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The effect of frozen storage time on the composition of the cell wall polysaccharide (CWP) of muskmelon (Cucumis melo) cultivars at different stages of maturity was investigated. Changes in composition, firmness, drip loss, and color of Cantaloupe and Honey Dew melon flesh were determined at three stages of maturity and for three periods of storage at -23°C. Relationships between firmness, drip loss, and other composition measurements, as well as the total CWP sugar composition, were also determined.

Cell wall polyssacharides were isolated and purified, and fractionations were performed using cyclohexane trans-1,2-diamine tetraacetate (CDTA), Na_2CO_3, guanidinium thiocyanate (GTC), and KOH. All fractions and residues were dialysed and then freeze-dried. Following hydrolysis of CWP fractions with trifluoroacetate (TFA), the alditol

acetate derivatives of neutral sugars from each CWP fraction were prepared and analyzed by gas chromatography, using myo-inositol as the internal standard.

TFA insoluble fractions were analyzed colorimetrically using phenol-sulphuric acid reagent. Uronic acid was determined using 0.15% m-hydroxybiphenyl for absorbance at 520 nm with galacturonic acid as the standard. It was determined that CDTA and Na_2CO_3 fractions were composed of typical pectic materials, containing mostly galacturonic acid with the neutral sugars arabinose, galactose, rhamnose, and a smaller amount of xylose. As maturity increased, CDTA fraction yields increased, though total neutral sugar CWP compositions decreased. GTC and KOH fractions were typical of hemicellulose, and contained principally xylose, glucose, galactose, mannose, and fucose, with very small amounts of uronic acid, arabinose, and rhamnose. Residue fractions contained principally glucose and galactose, with smaller amounts of mannose, xylose, arabinose, and fucose. With the exception of xylose and glucose, all neutral sugars decreased significantly ($p < 0.01$) as maturity increased in both the Cantaloupe and Honey Dew melons.

Total uronic acid did not change as maturity increased, except for Cantaloupe, where total uronic acid decreased from the ripe to overripe stages. The CDTA fraction yield increased and all neutral sugars decreased significantly ($p < 0.05$) as storage time was increased. Only the CDTA fraction yield was negatively correlated with

the firmness of both melons, and was positively correlated with drip loss as maturity and frozen storage time were increased. Firmness was positively correlated with Na_2CO_3 and GTC fraction yield in Cantaloupe, whereas for Honey Dew there was no correlation between firmness and Na_2CO_3 or GTC fraction yield as maturity increased. The KOH fraction was negatively correlated with firmness in Cantaloupe, whereas there was no correlation between firmness and KOH fractions in Honey Dew existed as maturity increased. The residue fractions increased in both melons only from the underripe to the ripe stages, and did not change from ripe to overripe. Firmness was positively correlated with total rhamnose, arabinose, mannose, and galactose as maturity increased, and the drip loss was negatively correlated with all total neutral sugars as storage time was increased.

During frozen storage, there was a significant decreases ($p < 0.05$) in total CWP sugars in relation to increased storage time. The decrease in total sugars was more dramatic during the 0 to 5 month period than the 5 to 10 month period of frozen storage. Galactose did not change in the Cantaloupe, whereas in Honey Dew it decreased 34.3% from 0 to 5 months then decreased only 13% from 5 to 10 months of storage.

The Effect of Cultivar Maturity and Frozen Storage Time on the Cell Wall Polysaccharide Composition of Muskmelon (*Cucumis melo*)

by

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INTRODUCTION

Ripening is the final stage of fruit development, representing the complex events which eventually provide fruits with those qualities which renders them attractive and desirable for consumption. Dramatic changes occur during the ripening processes of different plant species, including the accumulation and/or loss of pigment with resultant changes in color (Giovannoni et al., 1992) and loss of firmness, or softening (McCollum et al., 1989; Bennett et al., 1989). Since early in this century, numerous studies have been conducted to determine why and/or how fruits soften during the ripening process.

From studies in peaches (Ben-Arie et al., 1989), pears (Jermyn and Isherwood, 1956), tomato (Huber and Lee, 1986; Seymour et al., 1987), and kiwifruit (Redgwell et al., 1990, 1992) it has been generally assumed that fruit softening, for the greater part, may be attributed to the solubilization of pectin (Wallner and Walker, 1975) and increases in water soluble pectins.

During fruit ripening, modification of pectins and hemicelluloses have been reported for tomatoes, pears, and muskmelon. Huber (1983) reported that increases in the low molecular weight of pectins and hemicelluloses indicated that degradation of these polymers occurred as the maturity of tomato fruits increased. Jermyn and Isherwood (1956) reported a decrease in galactan, mannan, araban, and poly-galacturonic acid, and an increase in xylan, as pear fruit maturity increased. Whereas McCollum et al. (1989) reported that a decrease in the molecular size of pectins and hemicelluloses occurred during muskmelon ripening. (Unless otherwise indicated, discussion of methods and/or findings refers to both Cantaloupe and Honey Dew muskmelons, or to melon cultivars).

Changes in cellulose during the ripening process have been reported in pears by Jermyn and Isherwood (1956) and in peaches by Sterling (1961), whereas Ahmed and Labavitch (1980) reported that the cellulose composition of pear fruit did not change as fruits softened during ripening. Gross and Sams (1984) conducted a study of the cell wall compositions of 17 fruit types (included muskmelon) during ripening, and reported that changes were restricted to galactose and arabinose.

Reid et al. (1986) conducted a study on green beans, peaches, and strawberries placed in frozen storage. Loss in firmness was accompanied by the release of pectin. It

was also stated that there was a continuing decrease in tissue firmness as a consequence of extended frozen storage, a characteristic which was correlated with changes in cell wall structure. Decreases in uronic acid, a pectic component, and in the rhamno-galacturonan backbone were also observed.

There have been numerous studies on the effect of frozen storage on the firmness of thawed fruit, but investigations of cell wall composition as applied to differences in cultivars have been limited in extent and number. Moreover, no studies have been reported in relation to the effect of frozen storage upon the cell wall polysaccharide (CWP) composition of muskmelon (Cucumis melo). Therefore, an investigation is reported in this paper on the effect of cultivar, maturity, and frozen storage times upon the cell wall compositions of muskmelon. Relationships between changes in CWP fractions and firmness is also reported, as well as relationships between total sugars with respect to changes in firmness or drip loss.

The specific objectives of this study were as follows:

1. To determine the composition, firmness, and color of the edible flesh of fresh Cantaloupe and Honey Dew muskmelon at three different stages of maturity and after three periods of frozen storage.

2. To determine the CWP composition of Cantaloupe and Honey Dew muskmelon at three different stages of maturity and at three frozen storage times.
3. To determine the relationship between firmness and CWP fraction yields for both types of muskmelons with respect to increases in maturity and increases in frozen storage time for periods of 0, 5, and 10 months.
4. To determine the relationships between changes in firmness and drip loss with total sugars for both types of muskmelons with respect to maturity and frozen storage for 0, 5, and 10 months.

LITERATURE REVIEW

Cantaloupe and Honey Dew muskmelons, of the genus Cucumis and the species melo, belong to the cucurbit family, Cucurbitaceae. The muskmelons were so named because of the aroma of the ripe fruit. "Musk" is from the Persian, meaning a kind of perfume, and "melon" was derived from the Latin "melopepo," meaning "apple-shaped melon" (Seelig, 1967). The botanical name of Honey Dew is Cucumis melo inodorus "Honey Dew," and for Cantaloupe it is "Cucumis melo reticulatus," which includes all of the netted melons (i.e., reticulated).

Among various cultivars of Cucumis melo, there are a diversity of shapes, colors, and flavors. The shape may be spherical, oval, or oblong and usually there is a central cavity. The exterior may be netted or ribbed as well as smooth. Immature melons are usually green, and may retain a green coloration or may turn to yellow or reddish-brown at maturity. Honey Dew develops rapidly at first, then ceases to grow until it ripens and achieves senescence. There is no development of an abscission zone until the fruit is commercially overripe. In contrast, the Cantaloupe develops an abscission zone and falls from the vine, maintaining rapid growth up to the time of abscission

(Pratt, 1971). The abscission layer at maturity in the Cantaloupe provides a useful harvest index, and if the Cantaloupe is harvested prior to the development of the abscission layer, it never develops the full flavor.

Although melons are grown in almost all the countries of the world, the United States is the largest producer of melons, followed by Spain and Italy (Nirankar and Ranganna, 1977). Cantaloupe and Honey Dew melons are ranked 6th and 14th by weight of all fruits produced in the United States (Eitenmiller et al., 1985). Melons are a good source of vitamins A and C. For adults, a half-melon five inches in diameter provides an amount of vitamin A in excess of the recommended dietary allowance of vitamin A and 100% of the vitamin C recommendation. In addition, melons are ideal desserts for weight-reducers since they are low in calories, providing only 60 calories per 185 g of edible fruit (Seelig, 1973).

Composition, Firmness and Color of the Edible Flesh of Muskmelon

The composition, firmness, and color of muskmelons have been studied for the purpose of determining changes in the quality of melons during development, storage or ripening. Rosa (1928) stated that the sweetness and firmness of melons were the most important indicators of the quality of melons. The sugar or soluble solid (SS) content of melons,

as determined by refractometer, has also been used to estimate quality among muskmelon fruits (Currence and Larson, 1941). The Agricultural Marketing Service of the U.S. Department of Agriculture (1993, USDA) has established that commercial grades of Honey Dew shall contain not less than 8% SS, whereas U.S. No. 1 and U.S. fancy grades of Cantaloupe shall contain, respectively, not less than 9% or 11% SS (USDA, 1992).

With increases in the stage of maturity, firmness decreases and drip loss increases in muskmelon were reported by Reddy (1986) and Miccolis and Saltveit (1991). Evenson (1983) reported that SS contents for underripe, ripe, and overripe Cantaloupe were 14.4%, 15.9%, and 13.1%, respectively. However, Yamaguchi et al. (1977) stated that a high SS content does not by itself adequately define good melon quality, and from the results of sensory evaluations it was determined that the best flavors were dependent upon sweetness, which can be only partially correlated with SS content. At the same time, low SS content makes good quality unlikely.

Lester and Dunlap (1985), Reddy (1986), and Bianco and Pratt (1977) reported that with advancing maturity, SS and pH increased whereas titratable acidity (TA) decreased in muskmelon. Bianco and Pratt (1977) conducted a study on compositional changes during development of muskmelon (i.e., until abscission occurred in Cantaloupe and until

Honey Dew fruits were fully ripened on the vine), and reported that the SS content of fully-ripened Honey Dew could reach as high as 17%.

Variations may be due to differences in cultivars or in growing conditions, fertilizers in use (Davis et al., 1964), irrigation systems, or weather patterns. Pratt (1971), Gebhardt et al., (1982), and Eitenmiller et al. (1985) reported that the moisture content of mature Cantaloupe and Honey Dew melons ranged from 89.7% to 92% and from 87.0% to 89.7%, respectively. When Cohen and Hicks (1986) conducted a study on the effect of post-harvest storage on the quality and sugar content of muskmelon, it was reported that there was no effect for 2, 5, or 9 days of storage at either 5°C or 12.5°C, and no effect for either 2 or 5 days storage at 20°C for SS content and sucrose concentration.

Specific numerical values for the responses of the human eye to different light wavelengths are required to measure visual response to color. The Commission Internationale de l'Eclairage (CIE) has adopted a method for the measurement of color which includes the use of appropriate units to express color. Hunter (1958) developed a specialized photoelectric Color-Difference Meter, which has been adapted to grade a wide variety of products. Researchers have developed uniform color scales based on opponent-color scales that may be computed and read directly from instru-

mentation (Hunter, 1975). According to opponent-color theory, there is an intermediate signal-switching stage in the human eye between light receptors and the optic nerves. Greens generates red-to-green color dimensions are compared with red responses. Similarly, blue generates yellow-to-blue color dimensions are compared to yellow responses. Red-to-green and yellow to blue are represented by the symbols "a" and "b," whereas "L" (the third dimension) is a nonlinear function for "lightness."

Reid et al. (1970), in an investigation of chlorophyll and carotenoid change in developing muskmelon, reported that in the Cantaloupe the green/red ratio decreased linearly, reflecting changing balances between chlorophyll and carotenoid content within the flesh. Forbus et al., (1991) used delayed light emissions (DLE) to measure the maturity of netted muskmelons, and reported that DLE, firmness, the Hunter "a" value, and chlorophyll content decreased with increasing maturity. Lester and Dunlap (1985), Bianco and Pratt (1977), and Reddy (1986) reported that as the maturity of the muskmelon increased, there was an increase in both SS and pH, accompanied by a decrease in titratable acidity (TA).

Enzymes Associated with Cell Wall Degradation During Fruit Ripening

In a review of melons, Pratt (1971) stated that muskmelon tissues contained several types of enzymes, including peroxidase, oxidase (flavo-enzyme, copper-enzyme, and iron-enzyme), catalase, ascorbic acid oxidase, invertase, polyphenol oxidase, and proteinase, but also stated that polygalacturonase (PG) was not present. Lester and Dunlap (1985), Hobson (1962), and McCollum et al., (1989) also reported no PG activity in the muskmelon, whereas it was stated that Cx-cellulase and pectin methyl esterase (PME) were both present (Lester and Dunlap, 1985).

Lester and Dunlap (1985) dissolved 0.5% carboxymethylcellulose (CMC) in a 0.1 M acetate buffer at pH 4.5, used as a substrate for the Cx-cellulase activity assay and 2% sodium polypectate in a 0.1 M acetate buffer at pH 4.5 for the PG assay. After water bath incubation for 16 hours at 30°C, viscosity was measured and no PG activity was detected, although it was stated that the low levels of PG activity may have been undetectable with the viscometer. It was also observed that Cx-cellulase activity was highest in "Perlita" muskmelon in the youngest tissue, declining as maturity increased. Hobson (1968) also reported the presence of cellulase in tomato fruit during maturation and ripening, decreasing as maturity increased.

Cellulase activity has been shown to increase in a number of fruits during maturation and ripening (Buescher and Tigchelaar, 1975; Awad and Young, 1979; Babbitt et al., 1973), though changes in cell wall cellulose content has generally not been assessed. Other polysaccharide hydrolases, including glucanase, xylanase, and a number of the glycosidases, have been found in various ripening fruits and could be involved in the cell wall degradation process (Bartley, 1974; Wallner and Walker, 1975; Paull and Chen, 1983, Ahmed and Labavitch, 1980). Babbitt et al., (1973) reported that cellulase activity increased steadily during tomato maturation, increasing rapidly in detached fruits during the onset of ripening at 20°C and reaching higher levels than in the fruit ripened on the plant. The loss of firmness during this period suggests that softening was initiated by the action of cellulase, and that pectinolytic enzymes were then involved in subsequent changes in firmness.

Controversial results have been reported in studies of PME activity as tomato fruit ripens. Buescher and Tigchelaar (1975) concluded that PME activity remained relatively constant during ripening. Hobson (1963) reported an increase in PME activity, but Tucker and Grierson (1982) stated that PME activity decreased during ripening. Hobson (1963) and Ulrich (1958) reported that PME activity could be found even before the fruit reached the green mature

stage. It was concluded that PME was actively involved in softening tomato fruit, and it was suggested that PG activity occurs prior to PME deesterification. This was contrary to the reports of Koch and Nevins (1989), Bruinsma et al. (1989), and Watkins et al., (1988), in which it was suggested that PME activity first demethylated pectins prior to subsequent PG degradation, and that PME was not involved directly in the softening of tomato fruits during ripening.

Huber and Lee (1986) found that soluble pectins from ripe tomatos exhibited a lower degree of esterification than unripe fruits, suggesting the involvement of PME in the softening process. Using the titrimetric analysis for the determination of pectin methylesterase (PME), Lester and Dunlap (1985) reported that PME activity was highest in 10-day-old fruit and declined with age.

The function of peroxidase activity in fruit ripening remains an unknown factor. Ku et al. (1970) reported that a threefold increase in soluble peroxidase during tomato ripening was associated with loss of one isozyme and the formation of three new ones, suggesting that the enzyme may have been involved in ethylene synthesis during ripening. Mattoo and Modi (1969) investigated peroxidase activity during the ripening of mangoes, concluding that the climacteric and ethylene production were attended by a large increase in the peroxidase and catalase activity associated

with the disappearance of a heat labile inhibitor of these enzymes. An increase in these enzymes was also induced by treatment of mango tissue slices with ethylene.

Thus, although further study still needs to be undertaken, it is possible to consider that Cx-cellulase, PME, peroxidase, and/or other enzymes may be involved in the cell-wall solubilization and degradation which takes place in the muskmelon during ripening. Establishing sensitive methods may be necessary to confirm the presence of PG, as well as for determination of PME, peroxidase, and cellulase activity in muskmelons during the ripening process.

Enzymes, with the potential to catalyze the degenerative aspects of senescence, undergo substantial increases during the climacteric, a factor which has been determined to include such cell wall enzymes as PG in the tomato (Pressey and Avants, 1973; Tucker et al., 1980; Brady et al., 1987; Giovannoni et al., 1989; Pressey, 1987) and in the apple and clingstone peach (Pressey and Avants, 1978). Though endo-PG appears to be the key enzyme involved in wall solubilization (Wallner and Bloom, 1977; Pressey and Avants, 1978; Ahmed and Labavitch, 1980; Kramer et al., 1989), exo-PG activity has also been found in the tomato (Bartley, 1978; Pressey and Avants, 1978).

The appearance of PG activity may be the initial trigger for fruit ripening and for ethylene synthesis, as well as for other events which occur as a consequence of PG acti-

tivity (Tigchelaar et al, 1978). Hobson (1965), Poovaiah and Nukaya (1979) and Watkins et al. (1988) concluded that the softening of the tomato during the ripening process was closely related to PG activity. Though this research approach provided a number of apparent correlations, the fact remains that some fruits, including strawberries, plums (Boothby, 1983) and muskmelon (McCollum et al., 1989), exist without PG, but nonetheless continue to soften during the ripening process. It has also been observed that when the PG gene was introduced into a nonripening mutant tomato, the rates of softening, ethylene production, and color development did not increase markedly (Giovannoni et al., 1989).

Function and Chemistry of Plant Cell Walls

Outside the plasmalemma of plant cells, a tough coating of cells walls exist. Cell walls are classified as primary or secondary, depending on their properties and compositions. The primary cell wall is a dynamic structure encasing the cell during the period of rapid expansion that follows cell division. The most important components of all plant cell walls are the polysaccharide, followed by smaller amounts of glycoproteins and phenolic compounds (Bacic et al., 1988; Selvendran and Ryden, 1990; Northcote, 1972). The cell walls of edible plants are made up of the primary cell wall, whereas the secondary cell wall is a

mechanically static structure that determines the shape and size of the mature cell (York et al., 1985).

Secondary cell walls are absent from fruits, and would make the fruit or vegetable products too tough and fibrous as a foodstuff for humans if they were present (Nelmes and Preston, 1968). The main polysaccharide of the primary wall are divided into α-celluloses, hemicelluloses, pectins, and a small amount of proteins (Bartley and Knee, 1982; Goodwin and Mercer, 1983; Selvendran and O'Neill; 1987; Fry, 1988). The cellulose microfibrils are embedded in a matrix of pectic and hemicellulosic polysaccharide. Pectins are abundant in the middle lamella that joins the adjacent plant cell walls. Pectic substances make up about one-third of the dry matter of the primary walls of fruits and vegetables.

Chemistry of Pectic Polysaccharide

Historically, pectic substances have been considered to be those components of the primary cell wall of higher plants that are extractable with hot water, dilute acid, ammonium oxalate, or other chelating agents in which D-galactosyluronic acid is a principal constituent. In turn, the agent of extraction is dependent upon the plant source, and the galacturonan, rhamno-galacturonan, araban, galactan and arabinogalactan content (Steven, 1983).

Pectic composition and structure have been analyzed frequently (Bartley and Knee, 1982; Fry, 1988; O'Neill et al., 1990; Selvendran and O'Neill, 1987; Bacic et al., 1988) and, based on the composition of the pectic polysaccharide (Appendix A), are classified as one of three types: (1) homogalacturonan, (2) rhamnogalacturonan I (RG-I), or (3) a substituted galacturonan referred to as rhamnogalacturonan II (RG-II) (O'Neill et al., 1990; Fry, 1988). Homogalacturonan is a chain of 1,4-linked α-D-galactosyluronic acid residues in which some of the carboxyl groups are methyl esterified; RG-I is a polysaccharide which contains a backbone of the alternating disaccharide -α-D-GalpA-(1→2)-α-L-Rhap-; and RG-II is a polysaccharide composed of a 1,4-linked α-D-galactosyluronic acid backbone with aldehydo- and keto- sugar oligosaccharide side chains attached to C-2 and/or C-3.

In general, cell wall pectic polysaccharide are D-galactosyluronic acid-rich polymers (galacturonic acid and galactose) with side chains rich in arabinose. Rhamnose residues often produce "kinks" in the chain (Van Buren, 1979; Fry, 1988), and a variety of side chains, including arabinose, galactose, and xylose, can be found. Talmadge et al. (1973) and Bauer et al. (1973) have established the model structures for rhamnogalacturonan, with its side chains of galactose and arabinose residues.

The precise function of pectins remains unclear. Pectins are present in high concentrations in the middle lamella, where they presumably serve the function of cementing together adjacent cells (Bacic et al., 1988). Evidence for this consists of the rapid release of single cells from plant tissue by chelating agents. These consist of highly hydrophilic polysaccharide, and the water that they introduce into the matrix may loosen the wall and enable the skeletal cellulose microfibrils to separate, a process which is necessary for wall expansion (Rees and Wright, 1969). Water forms part of the gel structure of pectins, thus pectins are believed to be important for the structural support of plant tissue (O'Neill et al., 1990; Aspinall, 1980; Goodwin and Mercer, 1983).

Changes in the water content of the cell wall can cause changes in firmness of the cell wall matrix as the pectin change to viscous solutions. Pectic polysaccharide are tightly bound and are required to maintain conformance of the polyuronide chains. Water also acts as a solvent, as a transporter of salts and low molecular weight organic compounds, and as a suitable environment for glycosidases' enzymatic hydrolyzation of the cell wall polymers (Goodwin and Mercer, 1983). Pectins can form cross-links via Ca^{+2} bridges via the path of covalent bonds (Fry, 1988), and may also serve the opposite function of resisting the expansion of the wall. Pectins are also likely as a source of bio-

logically active oligosaccharides (Selvendran and Ryden, 1990).

Chemistry of Hemicellulose

Like pectins, hemicellulose consists of polysaccharide built from a variety of different sugars. It is not extractable in cold water, but can be effectively solubilized in alkali (Bartley and Knee, 1982; Selvendran and Ryden, 1990; Fry, 1988). The hemicelluloses, originally named for reason of a presumed chemical relationship to cellulose, are now known to include such polysaccharide as glucans, arabinoxylans, and arabinogalactans (Sturgeon, 1990). Many of these polysaccharide, together with the pectins, compose parts of the amorphous matrix of plant cell walls. Hemicellulose has xylose and mannose residues, characterized by Fry (1988), as follows (Appendix B):

1) Xylans have a backbone of ß-(1→4)-linked D-xylose pyranose (D-xyl*p*) residues, some of which carry single α-L-arabinofuranosyl (α-L-Ara*f*) and/or α-D-glucuronic acid pyranose (α-D-Gluc*p*A) residues attached to the 2- and/or 3-O-position. The proportion of the xylose residues that bear carbohydrate side chains varies between different xylans. Arabinosylated and/or glucuronosylated xylans tend to have greater water solubility because they cannot self-aggregate by hydrogen

bonds and are less able to hydrogen bond to cellulose. They can, therefore, be fractionated by affinity chromatography on a cellulose column, and can be eluted by a gradual increase in NaOH concentration.

2) Xyloglucans are the major components of the storage walls of seeds. Xyloglucans are polysaccharide with backbones identical to cellulose, a linear polymer of ß-(1→4)-linked D-Glucp residues. Side chains are attached to the 6-O-position of the glucose residues, and are often arranged in a definite order, forming blocks of three consecutive substituted glucose residues followed by one unsubstituted glucose. Xyloglucans contain acetyl-ester groups, apparently on the galactose residues.

3) ß-(1→3),(1→4)-Glucans are composed of an unbranched chain of ß-D-Glucp residues. The most common blocks are [(1→4)-(1→4)-(1→3)] and [(1→4)-(1→4)-(1→4)-(1→3)].

4) Callose is a second polymer of ß-D-Glucp residues, wherein all linkages are (1→3).

5) ß-Mannans occur in certain secondary cell walls, their presence in small amounts of mannose suggesting that the primary walls may also contain such polysaccharide; α-Mannose is a major compo-

nent of some glycoproteins. Mannans from cell walls possess a backbone of ß-(1→4)-linked D-Manno pyranose (D-Manp) residues.

Chemistry of Cellulose

Cellulose (α-cellulose) is named for a combination of cells, or cellulae, with the common "ose" suffix (Marchessault and Sundarajan, 1983). Cellulose is chemically similar to starch, but differs in solubility (Appendix C). The two are identical substances, but differ in states of aggregation. Cellulose is an unbranched polymer of D-glucopyranose residues joined by ß-(1→4) linkages (Sterling, 1961; Franz and Blascheck, 1990). Obtained as the insoluble residue following the extraction of pectin and hemicellulose, cellulose often contains traces of mannose and galactose. However, the presence of these substances may be owed to contaminant hemicelluloses. Cellulose is usually ca. 20-30% of the dry weight of the primary cell wall. The biological function of cellulose is presumed to be skeletal, providing shape and strength to the cell wall. Van Buren (1979) stated that cellulose has the function of providing rigidity and resistance to tearing, whereas the pectins and hemicelluloses contribute to plasticity and the ability to stretch.

Changes in Cell Wall Polysaccharide with Ripening

Ripening is the final stage of fruit development. Unripe fruits have smaller quantities of soluble pectins, but as fruits ripens, pectins in the fruit cell wall are changed, degraded and solubilized to render the fruits soft (Fry, 1988). In the tomato, cell wall polymer metabolism is involved in softening, reflecting lower quantities of high molecular weight polymer hemicelluloses and higher quantities of low molecular weight polymers at maturity (Huber, 1983; Hobson, 1963). These changes coincide with the degradation of pectic polysaccharide. An increase in the solubility of polyuronides occurs during ripening (Bartley and Knee, 1982), resulting in a net decrease in certain structural components (Gross and Sams, 1984). Labavitch (1981) stated that the change in cell wall composition is due to the loss of uronic acid polymers. Ahmed and Labavitch (1980) reported that the cellulose composition of pear fruit did not change as ripening fruits softened. Huber and Lee (1986) found that soluble pectin from ripe tomato fruit was rich in arabinose, galactose, and xylose, all of which decrease during early gel formation.

There is an absence of complete information on the softening of muskmelon during ripening. Gross and Sams (1984) reported a loss of cell wall galactose and arabinose during ripening in 14 of 17 fruit types examined, including

muskmelons. Mannose was reported to be constant, fucose and xylose increased, and rhamnose decreased during the ripening of the muskmelon. McCollum et al. (1989) observed that total polyuronides decreased, whereas soluble polyuronides increased during muskmelon ripening. In addition, rhamnose, mannose, and galactose decreased in proportion to increased maturity, while arabinose, xylose, and glucose increased from preripe to ripe stages and decreased from ripe to overripe stages. It was concluded that both the pectic and hemicellulose fractions of muskmelon cell wall had been modified during fruit ripening and softening, and the molecular weight of pectic muskmelon was changed in the absence of PG.

Isolation of Cell Wall Polysaccharide

In the earliest analyses of the cell wall, alcohol-insoluble residue (AIR) was used as a starting material (Jermyn and Isherwood, 1956). For analytical purposes, 500 g of frozen flour pear tissue was added to 2 L boiling 95% ethanol, and was then re-extracted with several changes of boiling ethanol. The final product was dried in air currents. Yields of from 15 to 18 g (3% to 3.6%) were reported from each batch.

A second modification of the AIR preparation was carried out by immersing the tissue in hot 90% alcohol for five minutes, disrupting the tissue by blending the mixture

then washing the residue with alcohol and acetone. Alcohol insoluble residues have been used by investigators as cell wall materials up to the present. For example, this technique was used by Ahmed and Labavitch (1980) to determine changes in carbohydrate-degrading enzymes in ripening Bartlett pears; by Gross and Sams (1984) to determine changes in cell wall neutral sugar compositions during fruit ripening for 17 fruit types; by Huber (1983) for the determination of polyuronide and hemicellulose modifications in ripening tomato fruit; and by McCollum et al. (1989) to determine polyuronide and hemicellulose fractions for muskmelons at three levels of maturity. Huber (1991) reported that ca. 11 to 13 mg of cell wall material was obtained from 1 g (1.1% to 1.3%) of pericarp tomato fruit using boiling ethanol as the extractive substance.

Buerger (1986) used an acetone extraction to prepare acetone insoluble solids (AIS) as cell wall materials. The AIS was prepared from three varieties of freeze-dried strawberry powder and from three varieties of fresh strawberries. For the freeze-dried strawberries, yields were in the range 13.8 to 19.2%. From the edible portions of muskmelon, Dinus (1967) obtained AIR in a range 0.75 to 1.66%. Lester and Dunlap (1985) conducted cell wall extraction from the Perlita muskmelon, using 6% NaCl with chloroform/methanol (1:1, v/v), which was then dried with acetone without report of yields.

To investigate differences in cell wall material yields from immature (i.e., slightly lignified) and mature (i.e., heavily lignified) runner bean pods and asparagus shoots, Selvendran (1975) compared the effect of solvent and ball-milling treatment. It was reported that the use of 80% aq. alcohol yielded higher amounts of cell wall materials (i.e., 5.15% and 5.40% for immature and mature beans, respectively) than cold water extraction (3.81% and 4.30%), 1% sodium deoxycholate (SDC, at 3.36% and 4.44%), phenol-acetic acid-water (PAW, 3.38% and 4.38%), and a mixture of PAW and 1% SDC (3.30% and 4.25%). The protein content of each cell wall material preparation was also investigated, and it was concluded that the sequential extraction of the fresh ball-milled tissue with 1% aq. SDC or 1.5% sodium dodecylsulphate (SDS) and PAW (2:1:1, w/v/v) provided cell wall preparations containing the smallest protein quantities.

With 1% SDC and 1.5% SLS, determined to be of equal effectiveness, a two-stage extraction method, based upon 1% SDC followed by PAW, was preferred to direct extraction with PAW. SLS was used to remove proteins, RNA and polyphenols from cell wall materials and PAW was used as a final precaution against the adverse effects of residual enzyme activity (Redgwell et al., 1988).

A number of subsequent investigations, including O'Neill and Selvendran (1983) for runner beans, Selvendran

and O'Neill (1987) for potatoes, Fry (1988) for a rose cell suspension culture, and Redgwell et al. (1988, 1991) for the CWP of kiwifruit, have adopted the Selvendran (1975) approach. All used aqueous inorganic solvents in place of aqueous alcohol.

To minimize enzyme activity, low temperature extractions should be maintained by blending frozen powders with SDC or SLS at 20°C. Using this method, Selvendran and O'Neill (1987) reported that 50 g fresh weight of potatoes yielded 0.6 g (1.2%) of dry cell wall material (CWM). Redgwell et al. (1988) isolated CWM from four different zones of the kiwifruit, reporting CWM of 0.96 g, 4.76 g, 0.7 g, and 1.6 g from 100 g each of, respectively, the outer pericarp, inner pericarp, locule wall, and the core. Reports on CWM yields differ from plant to plant. Differences in isolation methods and in the chemical extractants used also influenced the solubility properties of cell walls during fractionation.

Huber (1992) prepared cell walls from ripe tomato fruits using several methods. First, 100 g of tomato pericarp was homogenized in 0.04 M N-2-Hydroxyethylpiperazine-N-2-ethanesulphonate (HEPES) at pH 7.0. Following filtration, the residue was divided into two parts, one treated with PAW (2:1:1 w/v/v) and the second treated with Tris-buffered phenol (BP) at pH 7.5 containing 0.05% 8-hydroxyquinoline. After stirring and washing with 80% ethanol and

with 80% acetone, both cell wall materials were treated with chloroform:methanol (1:1 v/v), then washed with acetone before being dried at 34°C. A third method was identical, except that the phenolic solvent was not used. Huber (1992) reported that the solubilization of pectin from PAW treated AIS underwent more rapid and extensive depolymerization, whereas pectin from BP-treated walls exhibited no change in relative molecular mass. It was then argued that the low pH of PAW could have been the reason for the loss of 50% of tomato cell wall calcium, causing an increase in the solubilization of pectins and thus a decrease in chelator dependency for pectin solubilization.

To maintain the stability of the CWP preparation, Selvendran et al. (1985) suggested that following preparation, it was best to freeze dry an aliquot of the CWP for analysis of the constituent sugars and amino acids, and to store the bulk of it in a frozen state until required for fractionation studies.

Fractionation of Cell Wall Polysaccharide

Pectins

Pectic substances are the most readily extractable cell wall polymers. The most commonly used extractants for

pectic substances, subject to some degradation which may occur in the process, are water, chelating agents, and dilute alkali (Selvendran et al., 1985). Redgwell et al. (1990) reported that water washes and dialysis during the CWP purification served to remove most water soluble pectins. However, an increase in water soluble pectins during ripening of peaches was reported for fractionation performed with water (Ben-Arie et al., 1989) and pears (Jermyn and Isherwood, 1956). For the first fractionations of pear CWP, 12 hours of exposure to boiling water was used to effect complete removal of pectins. Ten g of AIR typically produced 2.5 to 3.5 g of dry pectin.

However, from more recent fractionations, it has become clear that insoluble pectic material, possibly joined by covalent linkages, is present in all types of cell walls, and that yield amounts are dependent upon the type of tissue. The use of acid reagents for pectin extraction from cell walls is avoided, due to their degradative effect upon polysaccharide, especially for those materials containing large amounts of araban (Jermyn, 1955).

Lester and Dunlap (1985) conducted the fractionation of Perlita muskmelon cell wall materials into pectin, determining total uronic acid by colorimetry using the alcohol carbazole method (i.e., polyuronide is considered to be pectin). Dinus (1967) also determined total pectins by using the same method, reporting that the range of total

pectic substances (calculated as galacturonic acid) from the muskmelon was from 82.33 mg to 238.77 mg per 100 g of the edible portion. This variation was due to the different variety of melons. O'Neill and Selvendran (1983) removed pectic materials with both water (pH 4.0, 80°C for 1 hr) and a 1% ammonium oxalate chelating agent (pH 5.5, 80°C for 2 hrs) from mature runner bean cell walls. Experiments determined that different fruit-type cell walls yielded differing proportions of pectic materials for the same chelating agents. For the runner beans, approximately 80% of the total pectins were extractable, whereas for apple and for sugar beets the proportions were approximately 50% and 30%, respectively (Selvendran et al., 1985). Degradation of the polymer cell walls during cell wall fractionation was also reported.

Recently, cyclohexanediamine tetra-acetate (CDTA) has been used for the extraction of pectins from the cell walls of celery petioles, cress hypocotyls, tomato, and cucumber pericarp (Jarvis, 1982), with the amounts of pectin derived determined colorimetrically for uronic acid. The total pectins (mg/g cell walls) were 82, 64, 100, and 151 for celery, cress, cucumber, and tomato, respectively. When an 0.05 M Na_2CO_3 solution was also used to extract the CDTA insoluble fraction, the pectins extracted (mg/g cell walls) were 123, 60, 105, and 72, respectively. This type of solution has been used to solubilize pectin galactans held

by ionic bonds from the middle lamella (Fry, 1988; Redgwell et al., 1991, 1992), and has also been used to de-esterify pectins while minimizing degradation (Fry, 1988; O'Neill et al, 1990). An increase in the poluronide fraction was reported in tomato fruits by Huber and Lee (1986) and Seymour et al. (1987), and for kiwifruit by Redgwell et al. (1990, 1992) as maturity of fruits increased.

Huber (1992) isolated pectins using two different methods from three different cell wall preparations. These included (1) standard (preparation of cell wall material without exposure to the phenolic solvents, (2) preparation of cell wall material with Tris-buffered phenol (BP), and (3) preparation of cell wall with phenol:acetic acid:water (PAW)):

1) Using 0.05 M Na-acetate at pH 6.5, it was reported that the soluble pectins were 24.4, 3.9, and 140 mg galacturonic acid equivalents for standard, BP-treated, and PAW-treated wall preparation, respectively.
2) Using 0.05 M CDTA in 0.05 M Na-acetate at pH 6.5, it was reported that the soluble pectins derived from standard, BP-treated, and PAW-treated wall preparation were 155, 135, and 170 mg galacturonic acid equivalents, respectively.

Hemicellulose

Hemicellulose, which consist of xylans, mannans, and galactans (Goodwin and Mercer, 1983), was extracted following the isolation of pectins using of 6 M guanidinium thiocyanate (GTC) and 4 M KOH (Selvendran et al., 1985; Selvendran and O'Neill, 1987; Redgwell et al., 1991, 1992).

For most tissues, some quantities of pectic substances are also extracted with the hemicellulose and some of this remains in the α-cellulose residues. Since potassium acetate formed during neutralization with acetic acid has greater solubility than sodium acetate, the use of KOH is preferred to the use of NaOH during the extraction of hemicellulose. Alkali solubilizes the polysaccharide by hydrolyzing ester linkages between the polysaccharide, and also between polysaccharide and non-carbohydrate components such as phenolic acids. Fry (1988) and Selvendran et al. (1985) stated that alkali has a "chaotropic" property which disrupts hydrogen bonding between hemicelluloses and hemicellulose fibrils. Other chaotropic agents such as urea or guanidinium thyocyanate (GTC) have also proved useful for the extraction of some of the mannose-rich polymers from the depectinated cell walls of apples (Selvendran et al., 1985). The degradation of hemicellulose can be minimized by the use of oxygen-free solutions and by the reduction of latent aldehyde groups with sodium borohydride.

Following the separation of pectic materials from alcohol insoluble solids (AIS), Dinus (1967) reported cellulose fractions in a range from 24.14 mg to 269.47 mg/100 g of the edible portions of muskmelon. It was noted that hemicellulose was included in the cellulose fractions. Gross and Sams (1984) directly hydrolysed the cell walls of muskmelon with 2 M trifluoroacetic acid (TFA), using gas chromatography (GC) to determine the amount of neutral sugars derived from noncellulosic polysaccharide.

Cellulose

The resultant residues are referred to as α-cellulose. Lester and Dunlap (1985) extracted hemicellulose sequentially in a discontinuous gradient from 0.01 M to 4 M KOH, yielding 17 fractions of hemicellulose polymers. Cellulose fractions were then determined by removing hemicellulose, lignins and xylans with acetic acid/nitric acid. The cellulose was dissolved in 67% H_2SO_4 and then diluted. Following the addition of anthrone, absorbance was recorded at 620 nm (Updegraff, 1969).

Lester and Dunlap (1985) reported an increase in cellulose fraction in the cell wall from 25 to 40% over the duration of 10, 20, 30, 40, and 50 days after pollination of the melons (50 days after the pollination date, melon fruits were categorized as full slip).

Redgwell et al. (1988) determined TFA insoluble fractions using phenol-sulphuric acid method (72% H_2SO_4 and 80% phenol) and absorbance at 490 nm was read (Dubois et al., 1956). Redgwell et al. (1988) also conducted a sequential fractionation of CWP from 4 tissue zones of the kiwifruit at harvest, using 0.05 M CDTA, 0.05 M Na_2CO_3, 6 M GTC, 4 M KOH and the residue (α-cellulose). It was reported that the percentages of CDTA, Na_2CO_3 (both were pectins), GTC, KOH (both were hemicelluloses), and cellulose fractions from the outer pericarp of kiwifruit were 8.9%, 20.2%, 6.9%, 21.2%, and 42.8%, respectively, whereas fractions from the inner pericarp compositions were 9.0%, 16.9%, 10.1%, 18.5%, and 45.5% for, respectively, CDTA, Na_2CO_3, GTC, KOH, and α-cellulose fractions.

Cell Wall Polysaccharide Sugar Composition During Ripening

Numerous investigations have sought to determine the sugar composition of CWP from different plant tissues at different maturity stages. Redgwell et al. (1991) reported the cell wall sugar compositions of two ripening stages of kiwifruit, observing variability in both amounts and sugar compositions. The CWM were fractionated as discussed above (Redgwell et al., 1988), and an increase in the proportions (mole %) of galactose, rhamnose, and arabinose in the CDTA and Na_2CO_3 soluble pectic during ripening was reported,

though a large decrease in total amounts was observed. It was suggested that the increased proportion of galactose in the pectic polysaccharide during ripening may have occured as a result of degradation and the release of unbranched pectic backbone. Redgwell et al. (1991) also reported that GTC and KOH soluble fractions (which contained most of the hemicelluloses) were more highly branched than the CDTA and Na_2CO_3 soluble polysaccharide.

Albersheim et al. (1967) warned that the laborious methods used to prepare samples for this type of process posed the possibility of experimental error. Jermyn and Isherwood (1956) previously noted that extensive degradation of araban occurred during similar experiments, and it was stated that there is no true boundary between pectins and hemicellulose. For example, araban and galactan, considered to be pectin components, also appear in hemicellulose, and considerable amounts of xylose and glucose have been found in pectin fractions. A moderate amount of pectic polymers, as evidenced by the presence of uronic acid and galactose in both the 6 M GTC and 4 M KOH fractions, were reported by Redgwell et al. (1988) for kiwifruit cell wall polysaccharide. This may have been due to the presence of highly branched pectic materials, cross-linked with polysaccharide associated with cellulose (Selvendran and Ryden, 1990). Thus, most investigations have focused sole-

ly upon changes in sugar composition within the pectin fractions.

Ahmad and Labavitch (1980) observed that substantial amounts of galacturonic acid and arabinose were lost from cell wall fractions as pear fruit ripened and as acidic polymers with side chain groups containing linked arabinose residues were recovered from the soluble fractions of homogenates. They also reported that most non-cellulosic sugars decreased as the fruits softened, and that the arabinose decrease was close to 50% of the neutral sugars of the wall. It was also noted that cellulose compositions did not change as the fruit ripened and softened. In turn, Seymour et al. (1987) reported that during the ripening of tomato fruit, there was a significant increase in soluble polyuronides and PG activity, as well as a loss of galactose and arabinose from the cell walls.

Processing of Muskmelons

Melons are consumed fresh as a natural dessert or in salads. Since melon production is seasonal, a means of preservation is required, usually accomplished by freezing. Wiegand and Onsdorf (1943) suggested two methods of preservation, the first being to dice or slice melon flesh for quick freezing in tightly covered containers for storage at 0°F. The second method was to mix sliced or diced melon flesh with sugar (4:1 w/w ratio), then freeze and store at

0°F in tightly covered containers. Winter and Leonard (1971) suggested that Cantaloupe and Honey Dew balls be immersed into a sugar syrup (at slightly greater strength than the Brix of the fruit itself), containing ascorbic acid (1%), and malic acid (0.25%), prior to quick freezing.

Angel and Preaud (1974) processed four varieties of Cantaloupe and Honey Dew into pretreated cubes soaked for three minutes in a sugar solution equal to the total SS of the muskmelon prior to freezing and packing. A second part was soaked in the same sugar solution with the addition of 10% ascorbic acid and 0.25% malic acid and the melons treated with both solutions were then exposed to two different freezing treatments to reduce their temperatures to -40°C: (a) air-blast freezing for 30 minutes and (b) plate freezing for 1.5 hours.

Following packing in three types of materials (polyethelene--regular pack in air, PVC laminate--vacuum pack, and PVC laminate--nitrogen flush), the results of the test were reported as follows: (i) The appearance of the cubes from the different varieties was not identical; (ii) for all varieties, the vacuum pack resulted in compression following thawing and the cubes appeared to be soft; (iii) taste tests showed that the four varieties reacted similarly to the various treatments; (iv) following one month of frozen storage, there was a loss of flavor in the polyethylene-packed fruits, or off-flavors resulted, whereas

the cubes in nitrogen and vacuum packs retained their flavor and did not develop off-flavors (Angel and Preaud, 1974).

Reddy (1986) infused melon balls with five different solutions prior to frozen storage at different temperatures and for different storage durations. He reported that mature melon balls were rated as superior in overall acceptability to sub-mature melons during sensory evaluations. There were no effects of freezing method or storage duration upon the mean overall acceptability scores. The texture of melon balls infused with 0.08% calcium cloride (Ca^{+2}) + 0.75% ascorbate was 100% and 33% greater than the texture of, respectively, mature raw and frozen melon balls, regardless of the freezing method used. Finally, it was stated that the melon balls that received infusion treatments with 0.75% ascorbate + 0.08% calcium cloride (Ca^{+2}) + 16% sugar solution were scored higher for overall acceptability, regardless of storage duration, freezing method or maturity.

In that Ca^{+2} is divalent, it can bind ionically to two free and subsequently cross-linked carboxyl groups of galactacturonic acids of pectin molecules (Goodwin and Mercer, 1983). In addition, the Ca^{+2} ion coordinates with neighboring hydroxyl (-OH) groups in the pectins (Fry, 1988). Thus, strong cross-links can be formed between pectin molecules to form the "egg-box" model (Jarvis, 1984;

Powell et al., 1982), and the firming action of calcium ions is used in firming canned tomatoes and pickled cucumbers and in the preparation of dietetic jams and jellies with low methoxyl pectin (Whistler and Daniel, 1985). However, little attention has been given to the role of calcium in the cell wall during fruit ripening.

Knee et al. (1977) reported that in unripe strawberries, more than half of the insoluble pectin was extracted by the chelating agent ethylene diamine tetra-acetate (EDTA), suggesting that pectins were stabilized in the wall by calcium. During ripening there was an increase in water soluble pectins in strawberry, suggesting that since strawberry has no endo-PG, the effect may have been due to the loss of calcium from the gel structure (Barnes and Patchett, 1976). In ripening tomatoes, solubilization of the cell walls and calcium release have also been observed (Rigney and Wills, 1981).

Freezing and Frozen Storage

Freezing has been a major factor in bringing convenience foods to the home, restaurant, and institutional feeding establishments. The proper freezing of foods preserves foods without causing major changes in size, shape, firmness, color, or flavor (Potter, 1986). Freezing of fruits or vegetables can be conducted by one of three major commercial freezing methods, including (i) air-blast freez-

ing, (ii) indirect contact with refrigerants, and (iii) direct immersion in refrigerant media.

Air-blast freezing is widely used to produce a great variety of quick frozen foods. The freezing rate of the product is influenced by the temperature of the air, the air velocity, and the type and shape of foods or packages (Desrosier, 1959). Blast freezing temperatures commonly range from -30°C to -40°C. If food packages are placed in contact with a cold metal surface cooled by circulating liquid coolants, the process is called indirect-contact freezing, and if the foods are submerged into cold liquids or cold liquids are sprayed onto foods, the process is called immersion freezing. Cryogenic freezing refers to the very rapid freezing achieved by exposing food items, either unpacked or thinly packed, to extremely cold freezants such as liguid nitrogen (-196°C) and dry ice or subliming CO_2 (-79°C) (Potter, 1986).

Foods such as meats, fruits, and vegetables have cellular structures with delicate cell membranes and cell walls. Water resides between and within the cells. When the water is frozen rapidly, it forms small crystals; however, when the water is frozen slowly, large ice crystals are formed. Larger ice crystals within or between the cells cause a greater degree of physical rupture and separation among the cells than do the smaller crystals.

To maintain the quality of frozen foods at a high level, it is necessary to maintain low temperatures during storage (i.e., -18°C or lower) and during distribution. Foods in the frozen state are not inert. If proper frozen storage temperatures are not met, then significant food deterioration can occur (Potter, 1986), either by chemical or physical means. For example, the degradation of pigments and vitamins, or an increase in the amount of drip loss from tissues, occurs as time of frozen storage is increased. Ice crystal formation or recrystallization that occurs in the frozen tissue can result in the physical disruption of relatively rigid plant cell structures, while "freezer burn" occurs due to the sublimation of ice during frozen storage if foods are packed improperly. Freezer burn irreversibly alters the color, firmness, flavor, and nutritive value of frozen foods.

Williams (1986) stated that at frozen storage temperatures as low as -20°C, changes were evident in vegetable firmness (due to pectic enzymes and cellulase activities), color (due to chlorophyllase, polyphenol oxidase, lipase, and protease activities), and nutritional quality (due to ascorbic acid oxidase and thiaminase activities). Lee (1958) also observed that autolysis continued to occur when vegetables were stored at -18°C.

Carr (1982) conducted a study of the effect of freezing and frozen storage on physico-chemical changes in

strawberries. It was observed that the texture of thawed strawberries was inversely proportional to frozen storage time and, as storage time was increased, there was a coincident decrease in the water solubility of pectic polysaccharide as well as total cell wall uronide content. It was argued that the increase in drip loss during long term frozen storage may be accounted for by the loss of water soluble pectic materials from strawberry cell walls. Carr (1982) stated that recrystallization could have been the driving force for frozen tissue damage and suggested that loss of firmness within the tissue may have been due to the release of pectic materials, thus resulting in diminished cell wall intercellular adhesion and structural rigidity.

Reid et al. (1986) stated that pectin is an important contributor to the firmness of tissues which have been frozen. Freezing causes severe damage to the cell membranes, and is therefore responsible for a loss of turgor. However, it was less clear whether or not cell wall compositions contributed to the loss of firmness in strawberries as a consequence of freezing and frozen storage.

MATERIALS AND METHODS

Two cultivars of muskmelon (*Cucumis melo*), Cantaloupe (Superstar) and Honey Dew (Volga), were planted at the Hermiston (Oregon) Research and Agriculture Extension Center. Cantaloupe was harvested on August 22, 1991 at three stages of maturity: half-slip, green full-slip, and yellow full-slip (Seelig, 1973; Evensen, 1983). Honey Dew was harvested on September 8, 1991. Determination of Honey Dew maturity was based upon skin color, aroma, and the softness of the blossom ends. In underripe Honey Dew, almost all skin color is green, and the blossom end of the fruit is not soft when pressed with the thumb. Green skin color is lost as the Honey Dew ripens and the blossom end softens. Overripe Honey Dew melons are yellowish in color with no green coloration, with the exception of some variety lines (Reed, 1991). All fruit was maintained in storage at 2°C prior to processing.

From all fruit harvested, 30, 48, and 30 Cantaloupes and 32, 54, 32 Honey Dew melon at, respectively, underripe, ripe, and overripe stages were used for this study. All cultivars of both fruits that were either underripe or overripe were randomly divided into two groups (replicate), processed separately into melon disks, and then mixed thor-

oughly prior to packaging. Each group of ripe melon fruit was divided into three subgroups, processed separately, and then maintained for 0, 5, and 10 months in frozen storage. A flow chart for the sample preparation of melon disks, composition, firmness, color analysis, and cell wall polysaccharide (CWP) is presented as Figure 1.

Preparation of Melon Disks

For the preliminary experiments, melon balls were prepared according to Reddy (1986), using a hand melon baller. Compared to disk preparation, melon ball preparation produces more juice. Lower yields are obtained and longer times are thus required to freeze the balls. The production of additional juice during preparation could be due to pressure exerted by the melon baller during the process of scooping. Therefore, for this study, melon disk samples were prepared.

Disk preparation was performed in a 10°C room, and the preparation steps are shown in Figure 2. For each cultivar, fruits at different stages of maturity were processed separately. The fruit was peeled, cut in halves longitudinally, and the seeds were removed. Using a knife and cutter, melon disks 1.2 cm in thickness and 2.2 cm in diameter were prepared.

Melon disks for each maturity were mixed thoroughly, then subsamples were used to determine composition, firmness, and color. For the purposes of freezing, a layer of melon disks was placed on a metal screen, covered by plastic, and then sprayed with liquid nitrogen until each disk was frozen individually. Plastic covers were used to keep the disks from sticking to the metal screen. Polyethylene bags were then filled with 500 g of frozen melon disks, flushed with nitrogen, and sealed with an automatic sealing machine. Depending on the maturity, bags of ripe Cantaloupe and Honey Dew were maintained at -23°C for 5 or 10 months of storage. For the fresh fruits (0 months storage), underripe, ripe, and overripe disks were maintained at -40°C prior to isolation of CWP. The CWP extracted within six days was considered to originate from fresh melons.

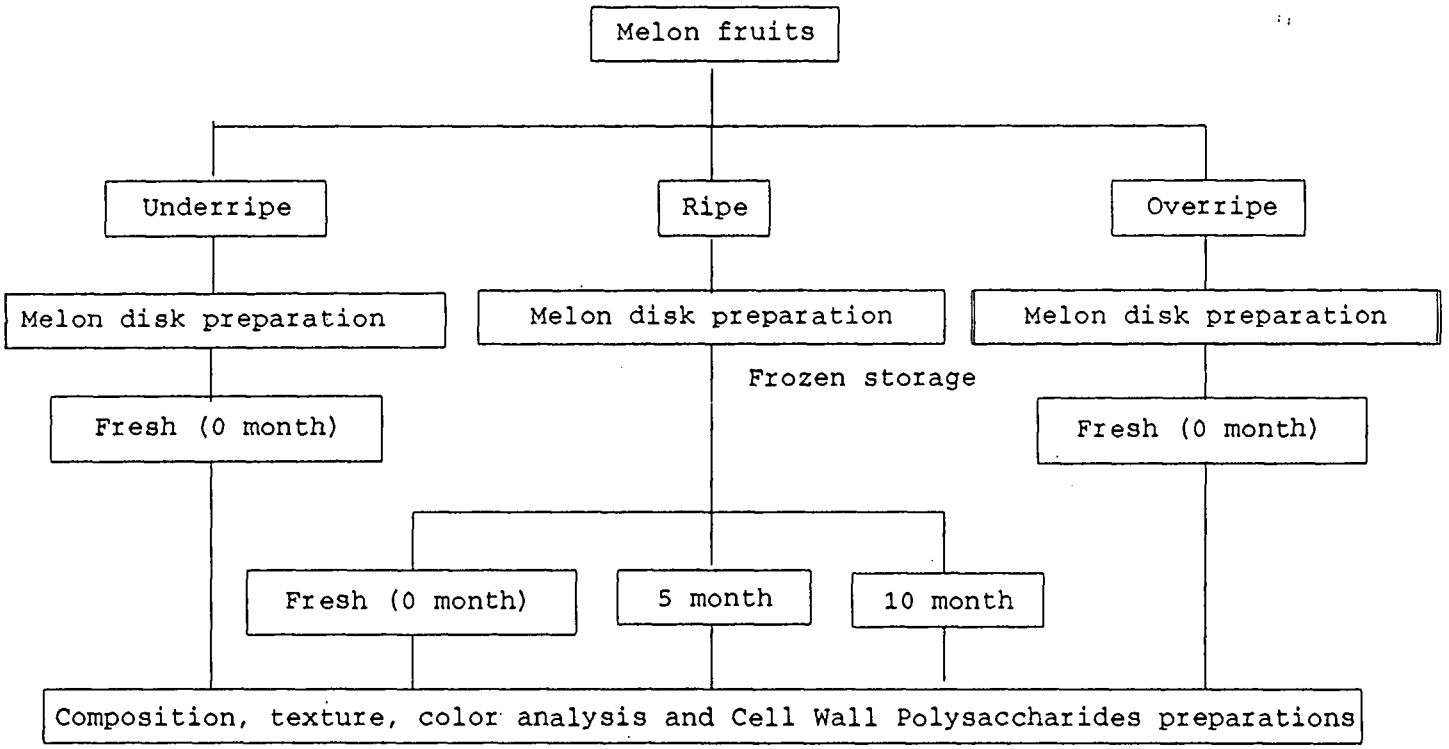


Figure 1. Sample preparation for melon disks, composition, color analysis, and cell wall polysaccharide.

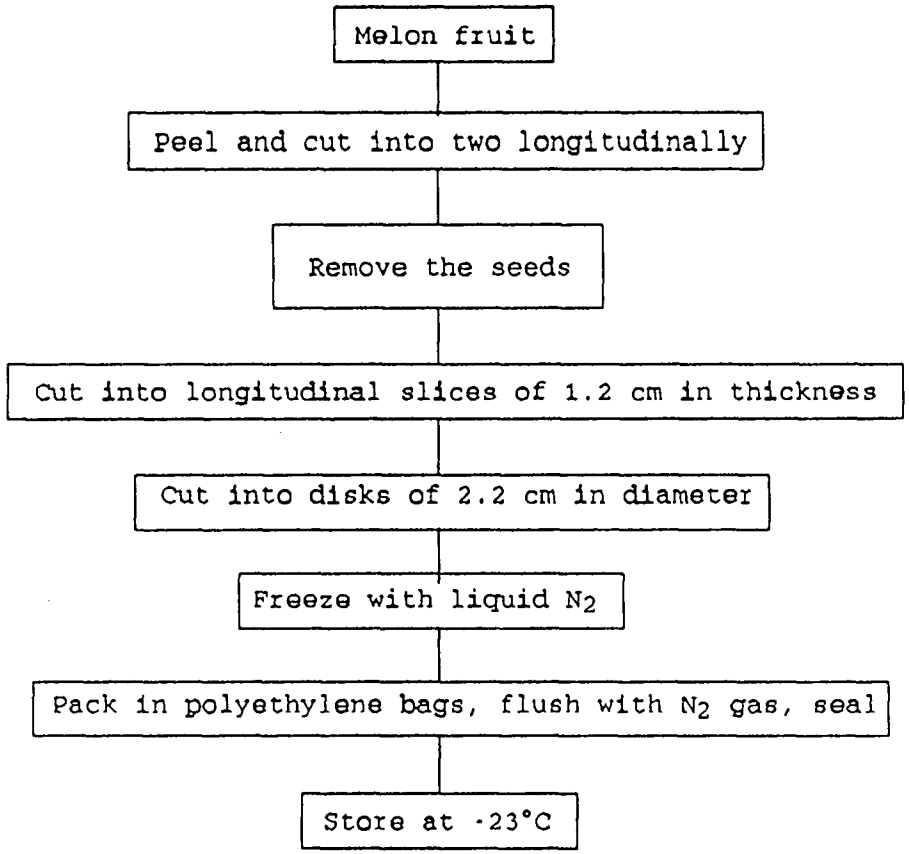


Figure 2. Preparation of melon disks.

Characteristics of Fresh Muskmelon

Composition, Firmness, Drip Loss, and Color Determination

Subsamples of fresh melon disks from both varieties for three maturity stages (without freezing) were analyzed for composition (pH, titratable acidity (TA), soluble solids (SS), moisture content), firmness, and color. For pH, TA, SS, moisture content, and color analyses, the melon disks were homogenized for 1.5 minutes using a Waring blender. For determination of firmness, unfrozen melon disks were used. Drip loss analysis was performed within six days following freezing of the fruit, which was then maintained at a temperature of -23°C.

Determination of pH

The pH of melon homogenates (in duplicate) was measured using a Metrohm 605 pH meter and an automatic titrator from Brinkmann-Metrohm, Herisau Inc., of Switzerland.

Determination of Titratable Acidity

Duplicate 10.0 g sample homogenates were mixed with 40 ml distilled water, from which 10 ml of solution was titrated with 0.1 N NaOH until a final pH of 8.1 was achieved using a TTA 80 titration assembly, an automatic titrator from Brinkmann-Metrohm, Herisau Inc. Total acid was then

calculated as follows:

$$\text{TA (\% citric)} = \frac{\text{ml NaOH} \times \text{N NaOH} \times \text{meq.wt of citric} \times 100}{\text{sample weight}} .$$

Determination of Soluble Solids

Soluble solids were determined (in duplicate) as °Brix (or % sucrose), using a Bausch and Lomb refractometer at 20°C temperature, connected to a temperature regulator. Melon homogenate was filtered and the juice was used for measuring the soluble solids.

Determination of Moisture Content

Ten g of melon disk homogenate (in duplicate) were put in a preweighed aluminium dishes, then vacuum dried in an oven at 60°C for 24 hr. The percentage of moisture was calculated as follows:

$$\text{Moisture (\%)} = \frac{\text{weight loss} \times 100}{\text{sample weight}} .$$

Determination of Firmness

Firmness of the fresh (unfrozen) melon disks, measured by force expressed in grams, was determined using a Sears Craftsman drill press penetrometer, model 335.25926, fitted with a tip of 0.5 cm diameter. Twenty melon disks were subjected to penetrometer testing. Two readings were re-

corded from each disk and the average was taken as the firmness value for the sample.

Determination of Drip Loss

Drip loss determination was performed on the 5th day following harvest for frozen Cantaloupe and Honey Dew at each of the three stages of maturity. Weights of 150 g (in duplicate) of frozen melon disks were placed on a #8 metal screen, and drippage was collected in a 50 ml glass cylinder. The sample was reweighed after two hours at room temperature and the volume of juice collected was also measured. Drip loss calculations were as follows:

$$\frac{\text{initial weight - final weight} \times 100\%}{\text{sample weight}}$$

or

$$\frac{\text{volume of juice collected} \times 100\%}{\text{sample weight}} .$$

Determination of Color

Color of the melon disk homogenate was determined (in duplicate) colorimetrically, using the Colorquest Hunterlab with the specular included mode of reflectance. The scale of Hunter "L" (lightness), "a" (redness to greenness), and "b" (yellowness to blueness) values were recorded.

Isolation of Cell Wall Polysaccharides

Cell wall polysaccharide were isolated following the methods of Redgwell et al. (1988), as modified. From each maturity group of both cultivars, 1000 g of frozen melon disks (in duplicate) were cryomilled (at -196°C) five times with one liter of liquid nitrogen in a commercial Waring blender, model 32BL39 (1 gallon stainless steel jar), at high speeds for 1.5 minutes, or until all melon disks were homogenized completely and reduced to a fine powder form. By freezing melon disks and using liquid nitrogen during cryomilling, this will prevent the activity of endogenous enzymes during the process of isolating the CWP. This treatment also allowed the rapid suspension of frozen tissues in 1.5% aqueous sodium lauryl sulphate (SLS) detergent to remove proteins, RNA, and polyphenols from the cell wall materials. A flow chart for this procedure is shown in Figure 3. The resultant powder was then blended with 1350 ml 1.5% aqueous SLS, containing 5mM sodium metabisulphite ($Na_2S_2O_5$), for two minutes at 2°C. $Na_2S_2O_5$ was added to the SLS solution to minimize the oxidation of phenolics and cross-linking as well as to inactivate enzyme activity. To prevent frothing, a few drops of octanol were added to each blend. Following blending, the suspension was centrifuged at 23,000 × g for 10 minutes at 2°C. The supernatant was decanted and the residue was washed twice in 400 ml

cold (2°C) distilled water (each time by centrifugation). The wash was then combined with the previous supernatant and filtered with glass fiber filter paper, type A/E Gelman Sciences, to recover all residues.

Residues were stirred with 400 ml phenol-acetic-acid-water (PAW = 2:1:1 w/v/v) for one hour in an ice bath to remove non-covalently bound proteins and glycoproteins (this treatment also prevented the adverse effects of residual enzyme activity), then washed four times in 400 ml of cold distilled water, as described above, followed by - centrifugation. All four supernatant washings of PAW treatments were combined and then filtered to recover residues. Iodine tests for the presence of starch in the CWP preparations were found to be negative. The residues were then dialyzed against cold distilled water (2°C), using a change of water each six hours on 10 repetitions. The dialysed materials were then centrifuged and the residues were freeze-dried for 75 hours at -70°C at 15 microns Hg vacuum, using a Labconco freeze-drier. The freeze-dried material was placed in a desiccator containing P_2O_5 prior to weighing. The CWP yield was recorded, then retained in a vial with a teflon screw cap at -40°C prior to fractionation.

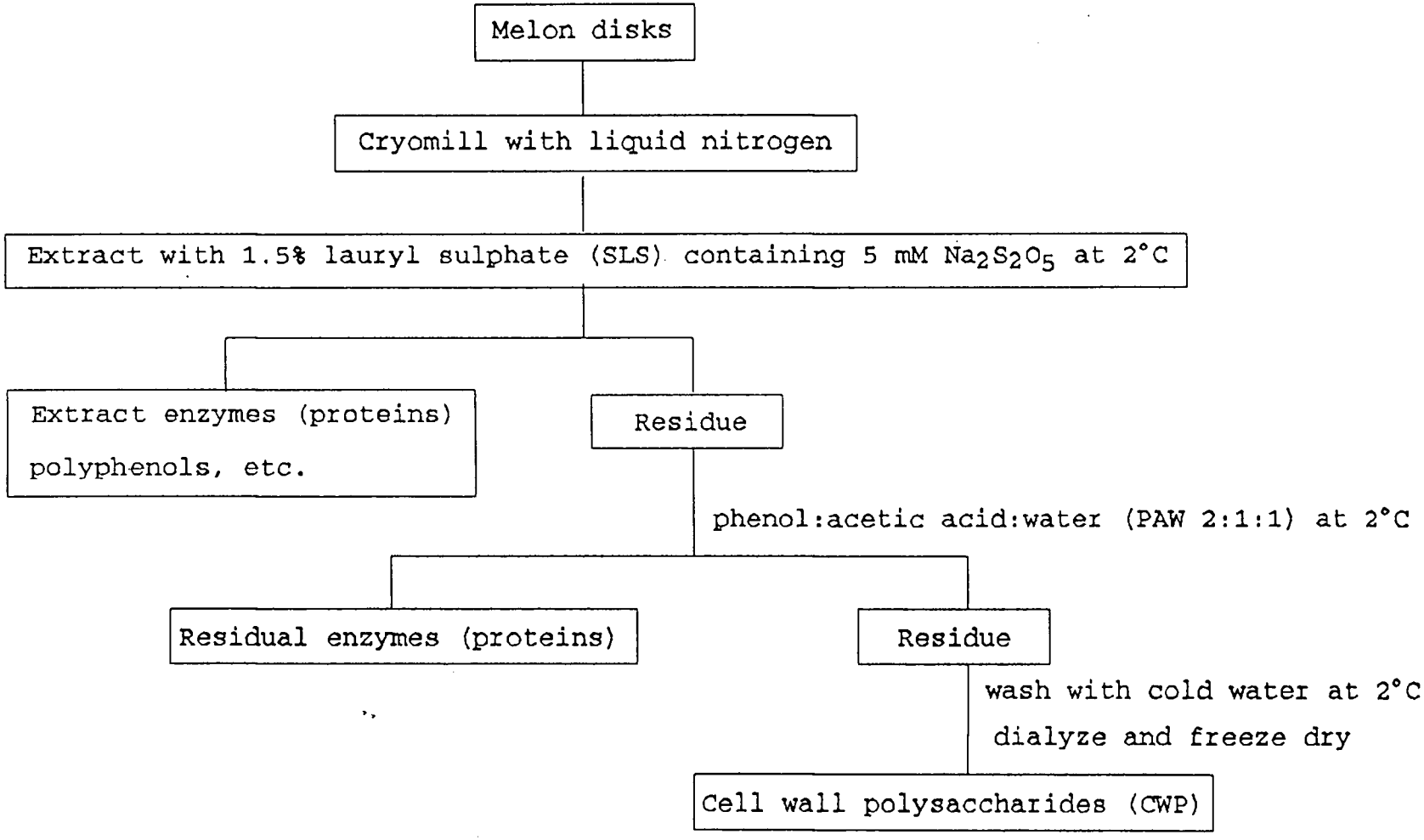


Figure 3. Isolation and purification of CWP (modified method of Redgwell et al., 1988)

Fractionation of Cell Wall Polysaccharide

Fractionation of CWP was based upon the modified method of Selvendran and O'Neill (1987). A flow chart for the procedure used is shown in Figure 4. Two grams of CWP was stirred with 200 ml of 0.05 M cyclohexane-trans-1,2-diamine tetra acetate (CDTA) at pH 6.5 for 6 hours at 20°C (to isolate the CDTA soluble pectins), then centrifuged at 23,000 x g for 10 minutes. The treatment with 200 ml 0.05 M CDTA was repeated, the solution was stirred for another 2 hours and then centrifuged. Both supernatants were combined. The residue was washed twice with 200 ml distilled water, and then centrifuged. Both washings were combined with the supernatant and filtered with glass fiber filter paper, type A/E Gelman Sciences (1 micron pores). The filtrate were then dialyzed, using spectra por (6000-8000 molecular weight cut off -- MWCO), from VWR Scientific), against distilled water at 2°C, changing the water each 6 hrs for 10 repetitions. Dialyzed materials were concentrated using polyethylene glycol (average MW = 10,000), redialyzed, then freeze-dried at -70°C in a 15 micron vacuum for 75 hrs, using the Labconco freeze-drier. Following the CDTA treatment, the residue was stirred into 200 ml 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$ at 2°C for 20 hours (i.e., to isolate the CDTA insoluble pectins), then stirred for an additional two hours at 20°C. The suspen-

sion was centrifuged and the supernatant was collected. Treatment with 200 ml 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$ was repeated at room temperature for two hours, followed by centrifugation. The supernatant was collected and the residue was washed twice in 200 ml distilled water. Washings were collected, combined with the supernatant and then filtered through glass fiber filter paper. The filtrate was dialyzed for Na_2CO_3 fraction, concentrated, dialyzed, and then freeze-dried.

Residue was stirred into 200 ml 6 M GTC at room temperature for 18 hours under nitrogen gas (i.e., to isolate the GTC soluble hemicellulose), centrifuged, and the supernatant was collected. Residue was resuspended in 200 ml 6 M GTC at room temperature for an additional two hours under nitrogen gas, centrifuged, and washed twice with 200 ml cold distilled water. The supernatant and washings were combined, and filtered. The filtrate was dialyzed, concentrated, redialyzed, and freeze-dried.

Residues were twice stirred into 200 ml 4 M KOH containing 20 mM $NaBH_4$ for two hours and centrifuged (i.e., to isolate GTC insoluble hemicellulose). Strong alkali, such as 4 M KOH, serves to disrupt the hydrogen bonds between hemicellulose and α-cellulose, thereby solubilizing the bulk of the xyloglucans and glucomannans from melon CWP. To minimize degradation of the hemicelluloses, an oxygen-free solution was used during the extraction (i.e., by

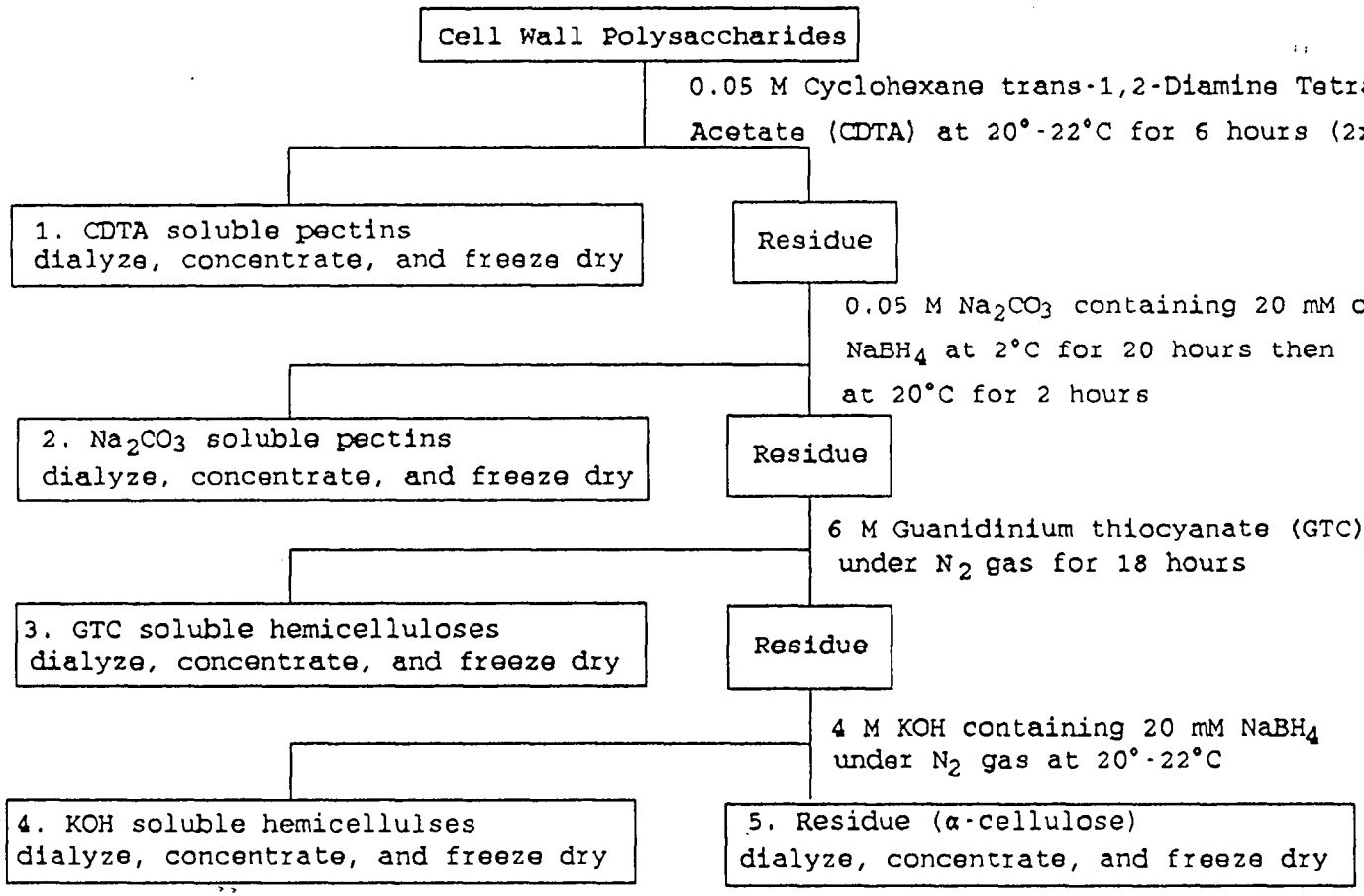


Figure 4. Fractionation of CWP (modified method of Selvendran and O'Neill, 1987).

stirring under nitrogen gas). Alkali also hydrolyses ester linkages between polysaccharide and such non-carbohydrate components as the phenolic acids. $NaBH_4$ was added to reduce the latent aldehyde group. The supernatant was collected and was washed twice in 200 ml distilled water. Supernatant and washings were combined and filtered. The filtrate was dialyzed, concentrated, redialyzed, and freeze-dried.

The α-cellulose residue was dialyzed and freeze-dried for 75 hours at -70°C in a 15 micron Hg vacuum. The resultant freeze-dried materials were placed in a desiccator containing P_2O_5 prior to final weighing. Each of the CWP fractions was retained in a vial with a teflon screw cap at -40°C prior to use for the analysis.

Analysis of Cell Wall Polysaccharide Fractions

Analysis of Neutral Sugars

Analysis of the CWP fractions for neutral sugars was based upon the method of Albersheim et al. (1967), as presented in three steps: (i) hydrolysis of the CWP fractions, (ii) reduction of neutral sugars to alditols, and (iii) derivatization of alditol acetate (Figure 5).

Duplicate 20 mg samples of the CWP fractions were hydrolyzed in 2 ml 2 M TFA in a culture tube, covered with a teflon screw cap, then heated at 121°C for one hour using

a heating block. The hydrolyzed materials were cooled, then filtered using glass fiber filter paper. TFA insoluble materials were analysed using the phenol-sulphuric acid method for sugar determination, based upon the glucose standard. The filtrate was then dried by filtered air at 40°C. One mg myo-inositol was added as an internal standard to 0.5 ml M ammonia containing 10 mg $NaBH_4$ to reduce sugars and produce alditols, and allowed to sit for one hour at room temperature. Excess $NaBH_4$ was removed by the dropwise addition of glacial acetic acid until effervescence had ceased. The mixture was then washed and dried with 5 × 1 ml methanol, using filtered air at 40°C. Finally, 1 ml acetic anhydride was added and heated at 121°C for three hours to derivatize alditols to alditol acetate. A series of standard sugars, including rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), galactose (Gal), glucose (Glu), each contain between 0.5 mg/ml to 2.5 mg/ml, was duplicated. One mg/ml myo-inositol was added as internal standard.

Each standard solution was also derivatized to alditolacetate by a method identical to that use to prepare the sample. Then duplicates of 1 μl alditol acetate (standard and sample) were injected onto a Varian aerograph series 1200 gas chromatograph (GC), in a stainless steel column (2 × 2.2 mm i.d.) packed with SP 2330 on 100/120 Supelcoport. The initial temperature of 150°C was maintained for

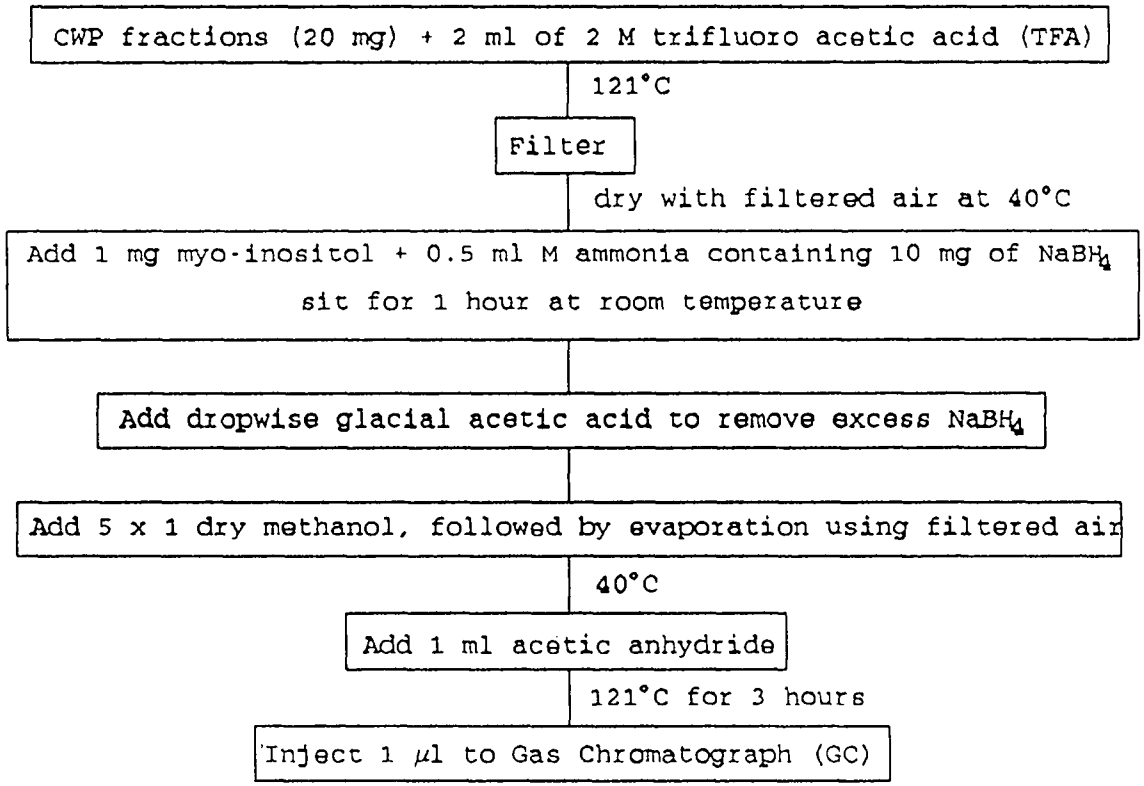


Figure 5. Alditol acetate preparation (modified method of Albersheim et al., 1967)

two minutes, then raised to 220°C at the rate of 20°C/minute, and retained at this temperature for an additional 20 minutes. Nitrogen gas, at the rate of 20 ml/minute, was used as the carrier and a flame-ionization detector (FID) with hydrogen gas was used.

The normalized area of the chromatogram was calculated with myo-inositol used as an internal standard and based on the chromatogram area of standard sugars. The sample sugars were calculated in mg/g CWP and mmole% as anhydro sugars. Calculation of anhydro sugar residues was performed to provide the total amount of glycans ("glucan," "galactan," "xylan," etc.) in terms of monomeric sugar units found in CWP. The anhydro uronic acid/anhydro sugars ratios were also calculated to find the closest number of uronic acid molecules as compared to the number of neutral sugar molecules in the cell wall polysaccharide. To form a polysaccharide (polymer), each molecule of sugar has one less molecule of H_2O.

Trifluoro-Acetic Acid Insoluble Fraction Analysis

TFA insoluble materials from each CWP fraction were determined by the phenol-sulphuric acid method for total sugar determination, following the method of Dubois et al. (1956). A glass-fiber filter paper containing TFA insoluble material (derived as discussed in the previous section)

was placed in a 250 ml glass beaker. Preliminary analyses indicated that 4 ml 72% H_2SO_4 was required to dissolve and hydrolyse the polysaccharide from the CDTA, Na_2CO_3, GTC, and KOH fractions, and 50 ml 72% H_2SO_4 was required to dissolve and hydrolyze the residue fractions. The beakers were shaken and then allowed to sit at room temperature for three hours. A duplicate one ml solution was diluted with 11.6 ml distilled water, then heated in a boiling water bath for two hours (Selvendran et al., 1979).

A series of five 2 ml standard glucose solutions were prepared containing, respectively, 10, 20, 30, 40, and 50 μg glucose. Then 50 μl (0.05 ml) of 80% phenol was added to each sample and the standard solutions, and 5 ml concentrated H_2SO_4 was added rapidly at the surface (not to the side) of the tube to obtain a good mixture. A blank was prepared using the same method, except two ml of distilled water in place of the sample solution. The tubes were allowed to stand for 10 minutes at room temperature, then were shaken in a water bath at 25°C for 20 minutes. Absorbance was then read at 486 nm, using a Shimadzu spectrophotometer, model UV160U. Sample absorbance was subtracted from the blank absorbance reading, and the amounts of su

gars were determined by reference to the standard curve for a glucose solution (Figure 6), calculated as follows:

$$\text{glucose sample (mg / g CWP)} = \frac{[(A - B) / C] \times D \times E}{F \times 1000},$$

where A = sample absorbance,

B = absorbance at Y intercept of the standard curve,

C = slope of the standard curve,

D = dilution factor,

E = CWP fraction (mg) recovered from 1 g of CWP,

F = CWP fraction (mg) used in the analysis, and

1000 = conversion factor of μg to mg.

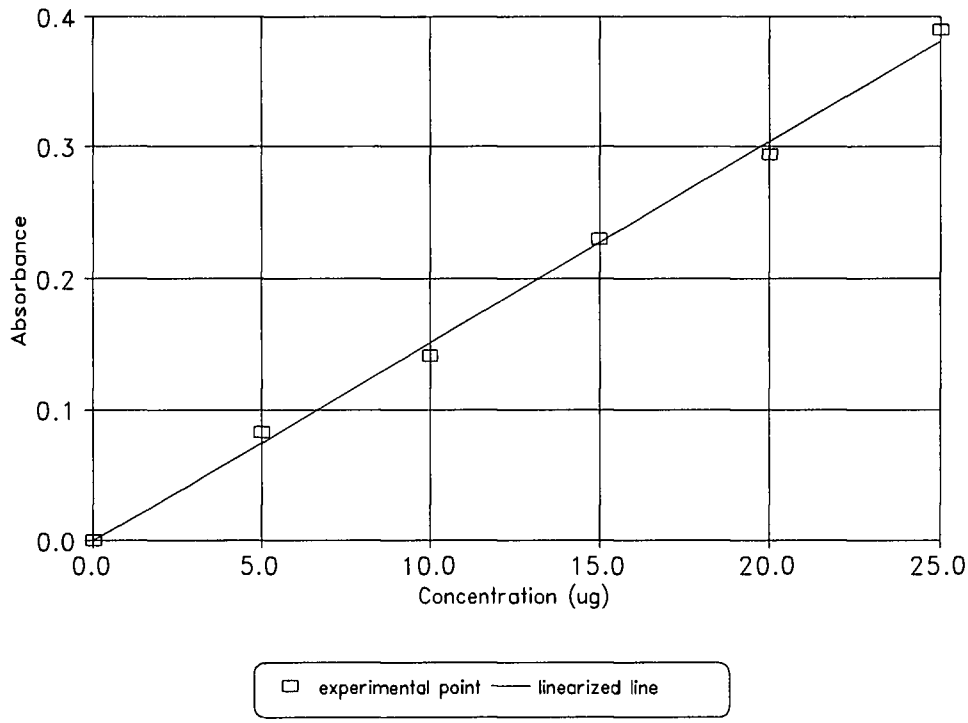


Figure 6. Glucose standard curve (modified method of Dubois et al., 1956).

Analysis of Uronic Acid

Analysis of uronic acid was carried out following the method of Blumenkrantz and Asboe-Hansen (1973), using galacturonic acid as a standard (Figure 7). Duplicate 10 mg CWP fractions were mixed with 0.5 ml 72% H_2SO_4 for three hours at 20°C, diluted with 6 ml distilled water, and filtered through glass fiber filter paper. A test tube (refrigerated in crushed ice) was filled with 1 ml of the filtrate, mixed with 6 ml 0.0125 M solution of sodium tetraborate ($Na_2B_4O_7$) in concentrated H_2SO_4, using a Vortex mixer. A series of standard galacturonic acid solutions (each ml of solution containing, respectively, 5.0, 10.0, 15.0, and 20.0 μg) were similarly treated with 6 ml 0.0125 M $Na_2B_4O_7$ in concentrated H_2SO_4. The tubes were heated in a water bath at 100°C for five minutes, then cooled in an ice-water bath, to which 0.1 ml of 0.15% m-hydroxybiphenyl in 0.5% NaOH was added.

A blank was also prepared using 1 ml of distilled water, treated with 6 ml 0.0125 M $Na_2B_4O_7$ in concentrated H_2SO_4, and in which 0.1 ml of 0.5% NaOH was used to replace the m-hydroxybiphenyl solution. The solution was shaken and absorbance was read within five minutes at 520 nm, using a Shimadzu spectrophotometer, model UV160U. Sample absorbance was subtracted from the blank absorbance. Using the standard curve for the galacturonic acid, the amount of uronic acid in each CWP fraction was calculated as follows:

$$\text{sample galacturonic acid (mg/g CWP)} = \frac{[(A-P)/Q] \times R \times S}{T \times 1000},$$

where A = sample absorbance,

P = absorbance at Y intercept of the standard curve,

Q = slope of the standard curve,

R = dilution factor,

S = CWP fraction (mg) recovered from 1 g of CWP,

T = CWP fraction (mg) used in the analysis, and

1000 = conversion factor of μg to mg.

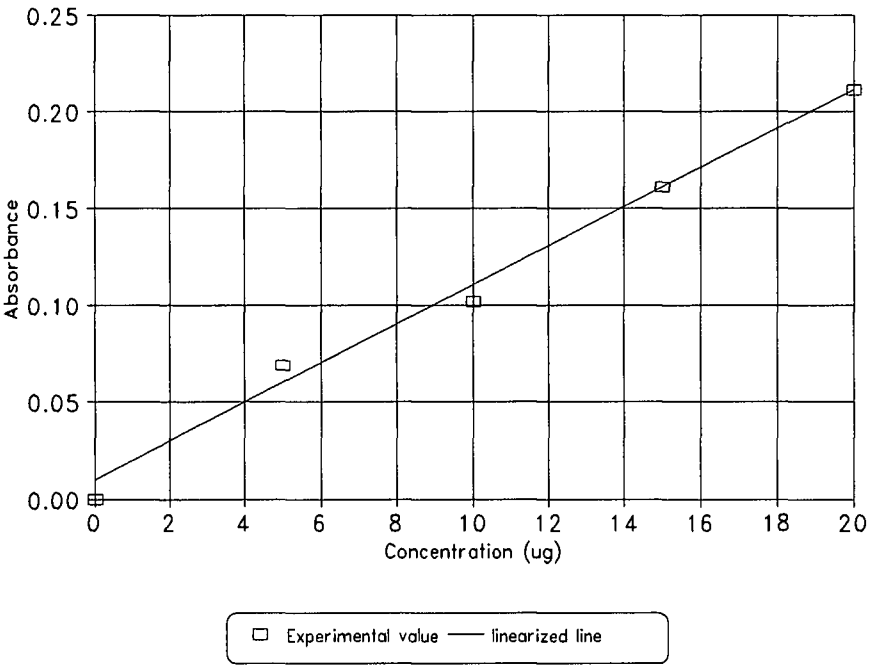


Figure 7. Galacturonic acid standard curve (modified method of Blumenkrantz and Asboe-Hansen, 1973).

Frozen Storage Characteristics of Melon

An identical treatment and analysis to that performed on fresh melon disks were also applied to the melon disks stored for either 5 or 10 months at -23°C, with the exception that moisture content and firmness determinations were not conducted. During frozen storage dehydration may occur, resulting in the miscalculation of the moisture content of melons. It is also inappropriate to measure the firmness of frozen melons. In summary, the following analyses were conducted as described in previous sections:

1. Composition, drip loss, and color determination:
   a) pH determination,
   b) titratable acidity determination,
   c) soluble solids determination,
   d) drip loss determination, and
   e) color determination;
2. Cell wall polysaccharide extraction;
3. Cell wall polysaccharide fractionation;
4. Analysis of cell wall polysaccharide fractions:
   a) TFA soluble fraction analysis,
   b) TFA insoluble fraction analysis, and
   c) uronic acid analysis.

Statistical Analysis

A two-way analysis of variance (ANOVA) was used to evaluate the results of composition, firmness, drip loss, color, CWP, CWP fraction yields, and sugar compositions of CWP fractions, comparing both cultivars of muskmelon as related to stages of maturity and storage duration.

RESULTS AND DISCUSSION

Characteristics of Fresh Melon Disks

The chemical and physical characteristics of two cultivars of muskmelon (Cucumis melo), including Cantaloupe (Superstar) and Honey Dew (Volga), were determined for three maturity stages (underripe, ripe, and overripe). The edible flesh of both cultivars was processed into disk form. Composition, firmness, and color data were obtained from observation of unfrozen melon disks and drip loss data was obtained from observation of frozen disks, stored at -23°C for less than six days (0 months). Cell wall polysaccharide (CWP) were extracted and then fractionated from disks stored at -40°C for less than six days (0 months).

Although all physical and chemical characteristics were evaluated in duplicate, this method did not provide for calculation of correlation coefficients. Because of biological sample variability, additional replicates would have been required to carry out these calculations. Therefore, graphical analysis was used to provide approximated trend change information for measurement of the characteristics considered for the two cultivars. This method of analysis forms the basis for discussion in this chapter.

Composition, Firmness, Drip Loss, and Color

The results of composition, firmness, drip loss, and color determination are shown in Table 1, whereas the two-way ANOVA for these results is shown in Table 2. Since interactions between cultivars and maturity were significant at $p < 0.05$. Table 3 provides information on changes in composition, firmness and color for both melons as maturity increased.

pH and Titratable Acidity

Honey Dew had a lower pH than did Cantaloupe, and for both cultivars there was a slight increase in pH from underripe, to ripe, and overripe stages of maturity (Table 1). It was also observed that titratable acidity (TA) was higher in the Honey Dew than in the Cantaloupe, decreasing for both as maturity increased from underripe, to ripe, to overripe stages. A similar increase in pH was also reported by Lester and Dunlap (1985) during the development and ripening of "Perlita" muskmelon fruits and a similar decrease in TA was reported for muskmelon by Reddy (1986). This effect was likely a consequence of a decrease in acid as the muskmelon ripened.

Soluble Solids

Soluble solids (SS) for the Honey Dew were higher than for the Cantaloupe, and for both cultivars there was an increase in SS coincident with increases maturity (Table 1). The SS range was from 8.45% to 10.1% for the three stages of maturity for Cantaloupe and from 9.45% to 12.7% for Honey Dew melon (Table 1). Variation in SS reported by previous investigators for muskmelon may have been due to differences in cultivars or in such growing conditions as soil, fertilizers, irrigation, or climatic conditions. Significant changes in SS ($p < 0.01$) were observed for both melons as maturity increased (Table 3).

Firmness and Drip Loss

Honey Dew had significantly higher firmness and lower drip loss in comparison to the Cantaloupe. For both types of melons, an inverse relationship between firmness and drip loss was observed (Figure 8). With increased maturity, firmness decreased and drip loss increased for both melons. Similar observations were also reported by previous investigators in muskmelon (Reddy, 1986; Miccolis and Saltveit, 1991).

Table 1. Composition, firmness, and color of melons with different maturity

Cultivar	Maturity stages	pH	TA % citric	SS (°Brix)	Firmness g (force)	Drip loss (%)	Moisture %	Color		
								"L"	"a"	"b"
Cantaloupe	Underripe	6.19	0.08	8.45	290	10.6	90.4	47.3	9.24	19.7
	Ripe	6.49	0.05	9.20	189	12.9	91.4	44.4	12.3	22.7
	Overripe	6.87	0.04	10.1	140	14.1	91.9	43.9	12.4	20.9
Honey Dew	Underripe	5.65	0.16	9.45	1290	10.2	90.4	58.3	-7.86	21.4
	Ripe	6.16	0.08	11.0	1201	11.2	89.2	54.9	-7.44	20.8
	Overripe	6.43	0.07	12.7	877	13.8	88.8	61.9	-7.06	20.2

Table 2. Effect of cultivar and maturity on the composition, firmness, drip loss, moisture, and color (F-ratios from two way ANOVA)

Source of error	d.f.	pH	TA	SS	Firm-ness	Drip loss	Mois-ture	Color: "L" value	Color: "a" value	Color: "b" value
C	1	290 *	600 *	1285 *	43 *	34.6 *	60370 *	20585 *	121205 *	40.4 *
M	2	268 *	345 *	497 *	1318 *	224 *	84 *	548 *	544 *	283 *
C * M	2	6.26 **	73.5 *	23.3 *	12.6 *	12.3 *	16969 *	700 *	247 *	551 *
M.S.-Error	6	0.002	1.25	0.007	1909	0.056	1.58	0.025	0.009	0.0066

C = Cultivar; M = Maturity; * = F-ratios significant at $p < 0.01$;
** = F-ratio significant at $p < 0.05$.

Table 3. Composition, firmness, and color change in different cultivar and maturity of melons (%)

Cantaloupe	pH	TA	SS	Firmness	Drip loss	Moisture	Color		
							"L"	"a"	"b"
Underripe → Ripe	4.84*	37.5**	8.88*	37.9**	21.7*	1.1*	6.13**	33.1*	15.23*
Ripe → Overripe	5.86*	20 **	9.78 *	25.9 **	9.3 *	0.54 *	1.13 **	0.813 *	7.92 *
Honey Dew									
Underripe → Ripe	9.03 *	50 **	16.4 *	6.9 **	9.8 *	1.33 **	5.83 **	5.34 *	2.8 **
Ripe → Overripe	4.4 *	12.5 **	15.5 *	27 **	22.3 *	0.45 **	12.75 *	5.1 *	2.88 **

* = % increase; ** = % decrease.

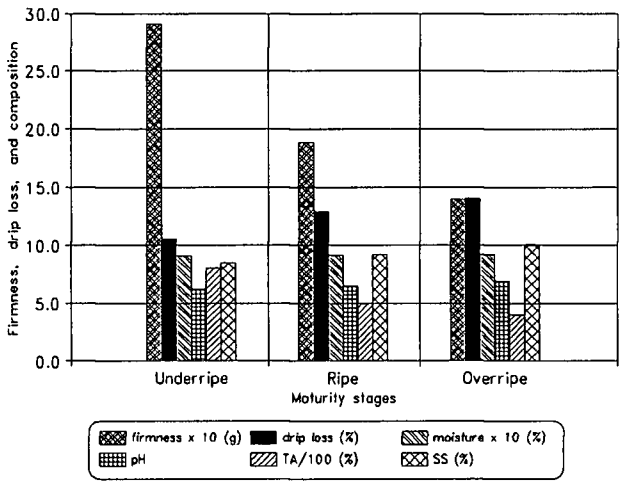
Moisture Content

The moisture content for Cantaloupe increased slightly, whereas that for Honey Dew decreased as maturity increased. However, differences in moisture content between the two cultivars for the different stages of maturity were quite small, and these values were close to those reported by previous investigators (Eitenmiller et al, 1985; Pratt, 1971).

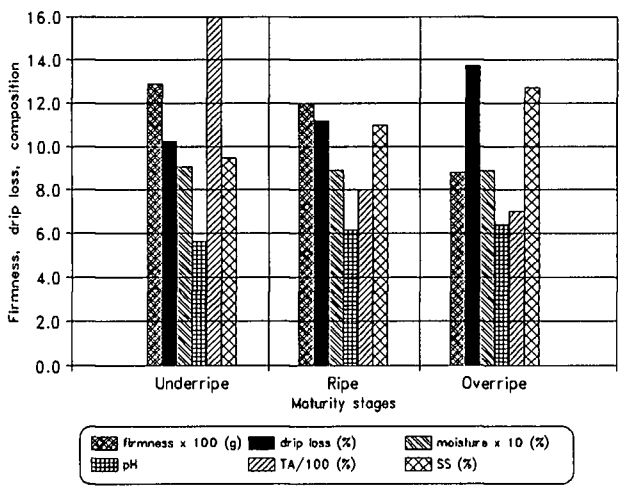
Color

For color evaluation, Hunter "L," "a," and "b" measurements were taken. The "a" values, representing red-to-green color dimensions, increased from underripe to ripe in both the Cantaloupe and the Honey Dew, whereas the "b" values, representing yellow-to-blue, decreased.

According to Hunter (1975), positive and negative "a" values indicate, respectively, the redness or the greenness of an object. Therefore, it was reasonable to observe a positive "a" value for Cantaloupe, which is orange in color, and a negative "a" value for Honey Dew melon, which is greenish in color (Table 1 and Figure 9). Cantaloupe redness increased, while Honey Dew greenness decreased from one stage of maturity to the next.



a) Cantaloupe



b) Honey Dew

Figure 8. Firmness, drip loss, and composition for different maturities of melons.

The "b" values for both cultivars were positive, providing a prediction of increased yellowness. Note that negative "b" values predict blueness, which is less relevant to the color of the muskmelon. Cantaloupe yellowness increased slightly from underripe to ripe stages, but there were no observable changes in color from the ripe to overripe stages. Honey Dew yellowness decreased as maturity increased.

Lightness ("L" value) decreased slightly in the Cantaloupe as maturity increased (i.e., became darker in color). "L" values represent light to dark (0 = white to 100 = black), where darkness is in relation to changes in the "a" and "b" values. For the Honey Dew there was a decrease in "L" value from underripe to ripe stages, followed by an increase from ripe to overripe stages. Whereas the darker in color in Cantaloupe was due to changes in "a" and "b" values.

These observations were reasonable since ß-carotene (orange in color) is subject to increase during the development and ripening of melons (Pratt, 1971; Lester and Dunlap, 1985). The decrease in green color in the Honey Dew was due to a decline in chlorophyll content as the fruit developed and ripened (Pratt, 1971).

Figure 9 shows the relationship between the color ("L," "a," and "b") values as maturity increased for both the Cantaloupe and the Honey Dew melon. There was no ap-

parent correlation between the "L" and "b" values as maturity increased. The "a" values demonstrated consistent increases for both Cantaloupe and Honey Dew as maturity increased. Therefore, the "a" value appeared to provide a good indicator of maturity.

Carbohydrates in Cell Wall Polysaccharide Fractions

Neutral Sugars in TFA Soluble Fractions

Duplicate alditol acetate derivative preparation was performed on the CWP fractions and each preparation was analyzed twice using a gas chromatograph. The normalized area of the chromatogram was calculated, using myo-inositol as an internal standard. A standard sugar solution, consisting of rhamnose, fucose, arabinose, xylose, mannose, galactose, and glucose, also derivatized to alditol acetate, subject to the addition of myo-inositol to the preparation. To minimize errors due to variation from day-to-day injections, an average of six days was used as the injection standard for the calculation of neutral sugars for each sample. Chromatograms of alditol acetate derivative of standard sugars are shown in Figure 10a. Whereas chromatograms of alditol acetate from CDTA, Na_2CO_3, GTC, KOH and residue fraction samples are shown in Figures 10b, 10c, 11a, 11b, and 11c respectively.

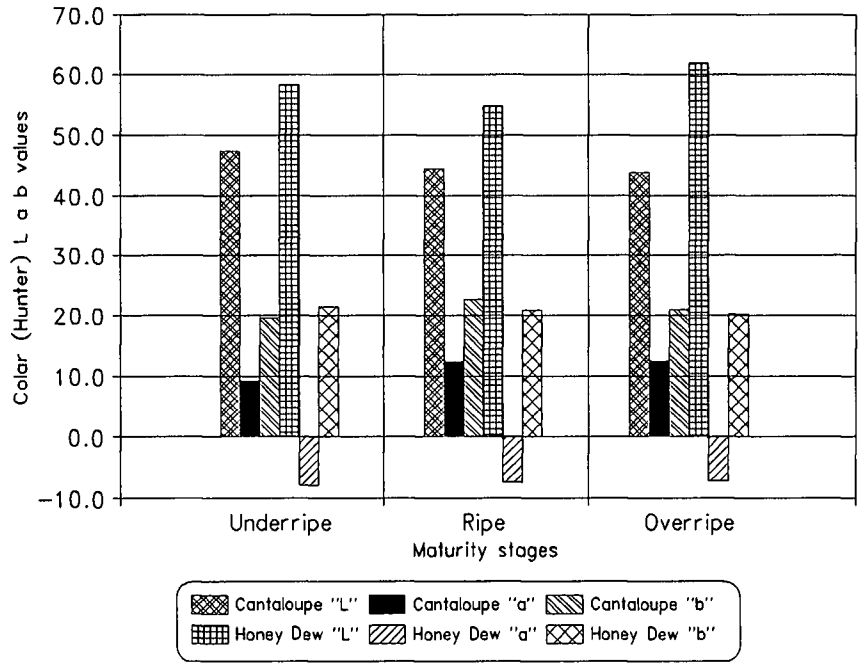


Figure 9. Color at different melon maturities (Hunter "L", "a", and "b" values).

Results summarized in Tables 4a and 4b (wherein proportions of neutral sugars are calculated as mg/g CWP) and Tables 5a and 5b (calculated as anhydro sugars in mmole%) indicate that arabinose, galactose, and rhamnose were the major components of the CDTA and Na_2CO_3 fractions, (subject to decrease as maturity was increased). These fractions also had the highest amount of uronic acid (Tables 4a and 4b). Thus, CDTA and Na_2CO_3 fractions were typical of pectic polysaccharide, results which are similar to the reports of Bartley and Knee (1982); Redgwell et al. (1990), and Selvendran and Ryden (1990).

Fucose, mannose and glucose were not detected in either the CDTA or Na_2CO_3 fractions. Moreover, though arabinose, xylose and galactose were found in all CWP fractions, arabinose was mostly found in the CDTA and Na_2CO_3 (pectin) fractions, whereas the greater part of the xylose and galactose were found in the hemicellulose and residue fractions.

Typical of hemicellulose components, xylose, glucose, galactose and mannose were the principal sugars found in the GTC and KOH fractions for both Cantaloupe and Honey Dew. This observation is similar to the reports of Goodwin and Mercer (1983), Fry, (1988), and O'Neill et al. (1990). The GTC fraction and the residue contained only a small amount of rhamnose, which was not detected in any quantity in the KOH fraction.

A moderate amount of pectic polymers were evidenced by the presence of uronic acid and galactose in both the GTC and KOH fractions. Uronic acids, rhamnose and arabinose were also detected in the residue (α-cellulose) fraction of both Cantaloupe and Honey Dew (Tables 4a and 4b). This may have been due to the presence of highly branched pectic materials, cross-linked with polysaccharide associated with hemicellulose and cellulose.

In the GTC and residue fractions, glucose content increased from the underripe to ripe stages of maturity, then decreased from the ripe to overripe stages for both Cantaloupe and Honey Dew. The glucose content of the KOH fractions did not change from the underripe to ripe stages, and decreased from ripe to overripe in Cantaloupe. For Honey Dew, there was a large decrease from the underripe to ripe stages and a nearly equal degree of change from ripe to overripe stages. Compared to GTC and KOH fractions, the residue fraction had the highest glucose content.

For Cantaloupe, total glucose in the TFA soluble fraction was highest, followed by xylose, galactose, and arabinose, in that order. For Honey Dew, total galactose was highest, followed by glucose and then xylose and arabinose. The amounts of TFA insoluble materials did not change as maturity increased for either melon cultivar, though the total sugars in TFA insoluble fraction of Honey Dew was slightly higher than for Cantaloupe.

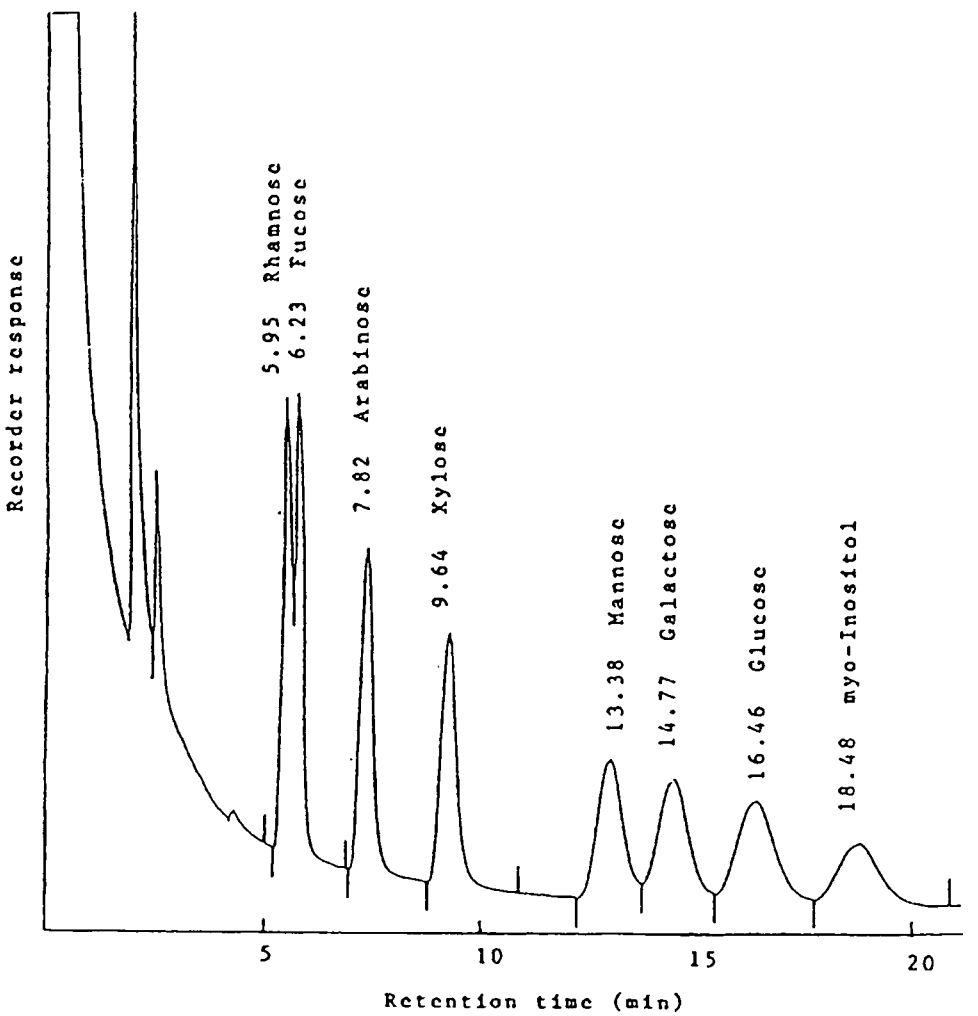


Figure 10a. Chromatograms of alditol acetate for standard sugars.

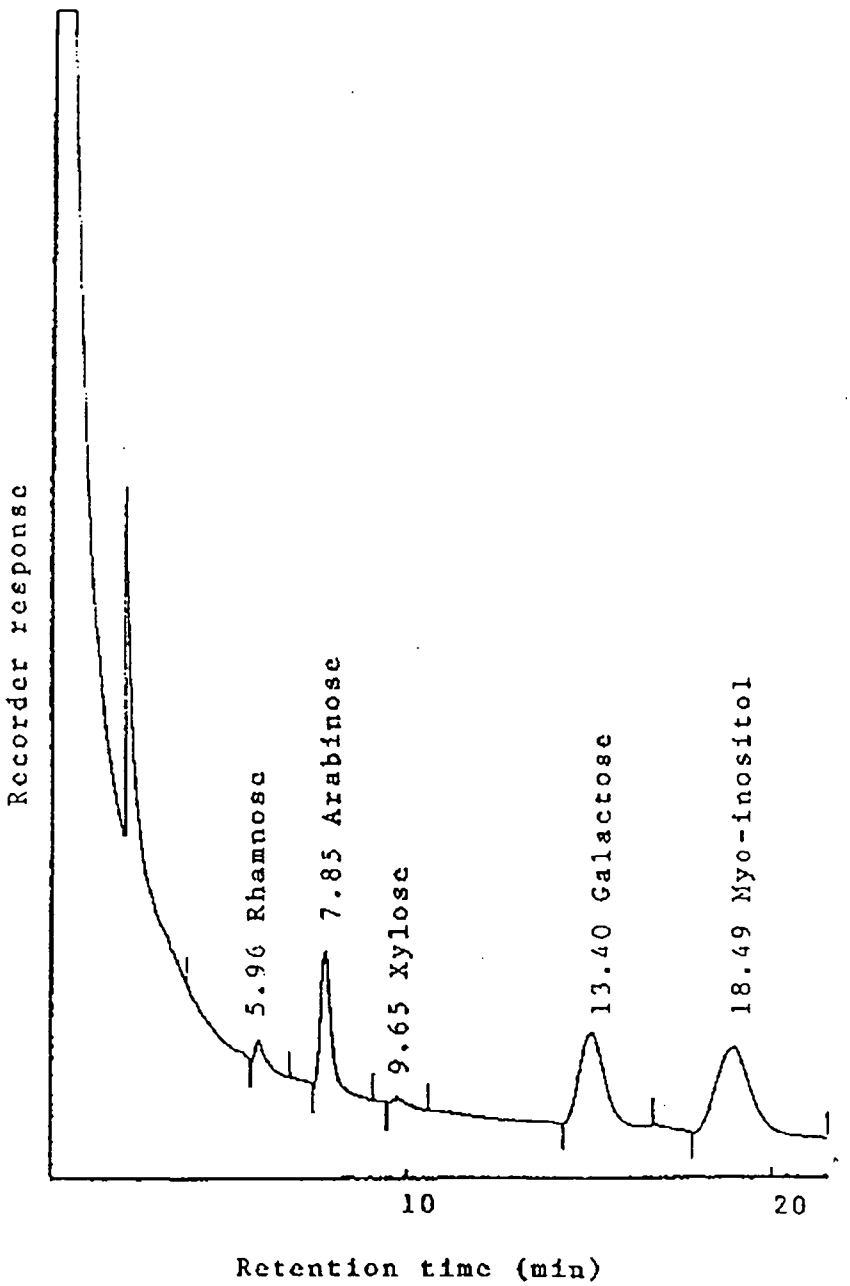


Figure 10b. Chromatograms of alditol acetate from CDTA (pectin) fraction sample.

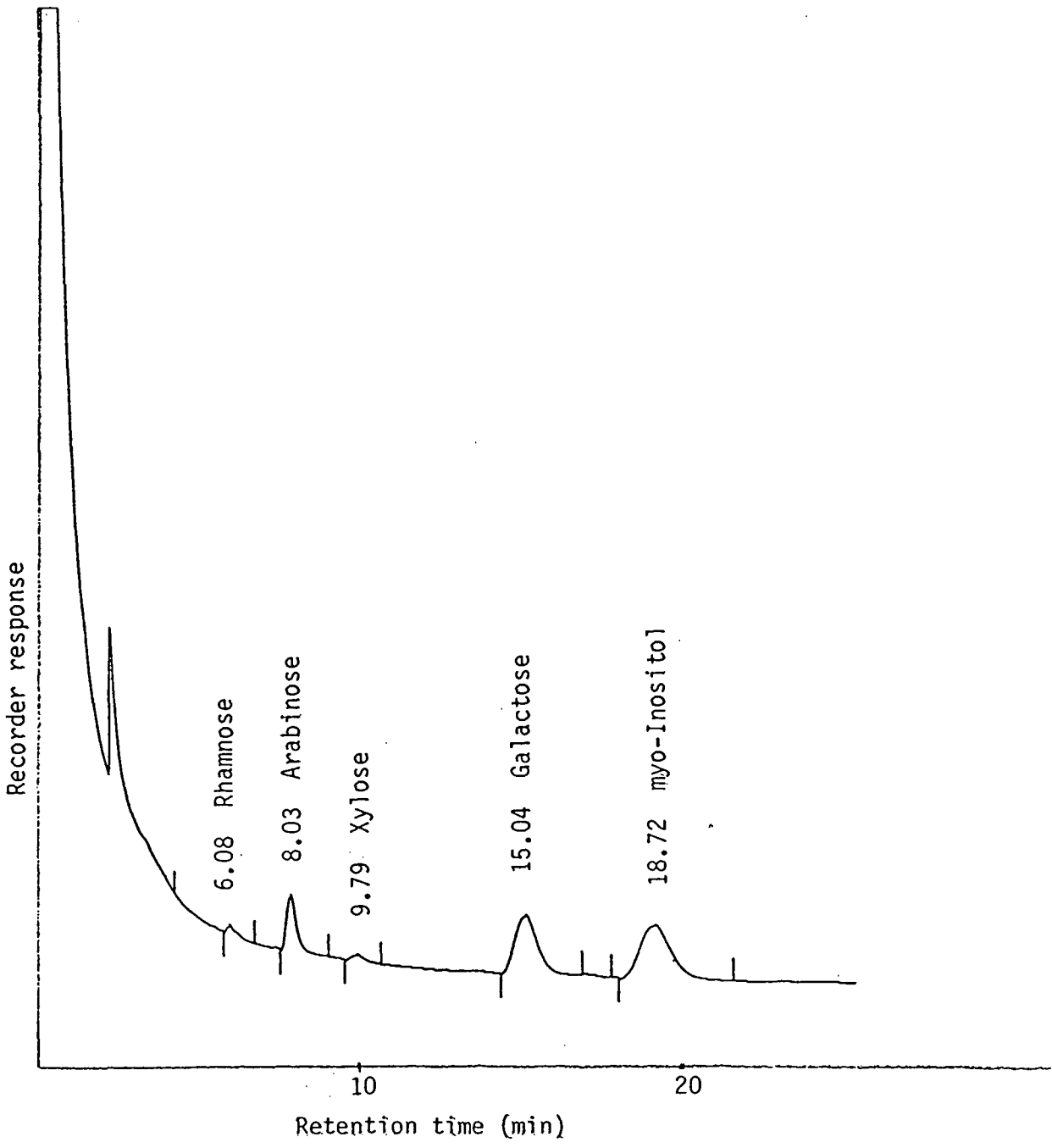


Figure 10c. Chromatograms of alditol acetate from Na_2CO_3 (pectin) fraction sample.

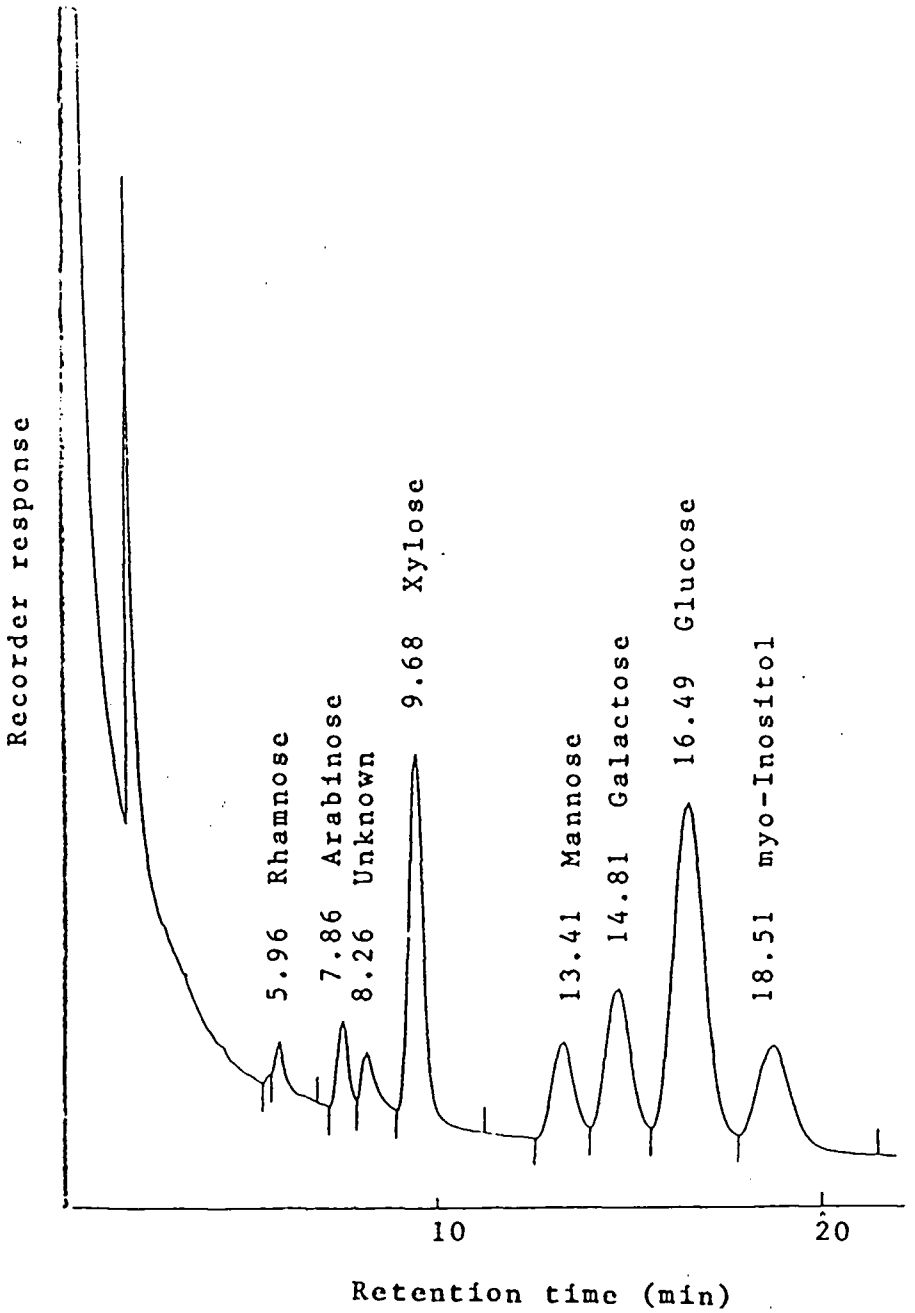


Figure 11a. Chromatograms of alditol acetate from GTC (hemicellulose) fraction sample.

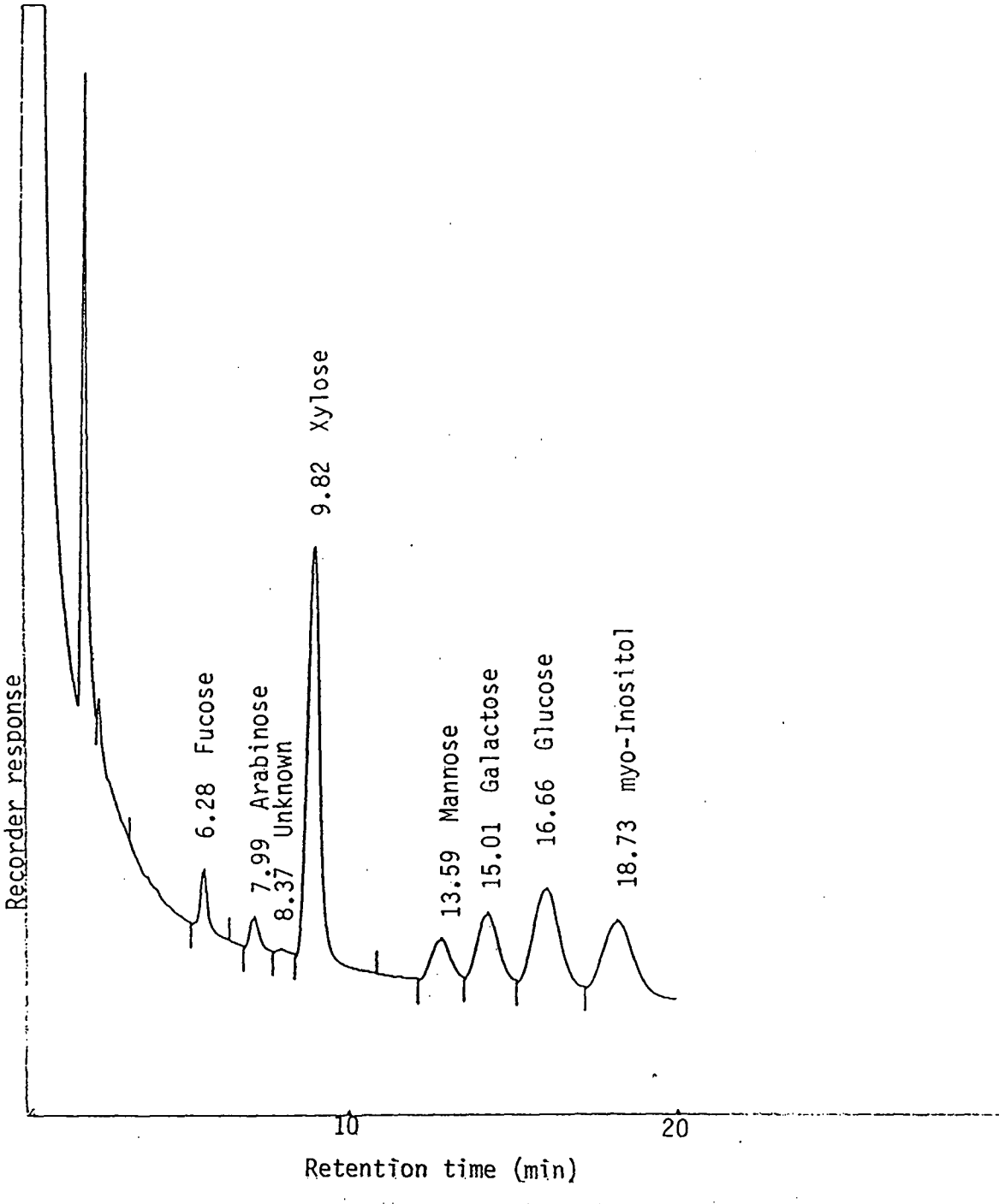


Figure 11b. Chromatograms of alditol acetate from KOH (hemicellulose) fraction sample.

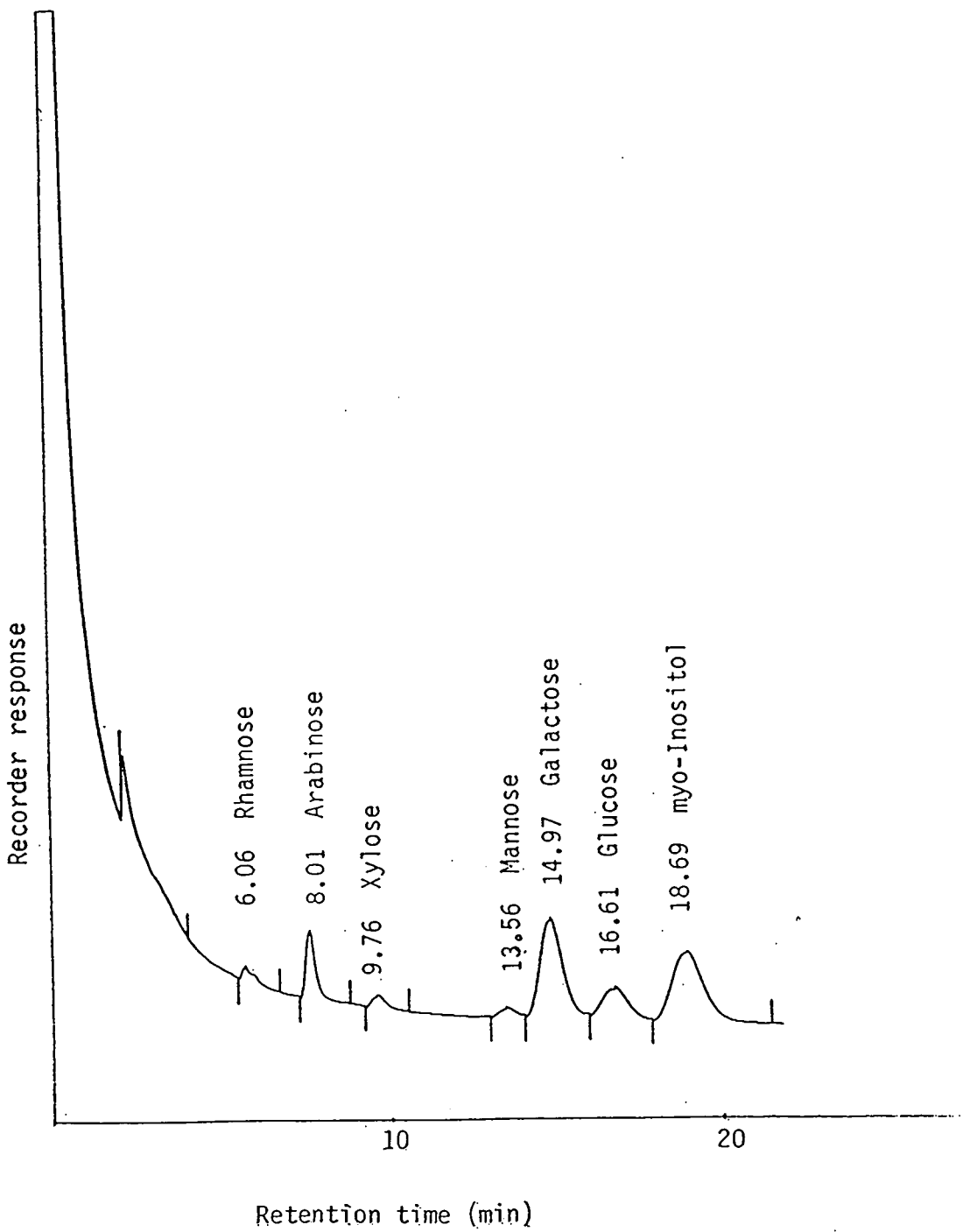


Figure 11c. Chromatograms of alditol acetate from residue fraction sample.

Unknown sugars, not previously reported in CWP literature, were observed in both Cantaloupe and Honey Dew. Since they were not one of the sugars used as standards during GC analysis, these sugars are classified as unknowns. In fact, the existence of other sugars in muskmelon is possible. According to York et al. (1985), many of the sugars that can be found in pectic polysaccharide are quite unusual, including 2-O-methyl fucose, 2-O-methyl-xylose, apiose, aceric acid (3-C-carboxy-5-deoxy-L-xylose), and 3-deoxy-D-manno-octulosonic acid (ketodeoxyoctulosonic acid (KDO)). These sugars are released complex RG-II as pectic polysaccharide from the primary cell walls of dicots, monocots and gymnosperms, including suspensions of cultured cells of sycamore (Acer pseudoplatanus), Black Mexican sweet corn, tomato, and soybean seedlings. The unknown sugars in the muskmelons were calculated as pentoses due to their retention times being between arabinose and xylose (both pentoses) and their area of the chromatogram effected the total area of sugar chromatograms.

Total TFA soluble Sugars

As the maturity of both Cantaloupe and Honey Dew increased, almost all sugars, including galactose, mannose, arabinose and rhamnose, decreased significantly ($p < 0.01$)

Table 4a. Sugar composition of CWP fraction (mg/g CWP) for different maturities of Cantaloupe

	CWP fractions	Neutral sugars in TFA soluble *								Total TFA soluble	TFA insoluble	Uronic acid	Total sugars
		Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unkn				
Underripe	CDTA	1.56	n	4.13	0.437	n	2.53	n	n	8.66	16.1	196	220
	Na_2CO_3	1.29	n	4.49	0.621	n	3.83	n	1.40	11.6	12.1	128	151
	GTC	0.253	0.921	1.51	10.9	1.82	3.72	5.36	n	24.5	3.35	25.8	53.6
	KOH	n	2.95	0.421	20.1	4.64	6.22	17.7	n	52.0	2.20	13.8	68.0
	Residue	1.02	t	2.88	2.24	2.59	6.40	16.2	n	31.3	316	88.8	437
	Total	4.13	3.87	13.4	34.3	9.06	22.7	39.3	1.40	128	350	452	930
Ripe	CDTA	1.05	n	3.67	0.600	n	2.49	n	n	7.81	14.6	222	245
	Na_2CO_3	0.704	n	3.12	0.584	n	1.56	n	1.01	6.98	7.95	107	122
	GTC	0.294	0.886	2.07	17.5	1.66	3.55	9.23	0.317	35.5	3.61	19.7	58.8
	KOH	n	2.25	0.343	20.2	3.57	5.54	17.7	n	49.6	2.47	11.1	63.1
	Residue	1.04	0.71	2.79	2.93	2.86	6.55	18.1	n	35.0	320	82.8	438
	Total	3.09	3.84	12.0	41.8	8.09	19.7	45.1	1.33	135	349	443	926
Overripe	CDTA	0.978	n	3.46	0.447	n	1.48	n	n	6.36	17.1	252	276
	Na_2CO_3	0.350	n	1.27	0.495	0.167	1.45	0.140	1.10	4.97	2.70	47.0	54.7
	GTC	0.343	0.50	0.897	5.02	1.82	2.45	5.50	n	16.5	1.54	15.9	33.9
	KOH	n	1.59	0.423	13.8	2.04	3.87	8.31	0.541	30.6	2.19	12.0	44.8
	Residue	0.808	1.39	2.26	2.48	2.05	6.70	12.5	n	28.2	313	75.0	416
	Total	2.48	3.48	8.31	22.2	6.07	15.9	26.4	1.64	86.6	336	402	825

* = The mean values for 4 injections; t = trace; n = not detectable; Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Unkn = Unknown sugar calculated as pentose.

Table 4b. Sugar composition of CWP fraction (mg/g CWP) for different maturities of Honey Dew

Honey Dew	CWP fractions	Neutral sugars in TFA soluble*								Total TFA soluble	TFA insoluble	Uronic acid	Total sugars
		Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unkn				
Underripe	CDTA	2.13	n	5.93	0.289	n	9.51	n	0.460	18.3	23.0	164	205
	Na_2CO_3	0.700	n	3.47	0.582	n	6.14	n	1.21	12.1	6.44	111	129
	GTC	t	1.18	1.53	7.59	3.57	8.07	13.5	n	35.5	2.09	20.1	57.7
	KOH	n	1.47	1.43	10.9	3.29	6.12	9.09	n	32.3	4.39	22.5	59.2
	Residue	0.519	t	4.49	1.93	2.73	15.7	18.9	n	44.3	362	101	507
	Total	3.35	2.64	16.9	21.3	9.58	45.6	41.5	1.67	142	398	418	958
Ripe	CDTA	1.45	n	5.14	0.951	n	7.86	n	n	15.4	28.5	191	235
	Na_2CO_3	0.306	n	1.33	0.195	n	2.49	n	n	4.32	5.01	60.7	59.1
	GTC	0.201	0.695	1.74	12.7	4.13	6.75	19.8	1.16	47.3	4.44	25.8	93.2
	KOH	n	0.465	0.459	7.59	1.31	2.39	3.41	0.107	15.7	1.92	7.40	25.1
	Residue	t	1.10	4.20	1.82	1.94	16.7	22.2	n	48.0	365	118	531
	Total	1.95	2.26	12.9	23.3	7.38	36.2	45.5	1.26	131	405	403	944
Overripe	CDTA	1.05	n	4.01	0.445	n	4.89	n	n	10.4	27.3	210	248
	Na_2CO_3	0.337	n	1.21	0.407	n	2.90	n	1.01	5.86	2.39	43.5	39.1
	GTC	0.053	0.535	0.870	8.45	1.08	3.86	9.44	0.050	24.3	1.99	18.8	57.7
	KOH	n	0.403	0.161	4.04	0.916	1.11	3.06	n	9.69	1.46	7.47	18.6
	Residue	t	1.19	3.24	3.93	3.79	11.6	21.9	n	45.7	364	117	527
	Total	1.44	2.13	9.49	17.3	5.78	24.4	34.4	1.06	96.0	397	397	890

* = The mean values for 4 injections; t = trace; n = not detectable; Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Unkn = Unknown sugar calculated as pentose.

Table 5a. Anhydro sugars (mmole %) for different maturities of Cantaloupe

	CWP fractions	Rham	Fuc	Ara	Xyl	Man	Gal	Gluc	Unkn
Underripe	CDTA	17.5	n	51.4	5.44	n	25.6	n	n
	Na_2CO_3	10.8	n	41.6	5.75	n	28.9	n	12.9
	GTC	1.03	3.73	6.75	48.7	6.66	13.6	19.6	n
	KOH	n	5.73	0.91	43.3	8.14	10.9	31.0	n
	Residue	3.46	t	10.8	8.44	7.95	19.6	49.7	n
Ripe	CDTA	13.1	n	50.7	8.27	n	28.0	n	n
	Na_2CO_3	9.80	n	48.0	8.99	n	19.5	n	13.7
	GTC	0.81	2.45	6.34	53.5	4.15	8.85	23.0	0.85
	KOH	n	4.58	0.77	45.4	6.55	10.2	32.5	n
	Residue	3.17	2.15	9.37	9.8	7.83	17.9	49.7	n
Overripe	CDTA	14.8	n	57.7	7.46	n	20.1	n	n
	Na_2CO_3	6.86	n	27.5	10.7	2.95	25.6	2.47	23.9
	GTC	2.12	3.11	6.13	34.3	10.1	13.6	30.6	n
	KOH	n	5.17	1.52	49.6	5.98	11.4	24.4	1.95
	Residue	3.04	5.22	9.42	10.3	6.95	22.7	42.3	n

* = Average of duplicate of fractionations. ** = The mean values from 4 injections.
Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose;
Gluc = glucose; Unkn = Unknown sugar calculated as pentose; n = not detected; t = trace.

Table 5b. Anhydro sugars (mmole %) for different maturities of Honey Dew

	CWP fractions	Rham	Fuc	Ara	Xyl	Man	Gal	Gluc	Unkn
Underripe	CDTA	11.8	n	36.3	1.77	n	47.4	n	2.18
	Na_2CO_3	5.80	n	31.9	5.34	n	45.9	n	11.1
	GTC	t	3.44	4.99	24.7	9.47	21.4	35.9	n
	KOH	n	4.62	4.97	38.0	9.32	17.4	25.8	n
	Residue	1.26	t	12.0	5.18	5.95	34.3	41.2	n
Ripe	CDTA	9.47	n	37.2	6.89	n	46.4	n	n
	Na_2CO_3	7.22	n	34.8	5.10	n	52.9	n	n
	GTC	0.44	1.52	4.19	30.7	8.11	13.3	39.0	2.79
	KOH	n	2.92	3.19	52.8	7.43	13.5	19.4	0.74
	Residue	t	2.46	10.4	4.51	3.92	33.7	45.0	n
Overripe	CDTA	10.2	n	42.7	4.74	n	42.4	n	n
	Na_2CO_3	5.76	n	22.8	7.69	n	44.6	n	19.1
	GTC	0.22	2.24	4.02	39.1	4.06	14.6	35.6	0.23
	KOH	n	4.18	1.85	46.4	8.57	10.4	28.6	n
	Residue	t	2.79	8.38	10.2	7.99	24.6	46.1	n

* = Average of duplicate of fractionations. ** = The mean values from 4 injection.
Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Gluc = glucose; Unkn = Unknown sugar calculated as pentose; n = not detected; t = trace.

(Tables 6 and 7). The exceptions were that xylose and glucose increased from the underripe to ripe, then decreased from the ripe to overripe stages. Since these sugars are components of both pectin and hemicelluloses fractions, these observation therefore suggest that both fractions of the melons were modified, solubilized, or degraded as maturity increased.

Total fucose did not change from the underripe to the ripe stages in Cantaloupe, whereas for Honey Dew fucose was subject to decrease. When sugars decreased, the effect was more obvious from the ripe to overripe stages than for the underripe to ripe stages. Gross and Sams (1984) reported only a decrease in galactose and arabinose in muskmelon and squash cell walls during maturation and ripening, while mannose, fucose, and rhamnose either remained constant or increased as melon maturity was increased.

The percentages of TFA soluble total sugars at each maturity stage for Cantaloupe and Honey Dew are shown in Table 8. Total sugars decreased in percentage terms from the underripe to ripe stages in both cultivars, then increased from the ripe to overripe. However, total CWP sugar weight decreased for both melons as maturity increased (Tables 4a and 4b).

Changes in the percentages of xylose and glucose were different for the two cultivars (Table 7). In Cantaloupe, there was a percentage increase from underripe to ripe,

then a decrease from ripe to overripe. In Honey Dew, however, percentages consistently decreased as maturity increased. On a weight basis, both xylose and glucose increased from underripe to ripe, then decreased from ripe to overripe for both melon cultivars.

TFA Insoluble Fractions

TFA insoluble fractions were analyzed to facilitate recovery calculations of CWP fractions. It was then possible to calculate total sugars (Table 4a and 4b) by adding together TFA soluble, TFA insoluble and uronic acid. Following the modified procedure of Dubois et al. (1956), total sugars in the TFA insoluble fractions were determined by the phenol-sulphuric acid method, using glucose as a standard. A standard glucose curve in the range 5-25 μg is shown in Figure 6. The sugars found in the TFA insoluble fraction was much larger than the sugars found in the TFA soluble fraction (which was 10 to 14% of the CWP weight). Total sugars in the TFA insoluble fractions for Cantaloupe and Honey Dew did not change as maturity increased (Tables 4a and 4b).

Generally, total sugars from TFA insoluble fractions were higher in Honey Dew than for Cantaloupe at the same level of maturity. This suggests that TFA insoluble materials may also contribute to the higher firmness of the

Table 6. F-ratios from two-way ANOVA (effect of cultivar and maturity on sugar composition of CWP of melon)

Source of error	d.f.	Neutral sugars								TFA insoluble	Uronic acid
		Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unk		
C	1	13.2 *	14.7 *	2.53	50.3 *	0.115	241 *	21.0 *	0.123	175 *	58.2 *
M	2	14.3 *	1.05	18.6 *	19.6 *	16.8 *	61.9 *	43.3 *	0.138	2.27	175 *
C*M	2	2.81	0.199	3.38	5.52 **	0.574	3.17	2.10	2.60	0.708	101 *
M.S. error	6	0.009	0.0172	0.0577	0.347	0.0274	0.126	0.159	0.019	2.07	1.06

C = Cultivar; M = Maturity. * = F-ratios are significant at $p < 0.01$; ** = F-ratios are significant at $p < 0.05$.

Table 7. Percentage decrease of total sugars during maturation

Cantaloupe from stage:	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Unk	TFA insoluble	Uronic acid
Underripe-->Ripe	25.2	0.775	10.4	21.9 *	10.7	13.2	12.9 *	5	0.286	2
Ripe-->Overripe	19.7	9.38	30.8	46.9	25	19.3	41.7	23.3 *	3.72	9.3
Honey Dew from stage:										
Underripe-->Ripe	41.8	14.4	23.7	9.39 *	23	20.6	14.8 *	24.6	2 *	2
Ripe-->Overripe	26.2	5.75	26.4	25.8	21.7	32.6	41.7	15.9	2	2

* = increase.

Table 8. Percentage of neutral sugars in TFA soluble of CWP of melons different maturities

Cantaloupe	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Unk
Underripe	3.22	3.02	10.5	26.8	7.07	17.7	30.6	1.09
Ripe	2.29	2.85	8.89	31.0	6.00	14.6	33.4	0.98
Overripe	2.86	4.02	9.59	25.7	7.01	18.4	30.5	1.90
Honeydew								
Underripe	2.35	1.85	11.8	15.0	6.72	32.0	29.1	1.17
Ripe	1.49	1.73	9.85	17.8	5.65	27.7	34.8	0.97
Overripe	1.51	2.22	9.88	18.0	6.02	25.4	35.8	1.10

** = The mean values from 4 injections.
Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose;
Man = mannose; Gal = galactose; Gluc = glucose; Unk = unknown.

Honey Dew (Table 1). However, there were no significant correlations between TFA insoluble fractions and firmness as maturity progressed.

Uronic Acids in CWP Fractions

The modified method of Selvendran et al. (1979) was used to hydrolyze CWP fractions. Using a spectrophotometer, uronic acid analysis was conducted according to the modified method of Blumenkrantz and Asboe-Hansen (1973). The standard curve for uronic acid is shown in Figure 7. The CDTA fraction, followed by the Na_2CO_3 and residue fractions, had the highest uronic acid content for the underripe and ripe stages of Cantaloupe and for the underripe of Honey Dew, whereas CDTA followed by the residue and Na_2CO_3 fractions was highest in uronic acid content in the overripe stage of Cantaloupe, in ripe and overripe of Honey Dew (Tables 4a and 4b).

McCollum et al. (1989) determined that the total polyuronides in ethanol insoluble powder (EIP, similar to AIR) from the cultivar Galia muskmelon for preripe, ripe and overripe stages were only approximately one-half of the total uronic acid measured in this study. Values for both studies were obtained using the Blumenkrantz and Asboe-Hansen (1973) method. The difference in these results may be due to different methods of isolating cell wall materials. Total uronic acids were determined by McCollum et al.

(1989) from extractions based upon an Na-acetate buffer containing 20 mM sodium ethylenediaminetetra acetate (Na_2-EDTA) at pH 5.0 and incubated for 24 hours at 23°C. For this study, total CWP uronic acids were drawn from all five CWP fractions measured.

Uronic acids in the CDTA fractions increased, but decreased for Na_2CO_3 fractions, whereas total CWP uronic acids did not change as maturity progressed for Honey Dew melon and not change in total uronic acid of Cantaloupe from underripe to ripe, but decrease from ripe to overripe. However, changes in total uronic acid in the Honey Dew were less obvious (Tables 4a, 4b, and 7). This observation is slightly different to that of Ahmed and Labavitch (1980) for ripening pear fruits. It was observed that the uronic acid content of fruits declined sharply as the fruit softened, suggesting that the initial metabolism of cell wall pectic polysaccharide caused a decrease in tissue firmness.

For the GTC and KOH fractions, compared to the other fractions, the amount of uronic acids were lower for both melons as maturity increased. The uronic acid in the KOH fractions of Cantaloupe increased from the underripe to ripe, then decreased in the overripe stage, whereas uronic acid in the KOH fractions increased as maturity increased in the Honey Dew.

The anhydro uronic acid/anhydro sugar ratios are shown in Tables 9a and 9b, indicating that uronic acid/xylose was very large in the CDTA and Na_2CO_3 fractions. This suggests that for each 335 mmole of uronic acid, there was only one mmole of xylose in the pectic polysaccharide. The anhydro uronic acid/anhydro neutral sugars ratios were calculated to find the nearest number of molecules in the real polysaccharide. To form a polymer, each molecule of sugar has less than one molecule of H_2O.

The uronic acid/arabinose ratio was the smallest in CDTA and Na_2CO_3 fractions, and the uronic acid/galactose and uronic acid/rhamnose ratios were higher for all maturity stages of Cantaloupe. In contrast, in Honey Dew the uronic acid/galactose ratio was the lowest, followed by uronic acid arabinose and uronic acid/rhamnose ratios. The uronic acid/xylose and uronic acid/glucose ratios were the lowest in both GTC and KOH (hemicellulose) fractions, then increased for uronic acid/galactose ratios, followed by uronic acid/mannose, uronic acid/fucose and uronic acid/arabinose ratios for both cultivars of melons. These results show that uronic acid was present in hemicellulose fractions, and changes in the uronic acid/sugar ratios also suggest that modification in the CWP of both melons occured as maturity increased.

Relationship Between Firmness and Total Sugars

The relationship between changes in firmness and the total sugar composition of CWP as maturity increased can be seen in Figure 12a through 12f. Firmness was inversely related to drip loss in both Cantaloupe and Honey Dew as maturity increased. With increased maturity, firmness decreased and drip loss increased as rhamnose, fucose, arabinose, mannose and galactose also decreased. This suggests that both pectin and hemicellulose were solubilized from muskmelon CWP as softening occurred. Prior to solubilization, polysaccharide degradation into smaller molecules may have occurred. It is likely that these molecules were discarded during the isolation and purification of CWP, causing a decrease in sugars as maturity increased.

Cell Wall Polysaccharide Fraction Yields

There were differences in CWP fraction yields between Cantaloupe and Honey Dew (Table 10). Analysis of variance indicated that the interaction between cultivars and maturity were significant at $p < 0.01$ (Table 11). Thus, in Cantaloupe the CDTA fraction (pectin) yield increased by 10% from underripe to ripe stages and by 14% from the ripe to overripe stages, whereas comparative increases for the Honey Dew melon were 21% and 33%. The increase in CDTA fraction yields suggests that modification and

Table 9a. The mmole anhydro uronic acid/mmole anhydro sugar ratios in CWP fractions of different maturities of Cantaloupe

Maturity stages:	CWP fractions	Rham	Fu	Ar	Xyl	Man	Gal	Gluc	Unk
Underripe	CDTA	104	n	35	335	n	71	n	n
	Na_2CO_3	82	n	21	154	n	31	n	68
	GTC	84	23	13	2	13	6	4	n
	KOH	n	4	25	1	3	2	1	n
	Residue	72	t	23	30	32	13	5	n
	CDTA	176	n	45	278	n	82	n	n
Ripe	Na_2CO_3	126	n	26	137	n	63	n	90
	GTC	55	18	7	1	11	5	2	53
	KOH	n	4	24	1	3	2	1	n
	Residue	66	97	22	21	27	12	4	n
	CDTA	214	n	55	423	n	157	n	n
Overripe	Na_2CO_3	112	n	28	71	259	30	310	32
	GTC	38	26	13	2	8	6	3	n
	KOH	n	6	21	1	5	3	1	17
	Residue	77	45	25	23	34	10	6	n

* = Values shows number of mole anhydro uronic acid for each mole anhydro sugars in CWP fractions.

Rham = rhamnose; Fuc = fucose; Ara= arabinose; Xyl = xylose;
Man = mannose; Gal = galactose; Gluc = glucose; Unk = unknown;
t = trace amount of sugars; n = not detectable.

Table 9b. The mmole anhydro uronic acid/mmole anhydro sugar ratios in CWP fraction of different maturities of Honey Dew

Honeydew	CWP fractions	Rham	Fuc	Ara	Xyl	Man	Gal	Gluc	Unk
Underripe	CDTA	135	n	21	n	n	16	n	267
	Na_2CO_3	132	n	24	n	n	17	n	69
	GTC	t	14	10	2	5	2	1	n
	KOH	n	13	12	2	6	3	2	n
	Residue	161	n	17	39	34	6	5	n
Ripe	CDTA	110	n	28	151	n	22	n	n
	Na_2CO_3	165	n	34	233	n	22	n	n
	GTC	106	31	11	2	6	4	1	17
	KOH	n	13	12	1	5	3	2	52
	Residue	t	90	21	49	56	7	5	n
Overripe	CDTA	165	n	39	354	n	39	n	n
	Na_2CO_3	107	n	27	80	n	14	n	32
	GTC	296	29	16	2	16	4	2	282
	KOH	n	15	35	1	8	6	2	n
	Residue	t	82	27	22	29	9	5	n

* = Values shows number of mole anhydro uronic acid for each mole anhydro sugars in CWP fractions.
Rham = rhamnose; Fuc = fucose; Ara= arabinose; Xyl = xylose;
Man = mannose; Gal = galactose; Gluc = glucose; Unk = unknown;
n = sugar was not detected;t = trace amount of sugars.

Table 10. Yields of CWP fraction for melons at different maturities

CWP Fraction	Underripe			Ripe			Overripe		
	mean ** (mg/g CWP)	stdv (mg)	mean (%)	mean ** (mg/g CWP)	stdv (mg)	mean (%)	mean ** (mg/g CWP)	stdv (mg)	mean (%)
Cantaloupe:									
CDTA	195	2.58	19.5	215	4.13	21.5	246	5.10	24.6
Na_2CO_3	145	4.25	14.5	113	2.26	11.3	89.2	3.03	8.92
GTC	112	2.77	11.2	79.5	2.49	8.0	68.3	4.38	6.83
KOH	128	2.41	12.8	131	2.88	13.1	138	4.80	13.8
Residue	461	12.1	46.1	499	13.4	49.9	504	16.8	50.4
Honey dew:									
CDTA	186	6.07	18.6	225	7.69	22.5	300	3.79	30.0
Na_2CO_3	132	2.90	13.2	82.5	2.74	8.25	83.6	2.56	8.36
GTC	76.8	2.74	7.68	136	3.61	13.6	73.8	3.79	7.38
KOH	168	6.50	16.8	78.5	2.50	7.85	89.3	2.93	8.93
Residue	466	15.9	46.6	516	17.3	51.6	515	13.2	51.5

** = From duplicate of fractionations.

solubilization of pectins occurred as the maturity of both melons increased.

The Na_2CO_3 (CDTA insoluble pectins) fractions for Cantaloupe decreased by 22% from underripe to ripe and by 21% from ripe to overripe, whereas the fraction decrease for Honey Dew was 37.1% from underripe to ripe with no change from the ripe to overripe stages of maturity. The decrease in Na_2CO_3 (CDTA insoluble pectins) fraction yields occurred because more pectins were isolated by CDTA as maturity increased.

The Na_2CO_3 soluble pectins have a more highly branched rhamnogalacturonan backbone than the CDTA soluble pectins (Redgwell et al., 1988). Contrary to the report of Redgwell et al. (1991), CDTA soluble pectins decreased and the Na_2CO_3 soluble pectins increased during ripening of kiwifruits. Huber and Lee (1986), without conducting the Na_2CO_3 extraction for acetate-EDTA insoluble pectins, reported an increase in acetate-EDTA soluble pectins in AIR from tomato pericarp as maturity increased.

The guanidinium thiocyanate (GTC) (hemicellulose) fraction decreased as maturity increased in the Cantaloupe, whereas Honey Dew was subject to an increase of 77% from underripe to ripe, followed by a decrease of 45% from ripe to overripe. In turn, the KOH (GTC insoluble hemicellulose) fraction did not change in the Cantaloupe as maturity increased, whereas there was a decrease of 52% between the

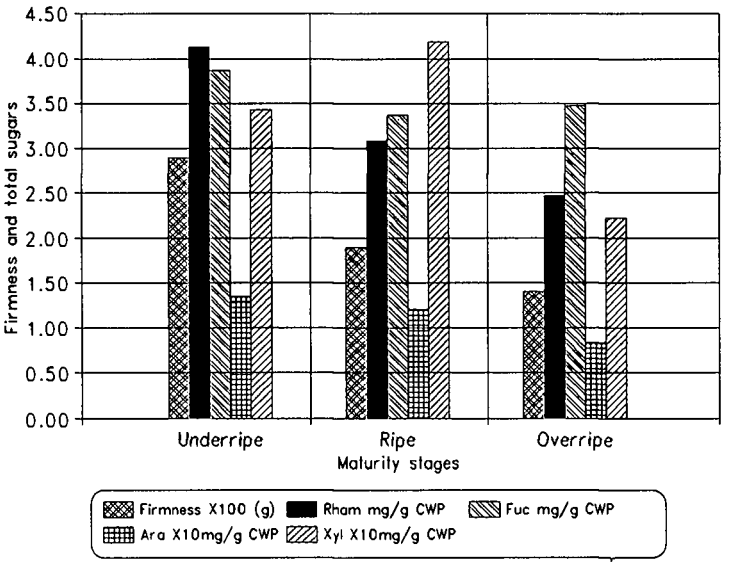


Figure 12a. Relationship of firmness (X100) to total sugars for Cantaloupe at different maturities.

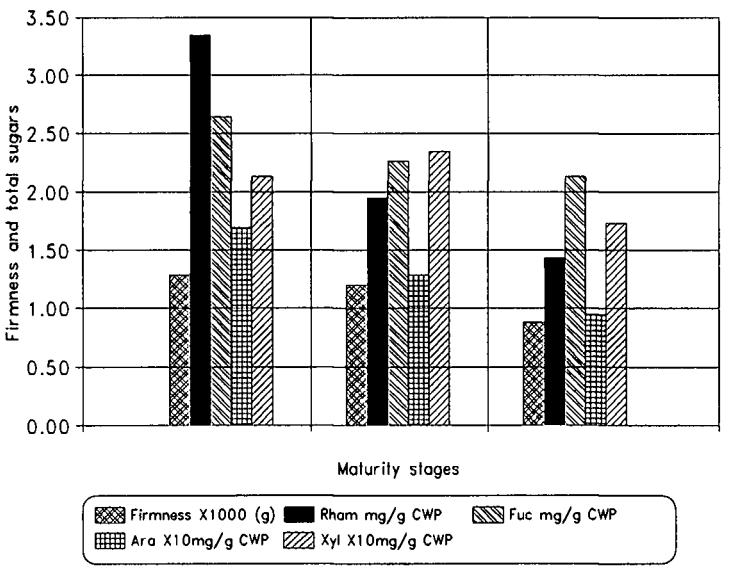


Figure 12b. Relationship of firmness (X1000) to total sugars for Honey Dew at different maturities.

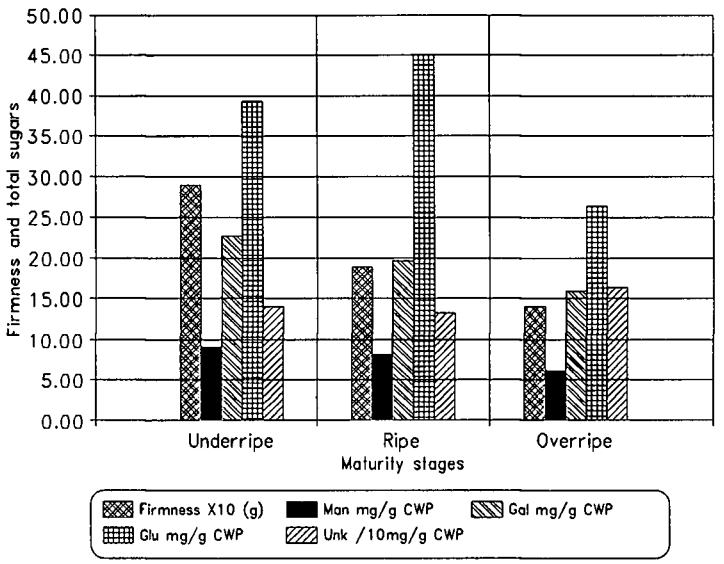


Figure 12c. Relationship of firmness (X10) to total sugars for Cantaloupe at different maturities.

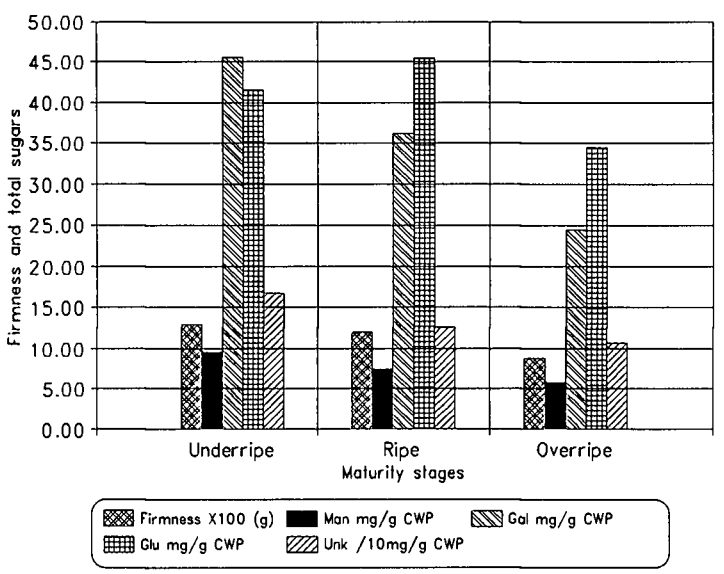


Figure 12d. Relationship of firmness (X100) to total sugars for Honey Dew at different maturities.

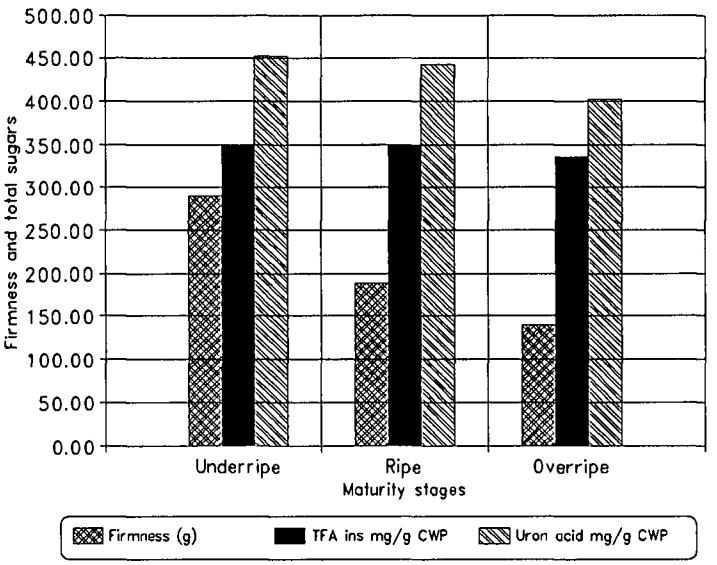


Figure 12e. Relationship of firmness to total sugars for Cantaloupe at different maturities.

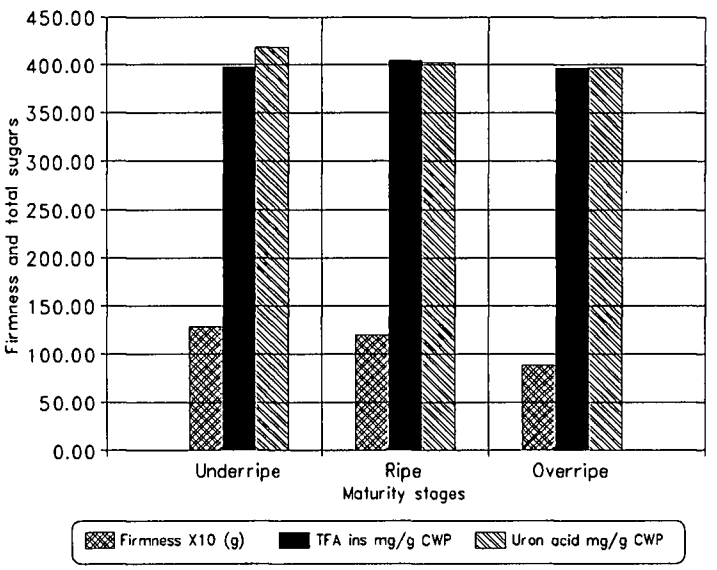


Figure 12f. Relationship of firmness (X10) to total sugars for Honey Dew at different maturities.

Table 11. F-ratios from two-way ANOVA (effect of cultivar and maturity on the CWP fraction for melons)

Source of error	d.f.	CWP fraction				
		CDTA	N_a2CO_3	GTC	KOH	Residue
C	1	36.8 *	87.8 *	21 *	75.7 *	1.67
M	2	262 *	322 *	123 *	133 *	11.9 8 *
C * M	2	37.9 *	18.5 *	188 *	175 *	0.158
MS-Error	6	26.8	9.16	11.3	15.8	222.3

C = Cultivar; M = Maturity; * F-ratios are significant at P<0.01.

underripe and ripe stages for the Honey Dew, followed by an increase of 13.7% from the ripe to overripe stages of maturity. This suggests that there was a difference between Cantaloupe and Honey Dew for hemicellulose, chemical properties and responses to GTC and KOH solutions as maturity progressed. The increase in the hemicelluloses solubilized by GTC and the decrease of KOH fraction in Honey Dew from underripe to ripe suggests that modification and solubilization of hemicelluloses occured when the ripe stage had been reached, whereas in Cantaloupe degradation to a smaller molecular weight may have occured after the solubilization of the hemicelluloses, as maturity increased (Huber, 1983; Redgwell et al., 1991). For the same reason, from ripe to overripe in Honey Dew, hemicelluloses may have been degraded, resulting in the decrease of GTC soluble hemicelluloses.

The KOH soluble hemicelluloses did not change in Cantaloupe as maturity increased, whereas in Honey Dew the decrease of KOH soluble hemicelluloses occured from underripe to ripe, and then increased from ripe to overripe. During ripening of kiwifruits, Redgwell et al. (1991) reported that both GTC and KOH soluble hemicelluloses increased. Huber and Lee (1986) reported a trend of consistent decrease in high-molecular-weight and increase in low-molecular-weight polymers for 4 M NaOH soluble hemicelluloses during the development of tomato fruits from immature green to ripe

(i.e., five maturity stages, including immature green, mature green, turning, pink, and ripe). Extraction with GTC was not performed and hemicellulose yield was not reported.

Strong alkali, such as 4 M KOH, serves to disrupt the hydrogen bonds between hemicellulose and α-cellulose, thereby solubilizing the bulk of the xyloglucans and glucomannans from melon CWP. To minimize degradation of the hemicelluloses, an oxygen-free solution was used during extraction (i.e., by stirring under nitrogen gas), and 20 mM $NaBH_4$ was added to reduce the latent aldehyde group. Alkali also hydrolyzed ester linkages between polysaccharide and such non-carbohydrate components as the phenolic acids.

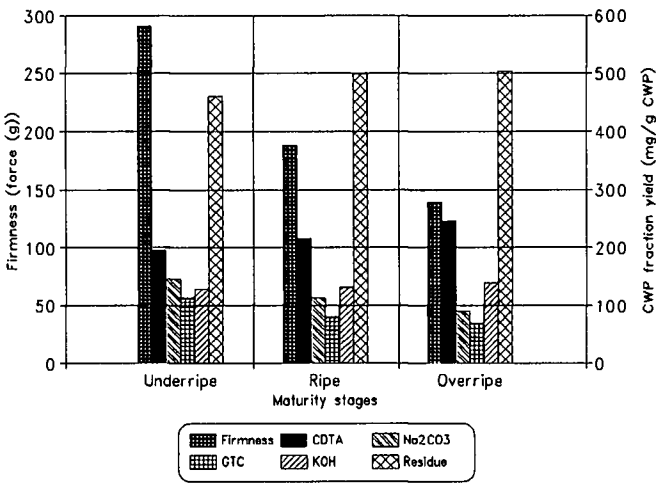
There was a slight increase in the weight of the residue fractions from underripe to the ripe stages, at 8.2% and 10.7%, respectively, for Cantaloupe and the Honey Dew. There were no further changes from the ripe to overripe stages. Though the residue (α-cellulose) fraction in the Honey Dew was only slightly higher than in Cantaloupe, this could have contributed to the higher firmness of the Honey Dew melons (Table 1). As previously noted, cellulose has the function of providing rigidity and resistance to tearing, while pectins and hemicelluloses contribute to plasticity and the ability to stretch (Van Boeren, 1979).

CWP were not fractionated with distilled water due to the fact that most water soluble pectins had been removed during the process of isolating and purifying CWP. The

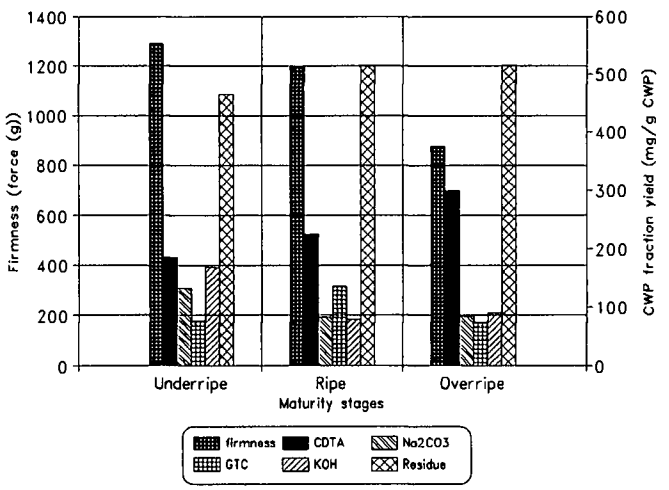
release of water soluble polysaccharide prior to the CDTA extraction seems to have been caused by modifications of the cell wall during drying and rehydration (Redgwell et al., 1990). In this study, it was determined that the use of 0.05 M CDTA solubilized the water-soluble as well as chelator-soluble pectins, resulting in an increase of the CDTA fraction as maturity increased. In subsequent extractions with 0.05 M Na_2CO_3 (containing 20 mM $NaBH_4$) at 2°C, pectins were deesterified, thus minimizing degradation by ß-elimination (Fry, 1988; O'Neill et al., 1990).

The firmness of both Cantaloupe and Honey Dew was inversely related to CDTA fraction yields (Figure 13); that is, when the firmness of Cantaloupe and Honey Dew decreased, the CDTA fractions increased. Increase in CDTA fraction yield could have been due to the degradation and solubilization of pectins, which increased as maturity increased.

In addition, the polysaccharide extracted from both Cantaloupe and Honey Dew with 6 M GTC and 4 M KOH proved useful for the extraction of the mannose rich polymers glucomannan, xylan, and galactan from melon CWP.



a) Cantaloupe



b) Honey Dew

Figure 13. Relationship between firmness and CWP fraction yields for different maturities.

Cell Wall Polysaccharide Yields

As shown in Table 12, CWP yields for Honey Dew were higher than for Cantaloupe. Dry CWP yields for Cantaloupe were 0.50%, 0.49%, and 0.49% of fresh weight for the three respective stages of maturity (underripe, ripe, overripe), whereas those for the Honey Dew were slightly higher (0.56%, 0.52%, and 0.51%).

Table 12. CWP yields at different maturities (g/1000 g of fresh melon disks).*

Cultivar	Underripe (g)	Ripe (g)	Overripe (g)
Cantaloupe	4.95	4.92	4.84
	(0.0518)**	(0.0597)**	(0.0558)**
Honey Dew	5.59	5.20	5.09
	(0.0567)**	(0.0577)**	0.0969

* = quadruplicate isolation yields; ** = standard deviation.

There were no significant changes in CWP yields as maturity increased for either fruit (Table 13). Since cellulose has the function of providing rigidity and resistance to tearing, whereas the pectins and hemicelluloses contribute to plasticity and the ability to stretch, higher CWP yields for the Honey Dew melon was a likely contributor to its higher firmness, especially since approximately 50% of the CWP consists of α-cellulose (Fig. 13a, 13b, and Table 10).

Table 13. Effect of maturity and cultivars on CWP melon yields *.

Source of error	d.f.	CWP
C	1	4.52
M	2	1.17
C * M	2	0.575
M.S. Error	6	1.29

C = Cultivar; M = Maturity; * = F-ratios from two-way ANOVA, not significant.

Huber (1991) reported that ca. 11 to 13 mg of cell wall material was obtained from 1 g (1.1% to 1.3%) of pericarp tomato fruit using boiling ethanol as the extractive substance. Buerger (1986) used an acetone extraction to prepare acetone insoluble solids (AIS) as cell wall materials. The AIS was prepared from three varieties of freeze-dried strawberry powder and from three varieties of fresh strawberries. For the freeze-dried strawberries, there were AIS yields in the range of 13.8 to 19.2%. From the edible portions of muskmelon, Dinus (1967) obtained AIR in a range of from 0.75 to 1.66%. The higher AIR yields compared to CWP yields from analyses conducted for this study may have been due to differences in isolation methods and in the fruit types used. Proteins, RNA, and phenolics are precipitated using alcohol to isolate cell wall materials (Fry, 1988; Selvendran and O'Neill, 1987).

Characteristic of Melon Disks Placed in Frozen Storage

An investigation of the effect of frozen storage time on the composition, drip loss, and color of frozen ripe melon disks was conducted. The CWP compositions of ripe melon disks were analysed following periods of 5 and 10 months of frozen storage.

Effect of Frozen Storage Upon Composition, Drip Loss, and Color

The results of composition, drip loss and color analysis of frozen melon disks are summarized in Table 14. Firmness and moisture content measures were not conducted due to the possibility of moisture penetration from the environment and of crystal formation, which would have caused incorrect measurements of firmness when using the penetrometer. However, drip loss determination, which has an inverse relationship to firmness, was performed. Figure 14a and 14b show the relationships between drip loss and compositon, and Figure 15 demonstrates relationships between color ("L", "a", "b" values) measurements and storage time. Relationships between total sugars in CWP fraction as well as the CWP fractions yields in relation to drip loss (i.e., as an indicator of firmness), were also determined (Figures 16a-16f, Figures 17a, and 17b).

pH and Titratable Acidity

During frozen storage of ripe melon disks, pH decreased and titratable acidity (TA) increased significantly ($p<0.01$) as storage time was increased from 0 to 5 and from 5 to 10 months for both Cantaloupe and Honey Dew melon disks (Table 14 through Table 16). This observation was contrary to pH trends observed during ripening of the two melons (Table 1). Reddy (1986) also reported that TA was significantly higher ($p<0.05$) at 6 and 12 weeks of frozen storage, compared to the TA at zero week storage.

BeMiller (1986) stated that pectins achieved maximum stability at about pH 4.0. Below or above this level, deesterification and depolymerization occurred concurrently. This has been found to occur during periods of prolonged frozen storage of melons with pH > 6.0. The solubilization of polyuronides during homogenization to a melon puree prior to the measurement of pH and deesterification may have contributed to the lower pH readings. Increased in the TA (or decreased in pH) might also be attributed to the dehydration during prolonged frozen storage, if packages were not impermeable or ice crystal formation on packaging of frozen melon disks.

Table 14. Composition, firmness, and color of melons disks in different frozen storage time

Cultivar	Month	pH	TA % citric	SS % °Brix	Drip loss	Firmness g (force)	Moisture (%)	Color		
								"L"	"a"	"b"
Cantaloupe	0	6.48	0.05	9.20	12.9	189	91.4	44.4	12.3	22.7
	5	6.26	0.07	9.30	15.1	-	-	54.6	12.0	21.6
	10	6.09	0.09	9.45	17.2	-	-	55.0	9.89	19.5
Honey Dew	0	6.16	0.08	11.0	11.2	1200	89.2	54.9	-7.44	20.8
	5	5.80	0.09	10.7	12.1	-	-	64.3	-7.11	20.8
	10	5.76	0.14	10.6	14.9	-	-	64.9	-6.67	19.8

Table 15. Effect of storage time on the composition, firmness, and color: F-ratios from two-way ANOVA

Source of error	d.f.	pH	TA	SS	Drip loss	Color:		
						"L" value	"a" value	"b" value
C	1	195 *	65.6 *	83.2 *	62.6 *	620 *	75258 *	21 *
ST.	2	79.3 *	58.2 *	10.8 *	452.8 *	280 *	69.6 *	48.3 *
C * ST	2	2.69	2.77	2.22	14.7 *	0.354	213 *	13.3 *
M.S.-Error	6	0.0021	4.58	0.03	0.04	0.486	0.014	0.098

C = Cultivar; ST = Storage Time; * F-ratios are significant at P<0.01.

Table 16. Effect of frozen storage time on percentage changes in composition, firmness, and color of melons

					Color		
Cantaloupe	pH	TA	SS	Drip loss	"L"	"a"	"b"
0 → 5 months	3.5**	40*	1.10	17.4*	23*	2.42**	4.85**
5 → 10 months	10.2 **	28.4*	1.6	13.3 *	0.733 *	17.6 **	9.72**
Honey Dew							
0 → 5 months	5.84 **	12.5 *	2.73	8.52 *	17.1 *	4.44 *	0
5 → 10 months	0.69 **	55.6 *	0.93	23.2 *	0.933 *	6.19 *	4.81 **

* = % increase; ** = % decrease.

Soluble Solids

Soluble solids (SS) of both Cantaloupe and Honey Dew melons not change during frozen storage. No study has been reported for the effect of frozen storage on the SS content of muskmelon, but Cohen and Hicks (1986) stated that there was no post-harvest effect on SS content for 2, 5 or 9 days at either 5°C or 12.5°C and no effect of storage time at 20°C for either 2 or 5 days; therefore, from the result of this study, it is reasonalbe that the melon disk SS did not change during periods of frozen storage at -23°C.

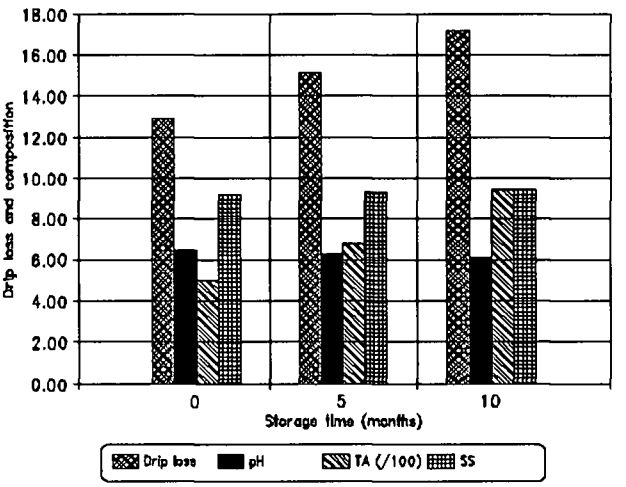
Drip Loss

Drip loss increased as storage time was increased for both Cantaloupe and Honey Dew (Figure 14a, 14b, and Table 16). The increase in drip loss could have been due to growth of ice crystals during storage, serving to disrupt the membrane cells. Thus, as storage time increased, an increase in crystal size would also have occurred, causing increased disruption to plant cell structure. Reddy (1986) also reported an increase in drip loss for frozen muskmelon as storage time was increased.

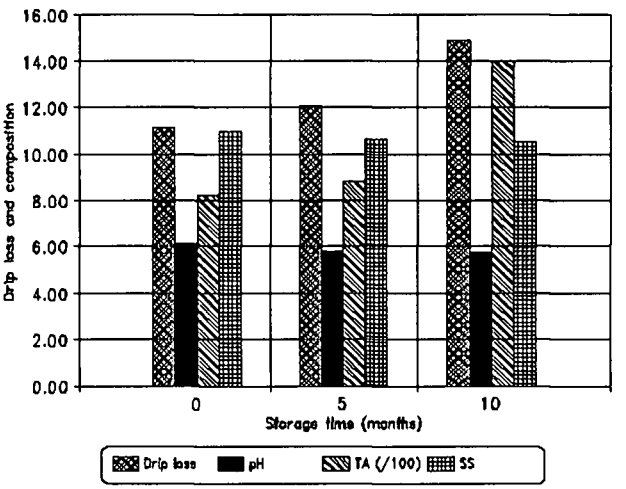
Color

The scale of Hunter "L" (lightness), "a" (represent redness to greenness), and "b" (represent yellowness to blueness) were evaluated. The "L" values increased as storage time was increased for both melon cultivars (Figure 15 and Table 14). "L" values represent light to dark (0 = white to 100 = black). The "a" values for Cantaloupe decreased, while those for Honey Dew increased with storage time. The "b" values for both Cantaloupe and Honey Dew decreased as the storage time increased. These observations suggest that the degradation of ß-carotene in Cantaloupe and the degradation of chlorophyll in Honey Dew occurred as frozen storage was increased.

As maturity increased, the orange color (i.e. an increase in the "a" value) for Cantaloupe also increased, though the changes were more obvious from the stages of underripe to ripe fruits than from ripe to overripe fruits. During frozen storage, the "a" value decrease for Cantaloupe was more obvious after 10 months of storage than for Cantaloupe subject to 5 months of storage. Decreases of chlorophyll in Honey Dew melon during some periods of frozen storage were proportionately equal from 0 to 5 months and from 5 to 10 months. This suggests that the green coloration of the Honey Dew was subject to more rapid degradation than the orange color of Cantaloupe during periods of frozen storage. Deterioration of ß-carotene effects the



a) Cantaloupe



b) Honey Dew

Figure 14. Relationships of drip loss to composition of different frozen storage times.

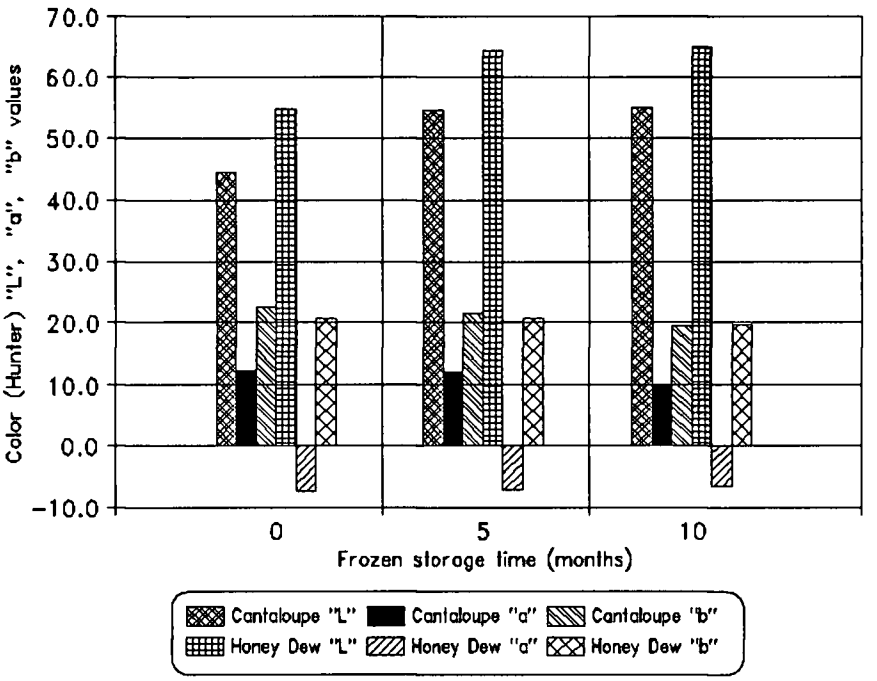


Figure 15. Relationships between color (Hunter, "L", "a", and "b" values) in different frozen storage time.

nutritional value and orange color of Cantaloupe. Decrease in chlorophyll and ß-carotene also reduce the attractiveness to the consumer.

Effect of Frozen Storage on the Sugar Composition of Cell Wall Polysaccharide Fractions

Neutral sugars in TFA Soluble Fractions

Neutral sugar composition for each of the CWP fractions is shown in Table 17a and 17b (mg of sugars/g CWP) and in Table 18a and 18b (mmole% in TFA soluble fraction).

During the frozen storage of ripe Cantaloupe and Honey Dew, there were significant decreases ($p<0.05$) in total sugars in relation to increased storage time (Table 19 and 20). However, the decrease in sugars was more obvious in the Honey Dew melon. For example, at 0 months frozen storage time, rhamnose, fucose, and mannose were present in the GTC fraction of Honey Dew; at both 5 and 10 months of storage time, rhamnose was not detected in the GTC fraction, and at 10 months rhamnose was not detected in Na_2CO_3 fraction. Though rhamnose was present in the Cantaloupe GTC fractions for all time periods, the period from 5 to 10 months reflected the sharpest proportionate decrease. Each of the total sugars in both melons decreased more dramatically during the 0 to 5 month period than during the 5 to 10 month period of frozen storage (Table 17a and 17b).

Galactose did not change in the Cantaloupe, whereas in Honey Dew it decreased 34.3 % from 0 to 5 months then decreased only 13% from 5 to 10 months of storage (Table 20). Changes in the proportion of neutral sugars (mmole%) of each CWP fraction were also noted as storage time increased in both Cantaloupe and Honey Dew (Table 18a and 18b). The mechanisms by which the CWP sugar compositions decreased during frozen storage in the two melons considered is not clear. There is a possibility that the disruption of cell membrane structures during ice crystal growth was the first step in the modification of the structures of pectins and the hemicelluloses. This could have been followed by the activity of such enzymes as pectin methyl esterase (PME), or Cx-cellulase. Furthermore, it was possible that pectins released from treated PAW (which has a pH 1.35) during the isolation of CWP underwent depolymerization. This effect also may have contributed to the degradation of polymers during the extended periods of frozen storage.

Monosaccharides in TFA Insoluble Fractions

The amount of sugars in the TFA insoluble fractions for both Cantaloupe and Honey Dew did not change during periods of extended frozen storage of melon disks (Table 17a and 17b). The exception to this observation was that Honey Dew melon which was subject to only a slight decrease (8.29%)

during the period from 5 to 10 months of storage. For both melons, total sugars in TFA insoluble fractions were highest in the residue fraction, followed in declining order by the CDTA, Na_2CO_3, GTC and KOH fractions. These observations also show that only a small proportion of the CWP that solubilized by the TFA (range from 10.4% to 14.6% for Cantaloupe and 9.24% to 13.5% for Honey Dew), whereas most of the polymers were only solubilized by concentrated (72%) H_2SO_4. The highest amount of sugars in CDTA as compared to Na_2CO_3, GTC, and KOH fractions during the frozen storage of melon disks suggests that most of the modified polymers in the CWP that underwent frozen storage was solubilized by the CDTA. The increase in the CDTA fraction yields in both melons also occured as frozen storage time increased (Table 21).

Carr (1982) argued that the decrease of water soluble pectic polysaccharides as well as total cell wall uronide in thawed frozen strawberries was due to increase in drip loss during long term of frozen storage. Whereas Reid et al. (1986) stated it was less clear whether or not cell wall compositions contributed to the loss of firmness in strawberries as a consequence of freezing and frozen storage.

Table 17a. Sugar composition of CWP fraction of Cantaloupe in different storage time

Storage time (month)	Sugars in TFA soluble fraction (mg/g CWP)*										Total in TFA mg/g	TFA insoluble glu/g CWP	Uronic acid mg/g	Total sugar mg/g
	CWP fractions	% of dry CWP	Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unkn				
0	CDTA	21.5	1.05	n	3.67	0.600	n	2.49	n	n	7.81	14.6	222	245
	Na_2CO_3	11.3	0.704	n	3.12	0.584	n	1.56	n	1.01	6.98	7.95	107	122
	GTC	7.95	0.294	0.886	2.07	17.5	1.66	3.55	9.23	0.317	35.5	3.61	19.7	58.8
	KOH	13.1	n	2.25	0.343	20.2	3.57	5.54	17.7	n	49.6	2.47	11.1	63.1
	Residue	49.9	1.04	0.707	2.79	2.93	2.86	6.55	18.1	n	35.0	320	82.8	438
	Total:	104	3.09	3.84	12.0	41.8	8.09	19.7	45.1	1.33	135	349	443	926
5	CDTA	24.0	0.921	n	3.00	1.29	n	2.34	n	n	7.56	15.5	234	257
	Na_2CO_3	12.9	0.335	n	1.81	0.607	n	1.77	n	0.311	4.83	9.67	62.9	77.4
	GTC	6.87	0.029	0.593	0.860	9.85	0.33	2.37	2.64	0.381	17.1	1.27	16.0	34.4
	KOH	12.1	n	2.54	0.911	18.3	3.64	6.32	9.74	n	41.5	1.88	14.7	58.1
	Residue	47.7	0.217	0.455	2.73	2.01	2.68	6.23	11.4	n	25.7	314	81.1	421
	Total:	104	1.50	3.59	9.31	32.1	6.65	19.0	23.8	0.692	96.6	343	409	848
10	CDTA	30.5	0.836	n	2.84	0.042	n	2.15	n	0.284	6.15	1.00	287	295
	Na_2CO_3	12.0	0.334	n	1.74	0.546	n	2.33	n	n	4.95	9.71	44.8	59.4
	GTC	6.10	0.071	0.239	0.766	6.62	0.54	1.89	2.17	0.980	13.3	1.64	7.46	22.4
	KOH	11.1	n	1.82	0.372	15.0	2.71	4.59	8.51	n	33.0	1.62	8.20	42.8
	Residue	44.9	0.285	0.572	2.56	3.22	2.49	7.19	11.4	n	27.7	312	58.5	398
	Total:	105	1.53	2.63	8.28	25.4	5.74	18.1	22.1	1.26	85.1	326	406	817

Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Gluc = glucose; Unkn = Unknown sugar calculated as pentose; n = not detected.
* = The mean values from 4 injection.

Table 17b. Sugar composition of CWP fraction of Honey Dew in different storage time

Storage time (month)	Sugars in TFA soluble fraction (mg/g CWP)*										Total in TFA mg/g CWP	TFA insoluble glu/g CWP	Uronic acid mg/g CWP	Total sugars mg/g CWP
	CWP fractio	% of dry CWP	Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unkn				
0	CDTA	22.5	1.45	n	5.14	0.951	n	7.86	n	n	15.4	28.5	191	235
	Na_2CO_3	8.25	0.306	n	1.33	0.195	n	2.49	n	n	4.32	5.01	60.7	70.0
	GTC	13.6	0.201	0.695	1.74	12.7	4.13	6.75	19.8	1.16	47.3	4.44	25.8	77.4
	KOH	7.85	n	0.465	0.45	7.59	1.31	2.39	3.41	0.10	15.7	1.92	7.40	25.1
	Residue	51.6	t	1.10	4.20	1.82	1.94	16.7	22.2	n	48.0	365	118	531
	Total:	104	1.95	2.26	12.9	23.3	7.38	36.2	45.5	1.26	131	405	403	939
5	CDTA	25.6	0.388	n	4.63	0.575	n	6.34	n	n	11.9	27.2	244	283
	Na_2CO_3	12.9	0.195	n	1.05	0.316	n	2.98	n	0.55	5.09	7.08	49.1	61.3
	GTC	7.74	n	0.487	0.12	2.13	2.74	0.45	14.0	0.17	20.1	1.09	9.35	30.5
	KOH	6.38	n	0.985	0.18	8.24	1.18	2.49	3.98	0.54	17.6	1.28	8.66	27.5
	Residue	51.1	0.114	0.246	2.64	3.15	1.82	11.6	16.7	n	36.3	374	71.7	482
	Total:	104	0.698	1.72	8.63	14.4	5.74	23.8	34.7	1.26	91.0	410	382	884
10	TA	31.3	0.246	n	3.61	0.466	n	5.62	n	n	9.94	29.1	192	231
	Na_2CO_3	7.87	n	n	1.75	0.177	n	2.20	n	0.09	4.22	7.18	97.9	109
	GTC	7.35	n	0.458	0.46	1.75	2.59	0.75	11.8	0.04	17.9	0.76	8.51	27.2
	KOH	6.47	n	0.747	0.32	6.85	1.09	2.89	3.95	0.92	16.8	1.26	15.6	33.6
	Residue	51.0	0.105	0.380	1.51	3.86	1.04	9.23	12.6	n	28.8	338	72.6	438
	Total:	104	0.352	1.58	7.66	13.1	4.72	20.7	28.4	1.07	77.6	376	387	840

Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose;
Gal = galactose; Glu = glucose; Unkn = Unknown sugar calculated as pentose;
n = not detected; * = The mean values from 4 injection;

Table 18a. Sugar composition of TFA soluble of Cantaloupe in different storage time (mmole%) **

Storage time (month)	CWP fraction	Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unkn
	CDTA	13.1	n	50.7	8.3	n	28.0	n	n
0	Na_2CO_3	9.62	n	47.2	8.8	n	19.1	n	15.3
	GTC	0.813	2.45	6.33	53.4	4.15	8.84	23.0	0.968
	KOH	n	4.58	0.772	45.4	6.55	10.2	32.5	n
	Residue	3.17	2.15	9.37	9.8	7.83	17.9	49.7	n
5	CDTA	11.8	n	42.6	18.4	n	27.1	n	n
	Na_2CO_3	6.77	n	40.4	13.6	n	32.3	n	6.95
	GTC	0.164	3.35	5.37	61.5	1.68	12.1	13.4	2.38
	KOH	n	6.11	2.42	48.8	7.89	13.7	21.1	n
	Residue	0.897	1.88	12.5	9.18	10.0	23.2	42.4	n
10	CDTA	13.3	n	50.0	0.73	n	30.9	n	5.00
	Na_2CO_3	6.72	n	38.8	12.2	n	42.3	n	n
	GTC	0.516	1.75	6.18	53.4	3.56	12.4	14.2	7.91
	KOH	n	5.50	1.25	50.1	7.39	12.5	23.2	n
	Residue	1.08	2.18	10.8	13.6	8.54	24.7	39.2	n

* = Average from duplicate of fractionations; **= The mean values from 4 injections.
Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Unkn = Unknown sugar calculated as pentose; ; t = trace; n = not detectable.

Table 18b. Sugar composition of TFA soluble of Honey Dew in different storage time (mmole%) **

Storage (month)	CWP fraction	Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unkn
	CDTA	9.47	n	37.2	6.89	n	46.4	n	n
0	Na_2CO_3	7.22	t	34.8	5.10	n	52.9	n	n
	GTC	0.438	1.52	4.19	30.7	8.11	13.3	39.0	2.79
	KOH	n	2.92	3.20	52.9	7.44	13.6	19.4	0.655
	Residue	t	2.46	10.4	4.51	3.92	33.7	45.0	n
5	CDTA	3.27	n	43.2	5.36	n	48.2	n	n
	Na_2CO_3	3.90	n	23.2	7.00	n	53.7	n	12.2
	GTC	t	2.61	0.722	12.6	13.2	2.21	67.6	1.01
	KOH	n	5.53	1.16	51.2	5.98	12.6	20.2	3.36
	Residue	0.337	0.73	8.6	10.3	4.83	30.7	44.5	n
10	CDTA	2.51	n	40.6	5.24	n	51.6	n	n
	Na_2CO_3	t	n	45.9	4.64	n	46.9	n	2.51
	GTC	n	2.75	3.12	11.7	14.0	4.10	64.0	0.325
	KOH	n	4.44	2.11	45.0	5.83	15.4	21.1	6.06
	Residue	0.389	1.40	6.17	15.8	3.46	30.7	42.1	n

* = Average from duplicate of fractionations; ** = The mean of anhydro values from 4 injections;
Rham = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose;
Glu = glucose; Unkn = Unknown sugar calculated as pentose; t = trace; n = not detectable.

Table 19. F-ratios from two-way ANOVA (effect of Cultivar and storage time on total sugar composition of CWP of melon

Source of error	d.f.	Sugars Rham	Fuc	Ara	Xyl	Man	Gal	Glu	Unk	TFA insoluble	Uronic acid
C	1	25.8 *	20.6 *	0.390	3052 *	9.91 **	81.7 *	12.9 **	1.19	115 *	50.0 *
ST	2	21.6 *	1.72	137 *	725 *	31.0 *	33.7 *	55.5 *	0.603	6.70 **	28.8 *
C * ST	2	1.19	0.611	4.84	4.62 **	0.131	2.74	3.46	0.257	9.51 *	9.83 *
M.S. error	6	0.006	0.0133	0.0064	0.0102	0.008	0.094	0.324	0.018	2.46	3.26

C = cultivar, ST = storage time, * = F-ratios are significant at P<0.01.
** = F-ratios are significant at P<0.05.

Table 20. Percentage decrease of total sugars during storage time increased

Cantaloupe:	Monosaccharides:									
From storage:	Rha	Fuc	Ara	Xyl	Man	Gal	Glu	Unk	TFA insoluble	Uronic Acid
0 --> 5 month	51.5	6.50	22.4	23.2	17.8	3.62	47.2	48.1	1.35	7.67
5 --> 10 month	19.7	26.7	11.0	20.9	13.7	4.74	7.14	82.6	4.96	0.73
Honey Dew:										
0--> 5 month	64.2	23.9	33.1	38.2	22.2	34.3	23.7	24.6	2.00	5.21
5--> 10 month	15.5	4.73	11.2	9.03	17.8	13.0	18.2	15.1	8.29	2.00

Uronic Acid in CWP Fractions

Total CWP uronic acid decreased significantly ($p<0.01$) from 0 to 5 months of frozen storage in both Cantaloupe or Honey Dew (Table 17a, 17b, 19, and 20). Total uronic acid for both melons did not change during the latter period of frozen storage.

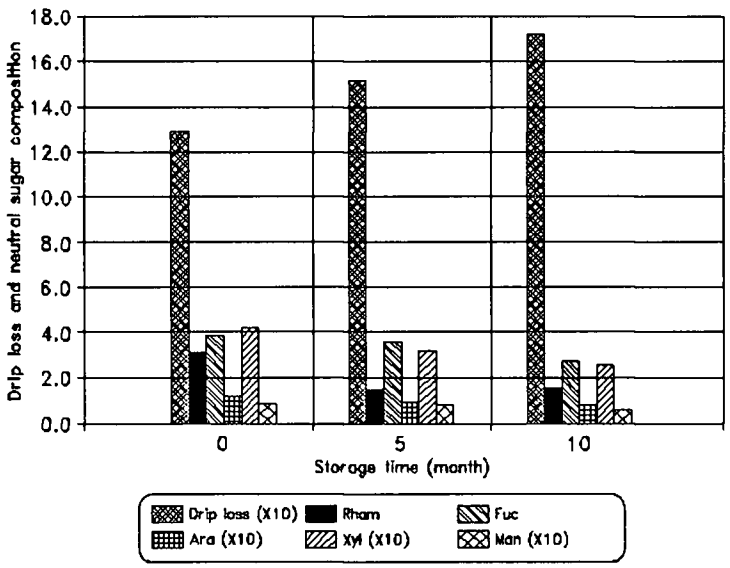
Total uronic acid in the CDTA fraction increased as frozen storage time was increased in the Cantaloupe, whereas there was an increase for Honey Dew from 0 to 5 months, followed by a decrease from 5 to 10 months of storage. It was also observed that as the amount of uronic acid increased in the CDTA fraction, the amount in the Na_2CO_3 fraction decreased. Conversely, when the amount of uronic acid decreased in the CDTA fraction, there was an increase in the Na_2CO_3 fraction. In addition, it was observed that uronic acid was present in all CWP fractions of both Cantaloupe and Honey Dew for all storage periods. These results were similar to those for both fresh (i.e., 0 months of frozen storage) Cantaloupe and Honey Dew melon during all three stages of maturity.

Relationship Between Drip Loss and Total Sugars

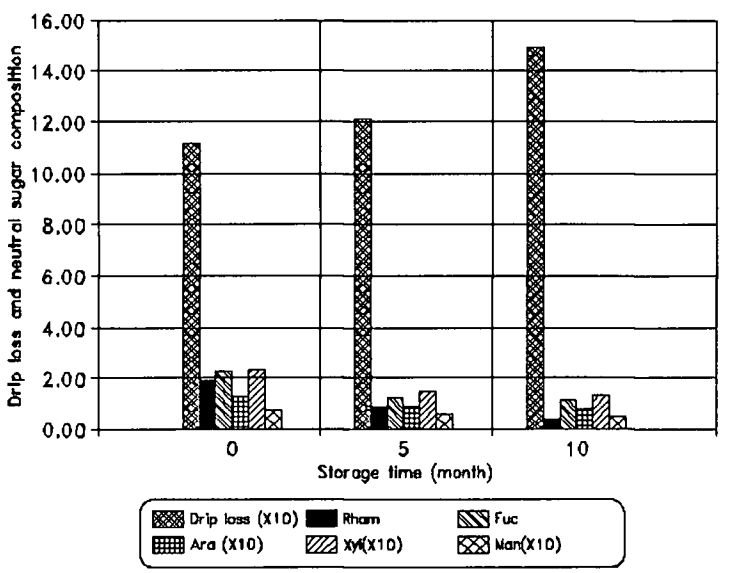
As previously noted, an inverse relationship exists between drip loss and the firmness of Cantaloupe as maturity increases. Therefore, experiments conducted for this

study were based upon the assumption that drip loss was representative of firmness in both Cantaloupe and Honey Dew melon during periods of frozen storage. Figure 16 through 19) demonstrate a consistent increase in drip loss for both Cantaloupe and Honey Dew melon as frozen storage time was increased. Although almost all of the sugars decreased in comparison to amounts present in CWP as storage time was increased, the decrease was more pronounced for the period 0 to 5 months than for 5 to 10 months of frozen storage. Maximum crystal formation is reached between 0 to 5 month storage which effects the disruption of cell walls.

Figures 16 through 19 show a positive correlation between drip loss and frozen storage time, and negative correlations for all total sugar compositions of CWP fractions, with the exception that total galactose showed no correlation with the drip loss.

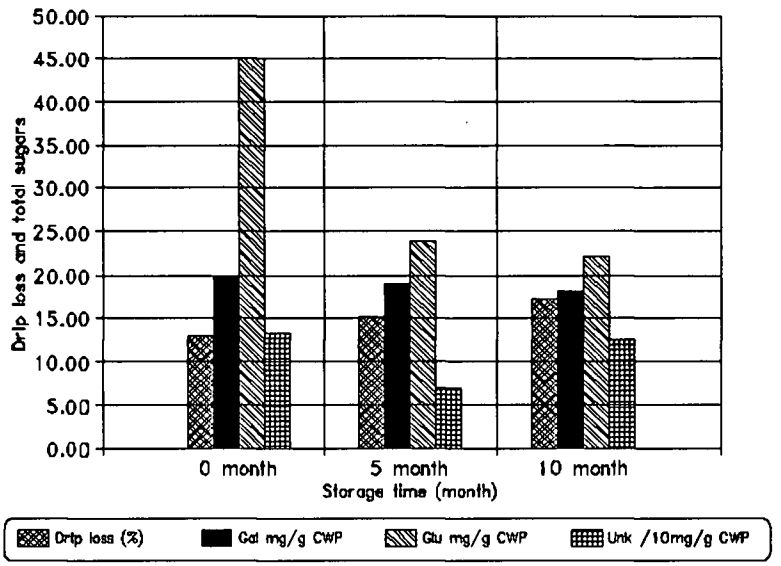


a) Cantaloupe

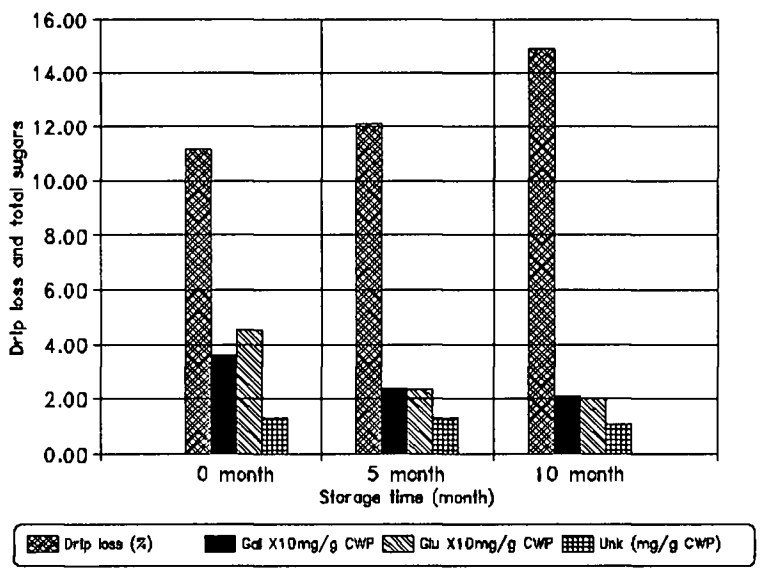


b) Honey Dew

Figure 16. Relationship between drip loss and total neutral sugars in different storage time of melons.

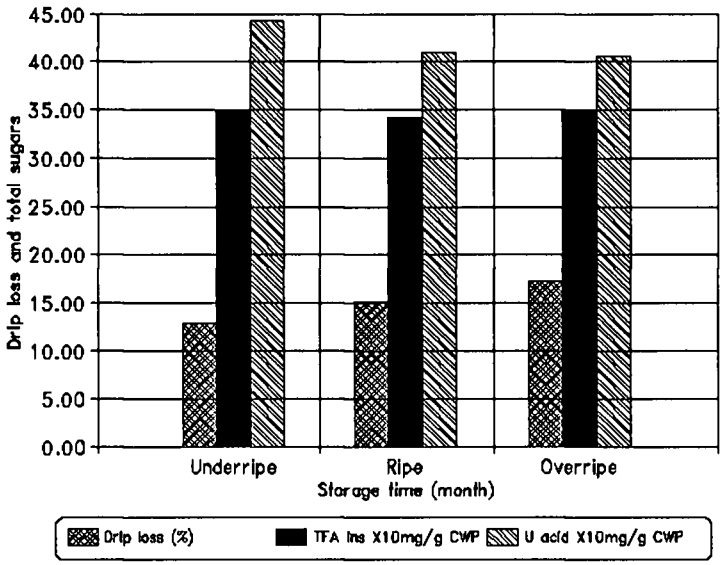


c) Cantaloupe

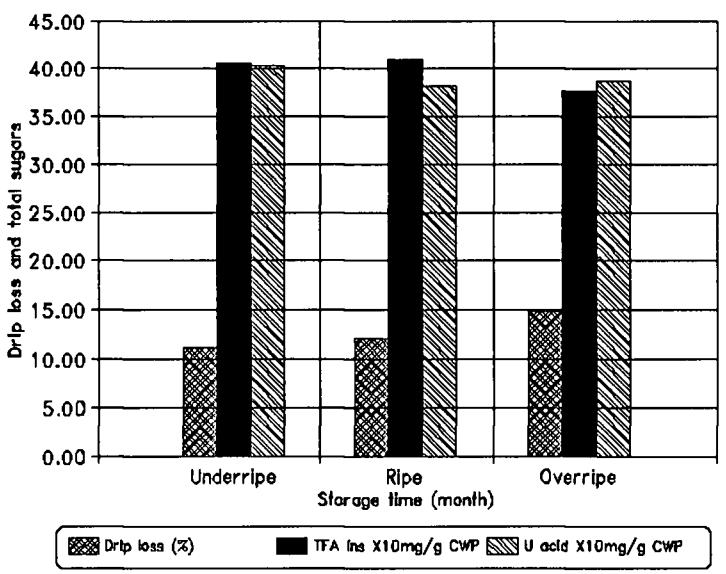


d) Honey Dew

Figure 16. Drip loss and total neutral sugars in different storage time of melons.



e) Cantaloupe



f) Honey Dew

Figure 16. Drip loss and total neutral sugars in different storage time of melons.

Effect of Frozen Storage Upon Cell Wall Polysaccharide Fraction Yields

The CDTA fraction yield increased significantly ($p<0.01$) for both Cantaloupe and Honey Dew melons in relation to increased frozen storage time (Table 21 and 22). In both Cantaloupe and Honey Dew, the Na_2CO_3 fraction increased during the period from 0 to 5 months of frozen storage (13% for Cantaloupe and 56% for Honey Dew), then decreased during the 5 to 10 month period of frozen storage (7% for Cantaloupe and 39% for Honey Dew). These observations suggest that pectin modification occurred during the prolonged frozen storage of melon disks.

GTC fractions decreased during 0 to 5 month period of frozen storage (13% for Cantaloupe and 43% for Honey Dew), and were not subject to further change during the 5 to 10 month period of storage. The KOH fraction for Cantaloupe decreased slightly, while in Honey Dew melon it decreased by 18% during the 0 to 5 month period of frozen storage. It is possible that the degradation of hemicellulose was so severe that the GTC and KOH fraction yields decrease and could not recovere during the period CWP fractionation was performed. Unfortunately, there have not been any other studies of frozen storage changes in CWP (specifically, no comparative hemicelluloses fraction yields) with which the results of this study may be compared.

Table 21. Yields of CWP fraction of melons in different storage time

CWP fraction	0 month			5 month			10 month		
	mean ** (mg/g CWP)	stdv	mean (%)	mean ** (mg/g CWP)	stdv	mean (%)	mean ** (mg/g CWP)	stdv	mean (%)
Cantaloupe:									
CDTA	215	4.13	21.5	240	5.20	24.0	305	6.70	30.5
Na_2CO_3	113	2.26	11.3	129	3.80	12.9	120	3.70	12.0
GTC	79.5	2.49	7.95	68.7	2.10	6.87	61.0	4.90	6.10
KOH	131	2.88	13.1	121	5.40	12.1	111	2.80	11.1
Residue	499	13.4	49.9	477	10.3	47.7	449	13.4	44.9
Honey Dew:									
CDTA	225	7.69	22.5	255	8.31	25.5	315	3.33	31.5
Na_2CO_3	82.5	2.74	8.25	129	2.99	12.9	78.7	0.63	7.87
GTC	136	3.61	13.6	77.4	1.97	7.74	73.5	1.12	7.35
KOH	78.5	2.50	7.85	63.8	2.27	6.38	64.7	1.72	6.47
Residue	516	17.3	51.6	511	13.9	51.1	510	14.1	51.0

** = From duplicate of fractionations; stdv = standard deviation.

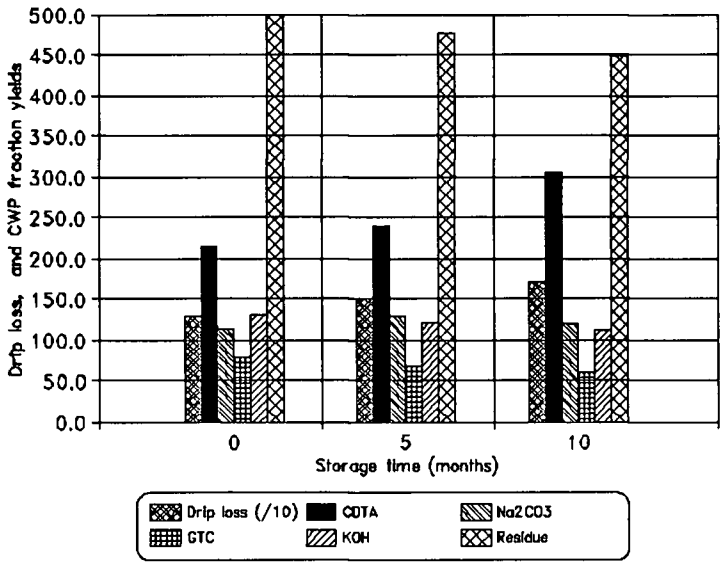
Table 22. F-ratios from two way ANOVA (effect of Cultivar and storage time on the CWP fraction yields of melons

Source of error	d.f	CWP fractions				
		CDTA	Na_2CO_3	GTC	KOH	Residue
C	1	10.6 **	203 *	2312 *	803 *	22.5 *
ST.	2	226 *	141 *	221 *	29.5 *	3.92
C * ST.	2	0.246	54.7 *	81.3 *	2,95	2.9
M.S.-Error	6	37.9	8.5	8.72	10.1	180.9

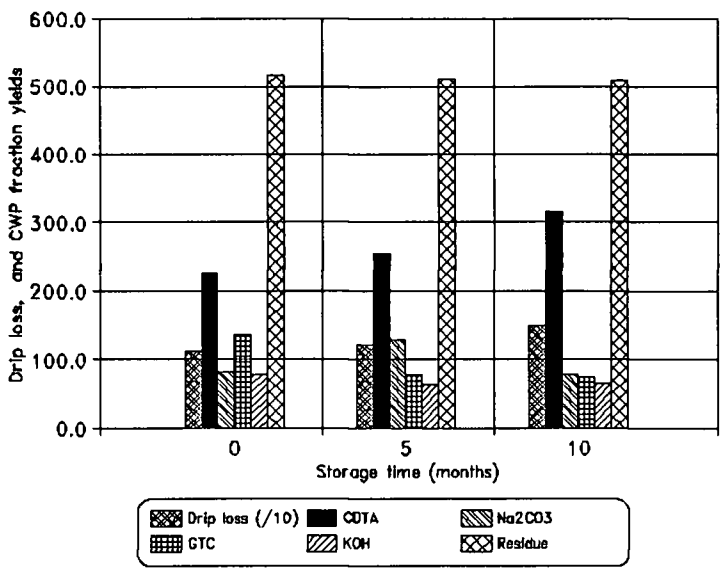
C = Cultivar; ST = Storage Time; * F-ratios are significant at P<0.01.

Relationship of CWP Fractions to Drip Loss

The relationship between CWP fraction yields and drip loss in the Cantaloupe and Honey Dew melon, as a function of frozen storage time, is shown in Figure 17a and 17b. Drip loss in the two muskmelons had a positive correlation with CDTA fraction yields, increasing for both melons in relation to increased frozen storage time. After 0, 5, and 10 months of frozen storage in the Cantaloupe, the combined (i.e., as pectins) CDTA and Na_2CO_3 fractions totaled 31.6%, 35.6%, and 40.6% and hemicelluloses totaled 20.3%, 18.3%, and 16.4%, respectively. Parallel measures for the Honey Dew, were 29.6%, 37.1%, and 37.8% for pectins and 20.7%, 13.6%, and 13.3% for hemicelluloses. The greatest proportion of changes in pectins were detected from the 0 to 5 month period of frozen storage, a factor which may be possibly attributed to the achievement of maximum ice crystal growth during this initial period of frozen storage. As previously established, the growth of ice crystals could have an effect upon both pectins and hemicelluloses, leading to cell separation followed by polysaccharide degradation.



a) Cantaloupe



b) Honey Dew

Figure 17. Relationships between drip loss and CWP fraction yields in different storage time of melons.

Effect of Frozen Storage Upon Cell Wall Polysaccharide Yields

For the Cantaloupe, CWP amounts decreased slightly (9.55%) as the frozen storage duration was increased from 0 to 5 months, but no changes were recorded when the period of frozen storage was extended from 5 to 10 months. For Honey Dew melons, CWP yields were not affected by the frozen storage time of melon disks (Table 23 and 24). There have been no other studies of frozen storage changes in CWP yields with which the results of this study may be compared. Reid et al. (1986) stated it was less clear whether or not cell wall compositions contributed to the loss of firmness in strawberries as a consequence of freezing or frozen storage, and no CWP yields were reported. Carr (1982) examined the effect of frozen storage time on the cell wall uronide content of strawberries (derived from alcohol insoluble solids, AIS), reporting a decrease in the total uronides as frozen storage progressed, but AIS yields were not reported.

Table 23. Yields of CWP (g/1000 g of frozen melon in different storage times

	0 Month	5 Month	10 Month
Cultivar	(g)	(g)	(g)
Cantaloupe	4.92	4.45	4.42
	(0.0597)**	(0.0310)**	(0.0374)**
Honey Dew	5.20	5.19	4.96
	(0.0577)**	(0.103)**	(0.0516)**

* = Yields of quadruplicate of isolations.
** = Standard deviation.

Table 24. Effect of Cultivar and storage time on the CWP yields of melons: F-ratios fromtwo way ANOVA.

Source of error	d.f.	CWP
C	1	109 *
ST.	2	625 *
C * ST	2	41.6 *
M.S.-Error	6	0.0013

C = Cultivar; ST = Storage Time
* F-ratio are significant at P<0.01

SUMMARY AND CONCLUSIONS

The effect of frozen storage upon two cultivars of muskmelon (Cucumis melo), at three stages of maturity, was analyzed. For Cantaloupe, when compared to Honey Dew melons, pH, drip loss, moisture content, and the Hunter (1975) color "a" values were higher, whereas titratable acidity, soluble solids, and firmness were lower for comparable maturity stages. As maturity increased, for both Cantaloupe and Honey Dew melons, pH, soluble solids, and drip loss increased, whereas titratable acidity and firmness decreased. It was determined that the color "a" values, which increased as maturity increased, could be used as an indicator of stage of maturity for both melons. The CWP yields was slightly higher in the Honey Dew than in the Cantaloupe, and CWP yields for both melons did not change as maturity increased.

Five fraction of CWP samples were also analyzed. The CDTA fraction in both Cantaloupe and Honey Dew increased as maturity increased. In the underripe Honey Dew, the CDTA fraction was lower than for the underripe Cantaloupe, but as maturity increased the CDTA fraction of the Honey Dew was higher than that of Cantaloupe. The Na_2CO_3 fraction of Cantaloupe decreased slightly as maturity increased, while in the Honey Dew it decreased sharply from the underripe to ripe stages and was not subject to further change in the

overripe stage. The GTC fraction also decreased in the Cantaloupe as maturity increased, whereas there was an increase from underripe to ripe stages followed by a decrease from ripe to overripe stages for Honey Dew. In Cantaloupe, the KOH fraction increased as maturity increased, while in the Honey Dew the GTC fraction decreased from underripe to ripe stages, then increased slightly from the ripe to overripe stages. In the Honey Dew, residue fractions increased from underripe to ripe stages, then were not subject to change from the ripe to overripe stages.

In both Cantaloupe and Honey Dew melons, most sugars, including rhamnose, arabinose, mannose and galactose, decreased significantly with increased maturity, subject to the exception that glucose and xylose increased from underripe to ripe stages, then decreased from the ripe to overripe stages. Total uronic acid decreased slightly in both the Cantaloupe and Honey Dew melons as maturity increased.

With increased maturity in both melons, there was almost no change in theTFA insoluble fraction, subject to the exception of a slight decrease from ripe to overripe stages. The TFA insoluble fraction was higher in Honey Dew than in Cantaloupe for all three maturity stages.

The Honey Dew was higher in CWP yields and TFA insoluble fraction might have contributed to its higher degree of firmness. Firmness was inversely related to drip loss in

both the Cantaloupe and the Honey Dew. Thus, drip loss increased as firmness decreased. In addition, firmness was inversely related to CDTA fraction yields for both Cantaloupe and Honey Dew CWP fractions, and was positively correlated with total sugars, including rhamnose, arabinose, mannose and galactose.

During periods of frozen storage, there were similar changes in composition, drip loss and color change for both Cantaloupe and Honey Dew, evidenced by a decrease in pH and an increased in TA and drip loss values as storage time was increased. Color determination indicated that the "a" value for Cantaloupe decreased, whereas for Honey Dew the "a" value increased with increased duration of storage.

The CWP yields were not affected by frozen storage time for either melon. In addition, similar trends were apparent for the CDTA, Na_2CO_3, and GTC fractions for both melons. As frozen storage time was increased, the CDTA fraction also increased, while Na_2CO_3 fractions increased from 0 to 5 months, followed by a decrease from 5 to 10 months of storage. For GTC fractions, there were decreases between 0 to 5 months of storage and no further changes from 5 to 10 months of storage. The KOH fraction decreased in the Cantaloupe with increased storage time, whereas for the Honey Dew a decrease occurred only between 0 and 5 months and there was no further change from 5 to 10 months of frozen storage.

Residue fractions were not affected by frozen storage for either melon.

Each of the total neutral sugars in both types of melon decreased by a greater magnitude from 0 to 5 months of storage in comparison to the decrease from 5 to 10 months. The decrease of sugars from the CWP of both melons could have been due to the disruption of polymers by ice crystal formation; that is, maximum crystal formation would have occurred between 0 and 5 months of frozen storage, disrupting the polysaccharide polymers. During prolonged periods of storage, minimal enzyme activity could also have provided a contribution, especially if PME and Cx cellulase were present. Treatment with PAW during the CWP isolation could have also contributed to the degradation of polysaccharides during periods of prolonged frozen storage.

Both the drip loss and the CDTA fractions increased, while the GTC fraction decreased with increased frozen storage time for both melons; other CWP fractions show no correlations with drip loss. Drip loss increased coincident with significant decreases in total sugars for all TFA soluble fractions as storage time increased, suggesting that frozen storage has an effect upon the solubilization and degradation of CWP. This process was progressive as storage time increased.

The two muskmelon cultivars considered had different composition, firmness, drip loss and color values. Canta-

loupe had higher pH and moisture, and lower titratable acidity, soluble solids, and firmness than Honey Dew for the same stages of maturity. For both cultivars, pH, soluble solids, drip loss, and Hunter (1975) color "a" values increased as maturity increased.

Decreased neutral sugars, including rhamnose, arabinose, mannose, and galactose, occurred in muskmelon CWP as maturity increased, suggesting that both pectins and hemicelluloses were modified during the ripening and softening of the two fruits. Total uronic acid did not change during muskmelon softening, except for a decrease between the ripe to overripe stages of Cantaloupe. Similarly, frozen storage of melon disks changed the composition, firmness, drip loss and color of the two melons, with the exception that soluble solids did not change as storage time was increased. The CDTA fraction of CWP increased and all total neutral sugars were decreased as storage time was increased.

Recommendation for future research possibilities include the following:

1. Due to the low amount of neutral sugars recovered in TFA soluble fractions in both studies of different maturity stages and of the effect of frozen storage, research should be undertaken to determine optimum temperatures and times for the

hydrolysis of CWP fractions with TFA for neutral sugar determination in the muskmelon.

2. Due to the PAW pH is very low (i.e., between pH 1.30 - pH 1.35), research should be undertaken to determine if there is an effect for PAW on the solubilization of pectic or hemicellulose materials.

3. Results of this study indicated that there were unknown sugars in the CWP of both Cantaloupe and Honey Dew melon. Studies should be undertaken to identify these sugars.

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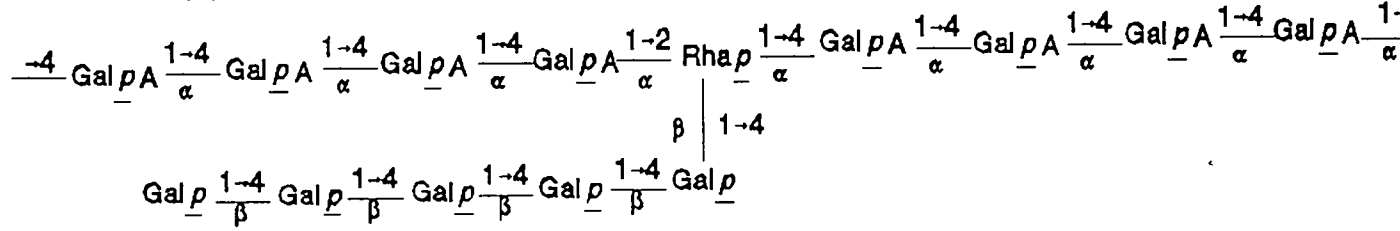
APPENDICES

Appendix A

Pectin Structures

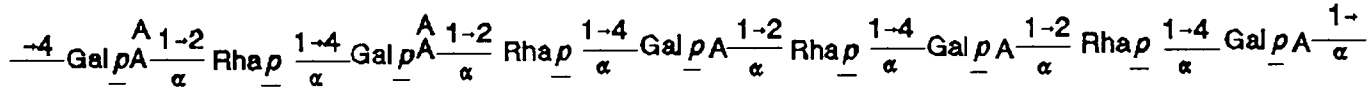
Appendix A. Pectins structures

(1)



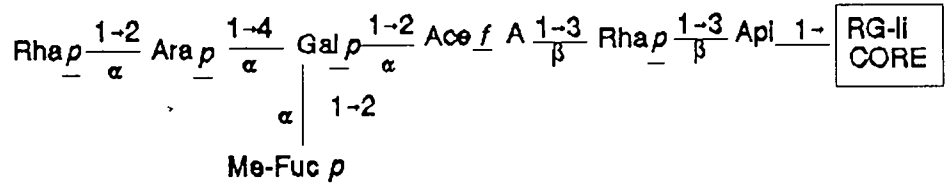
(2)

Rhamnogalacturonan (RG I)



(3)

Rhamnogalacturonan (RG II)

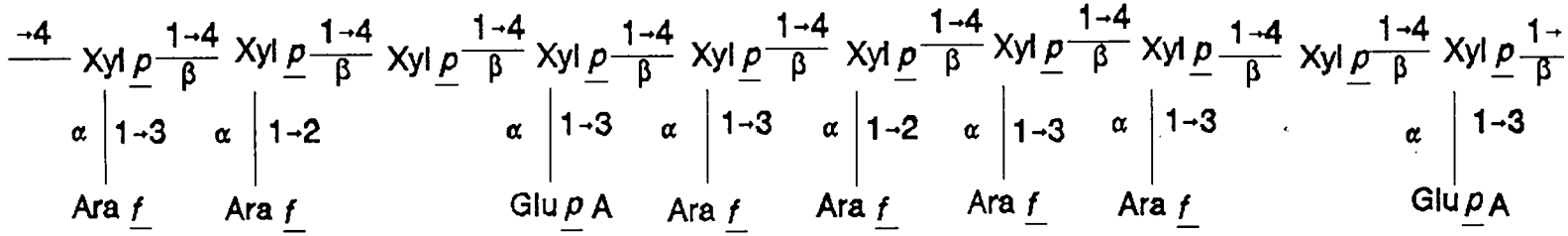


Appendix B

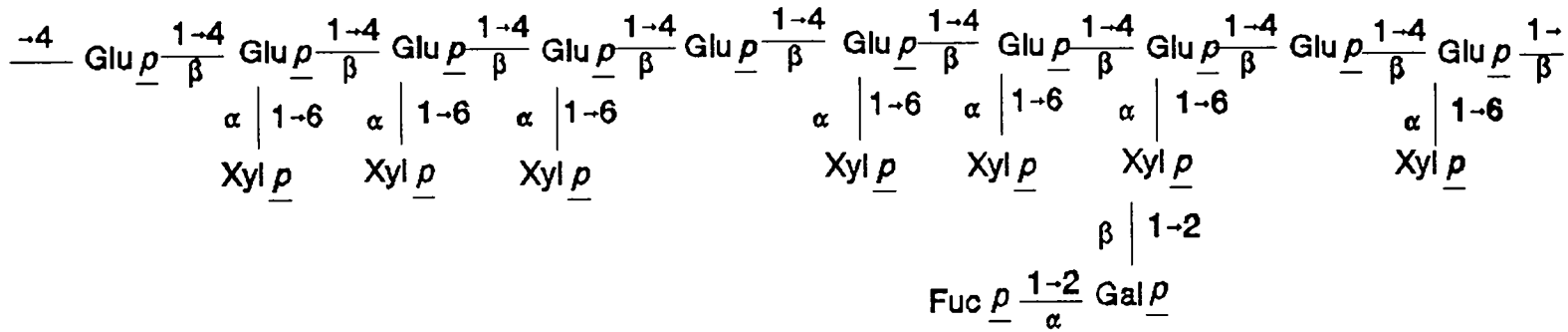
Hemicellulose Structures

Appendix B. Hemicellulose structures

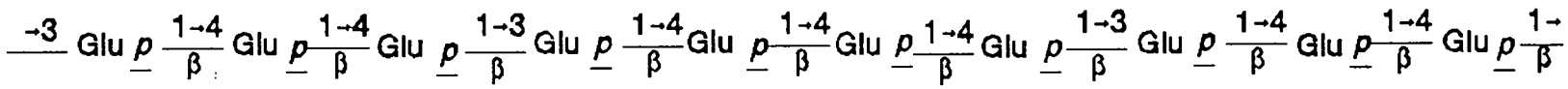
(1) Xylans



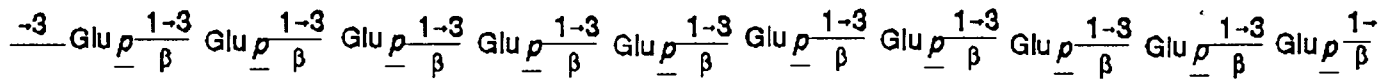
(2) Xyloglucans



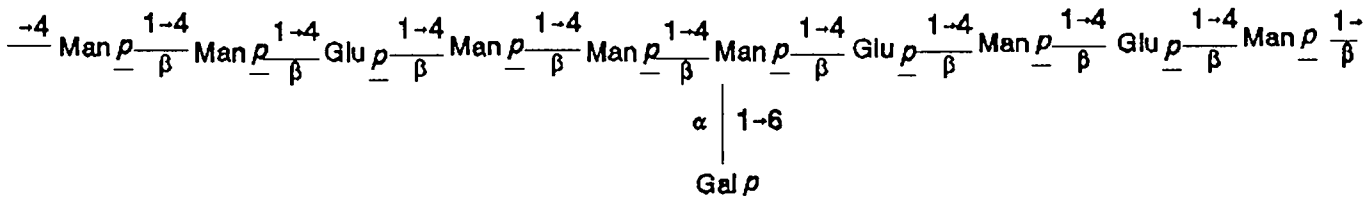
(3) β-(1→3), (1→4)-Glucans



(4) Callose



(5) β-Mannans



Appendix C

Cellulose Structures

Appendix C. Cellulose structures