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Cooking Quality of Lentils: The Role of Structure and Composition of Cell Walls

Rattan S. Bhatty

Crop Development Centre, Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

Good- and poor-cooking samples of Eston lentil, grown under field conditions, were compared for physical (microscopic) and chemical composition of seed, cell walls (CW), and pectin isolated from the CW. Hulled and dehulled, good- and poor-cooking samples of the lentil generally had similar hydration coefficients (water uptake). The poor-cooking sample had 44.1% lower seed phytic acid (PA) than the good-cooking sample. The CW were isolated in similar yields (3.8–4.2%) from the good- and poor-cooking samples and contained 5.8–6.5% protein, 0.5–1.2% starch, 1.2–1.7% lignin, and 17.7–18.1% galacturonic acid. Arabinose, glucose, galactose, and xylose were the major CW monosaccharides. Transmission electron micrographs of cotyledons and CW of uncooked poor-cooking lentil stained with $KMnO_4$ showed some evidence of a "lignification-like" mechanism at the cell junctions in the middle lamella. The CW of poor-cooking lentil appeared multilayered under a scanning electron microscope. Pectic substances of the two samples had similar methoxyl content, degree of esterification, and galacturonic acid content (colorimetric) and were thus of the low-ester variety. Seed PA content seemed to play a critical role in affecting the cooking quality of lentil.

Canada is now the fourth largest producer and second largest exporter of lentil in the world. Lentil, primarily used in human foods, has excellent nutritional quality. Although low in methionine and sometimes tryptophan, lentil protein complements proteins of wheat and rice with which it is commonly eaten in the developing countries. Lentil contains negligible levels of antinutritional factors, is low in flatulence-causing sucrose α-galacto-

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sides, and produces low postprandial glycemic response in normal and diabetic volunteers (Bhatty, 1988). A diet containing lentil may thus help in dietary management and control of diabetes.

A hard-to-cook (HTC) condition may develop in lentil grown under unfavorable growing conditions, unlike in *Phaseolus* bean where such a condition develops on storage under high moisture and temperature. However, lentils also deteriorate when they are stored under conditions of high temperature and moisture (Nozzolillo and De Bezada, 1984; Bhatty, 1989).

The influence of growth location and season (environment) on the cooking quality of lentil grown on farmer's fields across the Canadian Prairies has been reported previously (Bhatty et al., 1983, 1984). More recent studies conducted with lentil grown under diverse conditions of phosphorus availability or soaked in various solutions in the laboratory suggested that seed phytic acid (PA) was largely, if not entirely, responsible for the variability previously observed in the cooking quality of lentil grown under field conditions (Bhatty and Slinkard, 1989; Bhatty, 1989).

The poor-cooking lentil showed poor cell separation when observed under a scanning electron microscope (Bhatty et al., 1984). The cellular contents had dissolved or moved out, leaving behind, in many cases, an empty cell with a largely intact cell wall. Thus, lack of cell separation and cell wall dissolution is typical of a poor-cooking lentil, like other poor-cooking legumes (Kon, 1979; Sefa-Dedeh et al., 1979; Jones and Boulter, 1983). Whether this condition is directly the result of reduced PA in lentil seed or due to changes induced in the cell wall itself by the HTC condition is not known.

The role of cell wall in the HTC condition in lentil, grown under field conditions, has not been investigated. The present study, an extension of our previous research, reports further on differences in good- and poor-cooking samples of lentil grown under field conditions and, in particular, on the structure and composition of cell walls isolated from such lentils.

MATERIALS AND METHODS

Materials. Samples of Eston lentil, a small-seeded, yellow cotyledon licensed cultivar, were obtained from the 1985–1987 Lentil Cooperative Test described previously (Bhatty and Slinkard, 1989) and sized by passing through a Dockage tester (No. 10 screen). The sized samples were cooked for 30 min in boiling water at a seed to water ratio of 1 to 5, and shear force of the cooked samples was measured with a Kramer shear press as described previously (Bhatty et al., 1984). Good- or poor-cooking samples of lentil having shear force values less or greater than 4.0 kg/g, respectively, were combined to obtain, in each case, a composite sample. For analytical purposes, subsamples of good- and poor-cooking lentils were ground in a Udy cyclone mill to pass a 0.5-mm screen. Another set of good- and poor-cooking samples was dehulled on a tangential abrasive dehulling device (fitted with an A-36 carborandum disk), described by Reichert et al. (1986). The few remaining hull specks were removed manually, and the clean dehulled seed (cotyledons) was ground as described above and stored at 5 °C.

Lignin (indulin AT), *myo*-inositol, glucose, fucose, arabinose, and xylose were obtained from Sigma Chemical Co., St. Louis, MO; galactose was from Fisher Scientific, Edmonton, Alberta, Canada; rhamnose and a low-ester pectin (product 38052) were from the British Drug House, Saskatoon, Saskatchewan, Canada; erythrose was from Aldrich Fine Chemicals, Milwaukee, WI; another sample of pectin (Genu 41-21 AB) of a known degree of esterification was from Foodpro National, Montreal, Quebec, Canada.

Methods. Moisture contents of all samples were determined by drying at 130 °C for 1 h. Hydration coefficients (water uptake) of hulled and dehulled lentils were determined by soaking 3 g of seed or cotyledon (as is) in 10 mL of deionized water at room temperature (25 °C) for up to 30 h. At appropriate time intervals, the water was filtered off and the seed blotted dry and weighed to calculate the hydration coefficient. Phytic acid and total phosphorus (P) were determined by procedures described by Harland and Oberleas (1986).

Isolation of Cell Walls (CW). CW were isolated by the procedure of either Reichert (1981) or Shibuya et al. (1985). In the latter procedure, ground, defatted cotyledons were extracted with sodium dodecyl sulfate–mercaptoethanol to remove protein (three extractions) and the residue was washed twice with water to remove the solvent and then repeatedly extracted with dimethyl sulfoxide (DMSO) until free of starch (checked with iodine stain). The insoluble residue was dialyzed against distilled water for 24 h to remove DMSO and the nondiffusible fraction freeze-dried.

Composition of CW. Total nitrogen was determined by the micro-Kjeldahl method (AOAC, 1984), starch by a dual-enzyme procedure (Fleming and Reichert, 1980), lignin by digestion with acetyl bromide as described by Morrison (1972), and galacturonic acid by a colorimetric procedure (Ahmed and Labavitch, 1977). K^+, Ca^{2+}, and Mg^{2+} were determined, after sequential acid hydrolysis of the CW with nitric, perchloric, and hydrochloric acids, with an ICP spectrophotometer. Monosaccharides were determined after acid hydrolysis of CW, followed by reduction and acetylation (Blakeney et al., 1983). The alditol acetates were separated by gas–liquid chromatography on an SP 2330 fused silica column (Supelco, Oakville, Ontario, Canada), operated under the following conditions: carrier gas (nitrogen) flow rate adjusted to achieve retention time of about 13 min for the internal standard *myo*-inositol; injection port and detector temperatures, 250 and 300 °C, respectively; temperature program, 170–230 °C at 8 °C/min. Response factors were calculated for each sugar with authentic samples.

Isolation and Composition of Pectic Substances. Pectic substances were extracted from the CW by the procedure of Dever et al. (1968). The ethanol precipitates from the first and second extractions were combined and dried at 50 °C in vacuo to a constant weight. The degree of esterification (DE) and galacturonic acid content of the pectic substances, and for comparison of commercial pectin, were determined titrimetrically by procedures described in NRC–NAS (1972). Galacturonic acid was also determined colorimetrically (Ahmed and Labavitch, 1977). Methoxyl content was determined titrimetrically according to McCready (1970). In the titrimetric procedures used for the determination of methoxyl content and DE, V1 was the initial titer, V2 the saponification titer, V3 the amide substitution titer, and Vt the sum of the three titers. The DE was calculated by $V2/Vt \times 100$; galacturonic acid, $Vt \times 194.1 \times 0.05 \times 100/\text{sample weight}$; methoxyl content, $V2 \times 31 \times 0.05 \times 100/\text{sample weight}$, where 194.1 and 31 were the formula weights of galacturonic acid and methoxyl, respectively, and 0.05 was the normality of NaOH used in the titrations.

Microscopy. *Light.* Cooked lentil seeds were fixed for 24 h in 50 mM phosphate buffer (pH 5.0) containing 5% gluteraldehyde, dehydrated first with a series of ethanol solutions and then with propylene oxide and embedded in glycol methacrylate (historesin). Transverse sections (1–2 μm) were cut with a glass knife on a microtome (MT-1), stained with toluidine blue or periodic acid–Schiff reagent (PAS), counterstained with eosin or $KMnO_4$ (Feder and O'Brien, 1968), and viewed under a Phillips microscope.

Scanning. Freeze-dried CW was sprinkled on aluminum stubs, gold-plated with a "sputter" coater 5150B under vacuum and observed under a Phillips SEM operated at 30 kV.

Transmission. One-millimeter-thick specimens of cooked lentil were fixed in 2% osmium tetroxide in 50 mM phosphate buffer (pH 7.0) for 2 h. Excess osmium was removed by washing with the buffer. The specimens were then dehydrated with acetone and propylene oxide at 0 °C, infiltrated, and embedded in low-viscosity Spurr resin. Ultrathin sections (70–90 nm) were cut with a diamond knife with a Porter Blum microtome (MT-2), mounted on formavar-coated (200-mesh) copper grids, stained with $KMnO_4$ alone or with uranyl acetate and lead citrate (Dawes, 1979), and observed under a Phillips 300 electron

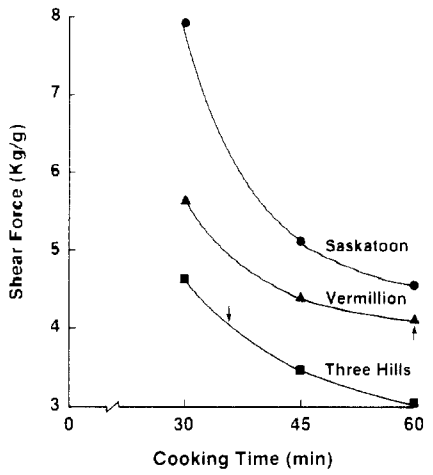

Figure 1. Relationship between cooking time and shear force (cooking quality) of Red Eston lentil grown at three locations.

microscope operated at 80 kV. In the case of CW, the material was suspended in 1% $KMnO_4$ in the phosphate buffer for 5 min. Excess $KMnO_4$ was then washed out with the buffer. The specimens were dehydrated, embedded, and sectioned as described above.

RESULTS AND DISCUSSION

Shear Force and Cooking Time. In the present study, as in previous ones published by me, shear force rather than cooking time was used as an index of cooking quality of lentils. Samples of Eston lentil were uniformly cooked for 30 min, those having shear force less or greater than 4.0 kg/g were considered good- or poor-cooking, respectively. The dividing value of 4.0 kg/g was based on cooking a large number of lentil samples of different cultivars for various times and, in addition, on taste panel data of cooked lentil reported previously (Bhatty et al., 1983).

Shear force of lentil, measured in a Kramer press, has a curvilinear relationship with cooking time, which is related to seed size. Although a varietal characteristic, seed size is influenced by environmental conditions during the growth of lentil (Singh et al., 1988). Figure 1 shows the difficulty in selecting an optimum cooking time for a few samples of lentil. Three samples of Red Eston lentil grown at three locations (Saskatoon, Vermillion, 3-Hills) and cooked for up to 60 min under identical conditions gave entirely different shear force values for each of the three cooking times. At a cooking time of 60 min, the sample grown at 3-Hills was overcooked (mushy), the one grown at Vermillion was cooked, and that grown at Saskatoon was undercooked. However, 60 min was a much longer cooking time for the Red Eston lentil as its seed size is only slightly larger than Eston lentil for which an optimum cooking time of 30 min has been established (Bhatty et al., 1984). Taking the shear force value of 4.0 kg/g as an index of cooking quality, the 3-Hills sample was cooked in 36 min or shortly thereafter (indicated by arrow) while the other two samples were undercooked at this time. This time related more closely to optimum cooking times established for other cultivars of lentil having different seed sizes.

In addition to seed size, other factors influencing cooking time are (a) absence of hull and (b) soaking of lentil prior to cooking. Dehulling of Laird lentil, a large-seeded cultivar, reduced cooking time to about one-tenth of that required for the hulled sample of the same cultivar (Bhatty and Slinkard, 1989). Soaking a small-seeded lentil sample in water or 1% $NaHCO_3$ for 16 h reduced cooking time to one-third and one-sixth, respectively (Singh et al., 1988).

Table I. Hydration Coefficients of Hulled and Dehulled Good- and Poor-Cooking Samples of Eston Lentil (Means and Standard Deviations of Duplicate Determinations)

	% dry wt basis	
soaking time, h	good-cooking	poor-cooking
	Hulled	
1	109 ± 2	120 ● 1**
2	130 ● 4	136 ± 1 ns
3	155 ± 5	157 ± 2 ns
5	172 ± 4	171 ± 2 ns
7	179 ± 3	177 ± 3 ns
9	184 ± 3	182 ± 1 ns
16	192 ± 4	187 ± 2 ns
20	195 ± 4	188 ± 3 ns
25	202 ± 5	192 ± 2*
29	203 ± 4	194 ± 2*
lsd (0.01)[a]	9	4
	Dehulled	
1	153 ± 4	168 ± 2**
3	187 ± 2	191 ± 3 ns
4	193 ± 2	194 ± 2 ns
7	195 ± 1	195 ± 2 ns
lsd (0.01)[a]	7	6

[a] Least significant difference for each column calculated from analysis of variance; two means in each row by a t-test. Key: *, $P = 0.05$; **, $P = 0.01$; ns = not significant.

Table II. Shear Force (Cooking Quality) and Phosphorus Fractions of Good- and Poor-Cooking Samples of Eston Lentil (Means and Standard Deviations of Duplicate Determinations)[a]

measurement	good-cooking	poor-cooking
shear force, kg/g	3.7 ± 0.3	5.1 ± 0.2**
seed phosphorus (P), %	0.52 ± 0.00	0.38 ± 0.01**
phytic acid (PA), %	1.11 ± 0.01	0.62 ± 0.01**
phytic acid P, %	60.2 ± 0.7	46.3 ± 0.9**

[a] Least significant difference for the two means in each row was calculated by a t-test: **, $P = 0.01$.

Water Uptake in Lentil. In *Phaseolus* bean the hard shell contributes to the HTC condition. The hard-shell beans fail to hydrate, and there is an inverse relationship between cooking time and the quantity of water imbibed (Antunes and Sgarbieri, 1979). A similar mechanism does not seem to operate in lentil used in the study. Data in Table I show that water uptake in both the good- and poor-cooking samples of hulled Eston lentil was similar between 2 and 20 h of soaking at 25 °C. The same was true for dehulled Eston lentil soaked in water for up to 7 h. Both the good- and poor-cooking hulled samples had absorbed most of the water by about 20 h; the dehulled samples, by about 4 h. Jones and Boulter (1983) had reported greater leaching losses from hard beans due to membrane impairment induced by unfavorable storage condition. In dehulled Eston lentil the solids leached out on soaking good- and poor-cooking samples for up to 7 h at 25 °C had generally similar weights (data not given). Thus, in lentil seed coat may have little, if any, role in affecting its cooking quality. It may simply act as water retardant, thereby prolonging water uptake as shown in Table I, as well as during cooking of lentil. In field-grown lentil, the HTC condition may originate entirely in the cotyledon without any participatory role by the seed coat. Radioautogram of HTC intact (hulled) and decorticated (dehulled) bean showed that hilum was the major rate-limiting barrier to water uptake. The water uptake by cotyledons was uniform and faster in both fresh and aged, dehulled bean (Varriano-Marston and Jack-

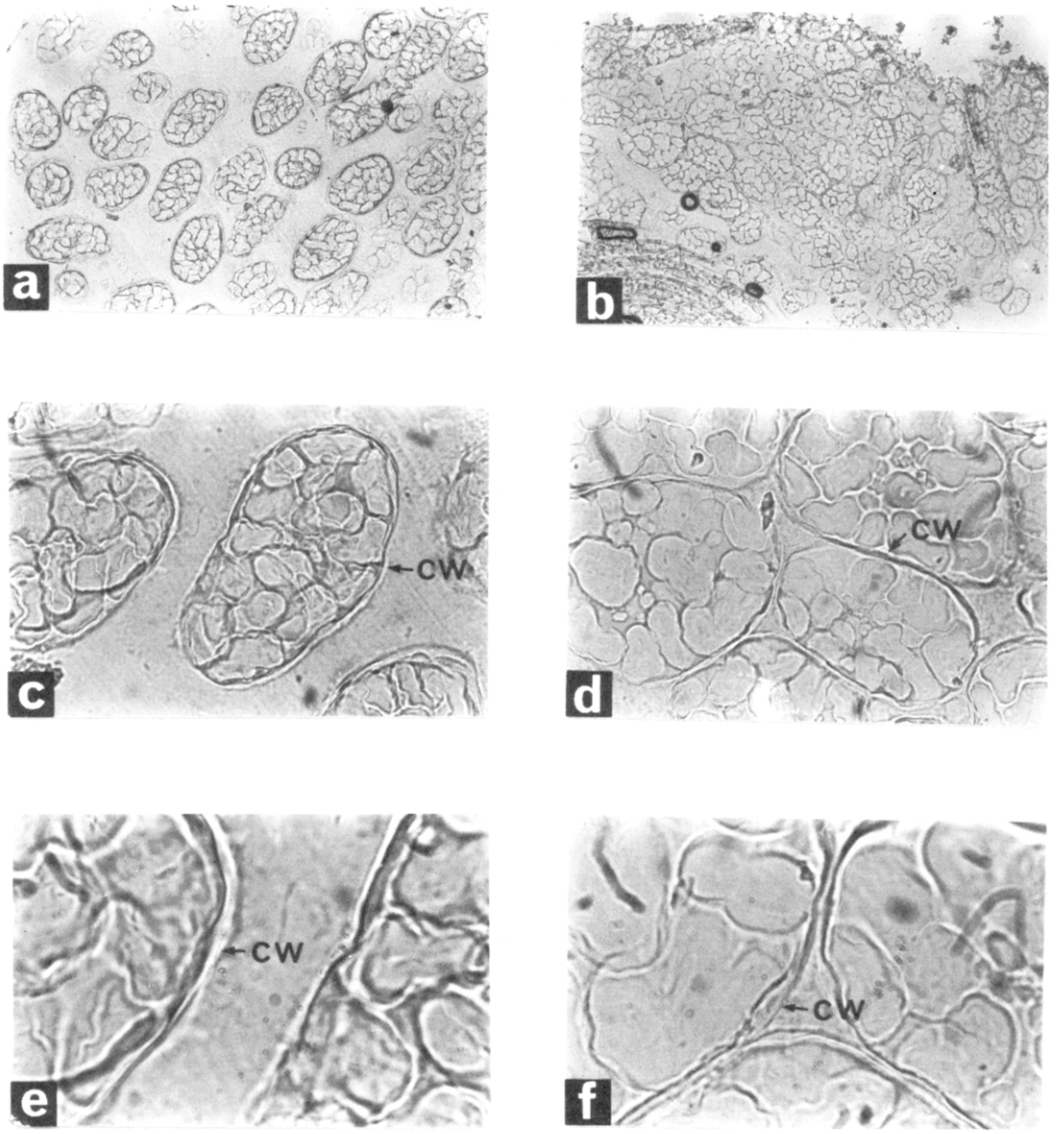

Figure 2. Light micrographs of good (a, c, e, clear cell separation) and poor (b, d, f, lack of cell separation) cooking samples of cooked Eston lentil. Magnification: a, b, 250; c, d, 400; e, f, 1000; CW, cell wall.

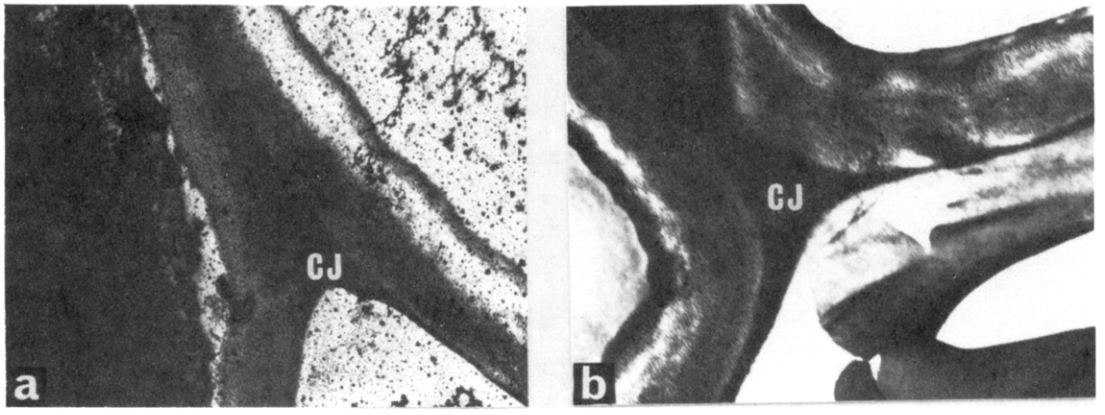

Figure 3. Transmission electron micrographs of cross-sections of good (a) and poor (b) cooking samples of uncooked Eston lentil cotyledons. Magnification: 20 000. The dark area at the cell junctions (CJ) suggests manganese dioxide deposition.

son, 1981). The hydration coefficient data of hulled and dehulled lentil (Table I) tend to support a similar conclusion.

Composition and Structure of Good- and Poor-Cooking Lentils. Table II shows the shear force and P fractions of the good- and poor-cooking samples of Eston lentil. The two samples varied in shear force by 37.8%. This increase in shear force in the poor-cooking sample was accompanied by 26.9%, 44.1%, and 23.1% reductions in seed P, PA, and PA-P, respectively. The lower PA content of the poor-cooking sample was in agreement with data reported previously (Bhatty and Slinkard, 1989). The role of PA in affecting the cooking quality of legumes has been suggested in a number of studies (Stanley and Aguilera, 1985; Vindiola et al., 1986; Bhatty and Slinkard, 1989). It has been postulated that PA che-

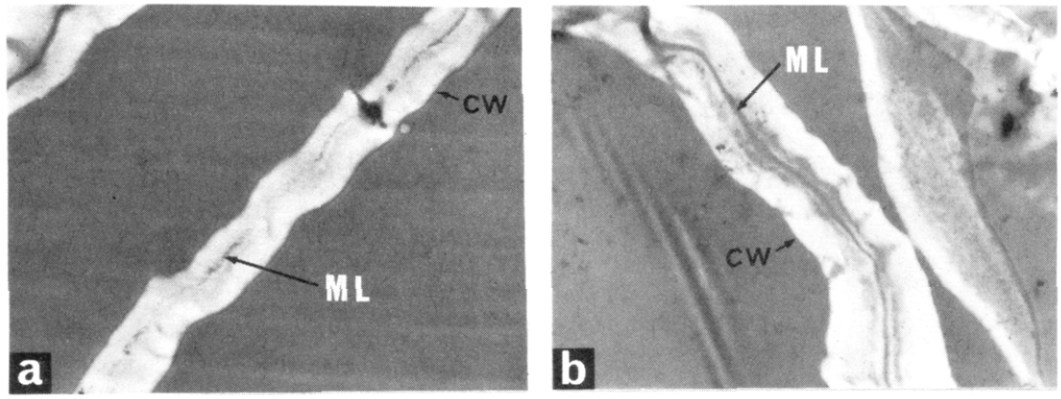

Figure 4. Transmission electron micrographs of cross-sections of good (a) and poor (b) cooking samples of uncooked Eston lentil cell walls isolated by the procedure of Reichert (1981). Magnification: a, 16 000; b, 10 000; ML, middle lamella; CW, cell wall.

lates Ca^{2+} and Mg^{2+}, preventing their binding to pectin in the cell wall for the formation of the insoluble calcium and magnesium pectates. In lentil, grown under field conditions, the critical level of PA sufficient to bind the divalent cations is not known. Nevertheless, reductions in PA content of lentil are likely to lower its cooking quality.

Light micrographs of the cooked samples, taken at three magnifications, showed clear cell separation in the good-cooking sample (Figure 2a,c,e), unlike in the poor-cooking sample where cells were not separated (Figure 2b,d,f). In Figure 2d,f, the tricellular junctions with intercellular spaces as well as the middle lamella were visible. As the middle lamella, predominantly containing pectin, binds adjacent cells together, cell separation in the good-cooking sample was a clear indication of pectin solubility. Obviously, the middle lamellar region in Figure 2b,d,f had reduced water solubility than in Figure 2a,c,e. Therefore, an explanation of the poor-cooking condition in lentil, grown under field conditions, may largely lie in altered solubility of pectin. The light micrographs of good- and poor-cooking lentil samples resembled those of hard and soft beans reported by other workers (Kon, 1979; Jones and Boulter, 1983).

Transverse sections of good- and poor-cooking uncooked lentil cotyledons were observed under the transmission microscope to determine lignification, if any, of these tissues. Cell wall lignification was implied as one of the participating factors in the HTC in *Phaseolus* bean (Hincks and Stanley, 1987). Phenolic acids such as *p*-coumaric and ferulic, the precursors for the formation of lignin, have been identified in lentil cotyledons (Sosulski and Dabrowski, 1984), where they are probably concentrated in the CW. Transmission micrographs of cotyledons from the poor-cooking sample showed heavier (darker) staining at the cell junction (Figure 3b), compared to Figure 3a, which is a micrograph of cotyledons of the good-cooking sample. Similarly, CW of the poor-cooking lentil showed more darker and regular staining of the middle lamella (Figure 4b) than that of the good-cooking sample (Figure 4a). The darker staining of these tissues was probably due to manganese dioxide formed on reduction of $KMnO_4$ by lignin. Lignin content of the CW determined quantitatively was significantly higher for the poor-cooking sample (Table III), although there was the difficulty of measuring it with precision. Hincks and Stanley (1987) had suggested that a partially lignified CW may reduce water permeation across the cells. In the present study this does not seem to be the case. Water uptake (hydration coefficient) of hulled and dehulled samples of good- and poor-cooking lentil soaked for various times was generally similar (Table I). Structural differences in CW of HTC bean, observed in electron micrographs, did not affect mode of water penetration into the cotyledons (Varriano-Marston and Jackson, 1981). Thus, although some degree of lignification of cell junctions and CW in the poor-cooking lentil may be apparent (Figures 3 and 4b; Table III), its implication in affecting the cooking quality of lentil was not clear. There was the difficulty, if not impossibility, of obtaining sections from the same general areas of the good- and poor-cooking lentil CW. Secondly, the extremely small size of the sections can easily lead to erroneous interpretation of the micrographs.

Table III. Yield and Composition of Cell Walls Isolated from Good- and Poor-Cooking Eston Lentil (Means and Standard Deviations of Duplicate Determinations)[a]

component	good-cooking	poor-cooking
yield, %	4.2	3.8
proximate, %		
protein (N × 5.8)	6.5 ± 0.0	5.8 ± 0.1*
ash	1.0 ± 0.6	1.6 ± 0.0 ns
starch	1.2 ± 0.0	0.5 ± 0.1*
lignin	1.2 ± 0.1	1.7 ± 0.1*
galacturonic acid	17.7 ± 0.5	18.1 ± 0.6 ns
minerals, μg/g		
potassium	966 ± 50 (746)	833 ± 74 (548) ns
calcium	1845 ± 50 (1710)	1762 ± 20 (1620) ns
magnesium	640 ± 10 (536)	501 ± 32 (440)*
monosaccharides, %		
arabinose	51.7 ± 4.4	61.8 ± 2.4**
erythrose	nd[a]	nd[a]
fucose	0.5 ± 0.1	0.5 ● 0.0 ns
galactose	8.3 ± 0.8	6.9 ± 0.5**
glucose	12.1 ± 3.1	11.3 ± 0.6 ns
rhamnose	0.8 ± 0.2	0.6 ± 0.1*
xylose	7.7 ± 0.7	8.8 ± 0.8*
total	81.1 ± 9.3	89.8 ± 4.4 ns

[a] Least significant difference between the two means in each row was calculated by a *t*-test. Key: *, $P = 0.05$; **, $P = 0.01$; ns = not significant. [b] Not detected.

Composition and Structure of CW. The CW were isolated in almost identical yields (3.8–4.2%) from the good- and poor-cooking samples of lentil, by the procedure of Shibuya et al. (1985). The preparations contained 5.8–6.5% protein (N × 5.8), which was within the range reported for dicotyledonous, primary CW (John and Dey, 1986), 1.0–1.6% ash, 0.5–1.0% starch (most likely contaminant), and 17.7–18.1% galacturonic acid (Table III). Some of these values, including lignin, were significantly different. Light micrographs of the CW preparations showed no evidence of starch granules, nor of protein poorly stained with Fast green (Figure 5a,b). The CW structures appeared similar in both the samples. Most CW were intact, although some were broken.

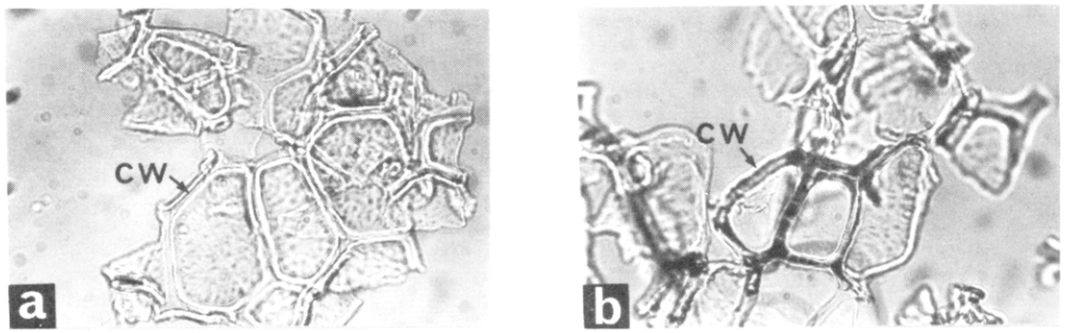

Figure 5. Light micrographs of cell walls isolated from good (a) and poor (b) cooking samples of uncooked Eston lentil by the procedure of Shibuya et al. (1985). Magnification: 400; CW, cell wall.

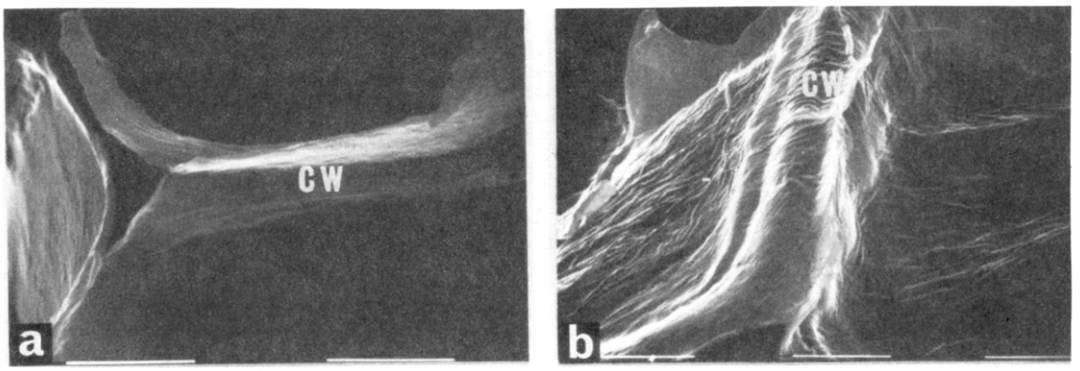

Figure 6. Scanning electron micrographs of cell walls isolated from good (a) and poor (b) cooking samples of uncooked Eston lentil by the method of Reichert (1981). Magnification: a, 2980; b, 2200; bar, 10 μm; CW, cell wall.

The CW isolated from the good- and poor-cooking lentil samples by the procedure of Reichert (1981) had generally similar proximate composition (not given in Table III). In this procedure, no chemicals are employed. The cellular contents are washed from swollen, slurried seed by repeated washings through a series of screens. The material retained on a 75-μm screen is freeze-dried. The CW contained 7.5–8.7% protein (N × 5.8), about 1.0% starch, and 1.1–1.3% lignin. These preparations were used for the scanning electron micrographs shown in Figure 6 and for transmission micrographs reported earlier (Figures 3 and 4). The CW of the poor-cooking lentil appeared multilayered and laminated (sheetlike) at the cell junction (Figure 6b), compared to a rounded structure observed in Figure 5a. The multilayered, sheetlike structure may be due to deposition of secondary CW materials. A similar structure was observed in the HTC bean by Hincks and Stanley (1987).

The CW were analyzed for Na^+, K^+, Ca^{2+}, and Mg^{2+} (Table III). Two separate preparations were used. The first analysis was done on small duplicate samples of the preparations, and the second singly on larger quantities of the material (values in parentheses in Table III). Values for Na^+ were ignored because of its easy contamination from external sources. Both sets of data gave lower values for the CW of the poor-cooking sample, although only the values for Mg^{2+} were significantly different. The minerals seemed tightly bound to the CW as they were not removed during its preparation, which required extensive washing. These data may appear contrary, as more Ca^{2+} and Mg^{2+} may be expected in the CW of the poor-cooking lentil to form the insoluble calcium and magnesium pectates, considered responsible for lack of cell separation seen in Figure 2b,d,f. There was the possibility of higher losses of these elements from the CW of the poor-cooking sample during its preparation, due to structural alterations observed in Figures 3, 4, and 6 or to lower PA content of this sample (Table II), which resulted in a reduced binding of the elements. Nor is it possible to predict the levels of Ca^{2+} and/or Mg^{2+} necessary for the formation of pectates, which may depend on reactive sites available on pectin.

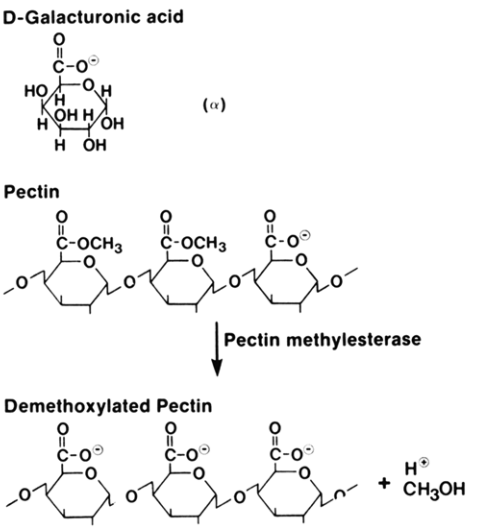

Figure 7. Mechanism for the demethoxylation of pectin by pectin methylesterase.

Arabinose was the major monosaccharide present in the CW of both the good- and poor-cooking samples; its concentration was considerably higher in the CW of the poor-cooking sample (Table III). The other major sugars were galactose and xylose; some of the glucose may have been derived from contaminating starch in the CW. The monosaccharide composition of lentil CW may suggest an arabinan or arabinogalactan types of polysaccharides, possibly with xylose side chains. Such polysaccharides, particularly arabinans, are commonly distributed in the primary CW of cotyledonous plants, although arabinogalactans may only be minor components of most

Table IV. Methoxyl Content, Degree of Esterification, and Galacturonic Acid Content of Pectic Substances Extracted from Cell Walls of Good- and Poor-Cooking Samples of Eston Lentil (Means and Standard Deviations of Duplicate Determinations)[a]

measurement	extracted pectin		commercial pectin	
	good-cooking	poor-cooking	given	found
yield, %	22.3	19.4**		
methoxyl, %	5.2 ± 0.3	6.3 ± 0.6 ns	6[b]	6.8–8.6
degree of esterification, %	59.3 ± 1.5	60.9 ± 0.8 ns	33–40[c]	36.4–38.5
galacturonic acid, %				
titrimetric	54.9 ± 2.0	64.5 ± 4.8**		
colorimetric	41.3 ± 0.3	42.7 ± 1.5		

[a] Least significant difference between the two means in the same row calculated by a t-test. Key: **, $P = 0.01$; ns = not significant. [b] Low ester apple pectin from BDH, Saskatoon. [c] Pectin from Foodpro National, Montreal.

plant CW (John and Dey, 1986). I am not aware of any data reported in the literature on the monosaccharide composition of lentil CW. The present data may be compared to those of pea and broad bean (*Vicia faba*) CW, isolated by a different procedure (Brillouet and Carre, 1983). Cell walls of both of these legumes contained arabinose, galactose, and xylose in the same order of concentration as obtained in the present study, although their proportions were different.

Pectic substances were extracted, with ammonium oxalate solution, from CW of the good- and poor-cooking samples. The yield varied from 19.4 to 22.3%, and was higher for the good-cooking sample (Table IV). Methoxyl content of pectin isolated from the good- and poor-cooking samples was not significantly different and was close to that of a low ester commercial pectin. The degree of esterification of the two extracted pectin samples was also similar. Like the methoxyl content, the degree of esterification of the commercial pectin, determined in the laboratory under the same conditions used for the extracted pectin, was within the range specified for the sample. Thus, the data suggested that lentil pectin was of the low-ester variety. Its galacturonic acid content varied significantly between the two samples when determined titrimetrically but was similar when determined by a colorimetric procedure (Ahmed and Labavitch, 1977). The similar galacturonic acid values obtained colorimetrically seemed more in agreement with methoxyl content and degree of esterification data of the lentil samples.

The low ester pectin may have implications of its role in affecting the cooking quality of lentil. Such a pectin need not be demethoxylated enzymatically to free carboxyl groups to bind Ca^{2+} and Mg^{2+}, as shown schematically in Figure 7. It may contain, in its natural state, enough of such groups for the formation of calcium and magnesium pectates. Thus, the role of the enzyme pectin methylesterase in the development of the HTC condition in lentil grown under field conditions may seem of questionable value. The similar degree of pectin esterification in good- and poor-cooking lentils found in this study, unlike in soft and hard beans (Jones and Boulter, 1983), may suggest a secondary role of CW in affecting the cooking quality of lentils grown under field conditions. However, a lower lignin content, higher manganese dioxide concentration in the TEM sections, and evidence for secondary cell wall formation in the poor-cooking lentil may suggest a "lignification-like" mechanism. Nevertheless, seed PA content seemed to be critical. However, its optimum level in affecting the cooking quality of lentil needs to be established.

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Registry No. K^+, 7440-09-7; Ca^{2+}, 7440-70-2; Mg^{2+}, 7439-95-4; P, 7723-14-0; PA, 83-86-3; lignin, 9005-53-2; low-methoxyl pectin, 9049-34-7; water, 7732-18-5.

Changes in Nonvolatile Acids, Sugars, Pectin, and Sugar Composition of Pectin during Peach (Cv. Monroe) Maturation

Glenn W. Chapman, Jr.,* and R. J. Horvat

R. B. Russell Agricultural Research Center, USDA—ARS, P.O. Box 5677, Athens, Georgia 30613

Changes in soluble sugars, nonvolatile acids, pectin content, and the sugar composition of isolated pectin were determined during Monroe peach maturation from 80 days after flowering (DAF) until about 2 weeks after the beginning of fruit drop (130 DAF). Sucrose and sorbitol showed the largest changes of all carbohydrates measured. Quinic was the major acid in immature fruit but rapidly decreased during maturation. Malic became the major acid during final growth stages, and the increase in malic acid to citric acid ratios closely paralleled the time at which sucrose reached maximum levels. Total pectin levels increased linearly in immature fruit but leveled off about the time sucrose began its second sigmoidal increase. The carbohydrate composition of pectin also changed more during the linear increase in total pectin but remained fairly constant after total amounts leveled off. The time at which sucrose levels and malic acid to citric acid ratios reached maxima and quinic acid levels were lowest could be used as reliable indices of peach physiological maturity.

Most research conducted on compositional changes during peach maturation has focused on soluble solids, total sugars, reducing sugars, and titratible acidity as reliable indices of physiological maturity (Sistrunk, 1985; Kader et al., 1982). With improved analytical techniques, individual sugars and nonvolatile acid components have been determined during maturation on a variety of peach cultivars (Ishida et al., 1985; Sandhu et al., 1983; Chalmers and van den Ende, 1975; Li and Woodroof, 1968). These studies give a more realistic approach to the biochemistry involved in fruit maturation and the relationship of the levels of sugars and acids to physiological maturity. However, there is very little information available in the literature on the changes in individual carbohydrates and nonvolatile acids relative to peach maturation in cultivars grown in the Southeastern United States. Also, information is lacking on the development of pectin content and composition during peach maturation. The purpose of this study was to monitor the changes in these components during maturation of Monroe peaches. Consistent relationships between one or more components may provide reliable indices of peach physiological maturity.

MATERIALS AND METHODS

Plant Material. Peaches were obtained from trees (cv. Monroe) grown at the University of Georgia Horticultural Farm (Watkinsville, GA). The trees were about 4 years old and received adequate rainfall and/or irrigation during the summer of 1988. Twelve peaches were hand-harvested at intervals during maturation (June 20–Aug 15), starting at 81 days after flowering (DAF) until about 2 weeks after the beginning of fruit-drop (130 DAF). Individual peach diameters (long axis) were measured with a vernier caliper to ensure sample uniformity. The average fruit weight (10–12 peaches) was also obtained.

Sugars and Nonvolatile Acid Determination. Two replicate samples of about 5–6 g of fresh mesocarp tissue were diced with a sharp knife and ground with a mortar and pestle in 75% ethanol. The tissue was allowed to extract for 10 min, brought to a final volume of 25 mL (75% ethanol), and then filtered through Whatman No. 4 filter paper. Aliquots of the extract (0.5 mL) were dried under a slow stream of dry nitrogen and sugars and nonvolatile acids determined by capillary GLC as previously described (Chapman and Horvat, 1989).

Pectin Extraction, Determination, and Carbohydrate Composition. Fresh mesocarp tissue (10–15 g) from each harvest was prepared as above and allowed to extract for 10 min in 150 mL of 75% ethanol. The extract was filtered and the pulp washed with three portions of 150 mL of 75% ethanol