the surfaces with wax reduced this loss, but full recovery was only obtained when tissue grinders with smooth surfaces were used.

The probable error of the glc methods is approximately 2% for macro and semi-micro purified PME and methoxy analysis, 6% for the macro and semi-micro crude PME analysis. Sensitivity is estimated as 3 ppm of methanol in the sample solution prior to nitrite conversion.

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Received for review June 7, 1971. Accepted September 3, 1971. Purdue University Agricultural Experiment Station, Journal Article No. 4412.

Firming of Potatoes: Biochemical Effects of Preheating

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When potato tuber tissue is preheated at temperatures between 60 and 70°C the fully processed tissue (boiled) is firmer and sloughs less than the untreated control. The effect is time dependent and approaches a maximum in about 2 hr. The native pectin methylesterase (PME) of the tissue is inactive at temperatures below 50°C. It is activated above this temperature and reacts with the pectins of the cell wall, as evidenced by the production of free methanol and a decrease in the methoxy content of the cell wall pectin. Above 70°C the enzyme is rapidly destroyed and exerts no effect on the cell wall material. The calcium and magnesium contents of the cell wall increase at the effective temperatures. These observations are consistent with the following interpretation concerning the mechanism of tissue firming. Heating at temperatures above 50°C leads to a loss of integrity of the cellular membrane (plasmalemma) allowing intracellular electrolytes (predominantly K) to contact the cell wall materials, thereby activating PME. The enzyme increases the amount of free carboxyl groups of the cell wall pectin, and Ca and Mg from the cell interior increase the number of metal bridges. This leads to an increased resistance of the tissue to further thermal degradation.

When potato tissue is held for some time at moderate temperatures (50–80°C) and subsequently boiled, it attains a firmer texture than samples that are boiled without pretreatment. This firming effect has been ascribed to the behavior of the starch. Thus, Reeve (1967) and Potter *et al.* (1959) believed that the effect is due to starch retrogradation which results in decreased swelling power of the starch granules. It is held that starch generally affects potato texture by causing a distension of the cell wall when the granules swell during gelatinization (Bettelheim and Sterling, 1955). According to this view a "swelling pressure" leads to a rounding off of the cells, thereby causing rupture of the middle lamella and separation of the cells. Essential to this concept is the existence of an internal pressure caused by the swelling of the starch, although no experimental evidence in support of its existence has yet been reported in the literature.

An alternative view is offered by Linehan and Hughes (1969), who postulated migration of amylose from the starch granules to the middle lamella. Infiltration by amylose into the cell wall fabric was believed to result in reinforcement of the mechanical strength of the cell wall and the middle lamella.

Firming effects have been observed in cauliflower, snap beans, and tomatoes (Sterling, 1955; Hoogzand and Doesburg, 1961; Van Buren, 1968; Hsu *et al.*, 1965). Since these tissues contain only traces of starch, it appears unlikely that the effect is due to starch retrogradation and, in these cases, activation of pectin methylesterase has been postulated as the more likely cause of firming. According to this view, de-esterification of the pectic substances in the cell wall promotes firming, either by reaction of the free carboxyl groups with divalent ions, or more directly by formation of gel-like structures of the pectinic acid produced by the enzyme.

The present investigation was undertaken for the purpose of establishing to what degree activation of pectin methylesterase occurs in potato tubers during pretreatments involving heat and to what extent this reaction is responsible for the firming effect.

EXPERIMENTAL

Field-grown tubers of the cultivar "Superior" stored at 4°C were used throughout this study. The physical effects of firming were observed by determination of firmness by penetrometry and of sloughing by a modified sloughing test (Le Tourneau *et al.*, 1962). The effect of preheating on firmness was studied by heating potato dice (0.5 in.) in water for

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30 min to 120 min at temperatures ranging from room temperature to 100°C. At least 40 dice were used per treatment. Half of the samples were cooled immediately in an ice bath for 10 min. The remaining dice were boiled for 30 min and similarly cooled. Firmness measurements were made with a penetrometer (Precision Universal Penetrometer, Precision Scientific Co.) immediately following the cooling period.

For determination of sloughing, potato slices (0.5-in. × 0.5-in. × 2 mm) were weighed and treated in water at temperatures ranging from room temperature to 100°C for 1 hr. Following pretreatment, the slices were boiled for 10 min while being stirred vigorously with a mechanical stirrer. The slices were placed on a No. 2 mesh screen and dipped ten times in distilled water to wash away sloughed material. The remaining slices were drained by leaving the screen in a tilted position for 2 min and then they were weighed. Sloughing was reported as percent change in weight.

Pectin methylesterase activity and free methanol content were determined in potato dice, pretreated for 1 hr as described above, according to procedures described elsewhere (Bartolome and Hoff, 1972).

Cell wall material was isolated according to Hoff and Castro (1969). All operations were quantitative and performed at temperatures not exceeding 4°C. Raw tubers and tubers which had been preheated for 90 min at 50, 60, 70, and 80°C were fractionated into cell wall material and starch. Since the surface tissue could have suffered some leaching during the treatment, a 30-g sample was taken from the tissue internal to the vascular bundles and homogenized with 100 ml of 0.1% cysteine solution for 10 min. When necessary, a few drops of hexanol were added to prevent foaming. The cell walls were collected by filtration through Miracloth, while starch was harvested from the filtrates. Slurries of the samples treated at 70 and 80°C were incubated with pancreatin at 40°C for 4 hr prior to the filtration for removal of gelatinized starch and denatured protein, since filtration was otherwise impossible. The isolated cell walls were washed with absolute alcohol, followed with diethyl ether, and dried at 40°C under vacuum. The first four filtrates through Miracloth were combined and the starch was allowed to settle. The starch was collected by filtration on paper, repeatedly washed with distilled water, and dried under vacuum at 40°C. Additional raw potato tissue was peeled, freeze-dried, and ground into a fine powder.

The calcium and magnesium content of the cell wall, starch, and intact tuber tissue were determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 290). For this purpose the samples were digested at 200°C with 2 ml each of concentrated nitric acid and perchloric acid. After cooling, the solutions were diluted to 10 ml with distilled water, and lanthanum chloride was (0.1%) added to suppress interference by phosphates. The amount of samples used was adjusted so as to give a final concentration of 1 to 10 ppm for calcium and 2 to 5 ppm for magnesium.

Part of the purified cell wall material was fractionated as described earlier (Hoff and Castro, 1969). The composition of the polysaccharides of the pectic substances isolated from the cell walls was determined by the gas chromatographic procedure of Albersheim *et al.* (1967).

RESULTS AND DISCUSSION

Preheating of potato dice at temperatures up to 80°C for 0.5 to 2 hr has little or no effect on the firmness of the tissue (Figure 1A). At temperatures above 80°C softening takes place at a rate which is temperature dependent. When the preheating treatment is followed by boiling for 0.5 hr, a pronounced softening occurs at the extremes of the temperature scale (Figure 1B), while samples pretreated in the temperature region of 60 to 70°C undergo smaller changes in texture. When the difference, ΔP, in penetrometer readings after and before boiling is considered, it is seen that in this region (Figure 1C) the effect is time dependent with respect to the preheating treatment to the extent that after 2 hr the change is almost negligible. Essentially similar results are obtained when tap water is used instead of distilled water as the heating medium (Figure 1D). The effect of preheating on sloughing (Table I) of potato slices is considerable. The preheated (60 and 70°C) samples gained in weight during boiling while the nontreated and the preboiled (100°C) samples suffered almost complete disintegration.

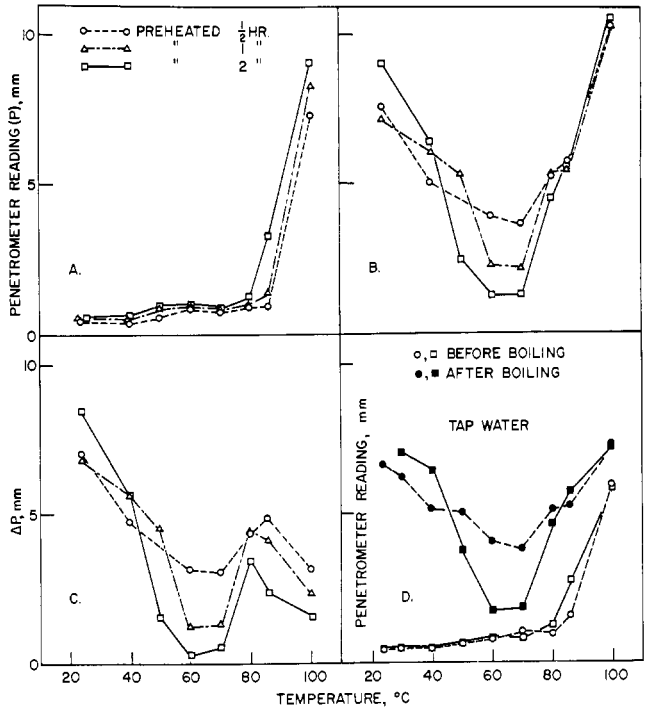

Figure 1. Effect of preheating on potato firmness

Table I. Effect of Preheating on Sloughing of Potato Slices

Preheating temperature, °C	% Slough[a,b]
25	−80.75 ± 19.40
60	2.89 ± 1.54
70	3.50 ± 3.10
100	−100 ± 0

[a] Mean of two determinations. [b] A negative value denotes weight loss.

These results confirm the observations made by others (Reeve, 1967; Potter *et al.*, 1959) that the firming effect exists. We observed that dice treated at 70°C contained very few ungelatinized starch grains, and that a viscous slurry resulted when the dice were homogenized. The interpretation for the firming effect given by the above authors, namely that marginal heat treatments result in starch retrogradation with consequent loss of swelling pressure, is not supported by this observation. The starch gelatinized normally, presumably quickly developed a "swelling pressure," if such exists, yet

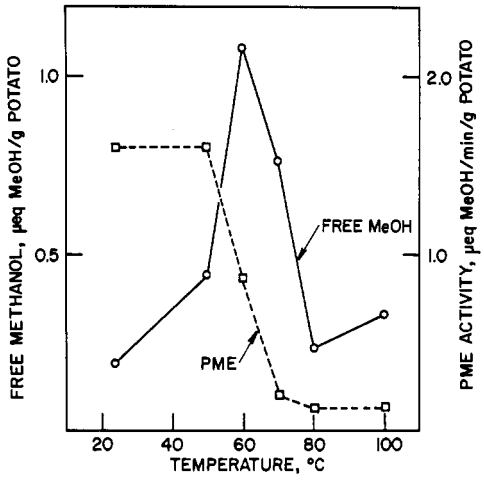

Figure 2. Effect of temperature on PME and free methanol in the tissue

Table II. Effect of Preheating on the Distribution of Calcium and Magnesium in Potato Tissue

Preheating temperature, °C	Component	Calcium content,[a] μg/g potato	Magnesium content,[a] μg/g potato
27	Whole tissue	53.8 ± 8.3	227.6 ± 13.3
50		46.8 ± 1.1	242.5 ± 18.1
60		63.2 ± 2.9	248.6 ± 16.9
70		65.9 ± 4.3	211.0 ± 2.0
80		52.2 ± 3.5	255.4 ± 23.8
		WSD[b] 39.4	WSD 56.0
27	Cell wall	20.5 ± 7.6	13.1 ± 4.5
50		12.7 ± 2.3	14.0 ± 5.1
60		20.9 ± 2.2	33.6 ± 1.3
70		31.6 ± 3.9	30.5 ± 1.3
80		15.8 ± 2.4	15.3 ± 10.6
		WSD 19.9	WSD 20.2
27	Starch	21.2 ± 8.1	30.1 ± 7.6
50		16.7 ± 6.1	28.0 ± 1.8
60		15.7 ± 1.0	34.8 ± 0.2
		WSD 20.7	WSD 17.2

[a] Values for 27°C are means of three determinations; all other values are means of two determinations. [b] WSD: wholly significant difference (Volk, 1958).

the tissue became increasingly resistant to shear as the treatment time increased.

These observations are, on the other hand, consistent with the proposal presented by Linehan and Hughes (1969) that intercellular adhesion determines potato texture and that the extent of amylose diffusing into the cell wall dictates the strength of the adhesion forces. However, other considerations, as presented below, lead us to favor an alternative hypothesis postulating the involvement of pectin methylesterase in analogy with phenomena observed in other vegetables (see introductory remarks).

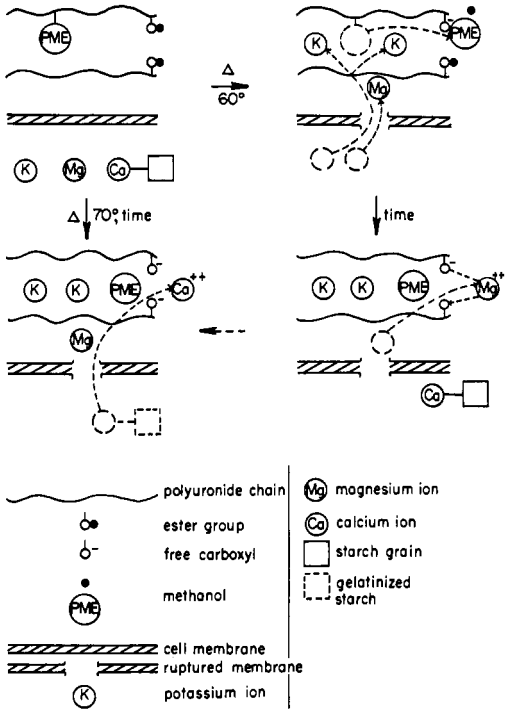

Figure 3. Proposed mechanism to account for the firming effect

The potato enzyme has a temperature sensitivity similar to that reported for tomato (Kertesz, 1951), suffering a loss of about half of its activity when heated in the tissue for 1 hr at 60°C and with negligible activity remaining when similarly treated at 70°C (Figure 2). As indicated by the release of free methanol, the enzyme, although present, is not active to any appreciable extent until the tissue is heated at temperatures above 50°C (free methanol, Figure 2; methoxy content, Table V). Thus, enzyme activation takes place in the temperature region where thermal inactivation sets in. This is also the region where the firming effect was noted. The calcium and magnesium contents (Table II) of the cell wall appeared to increase when the tissue was preheated at 60 to 70°C, although the changes were not significant at the 95% level. Noteworthy is the fact that the magnesium level (molar) is greater both in the cell wall and in the starch than the calcium level, and that the combined amount of calcium in the cell wall and in the starch accounts for approximately 80% of the total calcium in the tissue.

Table III. Effect of Preheating on Constituents of the Cell Wall (μM/g Cell Wall)

Temperature, °C	Ca	Mg	Ca + Mg	Methanol released	Methoxy	Increase[a] in Ca + Mg	Increase[a] in free methanol	Decrease[a] in methoxy
27	43.2	46.2	89.4	17.4	720			
50	27.8	51.8	73.3	39.3	690	−10.1	21.8	30
60	45.7	122.0	167.7	95.0	540	78.1	77.6	180
70	88.0	144.0	229.0	84.0	570	139.6	66.6	150
80	45.1	72.3	117.4	27.0	680	28.0	9.6	40

[a] As compared with the unheated control (27°C).

Table IV. Effect of Preheating on Potato Cell Wall Content and Proximate Composition

	Preheating temperature, °C					WSD
	27	50	60	70	80	
Cell wall content, % wet basis	1.19 ± 0.14[a]	1.14 ± 0.07	1.15 ± 0.18	0.90 ± 0.05	0.88 ± 0.06	0.43
Alkali-soluble hemicellulose, % of cell wall	3.98 ± 0.54	2.96 ± 1.80	3.24 ± 2.92	5.04 ± 0.80	3.80[b]	9.03
Cellulose and lignin, % of cell wall	21.45 ± 2.48[a]	21.39 ± 2.06	22.57 ± 0.88	28.08 ± 0.45	27.95[b]	7.64

[a] Mean of three determinations. [b] Based on one determination; all other values are means of two determinations.

Table V. Effect of Preheating on the Composition of Potato Cell Wall Pectic Material

	Preheating temperature, °C					WSD
	27	50	60	70	80	
Material solubilized by trifluoroacetic acid, %[a]	98.63 ± 0.95	100.00 ± 0.00	99.50 ± 0.71	98.50 ± 0.71	100.00	
Sugar, % of total pectic material[a]	49.40 ± 13.83	37.20 ± 6.29	46.40 ± 2.26	47.20 ± 14.35	67.10	
Polyuronide (as polygalacturonic acid, PGA), by difference	50.6	62.8	53.6	52.8	32.9	
Methoxyl content of PGA, %[b]	10.65 ± 3.33	9.02 ± 3.01	6.25 ± 1.22	5.33 ± 1.55	9.63 ± 1.70	2.78
Composition of sugars, %[c]						
Rhamnose	6.59 ± 0.48	7.10 ± 0.78	6.66 ± 0.28	6.38 ± 0.37	6.66	1.88
Fucose	0.12	0.21	0.09	0.00	0.00	
Arabinose	3.34 ± 0.76	3.42 ± 0.06	3.14 ± 0.28	3.24 ± 0.15	2.86	1.50
Xylose	0.35 ± 0.26	0.79 ± 0.21	0.63 ± 0.14	0.37 ± 0.03	0.3 ± 0.12	1.47
Mannose	0.17 ± 0.03	0.16 ± 0.05	0.00	0.00	0.00	
Galactose	89.50 ± 0.71	88.10 ± 1.27	89.50 ± 0.00	90.10 ± 0.14	90.00	2.67

[a] Number of determinations are 27°C, −3, 80°C, −1; all others −2. [b] Number of determinations are 27°C, −6, 50°C, −5, 60°C, −4; all others −3. [c] Corrected for glucose which was assumed to be due to starch contamination.

The molar increases in divalent metal and of free methanol per unit dry weight of cell wall material as a result of the various pre-treatments (Table III) are roughly similar and strongly suggest a causal relationship between the two. The increase of molar equivalents is of the order of 5–10% of the total carboxyl available in the cell wall. A similar decrease in the methoxy content is also observed. Whether metal bridge formation involving this quantity of galacturonic acid monomer can reasonably be expected to account for the textural changes may be questioned, but it has to be realized that this number represents an approximate doubling of the metal bridges originally present.

The cell wall content appeared to decrease with increasing temperature (Table IV), but the decline was not statistically significant. A similar nonsignificant increase occurred in the amount of cellulose/lignin in the cell wall, perhaps reflecting a corresponding decrease in pectic substances. The composition of the sugars in the pectic substances did not change with increasing temperature (Table V). These results indicated that a relatively minor portion of the cell wall solubilizes at temperatures exceeding 60°C, that the solubilized fraction is mainly composed of pectin, and that the composition of the remaining pectin does not change. Action of hypothetical hydrolytic enzyme systems (Hoff and Castro, 1969) acting on galactans and arabinans during the heat treatments is not supported.

Personius and Sharp (1938) discovered that the electrical conductance of potato tissue increases sharply when the tissue is heated to a temperature above 60°C and interpreted the phenomenon as an increase in permeability. Today, one would ascribe this to destruction of the cell membrane, followed by diffusion of intracellular solutes into the cell wall, thus establishing electrical contact between cells. We have observed (Bartolome and Hoff, 1971) that native PME adsorbed to the cell wall in the tissues is activated, probably desorbed, by a solute concentration of 0.15 *M*. Considering that the molar concentration of potassium alone in the cell sap is approximately 0.12 *M*, it seems reasonable to believe that when the cell membrane is disrupted by heating above 50°C, solutes from the cytoplasm and probably also from vacuoles diffuse into intercellular space and activate the enzyme. Given sufficient time, the enzyme interacts with accessible methyl ester groups on the polyuronide chains to produce additional free carboxyl groups. Diffusing divalent ions, either magnesium or calcium, finally establish cross-linkages between chains and render the tissue more resistant to further thermal degradation (Figure 3). Since a major proportion of the total calcium of potato tubers is bound in the starch, it is likely that the relative proportions of these metals interacting with uronide depend on the state of the starch granules. Release of calcium can probably not occur until the granules are fully gelatinized. The pre-heating temperature, whether 60 or 70°C, determines the extent of starch gelatinization and should therefore to some degree determine the extent of calcium involvement. Our results tend to support this concept (Table III).

If the proposed mechanism is correct, it seems likely that it generally governs at least in part the textural properties of fully processed potato products. The factors that determine the texture of processed potato tubers are, according to this view, the thermal lability of the cytoplasmic membrane, the ion concentration of the cell sap (predominantly K), the amount of PME present in the tissues, and the amount of available alkaline earths.

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Received for review June 9, 1971. Accepted September 3, 1971. Purdue University Agricultural Experiment Station Journal Article No. 4415.

Amino Acid Composition of Buckwheat

Yeshajahu Pomeranz* and George S. Robbins

Crude protein and 17 amino acids were determined in ten samples of genetically diverse buckwheats, in buckwheat fractions from a commercial mill, and in the germ and degermed groats. The buckwheat proteins were particularly rich in lysine (6.1%), and contained less glutamic acid and proline and more arginine and aspartic acid than cereal proteins. About 56% of glutamic and aspartic acids were in the form of amides. Whereas correlations among basic or neutral and acidic amino acids were positive, correlations between basic and acidic or neutral amino acids were negative. Dark flour and feed fractions contained more protein than the whole kernel or the groat, but the amino acid patterns differed little. Distribution of amino acids in buckwheat tissues differed significantly from distribution in tissues of cereal grains. The pattern of essential amino acids in buckwheat is compared to that of cereal grains and egg reference patterns. Chemical analyses of the buckwheat hydrolyzates indicated that the amino acid composition was nutritionally superior to that of cereal grains.

Buckwheat (*Fagopyrum esculentum* Möench) is not a true cereal. It belongs to the *Polygonaceae* (or buckwheat) family, but like the cereals, the grain of buckwheat is a dry fruit (Winton and Winton, 1945; Marshall, 1969). The black hulls of the triangular fruit are not suited for human food. Structurally, they have little in common with bran coats of the cereals. The seed proper (groat) is similar to that of cereals in that it consists of starchy endosperm and oily embryo.

Feeding experiments of Sure (1955) have shown that the proteins in buckwheat are the best known source of high biological value proteins in the plant kingdom, having 92.3% of the value of nonfat milk solids and 81.4% of whole egg solids. The proteins of buckwheat were shown to have excellent supplementary value to the cereal grains (Sure, 1955; Wyld *et al.*, 1958).

Sokolov and Semikhov (1968) fractionated proteins of diploid and tetraploid forms of buckwheat; globulins were a main component of both. However, seeds of the tetraploid form contained more globulins and less albumins. Polyacrylamide gel electrophoresis showed no qualitative differences in composition of albumin, globulin, and glutelin fractions of diploid and tetraploid seeds. Similarly, Jacko and Pleskov (1968) found little difference in albumins and globulins, separated by chromatography on tetraethylaminoethylcellulose and by electrophoresis in polyacrylamide gel, in diploid and tetraploid buckwheat.

Amino acid composition of whole buckwheat was determined by several investigators, including Lyman *et al.* (1956), who used a microbiological assay method, and more recently by Tkachuk and Irvine (1969), who used an ion-exchange procedure. Zebrok *et al.* (1966) found no significant difference in the amino acid composition of the total protein between diploid and tetraploid buckwheat.

Gross composition of milled buckwheat products was reported by Coe (1931) and by Watt and Merrill (1963).

The present work is concerned with the amino acid composition of buckwheat grown in the United States, of commercially milled buckwheat fractions, and of fractions from seeds dissected by hand.

MATERIALS AND METHODS

Ten buckwheat seed samples of various origins were obtained from H. G. Marshall, Research Agronomist of the United States Department of Agriculture, at Pennsylvania State University, University Park. Those samples are described in Table I.

The samples were from seed lots grown in different years at

National Barley and Malt Laboratory, Plant Science Research Division, Agricultural Research Service, U.S. Department of Agriculture, Madison, Wisconsin 53705.