4-linked galactans, pure 5-linked α-L-arabinofuranosyl residues and arabinogalactans of two bonding types (McNeil et al, 1984). However, it is not known how these polymers are linked within the primary cell wall. In strawberries, the firmer fruit appear to release arabinose-containing polymers more easily than do the softer ones. Aggregated homogeneous araban polymers have also been responsible for haze formation and preciptaion in apple and pear juices and wines (Babsky, 1985).

Molecular-weight distributions in this fraction indicate that a greater proportion of WSP in Selva fruit is composed of high molecular weight individual polymers, or pectins having a greater tendency to aggregate than WSP in the Benton variety. As O'Beirne and van Buren (1983) pointed out, fruit polyuronides have a tendency to form interpolymeric aggregates which influences their molecular size characteristics as revealed in gel permeation chromatography. The elution behavior of Selva polyuronides suggests that these polymers have the greatest affinity for aggregation due to interchain hydrogen bonding or to hydrophobic interactions (Oakenfull and Scott, 1984).

For pectins dissolved in distilled, deionized water, hydrogen bonding between carboxyl carbonyls and OH's of galacturonic acid is thought responsible for aggregation in polygalacturonic acid. Fishman et al (1984) reported extremely large radii of gyration for pectin and polygalacturonic acid (PG) in water which was attributed toa combination of elongated helices at low ionic strength and partial overlap of chains in side-by -side association. Neutral sugars in pectin can aggregate in water and buffered solutions, but uronic polymers only aggregate in water or solutions of lower pH. Hydrophobic interactions between methoxyl and acetyl ester groups could also contribute significantly to aggregation. In high-methoxyl commercial pectins, this contribution to the free energy of gelation is one half that from hydrogen bonding (Oakenfull and Scott, 1984). Thus, aggregation behavior of Benton WSP in solution may be lowered by kinking in the rhamnogalacturonide chain or by the distribution of neutral sugar side chains.

The chelator-soluble polysaccahrides (CSP) represents pectins bound into the primary cell wall by ionic associations with the divalent cation calcium. Approximately two-thirds of the soluble polyuronides in all samples were released into this extract. Significant differences in the ratios of uronic acids to neutral sugars are evident between varieties and appear related to strawberry firmness. Based on the Rees model of structural polysaccharides, in Benton berries the ionically bound polymers have acidic backbones with few rhamnose insertions to cause kinking.

CSP from all samples have approximately the same numbers of neutral sugars per rhamnose, an average of 5.6 to 8 sugar units per side chain if all sugars were linked to the pectin chains through rhamnose. However, not all neutral sugars in the CSP class are covalently bound to the rhamnogalacturonic pectin chains. Neutral polymers including arabinans, galactans, and arabinogalactans are also present which may be extricated from the cell wall matrix during the solubilization of ionically bound rhamnogalacturonates. Wesche-E. et al.(1985) found increased amounts of neutral sugars non-covalently bound touronic polysaccharides with ripening. The softer Bentons showed higher amounts of neutral sugars eluting in the void volume and in fractions just before the main uronic acid peak than the Totem profile had. Ion exchange chromatography of Selva-CSP (Lin, 1986) showed that most of the neutral sugars were eluted from the column with the main pectin peak. This peak required higher ionic strength for elution than CSP pectic polymers from ripe Benton and Totem had, indicating the polymers were larger and/or more highly charged.

Selva WSP and CSP extracts were the only samples to show trace amounts of apiose. This branched five-carbon sugar has been found as a disaccharide attatched at O-3 or O-2 to β-4 linked galactosyl uronic acid residues in duckweed (Hart and Kindel, 1970). Apiose is believed to be widely distributed in plant cell walls, usually in low concentrations (Robinson, 1983). Its distribution is poorly documented because in many analytical systems it elutes with rhamnose (Bell, 1962). This sugar readily decomposes under the harsh conditions used to hydrolyze the pectic fractions, which makes its quantitation difficult.

The molecular-weight distributions again showed more high-molecular weight polymers in the firmest berries. Selva-A samples had one peak eluting in the void volume, but Benton CSP had a wide distribution of substances, average molecular weights 70,000 to 500,000, with no peak maximum recorded. The peak of approximately 6000 Daltons present in all CSP samples may be the result of depolymerization reactions in the extraction process (Albersheim et al, 1960). If so, Selva-A was most resistance to breakdown. This work supports Huber's hypothesis (1984) that softening in strawberries results from decreasing associations between structural polysaccharides, by weakened ionic complexes, hydrogen bonding, or hydrophobic interactions, rather than by depolymerization of the polygalacturonide or rhamnogalacturonide backbone.

CONCLUSIONS

The significant findings of this study characterizing instrumental and sensory textural differences between Benton, Totem, and Selva strawberries in relation to cell wall characteristics may be summarized as follows:

1. The firmness rankings for strawberry varieties were Selva-A, Selva-B, Totem, and Benton in descending order from shear press measurements. Drip loss was inversely related to work of compression.

2. Selva strawberries have larger quantities of cell wall polysaccharides composing the dry weight of the fruit, as evidenced by the higher AIS values obtained. However, no major differences in the proportions of extracted pectic fractions from cell walls were detected.

3. Ratios of uronic acid to rhamnose and neutral sugars to rhamnose increased with firmness ranking in water-soluble polysaccharides and decreased in chelator-soluble polysaccharides. Water-soluble rhamnouronic polymers released from the firmest Selva berries appear to have longer uronic acid residues between rhamnose insertions, thus more linearity, than the other varieties. Chelator-soluble pectins in Selvas appear to have more neutral sugar polymers associated with them, covalently or non-covalently than do Totems and Bentons.

4. In free-run juice from thawed strawberries and in water-soluble pectins, the proportion of high molecular

weight polymers was higher in firmer fruits. Strawberry fruit firmness is related to the molecular size of the pectic polysaccharides or the greater tendency of the polymers to aggregate in solution.

5. Puree viscosity of thawed strawberries, in the temperature range of 10-60 C, was directly related to work of compression and sensory scores of textural integrity. This flow property of frozen-thawed strawberries shows potential as screening test for cultivar breeding programs and production quality control.

6. Techniques for profiling the texture of frozen strawberries were developed using magnitude estimation scaling. Descriptive and quantitative sensory evaluations of berry samples were directly related to work of compression by shear press and drip-loss volumes. The relative sensory firmness of Benton, Totem, and Selva-A strawberries was in the ratio of 1:2:4. Selva-A fruit was significantly higher in fibrousness and chewiness and lowest in juciness properties.

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APPENDIX

FROZEN STRAWBERRIES -- TEXTURE DEFINITIONS

-- All tests will be performed on halves of medium-sized berries, sliced from the stem side to the apex. If the half is larger than 2 cm. across the widest part, slice off the extra flesh.

-- All References = 50. Ratings are ratio estimates of the amount of the charateristic in the reference.

TEXTURE BY MOUTH

FIRMNESS: Reference -- Velveeta Cheese.
The amount of force needed to bite through the berry half, flesh side up, with your molars. Always bite at the same rate and on the same side of your mouth.

JUICINESS: Reference -- canned My-T-Fine mushrooms.
Juice Release -- the amount of juice released by a berry half after 3 chews.
Total Mouth Moisture -- the total amount of liquid in your mouth after 3 chews of a berry half.

FIBROUSNESS: Reference -- Del Monte 'Lite' sliced canned peaches, 2 cm. slice
The amount of fibers you feel up to the third chew of the sample.

CHEWINESS: no reference.
The number of chews required to prepare the sample for swallowing.
*For FRESH sample, cut the the berry half to a 2 cm. cube as before.

VISUAL CHARACTERISTICS

SIZE: How does the average berry size compare with the reference?

SHAPE MAINTAINED: How close is the sample shape to the expected shape of the fresh berry?

FIRMNESS: How firm do the berries appear to be?

TOTAL JUICE: How much free run juice is in the bowl compared to the reference?

SEEDS: Are the seeds more sunken (< 50) or more prominant (> 50) from the skin than the reference?

NAME: ____________________
DATE: ____________________

STRAWBERRY TEXTURE EVALUATION

For each characteristic listed, the Reference = 50. Rate each sample as a ratio estimate of the Reference.

Use 1/2 of each berry in the cups for testing, and cut the berry halves to 2 cm. widths, if they are too large.

FIRMNESS (cut side up)

cheese	50	50

FIBROUSNESS (after 3 chews)

			fiber description
peaches	50	50	

JUICINESS (after 3 chews)

	Juice Release		Total Mouth Moisture	
mushroom	50	50		

NAME: ____________________
DATE: ____________________

STRAWBERRY TEXTURE EVALUATION

CHEWINESS (# of chews)

sample

______	______	______
______	______	______
______	______	______
______	______	______

TAKE THIS SHEET INSIDE TO EVLAUATE VISUAL CHARACTERISTICS, please.

VISUAL CHARACTERISTICS

You may tip the bowls, but please don't touch the samples.

Sample #

	# ______	# ______	# ______
Size	50	______	______
Shape maintained	50	______	______
Seeds	50	______	______
Firmness	50	______	______
Total Juice	50	______	______

Comments ?

Thank you !