

# Determination of Amylose and Amylopectin of Wheat Starch Using High Performance Size-Exclusion Chromatography (HPSEC)

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A number of methods have been developed for the determination of percent amylose content of cereal starches. Those employing spectrophotometry (Williams et al 1970; Morrison and Laignelet 1983; Knutson 1986; Chrastil 1987; Jarvis and Walker 1993; Martinez and Prodollet 1996) are probably the most utilized, but more recently methods using differential scanning calorimetry (DSC) (Siefert and Holm 1993; Mestres et al 1996), HPSEC (Kobayashi et al 1985; Kennedy et al 1992; Flamme et al 1994; Batey and Curtin 1996) and enzymatic digestion (Sargeant 1982; Matheson and Welsh 1988; Yun and Mateson 1990) have been developed. With each of these methods sample preparation, solutions used, and results obtained differed widely. An HPSEC method was developed and tested on different cereal starches for the separation and determination of percent amylose and amylopectin. The method described uses 1.0M KOH, 6.0M urea, and 90 min heating at 100°C to completely solubilize the starch. The analysis uses deionized, distilled water as eluant, and separation of amylose and amylopectin is achieved within 90 min using one column.

## MATERIALS AND METHODS

### Samples

Starch, isolated from seven hard red spring (HRS) and four durum wheat cultivars, was prepared using the method of Grant (1998). Amylose and amylopectin were isolated from cv. Len HRS wheat starch according to the method of Montgomery and Senti (1958). Potato amylose (A0512) (Type III) and amylopectin (9765) were obtained from Sigma Chemical (St. Louis, MO).

### Sample Preparation

Isolated starches were defatted for 16 hr using methanol and a Soxhlet extraction apparatus. However, defatting was later found to be unnecessary for HPSEC (Fig. 1). Starch (20 mg) was solubilized by adding 4.5 mL of 1.0M KOH and 0.5 mL of 6.0M urea and heating at 100°C, under nitrogen, for ≈90 min (Morrison and Laignelet 1983). After heating, 1 mL of sample was neutralized with 1.0M HCL and filtered through a 13-mm dia., 45-μm hydrophilic nylon syringe filter before analysis.

### High-Performance Size-Exclusion Chromatography (HPSEC)

Amylose and amylopectin were separated on a Waters Ultrahydrogel Linear 6-13 μm, 7.8- × 300-mm column and ultrahydrogel guard column (Waters, Milford, MA) using a Hewlett Packard (HP 1090) high-performance liquid chromatograph (Agilent Technologies, Wilmington, DE), equipped with an auto sampler. A Hewlett Packard

1047A refractive index detector and PC with chemstation (HP ChemStation for LC Rev. A.04.01) were used for control and integration. All samples were analyzed at 45°C with filtered deionized, distilled water as eluant. Flow rate was 0.3 mL/min and injection volume was 20 μL.

## RESULTS AND DISCUSSION

### Sample Preparation

Wheat amylose and amylopectin do not solubilize readily at room temperature as does potato amylose and amylopectin, therefore, a heat treatment was used as described by Morrison and Laignelet (1983). Table I shows the optimum heating time required by wheat starch amylose and amylopectin to completely solubilize. Optimum heating times were determined for each fraction by calculating the total area for that particular fraction. Areas are shown for heating times of 30, 60, 90, and 120 min. The optimum peak area for the amylose fraction was reached with 30 min of heating (4820 refractive index units [RIU]), but it took 90 min of heating for the optimum peak area for the amylopectin fraction (4744 RIU). The percentage of amylose and amylopectin remained nearly constant for heating 30, 60, and 90 min for the amylose and nearly constant for heating 60, 90, and 120 min for the amylopectin. Therefore, heating 90 min was selected for all starch samples to assure complete solubilization of the amylopectin.

In the procedure of Morrison and Laignelet (1983), 0.5 mL of 6.0M urea was used to aid solubility. We conducted an experiment to see whether the urea was necessary for the HPSEC method. Table II shows the effect urea had on a HRS wheat starch heated for 30, 60, 90, and 120 min. For all heating times, the data showed larger peak areas for the samples with the urea added. These data confirm that the use of urea aids in the solubilization of the starch samples. Another benefit of the urea appeared to be a stabilizing effect. Urea breaks hydrogen bonds and a more stable conformation forms in its presence. The starch sample with urea added (Table

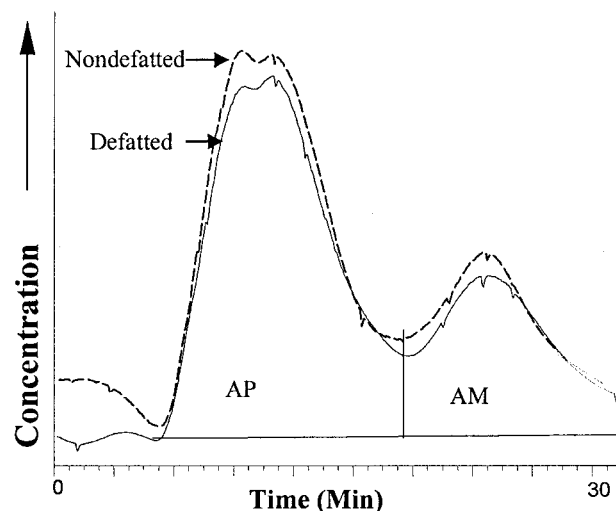


Fig. 1. Chromatograms showing elution patterns of nondefatted and defatted wheat starch using HPSEC.

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**TABLE I**  
Optimum Heating Time for Laboratory-Isolated Hard Red Spring (HRS) Amylose and Amylopectin<sup>a</sup>

Time (min)	Amylopectin Area (RIU) <sup>b</sup>	Amylose Area (RIU)	Total Area (RIU)	Amylopectin (%)	Amylose (%)
Amylose					
30	1,866	2,954	4,820	39.1 ± 4.3	60.9 ± 4.3
60	1,627	2,535	4,162	39.1 ± 0.6	60.9 ± 0.6
90	1,615	2,472	4,087	39.5 ± 0.7	60.5 ± 0.7
120	1,881	2,046	3,927	42.9 ± 1.7	52.1 ± 1.7
Amylopectin					
30	1,148	210	1,358	84.5 ± 0.1	15.5 ± 0.1
60	2,652	511	3,163	83.9 ± 0.1	16.1 ± 0.1
90	3,970	774	4,744	83.8 ± 0.9	16.2 ± 0.9
120	3,685	713	4,398	83.9 ± 0.6	16.1 ± 0.6

<sup>a</sup> Means and standard deviation of duplicate analysis.

<sup>b</sup> Refractive index units.

**TABLE II**  
HPSEC Analysis of Hard Red Spring (HRS) Starch With and Without Urea at Different Heating Times<sup>a</sup>

Treatment	Time (min)	Amylopectin Area (RIU) <sup>b</sup>	Amylose Area (RIU)	Total Area (RIU)	Amylopectin (%)	Amylose (%)
KOH <sup>3</sup>	30	1,908	1,162	3,070	61.9 ± 3.4	38.1 ± 3.4
KOH + urea	30	1,749	1,356	3,105	55.7 ± 3.0	44.3 ± 3.0
KOH	60	2,335	1,241	3,575	65.1 ± 4.3	34.9 ± 4.3
KOH + urea	60	2,511	1,560	4,071	61.6 ± 3.2	38.4 ± 3.2
KOH	90	3,013	942	3,955	76.1 ± 1.9	23.9 ± 1.9
KOH + urea	90	2,970	1,194	4,163	71.4 ± 1.0	28.6 ± 1.0
KOH	120	3,064	903	3,968	77.2 ± 2.3	22.8 ± 2.3
KOH + urea	120	3,127	1,229	4,355	71.8 ± 2.7	28.2 ± 2.7

<sup>a</sup> Means and standard deviation of duplicate analysis.

<sup>b</sup> Refractive index units.

**TABLE III**  
Amylose and Amylopectin of Hard Red Spring (HRS), Durum, and Other Normal and Waxy Starches Using HPSEC<sup>a</sup>

Starch Sample	Amylose (%)	Amylopectin (%)
HRS		
Grandin	25.0 ± 3.5	75.0 ± 3.5
Glupro	26.0 ± 1.9	74.0 ± 1.1
Prospect	25.0 ± 1.9	75.0 ± 1.9
Len	26.0 ± 1.2	74.0 ± 1.2
Stoa	27.0 ± 1.8	73.0 ± 1.8
Butte 86	24.0 ± 1.1	76.0 ± 1.1
Marshall	25.0 ± 2.3	75.0 ± 2.3
Durum		
Vic	26.0 ± 1.1	74.0 ± 1.1
Ward	24.0 ± 1.1	76.0 ± 1.1
Monroe	25.0 ± 2.6	75.0 ± 2.6
Cando	23.0 ± 1.8	77.0 ± 1.8
Other starches		
Corn	20.1 ± 1.6	79.9 ± 1.6
Potato	27.9 ± 2.1	72.1 ± 1.6
Rice	19.3 ± 1.9	80.7 ± 1.9
Waxy durum	0.0 ± 0.0	100.0 ± 0.0
Waxy corn	0.0 ± 0.0	100.0 ± 0.0

<sup>a</sup> Means and standard deviation of triplicate analysis.

II) maintained the peak areas for both amylose and amylopectin, whereas the starch sample without urea showed a decreased peak area for amylose indicating degradation.

### Wheat Starch Samples

Table III shows amylose and amylopectin means and standard deviations of triplicate HRS and durum wheat starch samples. The coefficient of variation (CV) values were 1.4–4.7%. The amounts of amylose and amylopectin were 23–27% and 73–77%, respectively. These values are in agreement with values reported for wheat by Medcalf and Gilles (1965). The HPSEC method was also tested with other cereal starches in addition to wheat starch. Table III also shows the mean values and standard deviations for corn, potato, rice, and two waxy starches (corn and durum wheat). Using HPSEC, the data clearly shows that the waxy starches contain 100% amylo-

pectin whereas the normal starches contain average amounts of amylose and amylopectin reported in the literature.

### Advantages of Method

There are three major advantages of using the described HPSEC method for the determination of percent amylose and amylopectin. 1) Accuracy. With HPSEC, a percent value for all normal and waxy cereal starches was established. Other methods reviewed gave an estimation of amylose content. 2) Safety. The described HPSEC method uses filtered deionized distilled water as eluant, whereas, other methods reviewed used caustic or carcinogenic solutions for solubilizing the starch and as eluants. 3) Time saving. Elimination of defatting the starch before analysis, using urea to aid solubilization of the starch, and utilizing one column versus a series of columns in the HPLC are all time-saving elements of this HPSEC method.

### Disadvantages of Method

The major disadvantage of this method is that amylose is not stable in water for more than a few hours. Therefore, 3–4 samples are neutralized at a time and placed into the auto-sampler of the HPLC. Conversely, the samples are stable in the KOH-urea solution so a number of samples can be prepared in advance and neutralized as needed. One other disadvantage, at the present time, is that the method only works well with starch as the starting material. Work is underway to find adequate sample cleanup so the method can be used with flour, semolina, or ground whole meal as starting materials.

## CONCLUSIONS

The HPSEC method described in this report showed improved precision over other methods reviewed. The method also eliminates the use of hazardous chemicals, is faster, more accurate, and reproducible than other methods reviewed. HPSEC analysis was not affected by the fat content in the starch samples, since integration of the peak areas of amylose and amylopectin is used in quantifying percentages. The improved precision of the method overcomes the fact that a relatively small number of samples (8–10) per day can be analyzed.

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