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Amylose and β-Glucan Content of New Waxy Barleys

Starch was isolated from four new waxy barleys and compared with normal and high-amylose barley starch. The waxy barley samples were selected lines from crosses of Swedish hulled and naked barley cultivars with the cultivar Azhul as donor of the waxy gene. The starches from the waxy barley samples were found to contain 0.7–2.6% amylose when determined iodimetrically by amperometric titration and 0.0–0.9% when determined by size exclusion chromatography after debranching. However, Sepharose CL-2B elution profiles of the starches detected by iodine staining showed that all four waxy samples were free from detectable amounts of amylose. The amylopectin starches were found to contain a small polysaccharide fraction with molecular size smaller than amylopectin, with an iodine staining λ_{max} range of 550–600 nm. The water extractable and acid extractable β-glucan contents in the waxy barley cultivars were generally found to be higher than those in normal barley.

Keywords: Amylose-free starch; β-Glucan; Barley

1 Introduction

Barley (*Hordeum vulgare* L.) is available in many different genotypes that vary in amylose content from high to very low [1]. Based on amylose content, barley starch is classified into normal (around 25% amylose), waxy (less than 15%) and high-amylose starch (more than 35%) [2]. A recessive *wax* gene located on chromosome 7HS controls the waxy endosperm character. In 1995, starch from a mutant of barley (M/5/) obtained by sodium azide treatment of a non-waxy hull-less barley cultivar was reported to be amylose-free [3]. In 1997, the amylose content of starch isolated from two lines of waxy hull-less barley (SB 94792 and SB 94794), obtained by intercrossing two waxy types was reported to be zero [4]. Amylose-free, hull-less barley starch has been reported to have higher stability, peak viscosity, paste clarity, freeze-thaw stability and water-binding capacity than waxy hull-less barley starch and waxy corn starch [4, 5]. This makes this barley starch useful for some unique food and industrial applications. Waxy cultivars tend to have high total dietary fibre content when compared to normal barley. They produce flour with excellent food thickening properties due to their high water absorption capacity [6, 7]. Total β-glucan content is highest for high-amylose barley but its water extractability has been reported to be relatively low (20.6–29.7%), when compared to normal (29.8–44.3%), zero-amylose waxy (34.0–52.5%) and waxy (36.7–52.7%) genotypes [8].

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It is difficult to determine whether a waxy starch is low in, or free from, amylose, because intermediate material may be present and different methods for amylose analysis may give variable results. In this study, starch was isolated from four new waxy barley cultivars. The amylose content of the starches was analysed using different methods and compared with one normal and one high-amylose barley cultivar. The content of extractable β-glucan in the barley samples was also analysed.

2 Materials and Methods

2.1 Materials

Four waxy barley samples (SW 28618, SW 28636, SW 18639, SW 28708), a normal barley sample (Golf), and a high-amylose barley sample (SW 2904) were provided by Svalöf Weibull AB (Svalöv, Sweden). Of these, SW 28618 and SW 28636 are sister lines from the cross Azhul × Meltan[5]. Azhul is an induced mutation received from the University of Arizona, USA. SW 18639 and SW 28708 are naked varieties, and are sister lines from the cross Azhul × SW 8775[5]. SW 8775 is a hull-less line where the gene for naked caryopsis originates from a Chinese cultivar. The high-amylose barley SW 2904 is a dihaploid line from the cross "Glacier AC 38" × Meltan[3]. Kernels (5–25 g) were ground in an ultracentrifugal mill type ZM 1 with a 0.5 mm ring sieve (Retsch, Haan, Germany), and starch was isolated according to the *McDonald* and *Stark* method [9]. All materials were prepared in at least duplicate and further analysed.

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2.2 Determination of amylose content

The amylose content of isolated starch was first determined iodimetrically by amperometric titration [10]. It was also determined by debranching the starch followed by fractionation on Sepharose CL-6B. For debranching isolated starch (6 mg) was dissolved in 1 mL dimethyl sulphoxide (DMSO) in a boiling water-bath for 30 min, and then transferred to an oven at 100°C for an additional 60 min. The starch was then precipitated with 9 mL ethanol (95%, v/v) and centrifuged for 5 min at 1000 × *g*. The precipitate was dissolved in 0.5 mL DMSO with heating, and incubated in 3.5 mL 0.06 M sodium acetate buffer (pH 3.6) and 5 μL isoamylase at 38°C in a shaking water-bath overnight. The debranched sample was then applied to a Sepharose CL-6B column (65 × 1.6 cm) which was eluted with 0.25 M aqueous KOH at a flow rate of 0.4 mL/min. Fractions of 2 mL were collected, and the amylose-amylopectin ratio was determined by carbohydrate detection using the phenol-sulphuric acid method [11]. The amylose, consisting of the long-chain α-1,4-glucans after debranching of the starch, comprised the first fraction eluted, including the void peak and the material before the beginning of the peak with amylopectin unit-chains.

2.3 Size exclusion chromatography of the starches

Isolated starch (6 mg) was defatted by dissolving in DMSO and precipitated in ethanol as described above. The precipitate was dissolved in 1 mL of 1 M aqueous NaOH with heating and 9 mL water added over a period of 4 h. The diluted sample was centrifuged for 5 min at 1000 × *g* and applied to a Sepharose CL-2B (70 × 1.6 cm) column which was eluted with 0.01 M NaOH at a flow rate of 0.4 mL/min. Fractions of 2 mL were collected and mixed with 0.1 mL of I_2-KI solution (2 mg I_2, 20 mg KI/mL) [12]. A spectrum of each fraction was measured between 300–800 nm (UV-VIS Spectrophotometer, Shimadzu, Kyoto, Japan) 20 min after addition of the I_2-KI reagent. The phenol-sulphuric acid method [11] also was used for detection of amylose and amylopectin in fractions of starch from SW 18639.

2.4 Determination of water-extractable β-glucan content

β-Glucan was extracted from barley samples (100 mg) using water (20 mL) containing $CaCl_2$ (0.28 mg/mL of $CaCl_2$) and thermostable α-amylase (50 μL, EC 3.2.1.1, 3000 U/mL, Megazyme, Wicklow, Ireland). The extractable β-glucan content and molar mass distribution determination was carried out according to *Rimsten* et al. [13].

2.5 Determination of acid extractable β-glucan content

β-Glucan was extracted from barley samples (100 mg) using water (9.9 mL) containing 100 μL α-amylase for 1 h followed by 10 mL of sulphuric acid (0.075 M) for 10 min. The acid extractable β-glucan content was determined according to Analytica EBC Method 3.10.2 [14].

3 Results and Discussion

The waxy samples had an amylose content of 0.7–2.6% as determined iodimetrically by amperometric titration (Tab. 1). Iodimetric method has been reported to overestimate amylose content, which could be due to the contribution of long amylopectin chains to complex formation [15]. In this investigation, amylose content of all the starch samples was also determined by debranching and fractionation on a Sepharose CL-6B column. The chromatograms showed a peak between 40–80 mL elution volume, which corresponds to amylose chains, and a second peak (between 81–140 mL elution volume), which corresponds to amylopectin chains. Only the Golf and SW2904 samples showed an amylose peak. All the waxy samples had an amylose content below 1%, which probably lies within the error limits of the experiment.

The chromatograms from iodine staining experiments on Sepharose CL-2B fractionated starch showed a peak between 40 and 70 mL elution volume (λ_{max} about

Tab. 1. Amylose content in starch isolated from four waxy barley, Golf and high-amylose barley (SW2904) cultivars, determined iodimetrically by amperometric titration and with size exclusion chromatography after debranching of starch. The water extractable and acid extractable content of β-glucan (% of dry flour) in the barley samples were determined enzymatically and fluorimetrically respectively.*

Sample	Amylose content [%]		β-Glucan content [%]	
	Iodine binding	Size exclusion chromatography	Water extractable	Acid extractable
SW 28618	0.7	0.0	1.7	6.0
SW 28708	0.9	0.0	2.5	7.5
SW 28636	1.2	0.9	1.4	5.6
SW 18639	2.6	0.7	2.2	6.9
Golf	28.6	29.0	1.5	4.7
SW 2904	40.7	37.6	1.4	6.3

* Values were expressed as a mean of duplicates and the variation between duplicates was less than 5%.

550 nm), which corresponds to amylopectin (Fig. 1). The peak corresponding to amylose appeared between 70 and 140 mL elution volume (λ_{max} about 650 nm) in Golf and SW2904. The chromatograms of SW28618, SW28708, SW28636 and SW18639 were devoid of this peak.

The λ_{max} of the 50 mL elution volume fractions of Golf and SW2904 was 550 nm, which corresponds to amylopectin, while that of the 90 and 120 mL fractions was 650 nm, which corresponds to amylose (Fig. 2). The 50 and 90 mL fractions of SW28618, SW28636, SW18639 and SW28708 showed λ_{max} of 550 nm, which corresponds to amylopectin. However, in the 120 mL fractions λ_{max} varied between 550 and 600 nm. The λ_{max} of all fractions is shown in Fig. 1. When compared to Golf, SW 2904 had very few fractions of pure amylopectin. The amylopectin peak appears to be overlapping with the amylose peak, as indicated by the early rise in λ_{max}. In SW28618, SW28708, SW28636 and SW18639 the majority of polysaccharides eluted had λ_{max} of 550 nm. However, the

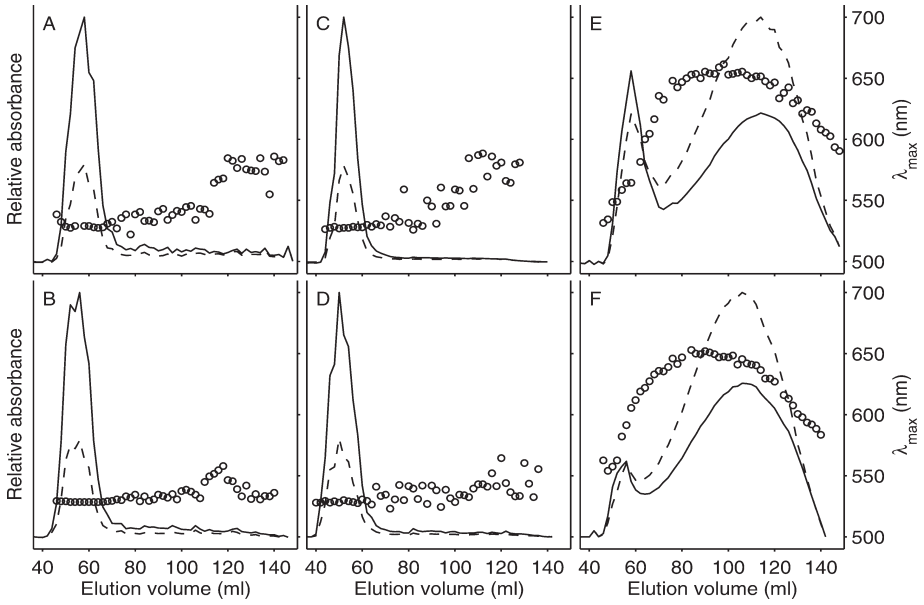

Fig. 1. Elution profiles of starches from (A) SW 28618 (B) SW 28708 (C) SW 28636 (D) SW 18639 (E) Golf (F) SW 2904 on Sepharose CL-2B detected at wavelengths 550 nm (—) and 650 nm (----). The λ_{max} of the fractions is shown with open circles.

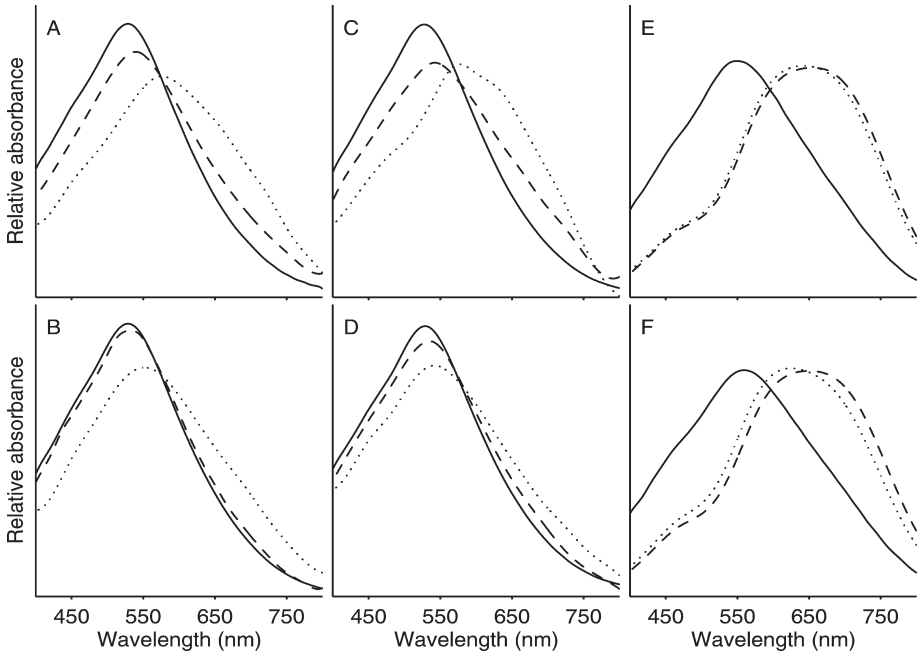

Fig. 2. Spectra of 50 (—), 90 (----) and 120 (· · · ·) mL fractions of (A) SW 28618 (B) SW 28708 (C) SW 28636 (D) SW 18639 (E) Golf (F) SW 2904 fractionated on Sepharose CL-2B.

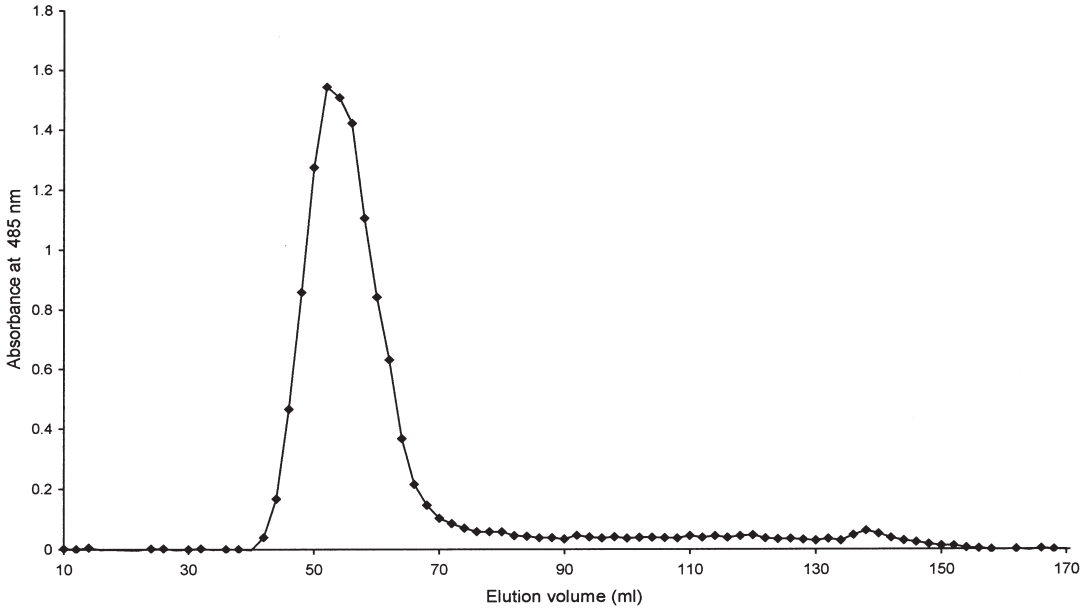

Fig. 3. Elution profile of starch from SW 18639 on a Sepharose CL-2B column detected by phenol-sulphuric acid method.

fractions from 74–140 mL elution volume showed small amounts of polysaccharides being eluted that had λ_{max} varying from 550–600 nm. Their iodine-binding spectrum indicated amylopectin of smaller molar mass rather than amylose. To confirm the presence of these fragments, the starch from SW18639 was fractionated on Sepharose CL-2B and the fractions were analysed with phenol-sulphuric acid. The chromatogram showed the presence of small amounts of polysaccharides of lower molecular size between 72–156 mL elution volume (Fig. 3).

Calcofluor staining experiments of barley endosperm have shown that Golf has overall thinner cell walls than waxy and high-amylose varieties [16]. The extractable β-glucan contents of all the samples tested were in accordance with this fact (Tab. 1). The water extractable β-glucan contents of the waxy barley samples except SW 28636 were higher than that of Golf. SW 2904 had a lower value than Golf on account of its low extractability [8]. The waxy and the high amylose varieties had higher values of acid extractable β-glucan compared to Golf. The calcofluor average molecular mass of the samples covered a range of $(1.6–1.9) \times 10^6$ g/mol, which was in agreement with that previously found for normal barley cultivars [17].

4 Conclusion

Selections made in crosses between waxy Azhul and hulled and naked Swedish barley varieties, resulted in lines with amylose-free starch. The amylopectin starches were found to contain small amounts of amylopectin-like polysaccharides of molecular size lower than the main amylopectin. The lines tested also had high β-glucan contents and hence would be of interest to the food industry.

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(Received: December 16, 2004)
(Revised: January 28, 2005)
(Accepted: January 28, 2005)

