Comparison of Alpha-Amylase and Simple Sugar Levels in Sound and Germinated Durum Wheat During Pasta Processing and Spaghetti Cooking[1]

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ABSTRACT

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Sugar and α-amylase levels of ungerminated and germinated durum wheats were compared at various processing stages. α-Amylase levels in the wheat germinated for 72 and 120 hr increased 155-fold and 320-fold, respectively. Processing into semolina and spaghetti decreased α-amylase levels in both ungerminated and germinated samples. When spaghetti was cooked, α-amylase was present in the germinated sample for at least 6 min of boiling. The levels of glucose and sucrose increased approximately 50% on germination, whereas the maltose level doubled. Conversion to the respective semolinas decreased sugars, whereas processing into spaghetti increased maltose and glucose. Cooking of spaghetti did not increase the total amount of a particular sugar in the solid and the cooking water combined. Increased cooking time, however, released more sugars as well as residue into the cooking water and decreased the sugars in the solid. Part of the cooking water residue was comprised of high molecular weight dextrins. Because spaghetti made from germinated wheat had a higher initial concentration of free sugars, loss of such sugars into the cooking water was correspondingly greater.

Preharvest sprouting is well known for its deleterious effect upon the bread-making quality of hard red spring wheats. The effect is attributable to the formation of the enzyme α-amylase and its subsequent degradation of starch. Much less is known concerning the effects of preharvest sprouting upon the pasta-making quality of durum wheats. Harris et al (1943) found very little effect of sprout damage on durum wheat quality, as did Dick et al (1974). More recently, Donnelly (1980) reported that the only effects of sprout damage were increased speck count in semolina and poorer shelf stability. Bean et al (1974) found, however, that sprout damage in soft white wheats caused stickiness in Japanese noodle doughs and that strands stretched and broke during drying. Although sprout damage is reported to have a deleterious effect on pasta quality (Maier 1980), laboratory results to date have not been conclusive.

One of the main effects of sprouting is the development of α-amylase and therefore, the present study was conducted to determine the extent to which this enzyme persisted throughout pasta processing and spaghetti cooking and the subsequent effect it had upon sugar levels and cooking water residue. Durum wheats germinated for three and five days were compared with a wheat having no visible sprouting.

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MATERIALS AND METHODS

Germination of Wheat Samples

Durum wheat kernels (*Triticum turgidum* L. cv. Wascana) were steeped for 2 hr, spread evenly on moist blotting paper, and

germinated at 18° C for 72 and 120 hr in a germination cabinet at 100% humidity. The samples were then air-dried on a bench top at 22° C. The final moisture content of the wheat kernels was 10.5%.

Semolina Milling

Wheat samples were washed, tempered overnight to 16.5% moisture, and milled in a three-stand Allis-Chalmers mill in conjunction with a laboratory purifier (Matsuo and Dexter 1980).

Spaghetti Processing

Semolina was processed into spaghetti by a micromethod described by Matsuo et al (1972). The spaghetti was dried for 29 hr with a controlled decrease in relative humidity at 38° C.

Spaghetti Cooking

Four grams of spaghetti was placed in 50 ml of rapidly boiling tap water in a 100-ml beaker. At various cooking times, samples were withdrawn and immersed in liquid nitrogen. The residual water was also rapidly frozen in liquid nitrogen. Both cooked spaghetti and water were then freeze-dried for subsequent sugar analyses. Cooked spaghetti samples for α-amylase determinations were not freeze-dried but kept frozen before analyses.

α-Amylase Activity

Extraction. Samples of durum wheat, semolina, and dry spaghetti were ground in a Udy cyclone mill. Two grams of ground sample was extracted with 5 ml of 0.2 *M* acetate buffer, pH 5.5, for 2 hr with gentle rotation on a Labquake rotator (Labindustries, Berkeley, CA), followed by centrifugation at 50,000 × *g*. Cooked spaghetti was extracted with 0.2*M* acetate buffer, pH 5.5, by grinding in a mortar and pestle, followed by centrifugation as above.

Determination of Activity. An automated fluorometric procedure for determining α-amylase was employed as described by Marchylo and Kruger (1978), using β-limit dextrin anthranilate as substrate. The analyzer was calibrated with fungal α-amylase (Calbiochem, Los Angeles, CA). The potency of the fungal α-amylase was checked from time to time with a reducing sugar assay, using reduced β-limit dextrin as substrate (Kruger and Marchylo 1972); results were expressed in terms of the milligrams of maltose hydrolyzed per minute at 35° C.

Analyses of Sugars

Extraction. Samples of wheat, semolina, and spaghetti were finely ground, using a Udy cyclone mill with an 18-mesh screen (UDY Corp., Boulder, CO). Two grams of sample was added to 20 ml of absolute ethanol at 80° C and gently shaken for 30 min in a water bath at 80° C. The suspension was then centrifuged for 20 min at 15,000 × *g*. This procedure was repeated twice more using 75% ethanol. The ethanol extracts were combined and evaporated to dryness overnight in a fume hood at room temperature. The solid was dissolved in 4 ml of acetonitrite/water (7:3, v/v) and filtered through an organic sample clarification kit with a fluoropore filter (pore size, 0.50 μm). Foreign material was removed from the sample using a minicolumn cleanup. The column was a 12 × 0.5-cm i.d. glass pipette containing a glass wool plug and packed with 0.32-cm Corasil AX and 1.43-cm Porasil B/C_{18} (Waters Associates, Milford, MA). The minicolumn was conditioned initially with acetonitrite/water (7:3, v/v). The sample was then applied to the minicolumn, with the first milliliter of eluate being discarded and the second retained for analyses.

High Pressure Liquid Chromatography. High pressure liquid chromatography (HPLC) was performed with a Waters model ALC/GPC chromatograph (Waters Associates, Milford, MA), consisting of a solvent reservoir, a Wisp automatic sample injector, a model 6000A pump, a model R401 differential refractomer, and a model 440 absorbance detector. Quantitative sugar analysis was performed on the refractometer attached to a computing integrator (Spectrophysics SP4000, Technical Marketing Assoc., Mississauga, Ont.), with digital interface and printer-plotter. Ultraviolet (UV)-absorbing impurities were detected at 254 nm on the absorbance detector attached to a Fisher recorder (recordal series 5,000, Fisher Scientific, Winnipeg) and connected in series with the refractive index detector. The HPLC column was a 30 × 0.39-cm μBondapak/carbohydrate column (Waters Associates).

The operating conditions consisted of a mobile phase of acetonitrite/water (85:15, v/v), flowing in the isocratic mode at 2.0 ml/min at room temperature (about 22° C). The refractometer was set at an attenuation of 8× and the recorder speed was 1 cm/min. The chromatograph was calibrated with standards of fructose, glucose, sucrose, and maltose. Injection volumes of sample solutions were generally 100 or 200 μl.

Molecular Weight Determination

To determine the molecular weight distribution of the carbohydrates in the residual cooking water, the chromatograph described above was used with a 60 × 0.75-cm Spherogel-TSK type of SW3000 column (Altex Scientific, Berkeley, CA) and water as eluant (1.0 ml/min). The column was monitored with the differential refractometer and absorbance detector at 280 nm as described. The column has an approximate molecular weight cutoff of 150,000 for dextrans. It was calibrated with the following dextrans obtained from Pharmacia Chemicals, Uppsula, Sweden: T-110 (mol wt 110 × 10^3), T-70 (mol wt 70.1 × 10^3), T-40 (mol wt 41.5 × 10^3), T-10 (mol wt 9.9 × 10^3) and employed a program on the computing integrator designed to give the molecular weight distribution. Samples were extracted in water heated to near

TABLE I
Alpha-Amylase Levels (maltose, mg/min/g × 10^3) in Ungerminated and Germinated Wheat at Various Stages of Processing[a,b]

	Length of Germination (hr)		
Processing Stage	**0**	**72**	**120**
Wheat	8.2	1,272	2,626
Semolina	4.0	990	1,838
Spaghetti			
Dry	1.71	798	1,321
Cooked (min)			
1	0.21	293	718
2	0.03	206	489
3	...	144	424
4	...	81	195
5	...	49	138
6	...	39	106
9	...	...	...
12	...	...	...

[a] 14% moisture basis.
[b] Samples having no value indicate that α-amylase was not detectable under the assay conditions used.

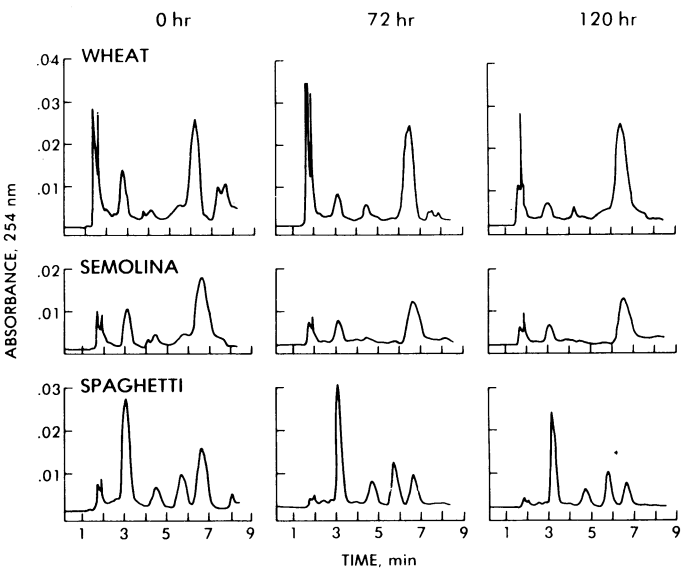

Fig. 1. High pressure liquid chromatograms of sugars in durum wheat, semolina, and spaghetti with ultraviolet-absorbance detection at 254 nm.

boiling and were centrifuged at 2,000 × *g* for 20 min to remove particulate matter.

RESULTS AND DISCUSSION

α-Amylase Levels

Comparison of the α-amylase levels in ungerminated wheat, in wheat germinated for 72 and 120 hr, and at various processing stages is shown in Table I. The level of α-amylase in durum wheat increased 155- and 320-fold on germination for 72 and 120 hr, respectively. On conversion to the respective semolinas, α-amylase activities decreased, reflecting the removal of the enzyme in the outer branny layers during milling. Conversion of the various semolinas into spaghetti also effected a loss of α-amylase. Because α-amylase is known to have a high thermostability, being stable to a temperature of 70° C in an amylograph heating cycle (Fleming et al 1961), the normal temperature of 38° C during the drying cycle would probably not be responsible for this decrease.

The decrease in enzyme activities on cooking of the spaghetti was quite surprising. After cooking for 6 min, 8% of the α-amylase remained in spaghetti made from germinated wheat. Although the α-amylase appeared to be inactivated earlier in the ungerminated spaghetti, this may be due to the inability of the assay to detect such low levels. The residual α-amylase activity at each cooking time would presumably be in the core area of the spaghetti.

Wheat Sugars

HPLC analyses of the sugars in durum wheat and its processed products presented certain advantages and disadvantages. The greatest advantages were the rapid separation of the sugars, which occurred in less than an hour, and the elimination of corrosive buffers used in the ion-exchange method (Kessler 1967). The main disadvantage of the HPLC method, aside from the high cost of the equipment, was the occurrence of UV-absorbing polar impurities, perhaps amino-sugars, which could not be removed with the minicolumn cleanup. These impurities eluted very early in the chromatogram (Fig. 1) and may have contributed to the refractive index of the earlier-eluting sugar. Fructose, in particular, may have been affected, and consequently the results for this sugar were not reported for the wheat and semolina. The problem appeared to be much less in the case of cooked spaghetti; the fructose concentration could be readily determined. Germination had little effect on the level or number of the major UV-absorbing impurities (Fig. 1). Semolinas had very similar types of impurities, but the levels were slightly less than those of the whole wheat. Pronounced differences occurred in the chromatographic profiles, however, upon conversion of semolina into spaghetti. Future investigations on the nature of this phenomenon might be interesting because of its possible effect upon quality.

Typical HPLC chromatographic profiles for the wheats, semolinas, and spaghetti are shown in Fig. 2. The initial large peak is due to solvent. The further major components were attributable to glucose, sucrose, maltose, and two unidentified components designated I and II. Retention times for the peaks indicate that they possibly consist of glucodifructose and raffinose or maltotriose. In the earlier part of the chromatogram (up to 7 min), a number of

TABLE II
Simple Sugars (mg/g) in Ungerminated and Germinated (72 and 120 hr) Durum Wheat at Various Stages of Processing

Processing Stage	Glucose in Wheat Germinated (hr)			Sucrose in Wheat Germinated (hr)			Maltose in Wheat Germinated (hr)		
	0	72	120	0	72	120	0	72	120
Wheat	0.95	1.3	1.5	9.5	15.2	15.9	1.3	1.3	2.4
Semolina	0.85	1.1	1.1	3.9	4.0	5.1	0.8	1.1	1.6
Spaghetti	0.95	4.1	4.4	4.2	4.1	5.2	15.8	37.1	33.2

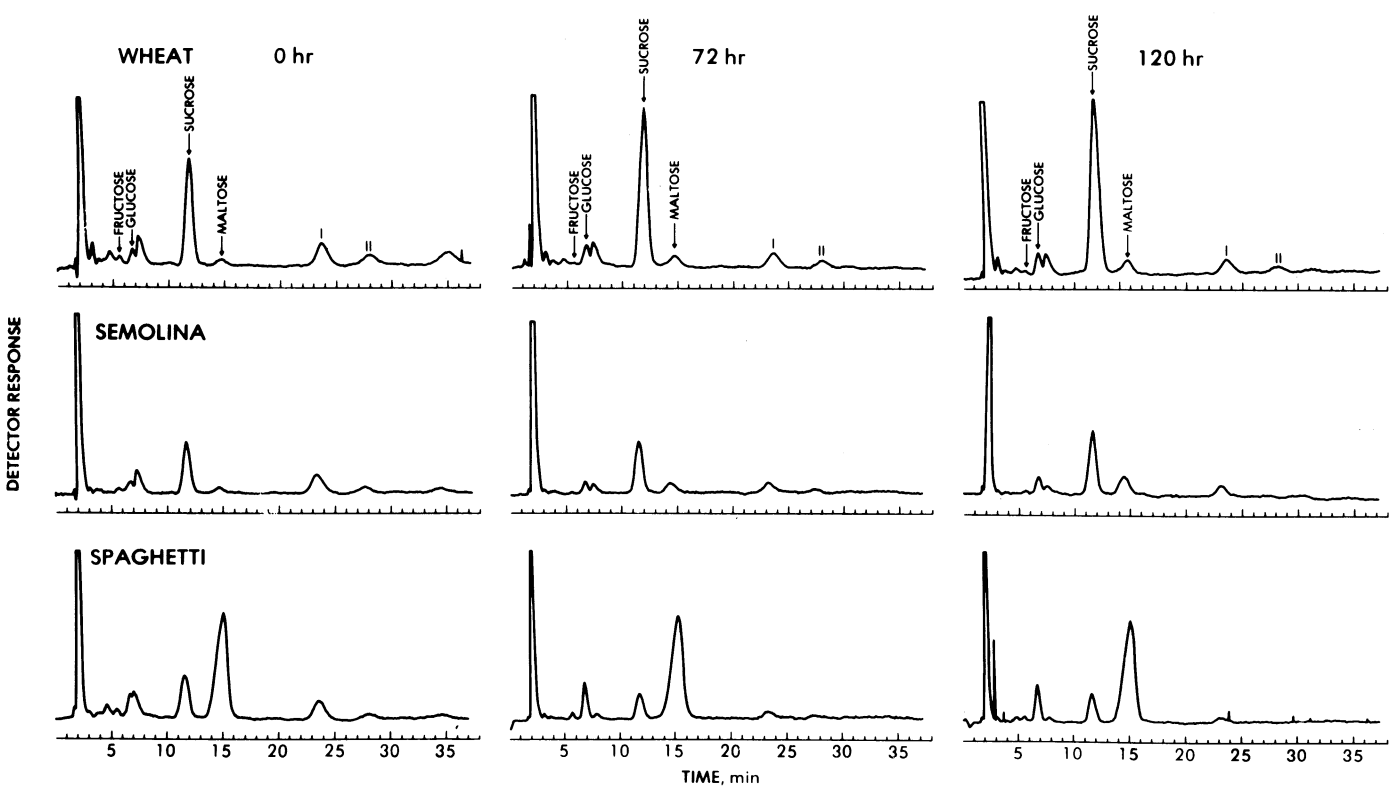

Fig. 2. High pressure liquid chromatograms of sugars in durum wheat, semolina, and spaghetti with refractive index detection. **I** and **II**, Unidentified components.

TABLE III
Simple Sugars (mg/g) in Spaghetti from Ungerminated and Germinated (72 and 120 hr) Durum Wheat Following Cooking

Cooking Time (min)	Phase	Fructose in Wheat Germinated (hr)			Glucose in Wheat Germinated (hr)			Sucrose in Wheat Germinated (hr)			Maltose in Wheat Germinated (hr)		
		0	72	120	0	72	120	0	72	120	0	72	120
3	Solid	0.5	0.55	0.75	1.4	2.1	2.7	2.3	3.6	3.6	12.3	24.7	19.0
	Water residue	0.2	0.30	0.20	0.62	1.6	2.3	1.7	1.6	1.9	6.3	10.2	10.3
	Total	0.7	0.85	0.95	2.02	3.7	5.0	4.0	5.2	5.5	18.6	34.9	29.3
7	Solid	0.35	0.4	0.75	1.08	2.6	1.9	1.9	2.5	3.0	8.3	16.8	15.2
	Water residue	0.25	0.25	0.25	0.60	1.6	2.3	1.3	1.9	2.3	5.5	11.4	15.4
	Total	0.6	0.65	1.0	1.68	4.2	4.2	3.2	4.4	5.3	13.8	28.2	30.6
12	Solid	0.25	0.8	0.8	0.93	1.3	1.5	1.5	2.2	2.0	6.3	13.7	11.6
	Water residue	0.35	0.3	0.3	1.2	2.7	3.4	2.1	2.6	2.9	8.5	13.4	16.8
	Total	0.6	1.1	1.1	2.13	4.0	4.9	3.6	4.8	4.9	14.8	27.1	28.4

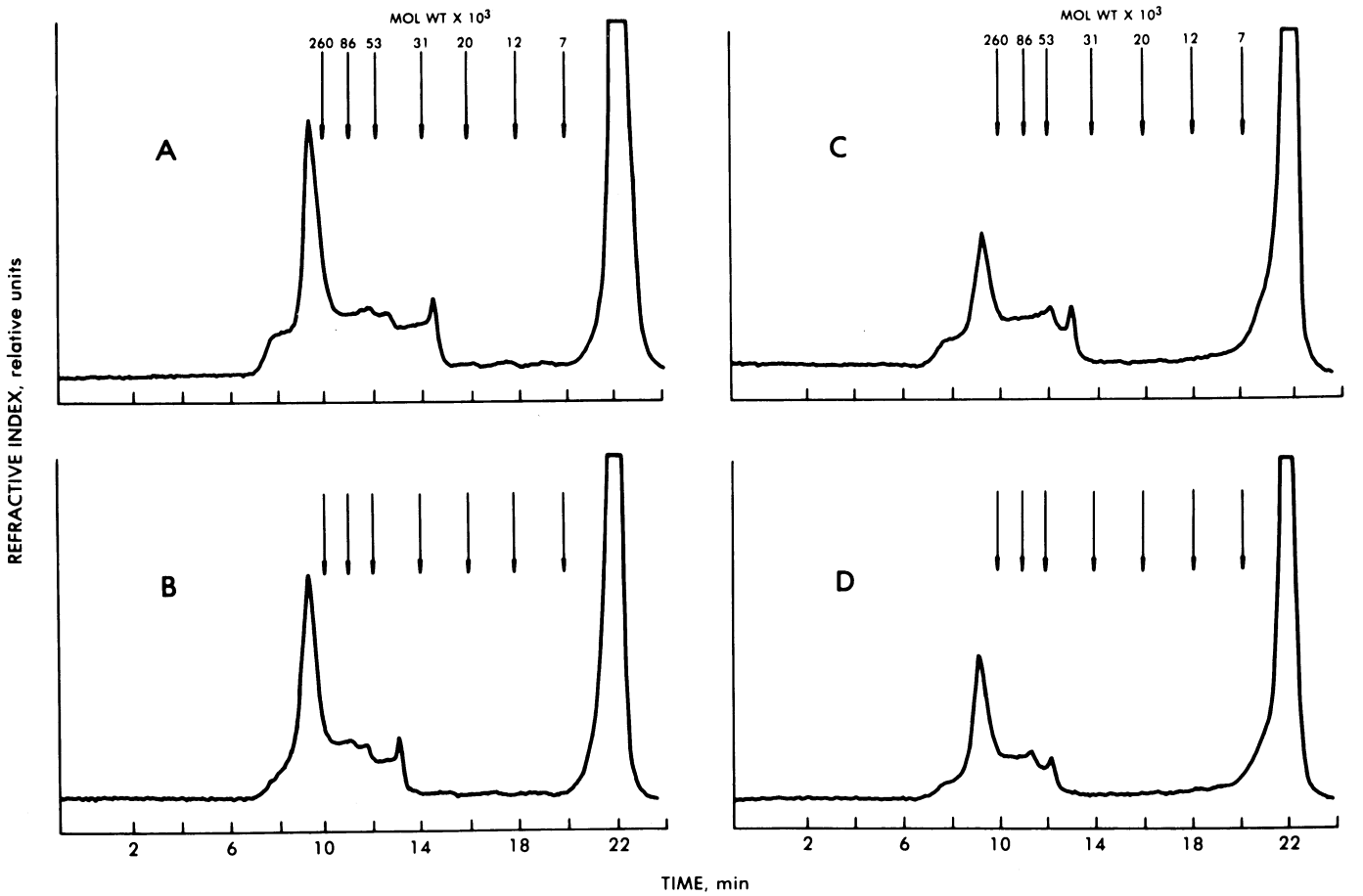

Fig. 3. Molecular weight distribution using high pressure liquid chromatography of components in spaghetti cooking water residue with refractive index detection. Spaghetti made from: **A**, ungerminated wheat, 3-min cooking; **B**, ungerminated wheat, 12-min cooking; **C**, wheat germinated 120 hr, 3-min cooking; **D**, wheat germinated 120 hr, 12-min cooking.

minor sugar components were present, including arabinose, xylose, and fructose. Some of the peaks could be attributed, however, to the UV-absorbing peaks illustrated in Fig. 1. This indicates that a UV detector should be used in addition to the refractive index detector to eliminate misinterpretation of results.

Glucose, sucrose, and maltose content in ungerminated and germinated wheats at various processing stages are shown in Table II. The levels of glucose, sucrose, and maltose in the ungerminated wheat were 0.95, 9.5, and 1.3 mg/g, respectively. Levels of 0.3, 7.4, and 1.8 mg/g, respectively, were reported by Menger (1971) for the same sugars. Glucose levels of 0.9 mg/g and sucrose levels of 8.4 mg/g were reported by MacLeod and Preece (1954) for English-grown wheat. On germination, levels of glucose and sucrose increased by approximately 50%, whereas the maltose level approximately doubled. Taufel et al (1959) reported a twofold increase in sucrose and a 60-fold increase in maltose. The large differences in levels of maltose could be the result of α- and β-amylase attack during the extraction procedure used by these workers. The present results indicate that on germination of wheat, the breakdown of starch does not readily proceed to the simple sugar stage and starch may remain as higher dextrins. Alternatively, part of the maltose, as it is formed, may be broken down by maltase, with the resulting glucose transferred and metabolized in the embryo immediately.

Semolina Sugars

The levels of simple sugars decreased in both ungerminated and germinated wheat samples on conversion to semolina. The largest decrease occurred with sucrose. This would be expected because the level of this sugar is greatest in the bran layers (Saunders and Walker 1969) of the wheat and would be removed by milling.

Spaghetti Sugars

Processing of the semolina into spaghetti did not affect the levels of sucrose. Large increases (19–33-fold) occurred, however, in the amount of maltose. Although the glucose level did not change appreciably in spaghetti made from ungerminated wheat, a fourfold increase was observed in spaghetti made from germinated semolina. Lintas and D'Appolonia (1973) have also indicated that an appreciable amount of maltose is formed in the processing of ungerminated semolina into spaghetti. The largest change appeared to be in converting the dough into wet spaghetti. Further conversion to dry spaghetti caused no further change in maltose, but glucose levels increased, presumably at the expense of any further maltose that was formed. In commercial practice, the higher spaghetti drying temperature might be expected to affect glucose levels to an even greater extent.

TABLE IV
Residue (mg/g) Present in Cooking Water of Ungerminated and Germinated Spaghetti

Cooking Time (min)	Length of Germination (hr)		
	0	72	120
3	32.6	40.4	53.8
7	38.1	54.1	77.4
12	39.6	65.1	72.5

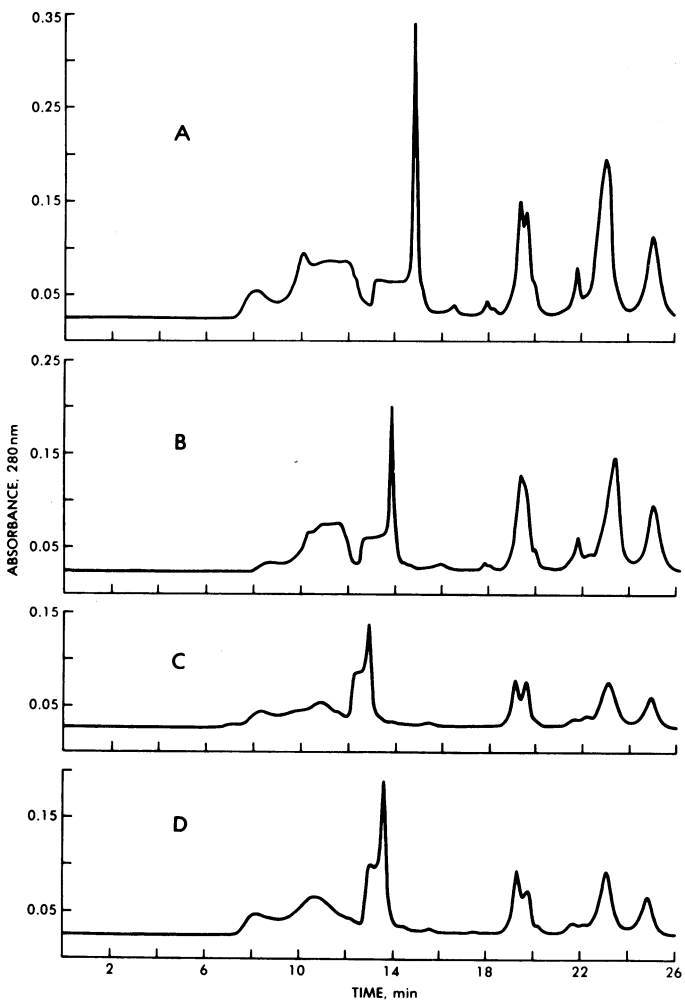

Fig. 4. Molecular weight distribution using high pressure liquid chromatography of components in spaghetti cooking water residue with ultraviolet absorbance detection at 280 nm. Spaghetti made from: **A**, ungerminated wheat, 3-min cooking; **B**, ungerminated wheat, 12-min cooking; **C**, wheat germinated 120 hr, 3-min cooking; **D**, wheat germinated 120 hr, 12-min cooking.

Sugars in Cooked Spaghetti

Fructose, glucose, and sucrose levels in spaghetti and water residues are shown in Table III. With a few exceptions, a number of generalizations can be made. Spaghetti cooking did not increase the total amount of a particular sugar in the solid and the water residue combined. This was expected, particularly with spaghetti made from germinated wheat. In some cases slight decreases in sugar concentrations were found, perhaps reflecting binding of the sugars to other biochemical components. Even the ratio between the combined level (total) of a particular sugar in the ungerminated-wheat spaghetti and in a germinated-wheat spaghetti did not vary greatly upon cooking.

The amount of sugar in the solid generally decreased during the early stages of cooking and sugar content in the water residue increased. Thus, with maltose, the amount of sugar in the water residue at 3, 7, and 12 min of cooking was 33, 42, and 55%, respectively, of the total recovered sugar. With sucrose, the corresponding sugar at 3, 7, and 12 min of cooking was 36, 44, and and 57% of the total recovered sugar.

Use of spaghetti made from germinated wheat was inconsequential in influencing the amount of sugar formed during cooking. Evidently the α-amylase that remains active in germinated spaghetti during the early cooking stages must be in the core area and unable to react with the substrate. On hydration and solubilization, it is rapidly inactivated before enzymic hydrolysis can occur. However, because more sugars are present in spaghetti from germinated wheat, a greater amount of sugar is lost to the cooking water. For example, 4.9–8.3 mg of additional maltose was lost for each gram of germinated-wheat spaghetti cooked for 12 min.

Cooking of germinated-wheat spaghetti also resulted in substantially more solids being released (Table IV). Fructose, glucose, sucrose, and maltose accounted for one fifth to one third of this residue. To elucidate the nature of the remaining portion of the residue, samples were extracted in water and analyzed for their molecular weight distributions by HPLC. This did not comprise the whole extract, however, because part of the extract was a milky suspension, possibly starch, which was removed by centrifugation. Similar chromatographic profiles were obtained for all the samples analyzed. Representative chromatograms for spaghetti from wheat germinated 0 and 120 hr with cooking times of 3 and 12 min, respectively, are shown in Figs. 3 and 4. Two major peaks are present in Fig. 3. The earlier eluting peak, at between 8 and 10 min, is comprised of very high molecular weight material, at least 250,000 mol wt. The fact that little UV absorption is associated with it (Fig. 4) indicates that it is probably composed of high molecular weight dextrins. The other major component, which eluted at about 22 min, is evidently the low molecular weight sugars. Although a fair amount of highly refractive components with molecular weights of 31,000 and above were found, the fact that smaller molecular weight dextrins were not present is extremely surprising.

CONCLUSIONS

The use of germinated durum wheat in pasta processing results in increased levels of sugars, particularly maltose, in spaghetti and a subsequent greater loss of solids during spaghetti cooking. This solids loss is comprised of simple sugars and of high-molecular weight dextrins. α-Amylase levels were substantially higher in germinated than in sound durum wheat, and the enzyme is probably a strong contributor to the observed effects. However, the effects of other enzymes such as protease, which could break down the protein structure and make the carbohydrates more susceptible to leaching in the cooking water, cannot be ruled out. In any event, use of germinated wheat in pasta processing has an adverse effect upon quality because during spaghetti cooking more solids are lost and thus become unavailable for human consumption.

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