Effect of Calcium and Magnesium on Cell Wall and Starch of Dehydrated Potato Granules

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Solubilization of pectic substances in isolated potato cell walls was suppressed during precooking by addition of Ca but not Mg and was highest when Ca equivalents stoichiometrically corresponded to or exceeded the pectin free carboxyl groups. Furthermore, unlike Mg, Ca bound to starch phosphate groups profoundly affected cell wall pectin solubilization. Potato tissue firming was appreciable up to 100 ppm of added Ca or Mg, implying that starch, in addition to cell wall pectins, was responsible for the firming phenomenon. A gradual substitution of hydrogen or alkaline cations in starch phosphate groups with Ca or Mg decreased starch swelling power, water binding capacity, viscosity, and solubilization. Processing of tubers using added Ca resulted in dehydrated granules with both improved integrity of cell wall and controlled swelling power of the starch matrix.

Quality characteristics of dehydrated mashed potato granules include, among others, bulk density, intact and broken cell counts, contents of soluble amylose, reducing sugars and ash, swelling power, and the rate of water uptake upon reconstitution. While the consistency of some of these characteristics is readily maintained in year-round production, swelling power and the rate of water uptake show a seasonal variation related to the hardness of water used in precooking and cooling steps of processing. The variation is aggravated by the fact that potatoes are low in Ca content and, as such, respond to a decrease in water hardness with an unfavorable rise in the swelling power of the reconstituted end product.

The swelling power, reflected by the volume attained when granules are placed in cold water under standardized conditions, is greatly influenced by the extent of starch retrogradation during processing, and by the ash content of the tuber. Tailoring starch solubility and swelling in the holding or tempering step of a granule process is well documented (Potter, 1954; Hadziyev and Steele, 1979). A decrease in gelling strength and viscosity of potato starches by polyvalent cations has also been observed (Whittenberger and Nutting, 1948; Winkler, 1960). In the process patented by Nelson et al. (1962), potatoes were precooked with demineralized water containing 17–85 ppm of Ca, the sole essential mineral ingredient. Though the patent was aimed at avoiding interaction of Fe with potato phenolics, the presence of Ca during precooking provided a product of superior and consistent texture. However, this observation was not elaborated upon.

The behavior of potato cell wall (CW) pectins at 100 °C in the presence of Ca and starch was reported by Keijbets et al. (1976), while the role of pectins in potato texture was recently reviewed by Reeve (1977). Part of the CW pectins serves as a cement in the middle lamella, while a part is located in the CW proper. According to Albersheim et al. (1975), this pectin was covalently bound to a monolayer of hemicellulose, which in turn, through H-bonding, covered the cellulose fibrils of the wall. A precooking step, followed by cooling and steam-cooking, brought about a firming of the potato tissue. The firming was ascribed to deesterification of pectin and to its decreased solubility resulting from an increase in CW Ca and Mg bridging (Bartolome and Hoff, 1972). Addition of Ca to cooking water, or its presence in a precook–soak treatment, resulting in toughening of cooked potato tissue, was also ascribed to Ca interaction with CW pectin.

Our observations during granule production suggested that Ca simultaneously interacted with CW pectin and gelled starch, and that both reactions were responsible for the count of intact sound cells (i.e., cells which are encircled by undamaged CW and which are clearly separated from the starch matrix), while the reaction with starch affected the swelling power. To substantiate these observations, we designed a study which involved simulated add-back (A-B) precooking and cooling steps, using CW and starch model systems, with or without Ca or Mg, as well as whole potato tissue processed further into granules by a freeze–thaw (F-T) process. Since most of the changes found were microscopically observable phenomena, micrographs were used to corroborate the findings.

EXPERIMENTAL SECTION

Raw Potatoes. The cultivar used was Netted Gem (Russet Burbank) of specific gravity 1.096 ± 0.002, grown under irrigation in Southern Alberta. The potatoes were stored at 4 °C and were reconditioned at 18 °C for 10 days before processing.

Dry Matter Content. The tuber dry matter content was determined by heating 10-g samples at 55 °C for 5 h and then at 105 °C for an additional 2 h.

Ash Content. Freeze-dried and ground samples of 2–5 g were dry ashed in a Vycor dish. After initial charring on a hot plate, samples were placed in an oven at 500 °C for 2 h. After cooling, then wetting with a few drops of concentrated HNO_3, the dishes were returned to the oven for an additional 30 min. The residue was then cooled in a desiccator and weighed. The mineral composition of the ash was determined after solubilization in 6 N HCl under gentle boiling for 30 min. The solution was made up to volume, and a diluted aliquot was analyzed in the presence of lanthanum chloride for Na, K, Ca, and Mg by using a Perkin Elmer M 303 atomic absorption spectrophotometer. The content of P was determined essentially by the method of Fiske and Subbarow (1925).

Processed Potato Samples. F-T potato granules were prepared in a semi-pilot scale, stirred fluidized bed dryer using a process which essentially involved steam-cooking, mashing, freezing, thawing, predrying, granulation, and drying.

A-B granules were taken from the production line of a processing plant. Details of these processes were given elsewhere (Moledina et al., 1978a). Precooking and cooking steps were designed to simulate the conditions of an A-B

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process. The peeled potatoes were cut into 6.4-mm thick slices and washed in 0.5% $NaHSO_3$. The slices were precooked at 70 °C for 30 min in 3 parts by weight of deionized water, with or without calcium acetate or magnesium acetate. Then they were cooled to 25 °C for 20 min in 6 parts by weight of deionized water, again with or without minerals. The precooked and cooled slices were then steam-cooked for 35 min and processed into dehydrated granules by a laboratory F-T process.

Mineral Gains and Losses by Slices during Precooking. The gain of Ca and leaching of K, Ca, or Mg during precooking and/or cooling of potato slices were studied in the presence of 0–500 ppm Ca^{2+} in deionized water. The weight ratio of slices to water was 1:3 in precooking and 1:6 in cooling. In precooking water clarified by centrifugation at 14000*g* for 10 min, the Ca content was determined by 0.02 N EDTA titration at pH 14.0 with Eriochrome Blue Black R as an indicator (Standard Methods, 1970). Corrections were required for P interference (20 ppm P was leached out in precooking, and 2.5 ppm during cooling). In addition, clarified aliquots were evaporated to dryness in Vycor dishes, wetted with concentrated HNO_3, and ashed. The residue was dissolved in 2 N HCl and analyzed for Ca, K, and Mg by atomic absorption spectroscopy.

Texture Measurement. The cooked 6.4-mm thick slices were cooled to 22 °C, and a random selection was made for penetration force measurement. The instrument described by Ooraikul (1974) was used, except that the probe consisted of two concentric annular rings connected at 90° radii. The inner ring was a cylinder of 8-cm length and 1.8-cm o.d. There were two venting slots of 1.6 × 6 cm on the sides of the cylinder. The outside ring was 4.4 cm in o.d. and 0.5 cm in height. The rings and the connecting radii were constructed of 0.457 mm thick 26-gauge stainless steel.

The penetration force was measured by driving the probe vertically on to the slice until the rings and the connecting radii penetrated through the surface. The force was recorded, and the maximum height of the peaks, calibrated in terms of g-force, was designated as the penetration force.

Microscopy. Samples for scanning electron microscopy (SEM) were prepared from the external phloem region of raw, precooked, or cooked potato tissue. The samples were fixed by glutaraldehyde in a 0.1 M potassium phosphate buffer, pH 7.0, postfixed with osmium tetroxide, and then dehydrated through a graded ethanol series (50–100%), followed by freeze-drying and coating with 20 nm of gold. Details of the procedure were outlined by Fedec et al. (1977). The samples were examined by a Cambridge "Stereoscan" microscope at an accelerating voltage of 15 kV.

Potato granules were also examined with transmitted light microscopy to obtain the count of intact sound cells. For this purpose, 1 g of dehydrated granules was suspended in 20 mL of deionized water with gentle stirring for 1 min and left to stand 2 h. A portion was spotted on a microscope slide and stained with a drop of either 0.1% aqueous methylene blue or 0.02 N KI_3 and viewed at 60–200× magnification using a phase contrast Olympus Model BH microscope.

Starch. *a. Isolation.* Several washed and peeled tubers were immersed in ice-cold water containing 100 ppm $NaHSO_3$, diced, and then homogenized in a Waring Blendor with 2 volumes of ice-cold deionized water or 1% ammonium oxalate. The slurry was squeezed through a 100-mesh polyester sieve cloth, and the homogenate was centrifuged at 2500*g* for 10 min. The upper light-brown layer of protein was removed from the sediment, and the lower layer of starch was resuspended in water and recentrifuged. This was repeated several times until no impurity was evident under the microscope. This undefatted preparation was dried in a vacuum oven at 25 °C until constant weight. The yield was 55% of the tuber dry matter.

b. Ash Content. The ash content and mineral composition of the starch was determined by both dry and wet ashing. In addition, starch cations were collected by ion-exchange using 0.1 N HCl as eluant (Winkler, 1960). Contents of Na, K, Ca, and Mg were then determined by titration with 0.02 N EDTA (Standard Methods, 1970) and/or by atomic absorption spectroscopy. The P content was determined by the method of Fiske and Subbarow (1925).

c. H (Ca, K, Mg)-Starch. The H-starch was prepared from native starch by ion exchange. Starch (20 g) was treated on a 25–40-μm sintered glass funnel intermittently by 0.1 N HCl and deionized water, as recommended by Winkler (1960). Alkalimetric titration of such H-starch provided starch-bound phosphoric acid.

K-, Ca-, and Mg-starches of 25, 50, 75, and 100% were prepared from H-starch by neutralization of its primary phosphoric acid hydrogen with corresponding base equivalents. All starch samples were dried at 25 °C in a vacuum oven and stored in a desiccator over P_2O_5.

d. Gelatinization Temperature. This was determined by visualization of starch granule outlines and by the loss of birefringence under plane polarized light. A Kofler programmed microscope hot stage fitted on a polarizing microscope was used. A drop of aqueous 0.2% starch slurry was spotted on a slide, and then it was surrounded by a ring of Nujol mineral oil. A cover glass was used, avoiding entrapment of air bubbles, and the slide was mounted on the hot stage. Heating started at 40 °C, and was programmed for a rise of 2 °C/min. The initial gelatinization temperature was recorded when 2% of the granules lost their birefringence, while the end point was at 98%. Five runs were averaged to ensure consistency and precision.

e. Swelling Power and Solubility. A single point characterization was done at 85 °C. A starch sample of 0.5 g was suspended in 180 g of distilled water in a 250-mL centrifuge bottle. The suspension was stirred at 200 rpm at 22 °C for 3–5 min with a rectangular stainless-steel paddle (1.9 × 3.8 cm) and then for 30 min at 85.0 ± 0.2 °C. The content in the bottle was then adjusted to 200 g, and the bottle was closed, gently mixed, and centrifuged at 2200 rpm (1200*g*) for 15 min in a swinging bucket rotor of an IEC centrifuge. The clear supernatant was removed and the sedimented, swollen paste weighed. The soluble starch was determined from a 50-mL aliquot of supernatant after evaporation of water at 100 °C and drying of the residue at 110 °C for 6 h. Calculation and details of the procedure were outlined by Schoch (1964).

f. Viscosity Measurement. The optima for starch gel concentration and for the speed of the rotor of the NV sensor system of the Haake Rotovisco Model RV-3 (Haake, Karlsruhe, Germany) were found to be 0.3% (starch dry weight/volume) and 90.5 rpm. The gel was prepared by refluxing starch in deionized water for 5 min, followed by cooling to 25 °C. A 20-mL aliquot was taken for assay. The sensor system and the cup were kept at 25 °C by means of a circulating water bath. Viscosity was measured after running the rotor at 90.5 rpm for 3 min and was calculated in centipoise (cP). Average values were obtained

from triplicate readings for each starch sample.

Cell Wall Isolation. CW preparations were obtained from freeze-dried potato powder by removal of starch with deionized water, as outlined earlier (Hoff and Castro, 1969; Moledina et al., 1978b). Preparation purity was examined by plane polarized light and scanning electron microscopy. The yield was 5.17 ● 0.14% of the tuber dry matter.

H-cell walls were prepared as outlined by Keijbets et al. (1976). The preparation had 16.1% pectin as anhydrogalacturonic acid (AGA), 373 ± 8 μmol of free COO^- groups/g of CW, and an average degree of esterification (DE) of 55.3%.

Pectic Substance Determination. *a. Carbazole Reaction.* The method of McComb and McCready (1952) was applied for deesterified solutions of pectic substance. The interfering sugar content was determined by the phenol–H_2SO_4 method and was expressed as galactose (Dubois et al., 1956). The calibration curve for the galactose–carbazole reaction was then used to correct the anhydrouronide results.

b. Degree of Esterification. The CW pectic substances DE with methanol was determined by the Cu^{2+}-ion exchange method described by Keijbets and Pilnik (1974).

Precooking and Cooking with Cell Walls. CW preparation (20–50 mg) was suspended in capped culture tubes in 10 mL of 0.02 M Tris–Pipes buffer pH 6.1 [Pipes = piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)]; Sigma Chem. Co., St. Louis, MO). Then $CaCl_2$ or $MgCl_2$, or Ca(Mg)-starches were added, and the mixture was gently shaken and heated at 60, 65, 70, or 100 °C for 30 min. The Ca(Mg) equivalents were added in amounts corresponding to 0, 0.5, 1, and 5 times the equivalents of free COO^- groups present in CW, while Ca(Mg)-starches were used in amounts simulating the original starch (76.06 ± 1.16%) and CW (5.17 ● 0.14%) content in the tuber. Heating was terminated by immersing the tubes in ice-cold water. The mixture was filtered through a 25–40-μm sintered glass funnel. The solubilized pectic substances were determined in the filtrate and the bound cations in the CW retained on the filter.

Solubilized starch, which interferes in pectin analysis, was removed from the filtrate by a preliminary F-T step at −10 °C, followed by addition of acetate buffer, pH 4.8, and centrifugation at 14000*g* for 10 min. The remaining starch was hydrolyzed enzymatically at 55 °C for 30 min by using amyloglucosidase (Sigma, grade II; 20 EU/5-mL aliquot), and completion was checked by a spot test with 0.005 N KI_3. The glucose generated was further oxidized under constant aeration at 35 °C for 3 h to gluconic acid by glucose oxidase (Sigma; 18 EU/5-mL aliquot), with completion checked by a glucose reagent strip. The enzymes were coagulated by boiling for 5 min, and then the solution was cooled to 25 °C, made up to volume, and centrifuged at 10000*g* for 10 min. The supernatant was collected and analyzed for uronide and galactose contents.

RESULTS AND DISCUSSION

Tuber Cells as Viewed by SEM. Scanning of a cross-section of a whole tuber (not shown) revealed potato peel averaging 11 cells in depth, followed by a single layer of phellogen (cork cambium) and phelloderm cells. Next came the external phloem region, a ring of disconnected vascular bundle regions, the internal phloem (which occupied close to 60% of the total tuber volume), and, finally, in the center of the tuber, the pith. The architecture of a section of tissue cut from the outside of the tuber and bounded by peel and external phloem is shown in Figure 1. The structure of a single potato cell from the internal phloem is shown in Figure 2a. Cell dimensions averaged

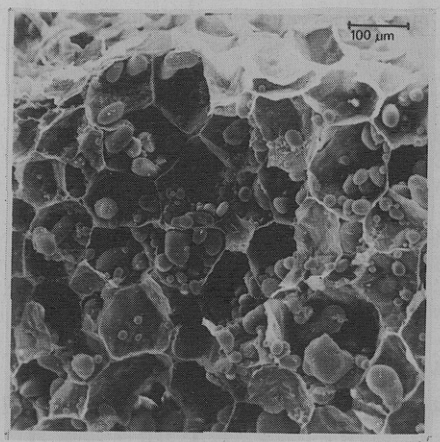

Figure 1. SEM micrograph of raw potato tissue cut from the outer portion of the tuber. The peel and adjacent cells are shown.

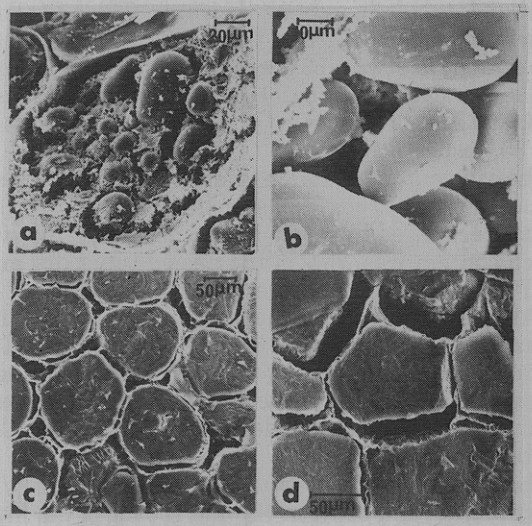

Figure 2. SEM micrograph of potato cells before and after cooking. Before cooking: (a, b) an overall view of a single cell from the external phloem region. After cooking: in the absence (c) and in the presence (d) of Ca^{2+}. Note the gap of 2.20 μm between the CW and gelled starch.

136 × 169 μm. The thickness of one CW was 0.24 ± 0.02 μm. The cytoplasm, layered between CW and vacuole, had a width of 0.5 μm. This was stretched to 1.1 μm when mitochondria were present. The majority of medium and large oval-shaped starch grains were found not in cytoplasm, but within the "huge" vacuole.

The average size of starch grains in micrometers in the cells of external phloem (32 × 54), vascular bundle (16.8 ± 4.6), inner phloem (20 × 38 to 32 × 54), and pith regions (12.4 ± 5.3) followed the same trend as that of dry matter in these regions. The dry matter content was 20.0% in peel, 24.5% in the external and 22.5% in the internal phloem, and only 18.8% in the pith.

Micrographs of starch grains (b) before and (c, d) after steam-cooking revealed that the grains, once swollen and gelatinized, were fused together, occupying nearly the entire volume of the cell. The extent of occupation, however, depended greatly on the mineral composition of the starch and of the water used in the precooking step of the potato granule process.

Factors such as pH, buffer, tonicity, temperature, etc., which affect the quality of potato cell fixation with glutaraldehyde and postfixation with osmium tetroxide, were optimized in order to effectively prevent swelling, shrinkage, or distortion of the cells. Thus, the results of SEM should be considered as close to those "in situ" (Hayat, 1970).

Table I. Mineral Composition of Peeled Potato Tuber and Its Starch and Cell Wall[a]

tuber size[b]	ash, %	P	Ca	Mg	Na	K
A	2.68 ± 0.30	156.7	34.8	106.2	33.8	1812
B	4.91 ± 0.13	192.6	42.2	126.8	34.1	1850
C	3.55 ± 0.11	141.3	36.9	123.0	37.9	1871
av ± $\overline{SD}$	3.71 ± 1.12	163.5 ± 26.3	37.9 ± 3.7	118.7 ± 10.9	35.3 ± 2.3	1844 ± 30
			Starch			
A	0.36	70.6	18.7	18.4	23.8	15.1
B	0.37	72.3	14.4	15.3	28.2	17.8
C	0.36	69.1	10.2	18.2	22.3	20.6
av ± $\overline{SD}$	0.36 ± 0.09	70.7 ± 1.6	14.5 ± 4.3	17.3 ± 1.7	24.8 ± 3.1	17.8 ± 2.7
			Cell Wall			
B	av ± $\overline{SD}$	9.3 ± 0.2	124.0 ± 8.7	130.0 ± 4.4	60.0 ± 3.5	121.0 ± 14.5

[a] In mg/100 g of dry matter (cv. Netted Gem, Southern Alberta). [b] A, 110-168 g (4-6 oz); B, 169-224 (6-8), and 225-336 (8-12).

Mineral Composition. The mineral composition of tuber and its starch and CW is given in Table I. The ash content averaged 3.71% of the peeled tuber dry matter. The content of P was 163.5 ± 26.3 mg/100 g of dry matter. The major cations, in mg/100 g of dry matter, were K, 1844, and Mg, 118.7, while Ca and Na were minor at 37.9 and 35, respectively.

The ash content of starch grains was 0.36%. The content of P was 70.7 mg/100 g of starch dry matter, while the cations ranged from 14.5 to 24.8 mg. Since the average starch content was 76% of the peeled tuber dry matter, the P bound to starch represented 32.4% of total tuber P. Also, about one-third the Ca content and only 11.1% of the Mg of the tuber were bound to starch. Only 0.74% of the tuber K content was found in starch. In the case of starch isolated from tuber by the aid of aqueous ammonium oxalate, a tenfold decrease was found for Ca and Mg bound to starch.

Thoroughly purified CW contained 9.3 mg of P/100 g of dry matter, and between 121 and 130 mg of Ca, Mg, and K. Since the average CW content of the peeled tuber dry matter was 5.2%, the P bound to CW represented only 0.3% of the total tuber content and that of Ca 17% and Mg 5.7%.

Some of these results were similar to those reported for European cultivars. Mič a (1976) found 101.6 mg % tuber P content after harvest, and 112 and 128 mg %, respectively, for tubers stored at 2 or 10 °C. Close to 49% of this amount was found in starch after harvest, the percentage declining slightly with storage at 2 °C, but not at 10 °C. However, K and Ca contents of starch differed significantly. The former was 2.5-fold lower and the latter 3.5-fold higher than the contents in the Alberta-grown Netted Gem cultivar.

The mineral content of cultivars grown in the United States was given by True et al. (1978). The results showed that the mineral composition of the Idaho- or Washington-grown Russet Burbank cultivar was close to Alberta-grown Netted Gems, while other cultivars were generally low in Ca. The cv. Superior had only 9.2–10.0 mg % Ca in the whole unpeeled tuber. As found by Bartolome and Hoff (1972), in such a cultivar Ca is preferentially (up to 80%) bound to tuber CW and starch. Mg contents in such tubers, though 90 ± 12 mg %, were only 1.7-times the equivalents of Ca in CW and starch.

The content of P in starch (70.7 ± 1.6 mg %) was obtained by an ion-exchange method. Wet ashing with $HClO_4$ gave values of 80.4 ± 0.8 mg %. Neither value was influenced by the weight of the tuber from which the starch was isolated, nor by starch grains in the size range of 20–60 μm. Close results to ours were those for native starches from potatoes grown in Germany (Winkler, 1960).

Table II. Degree of Esterification (DE) of Pectin and Its Content of Anhydrogalacturonic Acid (AGA %) in Potato Cell Wall Preparation

cell wall prep[a]	Cu^{2+}-ion exchange method		HCl-hydrolysis-carbazole method
	DE	AGA, %	AGA, %
I	55.71	16.10	16.17
II sonicated	55.13	16.40	16.25
III	54.55	16.28	16.08
IV sonicated	55.80	15.87	15.95
av ± $\overline{SD}$	55.30 ± 0.58	16.16 ± 0.23	16.11 ± 0.13

[a] Preparations had 373 ± 8 μM free COO^- group/g of cell wall dry matter.

The ion-exchange method gave 77.8–85 mg % P, while the gravimetric method inflated the results by 4 mg %.

The sum of milliequivalent cations (3.68) bound to starch surpassed that of primary phosphate groups (2.28). Nevertheless, their ratio of 1.6 still strongly suggested that the primary metal phosphates were predominant in native starch.

Tuber CW Pectin Characteristics. The potato CW pectin content is presented in Table II. The Cu^{2+}-ion-exchange and acid hydrolysis carbazole methods gave similar results. Since starch interfered in the ion-exchange method, the CW preparations were thoroughly washed before analysis. Destarching with Me_2SO (Ring and Selvendran, 1978) was avoided in order to retain "in situ" characteristics of the CW needed for model system assay. In such a CW, 55% of the total carboxyl groups were methylated, while the free carboxyls amounted to 373 ± 8 μM/g of CW dry matter. The combined Ca and Mg content (see Table I) was 170 μequiv, which implied that every second carboxyl group was bound to a divalent cation. The monovalent cations neutralized only 57 μequiv of the available carboxyl groups. All these cations were readily ion-exchangeable with H^+ ion.

CW prepared in tap water containing 40 ppm each of Ca and Mg had 12 mg of Ca/g of CW. This value was slightly less than double the theoretical value of 7.4 mg that would be expected with saturation of the free carboxyl groups of the CW. Ca incrustation of the CW was observable with electron microscopy (Figure 3). Yet to be clarified is whether the excessive Ca uptake is due to the phosphoric acid of the amylopectin starch moiety left enmeshed in CW (Ring and Selvendran, 1978) or to adherence of phytic acid to CW, which would then readily account for discrepancies observed.

Tuber Starch Characteristics. *a. Gelatinization as Affected by Starch Cations.* As seen from Table III, native starch gelatinization began at 56.8 °C and ended at 68.5

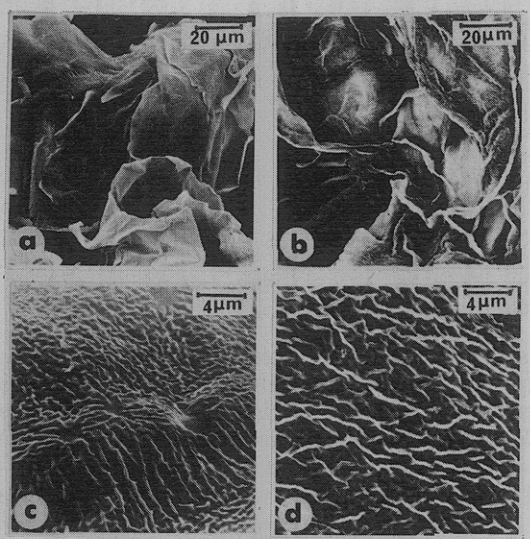

Figure 3. SEM micrograph of pure CW preparations isolated from raw tuber in the absence (a, c) and presence (b, d) of 50 ppm Ca^{2+}. The average thickness of two walls cemented by the middle lamella was close to 1 μm. Primary CW structure is revealed under high magnification (c, d).

Table III. Effect on Starch Gelatinization Temperatures of Starch Phosphate Neutralization by Some Tuber Cations[a]

starch P neutralization %	K		Ca		Mg	
	start	end	start	end	start	end
25	55.9	68.0	56.2	68.8	56.7	68.8
50	54.5	67.6	55.8	68.5	55.6	68.3
75	54.4	67.5	55.7	68.5	55.5	67.8
100	54.3	67.5	55.7	68.5	55.5	67.8

[a] Start and end °C for H- and native starch were 59.6, 69.4, and 56.8, and 68.5 °C, respectively.

°C, reflecting the wide size range of the starch grain population within the tuber. When native starch cations were replaced by H^+ ions (giving H-starch, pH of a 1% water suspension of 3.15, phosphate moieties all in the form of phosphoric acid), the range was 59.6 to 69.4 °C. At 25% neutralization of H-starch with Ca or Mg, the gelatinization temperature was close to that of native starch. Further decreases occurred as the degree of neutralization was increased. K brought about the lowest gelatinization temperature.

b. Solubility, Swelling, and Viscosity Characteristics as Affected by Starch Cations. The native starch solubility and swelling power averaged 30.1% and 205, respectively (Table IV). Starch devoid of metal cations (H-starch) was 2.5-times more soluble and had a 2.3-fold greater swelling power. A gradual increase in the content of metal cations, as illustrated by K, brought about a drop of starch solubilization from 94.1 to 59.7% and of swelling power from 447 to 287. Much greater suppression was found with divalent cations, especially when neutralization was over 50%. The similar values found for both Ca and Mg implied a common mechanism in their ability to decrease starch solubilization and swelling power.

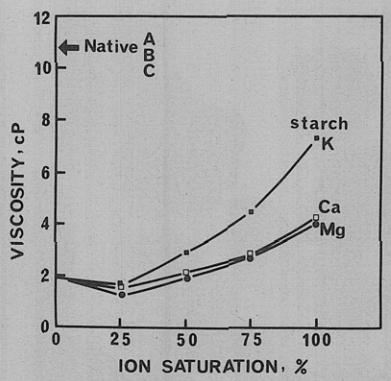

Figure 4. Viscosity of potato H-starch neutralized to different levels with K, Ca, and Mg. The values plotted had SD's at n = 3 ranging from 0.04 to 0.39, with an average of 0.18.

This conclusion could also be drawn from starch viscosity curves (Figure 4). The fluidity of native starch after gelatinization was lowest (viscosity in cP close to 11) and that of H-starch the highest (cP = 2). Neutralization of H-starch resulted in a fluidity decrease, the extent of which depended on the cation valence. Practically equal decreases were obtained with Ca or Mg, and their fluidity decrease effect was much less than that of K.

Some of the starch data obtained were expected since they reflected, in part, influence of phosphoric acid content on the behavior of starch. In ionized form, phosphates present in the branched amylopectin fraction, and separated from each other by 200–300 glucose units, introduce repulsive force among the starch molecules (Winkler, 1960). The force is diminished by neutralization of phosphate into its primary salt, as occurs in potato tuber at its natural pH of 6.1, since secondary salts are formed only at pH 8–9. Hence, salts of divalent cations bring about cross-linkages between two phosphoric acid ester groups, either in the same glucose chain or between two adjacent chains. Preferential bridging between two chains is the preferred view. Such cross-linking would then account for viscosity changes and contraction of the swollen gelatinized starch grain and for lower solubility of the starch (Winkler, 1960; Hollő et al., 1962). Additional pertinent data are those of Putz and Tegge (1976). They found that it is the cultivar which predetermines the ash and phosphoric acid contents, and starch viscosity, not the tuber weight or specific gravity. Moreover, they found that starch characteristics could not be tailored by fertilizer treatment. Our findings for starch isolated from Netted Gem tuber (Table I) and viscosity data support the former conclusion.

Granule Process. Model System. When tuber tissue, with mineral composition as given in Table I, was subjected to precooking and cooling in water with no Ca, followed

Table IV. The Effect on Starch Solubility (sol %) and Swelling Power (SP) of the Level of Starch Phosphate Neutralization by Some Tuber Cations[a]

starch P neutralization %	K		Ca		Mg	
	sol %	SP	sol %	SP	sol %	SP
25	94.1 ± 0.5	447 ± 45	87.4 ± 0.5	380 ± 33	88.0 ± 0.6	413 ± 40
50	83.8 ± 1.0	393 ± 36	80.6 ± 0.3	325 ± 19	81.4 ± 0.4	354 ± 17
75	69.8 ± 1.3	325 ± 22	53.2 ± 1.9	176 ± 12	58.4 ± 2.0	199 ± 16
100	59.7 ± 2.5	287 ± 23	38.2 ± 0.9	108 ± 2	40.1 ± 0.6	118 ± 2

[a] Solubility and swelling power for H-starch were 96.8 ± 0.6, 478 ± 93 and for native starch 38.1 ± 1.8, 205 ± 8, respectively.

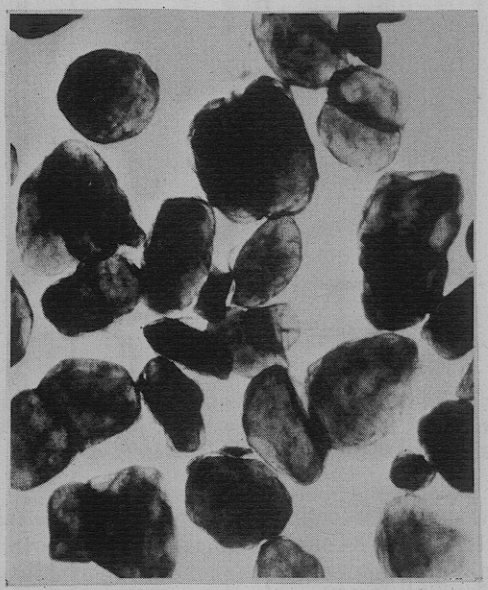

Figure 5. Photomicrograph of dehydrated potato cells obtained by a F-T granule process which included a precooking step in deionized water. The cells were rehydrated in cold water prior to staining by methylene blue.

Table V. Potato Cell Wall Pectin Solubilization and Ca Uptake as Affected by Ca during Precooking

precooking temp, °C	Ca^{2+}/COO^- equiv	AGA, %	Ca uptake by cell wall (μequiv/g of cell wall)
60	0	7.31	0
	0.5	1.22	183
	1.0	0.33	350
	5.0	0.29	880
65	0	8.84	0
	0.5	1.21	203
	1.0	0.31	296
	5.0	0.24	900
100	0	14.01	0
	0.5	0.62	192
	1.0	0.49	280
	5.0	0.36	925

by steam-cooking and drying steps of a granule process, the end product, after reconstitution in cold water, appeared as in Figure 5. The granules, corresponding to individual cells, appeared to be devoid of surrounding CWs and the starch matrix occupied the entire cell volume. This suggested that, in the absence of divalent cations, the CW pectin was either partially solubilized and the CW lost its rigidity, collapsing onto the starch matrix, or the CW remained rigid, but swelling of starch closed the void space between the CW and starch matrix. In order to check CW pectin solubilization, a model system consisting of isolated CW and Ca was subjected to precooking and cooling steps simulating those of a granule process.

a. Model Systems of CW and Ca. Results for potato CW pectin solubilization and the ability of CW to bind the Ca present in precooking water are given in Table V. As seen from the data, during precooking at 60 and 65 °C, followed by cooling, H-CW pectin solubilization was greatly influenced by the Ca present in water. The least solubilization occurred when Ca equivalents matched or surpassed those of pectin free carboxyl groups. In the absence of Ca, nearly half of the pectin content was lost, and, when CWs were cooked at 100 °C in the absence of Ca, pectin losses increased to 87%. Proportionally to the retained pectin and Ca level in water, Ca uptake by CW increased from 0 to close to 0.9 mequiv/g of CW. The Ca bound to

Table VI. Effect of Ca Starch during Precooking on Cell Wall Pectin Solubilization

starch P neutralization with Ca^{2+}, %	temperature					
	60 °C		70 °C		100 °C	
	AGA, %	rel sol, %	AGA, %	rel sol, %	AGA, %	rel sol %
H- starch	6.03	100	6.29	100	13.97	100
25	3.36	55.7	2.75	43.7	8.21	58.8
50	2.18	36.6	1.83	29.1	6.64	47.5
75	2.05	34.0	1.58	25.1	3.10	22.2
100	1.62	26.9	1.31	20.8	2.40	17.2

CW at 60 °C (up to 0.5 and 1.0 ratios of Ca/COO^- equivalents) reflected the preferential binding by available free carboxyl groups, while that at a ratio of 5.0 increased the Ca uptake beyond double that theoretically expected if all carboxyls of the pectin were free.

All the Ca bound to CW was in an ion-exchangeable form and was readily replaced by ethanolic 0.7 N HCl. This amount of ion-exchangeable Ca plus that of starch accounted for the cold 1 N HCl-extractable Ca of tuber reported by Bretzloff (1971).

When Ca in precooking water was replaced by Mg in a $cation/COO^-$ equivalents ratio between 0 and 5.0, no suppression of pectin solubilization was obtained. Nor was a decrease in solubilization beyond that already imparted by Ca alone obtained in the presence of both cations.

Thus, the extent of CW pectin solubilization should depend on the hardness of water used in precooking and cooling steps of a granule process. However, in addition to the external Ca source required to prevent pectin losses, the CW had its own available internal source (124.0 ± 8.7 mg/100 g of CW) which could cement only each sixth free carboxyl group of the pectin. Therefore, in subsequent model system assays, starch, with its 29% of the total Ca of the tuber, was tested as a potential internal source.

b. Model Systems with CW and Starch. Table VI lists the results of CW pectin solubilization in a model system of H-CW and H-starch in a weight proportion simulating that in tuber. Starch was neutralized to different degrees with Ca and as such was a Ca source for CW material. Precooking with H-starch alone did not bring about a drop in pectin loss when compared to blank tests with starch omitted. At 100 °C at a loss of 86% of the CW total pectin was obtained in the presence of H-starch, this loss again coinciding with those of blanks. An efficient pectin solubility decrease was provided by starch when only a quarter of its phosphoric acid primary hydrogens were neutralized by Ca. Higher starch Ca content brought about further lowering of pectin losses.

As expected, Mg-starch, regardless of the neutralization extent, did not effect pectin loss. Of interest was the finding that consistently less pectin was solubilized at 70 than at 60 °C. This implied that complete starch gelatinization was needed to take full advantage of Ca bound to starch. As suggested from results in Table III, for complete starch gelatinization and metal cation release, the optimum precooking temperature would be 68.5 °C, close to the 70 °C applied commercially in the add-back granule process. Also, the finding implied that starch gelatinization within potato cell should bring about losses of divalent cations of starch, and consequently changes in its solubility, viscosity, and swelling characteristics similar to those given in Table IV. Moreover, thermal diffusion of starch cations toward the CW and out of the cell would result in extensive leaching of tuber minerals other than Ca. This suggestion was proved in subsequent assays when the model system was replaced by whole tuber tissue in the precooking step.

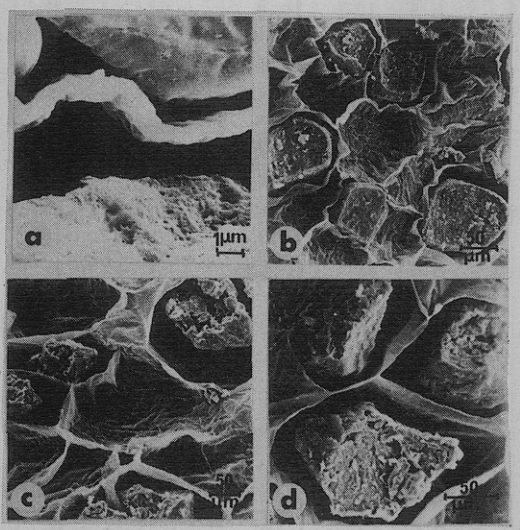

Figure 6. SEM micrograph of precooked potato cells. The gap width between CWs and gelled starch depended on the Ca^{2+} content in precooking water. For details see text.

Table VII. Effect of Ca in Precooking and Cooling Water on Some Tuber Mineral Constituents[a]

Ca^{2+} ppm in water	precooking[b]			cooling[b]		
	K	Ca	Mg	K	Ca	Mg
0 (deionized water)	−23	−14 ± 1	−29	−5	−5 ± 1	−5
10		−6 ± 1			4 ± 2	
50[c]	−24	−5 ± 1	−27	−4	16 ± 3	−8
100	−21	22 ± 3	−31	−6	36 ± 3	−7
200	−27	72 ± 3	−27	−3	46 ± 4	−5
300	−20	90 ± 4	−29	−6	55 ± 5	−6
400	−21	103 ± 6	−36	−8	66 ± 10	−5
500	−24	180 ± 7	−33	−6	79 ± 12	−6

[a] Uptake (+) was calculated as percent gain, while leaching (−) as percent loss of the original cation content of the tubers used (462 mg of K, 32 mg of Mg, and 10 mg of Ca per 100 g tuber fresh weight). [b] Results for Ca^{2+} are based on at least three runs, while the rest of averages of duplicates differing by less than 10%. [c] The results for percent change in tuber Ca^{2+} content after precooking in the presence of 60–90 ppm Ca^{2+} were −2 ± 1 (60), 2 ± 1 (70), 3 ± 1 (80), and 8 ± 2 (90).

Granule Process. Whole Tuber Tissue. *a. Role of Ca in Precooking.* The results so far obtained were for model systems in which CW pectin and starch behavior provided data necessary to better understand the changes in potato granule quality caused by water hardness. Cells of potato tissue slices precooked and cooked in water with varying levels of Ca are shown in Figure 6. The gap width between the Ca-cemented CW and swollen gelatinized starch matrix (Figure 6a) was negligible in the absence of Ca and large in the presence of high levels of Ca. An intermediate width was obtained at 50 ppm (Figure 6b) and a much greater width at 100 ppm or higher (Figure 6c,d). In all runs the swelling power suppression, as reflected by starch shrinkage within the cell, correlated well with increasing levels of Ca.

Ca uptake or leaching at various levels of Ca in water and its effect on Mg and K content of the tuber tissue are given in Table VII. Precooking in deionized water and water containing up to 50 ppm Ca resulted in leaching, while above 100 ppm there was an uptake of Ca. The equilibrium occurred at 60–70 ppm Ca in precooking and at 5 ppm in cooling water. Mg and K leaching was 30 and 23%, respectively, in precooking, and 6 and 5% in cooling water, relative to their original contents in raw tuber. The extent of leaching of both cations was independent of Ca level.

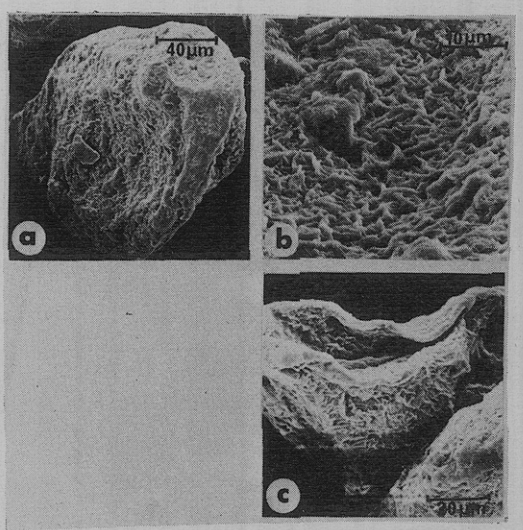

Figure 7. SEM micrograph of a dehydrated potato cell obtained by a F-T granule process which included a precooking step in deionized water. (a) A dehydrated intact cell. (b) The fine surface structure. (c) A burst cell.

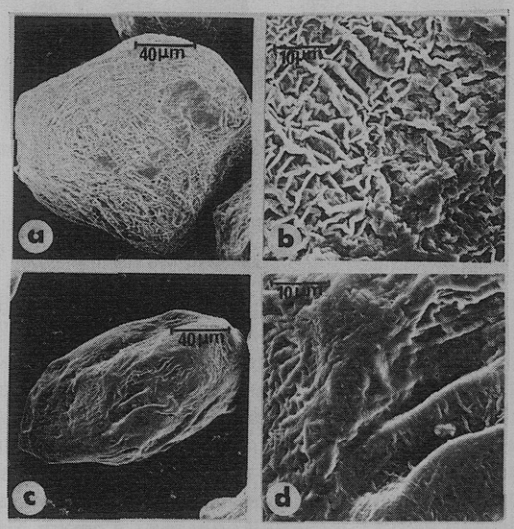

Figure 8. SEM micrograph of dehydrated potato cells obtained by a F-T granule process which included a precooking step in water containing 100 ppm Ca^{2+} (a, b), or 100 ppm Mg^{2+} (c, d).

When precooked, cooled, and steam-cooked potato slices were dried into granules by a F-T process, the appearance of the granule surface depended on the level of Ca in water. Precooking and cooling in deionized water provided granules as shown in Figure 7. The cell surface was a fused network of ridges, while some of the cells burst and were devoid of retrograded starch matrix. Also, sloughing of tuber was high, implying that the extent of sloughing was related to absence of divalent cations, as suggested by Zaehringer and Cunningham (1971). In the presence of 100 ppm Ca as the sole mineral ingredient in water, the granule surface appeared as a sharp, distinct network of ridges, most probably indicating the presence of cemented and firmed CW pectin (Figure 8a,b). When Ca was replaced by 100 ppm Mg, the network of ridges regained its fused form, and, in addition, the cell surface developed deep invaginations (Figure 8c,d), implying that the CW was not cemented or firmed, and had collapsed and adhered to the shrunken starch matrix.

b. Effect of Ca and Mg on Tissue Firming. The overall firming of the cells, as imparted by Ca and Mg, was

Table VIII. Effect of Ca and Mg in Precooking and Cooling Water on Penetration Force Required to Puncture the Steam-Cooked Potato Tissue[a]

cation, ppm	0	50	60	70	80	90	100	300	500
Ca^{2+}	2192 ± 58	2233 ± 57	2397 ± 33	2493 ± 37	2627 ± 23	2700 ± 30	2807 ± 63	2688 ± 12	2583 ± 87
Mg^{2+}	2192 ± 58	2367 ± 33					2633 ± 67	2533 ± 117	

[a] Acetate salts were used as the source of cations. Values for penetration force (g) were averages of three sliced potato samples.

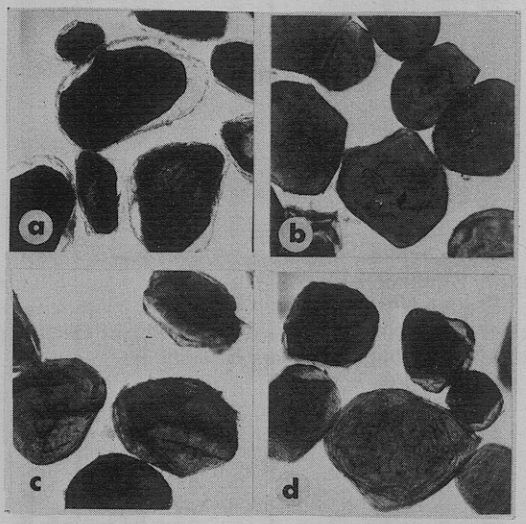

Figure 9. Photomicrograph of dehydrated potato cells obtained by a F-T granule process. For details see text.

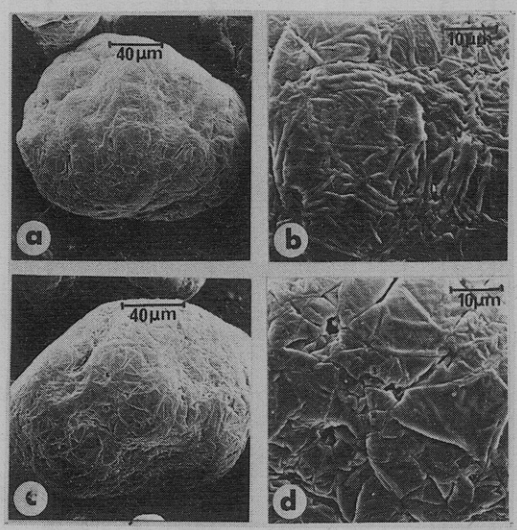

Figure 10. SEM micrograph of dehydrated potato cells obtained by the add-back granule process from Canadian plants A (a, b) and B (c, d).

measured by the penetration force required to puncture the precooked, cooled and steam-cooked potato tissue before mashing. As seen from results in Table VIII, there was an increasing firming effect up to the level of 100 ppm Ca, beyond which no additional firming occurred. Thus, 100 ppm Ca in precooking water of a granule process using 6.4 mm thick potato slices appeared to saturate both the available free carboxyl groups of the CW pectin and the starch phosphoric acid, the two major cell constituents accounting for firming. That starch should not be omitted in consideration of firming was demonstrated by processing the slices in water containing only Mg. Though Mg had no effect on CW pectin solubilization and did not have the ability to cement the CW, it did bring about tissue firming which paralleled that of Ca. The firming was highest at 100 ppm, but was still lower than that of Ca. The difference in penetration force appeared merely to reflect the inability of Mg to firm the CW.

Corroborating the above findings are the micrographs provided in Figure 9. Dehydrated cells precooked in the presence of 100 ppm Ca (Figure 9a,b) or 100 ppm Mg (Figure 9c,d) were rehydrated in cold (Figure 9a,c) or hot water (Figure 9b,d) and were then iodine stained. Unlike processing in the presence of Ca, Mg did not cause separation of CW and starch since the CWs adhered to the starch matrix (Figure 9c). Distinct separation in Ca-treated granules vanished only after rehydration in hot water (Figure 9b).

Thus, the count of intact sound cells (a quality parameter) in a granule process is related to the starch swelling power and cell wall firming and can be tailored in the precooking step of the process.

CONCLUSION

On the basis of all the findings on model systems and whole potato tissue, and on experience gained by semi-pilot scale granule production, the commercial add-back process is now performed with strict regulation of water hardness, paying particular attention to Ca content. The granules

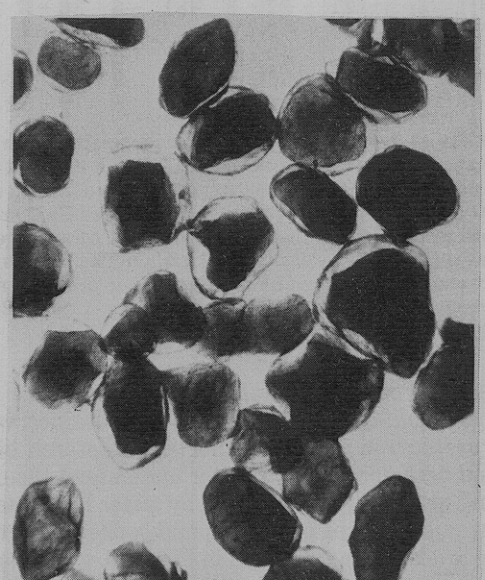

Figure 11. Photomicrograph of dehydrated potato cells treated as in Figure 5, but precooked in the presence of 100 ppm Ca^{2+}.

produced do not have a surface with a sharp network of ridges, but, rather, appear polished due to high shear and abrasive forces applied in the process (Figure 10). Nevertheless, cell walls are well firmed and are separated from the starch matrix to an extent of not less than 60% of the total cell count (Figure 11), unless market specifications require otherwise. In addition, such granules have favorable swelling power, rate of water uptake, contents of soluble amylose and ash, and broken cell count.

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Quality of Tall Fescue Forage Affected by Mefluidide

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Tall fescue (*Festuca arundinacea* Schreb. "Ky 31") was treated with mefluidide {*N*-[2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide} at 0, 0.28, and 0.56 kg/ha on 29 April in 1975 and 1976. Forage was sampled 20 May in 1975 through 24 June 1976, and cellulose, total sugar, and crude protein content and dry matter yield were determined. Within 14 days after mefluidide treatment, percent cellulose was decreased, whereas percent sugar and crude protein were increased in tall fescue. Dry matter yields obtained 21 days after treatment were reduced by mefluidide treatments. Yield reduction was the result of inhibition of floral development by the mefluidide application. However, by the second harvest date in both years, mefluidide treatments did not affect yields.

Tall fescue (*Festuca arundinacea* Schreb.) is the predominant cool-season pasture species in the transition zone that separates the northern and southern regions in the eastern half of the United States. "Kentucky 31" is the predominate cultivar. The popularity of tall fescue arises from its adaptability and many outstanding agronomic attributes (Bush and Buckner, 1973). Templeton and Taylor (1966) reported consistent dry matter yields of 7–9 metric tons/ha from well-fertilized stands of tall fescue. Tall fescue pastures were observed to carry animals 78 days longer each year than orchardgrass (*Dactylis glomerata* L.) (Blazer et al., 1956, and 1969). The quality of tall fescue, however, is often inadequate as a feed for maximum production of lean meat when grazed by ruminants. Poor forage quality is especially noted from the onset of reproductive growth until maturity (Blazer, 1964; Norman and Richardson, 1937). Total sugar and digestible energy tends to be lowest during the summer when tall fescue is in the reproductive stage (Sullivan, 1969). Phillips et al. (1954) reported increased cellulose content as tall fescue matured. Therefore, inhibition of maturation might maintain tall fescue in the vegetative stage and consequently maintain high-quality forage as indicated by sugar and cellulose composition. It was found that frequent cuttings (14-day intervals) prevented the onset of reproductive stage and produced a high-quality forage (Burrus, 1957). However, dry matter yields were severely suppressed. Maleic hydrazide retarded maturity and increased the water soluble carbohydrate content of orchardgrass, but many of the plants became chlorotic and necrotic and dry matter yields were reduced (Brown and Blazer, 1965).

Mefluidide {*N*-[2,4-dimethyl-5-[[(trifluoromethyl)-sulfonyl]amino]phenyl]acetamide}, previously known as MBR 12325, is a plant growth regulator that has been found to inhibit seedhead production (Chappell et al., 1977; Freeborg and Daniel, 1975; Gates, 1975; Hield and Henstreet, 1975) and enhance color and root growth of many cool-season grasses (Gates, 1975). Increased amounts of recoverable sugar from sugarcane (*Saccharum officinarum* L.) with application of mefluidide have been reported (Gates, 1975). Therefore, in an attempt to improve the quality of Kentucky 31 tall fescue forage this study was initiated using mefluidide to regulate growth. Cellulose, total sugar, crude protein, and dry matter yields were used to evaluate plant response to mefluidide.

MATERIALS AND METHODS

Kentucky 31 tall fescue was treated at the preboot stage with mefluidide at 0, 0.28, and 0.56 kg/ha on 29 April, 1975

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