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Survey of Starch Amylose Content in Naked Barley (*H. vulgare*. Nudum)

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Abstract: Starch of two lines of naked barley were extracted by three different methods of washing. The apparent amylose content were determined with the same standard method which recently has been modified for use with samples of 2-3 seeds of cereal. In the present experiment factorial design (2x3x3) was used. The starch extracted by soaking the seeds overnight in dilute ammonia solution grinding in NaCl solution in a microfuge tube with an appropriate pestle and decanting the starch slurry. Then it was washed in 4M NaCl, SDS and acetone. The results of apparent amylose content of this method statistically were better than the two others. With present selected method the apparent amylose content ranged from 18 to 34% was determined for 145 accessions of naked barley. The lowest amylose content was related to the line of 4062 from Pakistan and the highest one was related to the line of 743 from Turkmenstan. This range is considered sufficiently broad to allow amylose content to be further diversified through the working with more lines and breeding.

Key words: Amylose, starch, cereal seed, naked barley

Introduction

Barley cultivars with a wide range of different characteristics have been developed, including barley with low amylose starch, which has been shown to have interesting properties for human and animal food as well as for industrial uses. Variation in the amounts of amylose can affect the physico chemical and functional properties of starch, which may turn affect its utilization in food products or industrial applications (Kobayashi *et al*., 1986; Yan *et al*., 1993). An excellent freeze/ thaw stability of waxy barley starches has been reported (Bahatty and Rossangel, 1997), indicating that they may become a substitute for chemically modified maize starch, which is presently used in frozen foods. High-amylose starches from hydrogen-bonded, in soluble aggregates, which are suited for use as a source of dietary fibere, whereas waxy starches from clear, stable gels with little retrogradiation and particularly suited for use as thickeners or gel-foaming agents (Whistler, 1984).

Numerous reports have shown that in humans and other monogastric animals, amylose is more slowly digested than amylopectin, so blood glucose and insulin levels are lower after a meal high in amylose, satiety is maintained longer and the next meal is likely to be smaller (Heijnen *et al*., 1995; Holt and Brand Miller, 1995). Increasing the amylose content of the diet is thus likely to be beneficial for many members of society.

A substantial level of genetic variability in amylose content has been reported in both barley (Morrison *et al*., 1986; Salomonsson and Sundberg, 1994) and rice (Sano *et al*., 1986; Nakamura *et al*., 1995) the amylose content varied from 0 to 40%.

The amylose content of starch may be determined in several ways. The most economical and rapid, and hence probably the most commonly used, is based on the colorimetric measurement of the blue amylose-iodine complex. This has been applied widely in rice and wheat breeding programmes (Juliano, 1971; Welsh and Blakeney, 1992; Mohammadkhani *et al*., 1998; Mohammadkhani *et al*., 1999). It is; however, subject to interference from lipids bound to the amylose and true amylose content is determined only following a lengthy defatting process without which it is more correct to describe the result as " apparent" amylose (South and Morrison, 1990).

Here to find a method for the evaluation of apparent amylose in samples of 2-3 grains of barley, we compare three different methods of washing during extraction of starch. The selected method was used to survey naked barley for variation in amylose content that could be applied in plant breeding programmes.

Materials and Methods

Standard samples were maize starch as 0% amylose (waxy), 27% amylose (normal) were supplied through the courtesy of Goodman Fielder Ltd., and 95% amylose (high amylose) was from ICN Biomedicals Inc., (lot number 55172) Aurora, Ohio, USA.

A revised method was necessary for extraction and purification on the starch granules; therefore the factorial design (3x2x3) with three different methods of washing and two lines of naked barley were used with three replication . The indicated methods are as follow:

Method 1: Two to three grains of barley were soaked overnight in 0.2 Mammonia solution, drained, resuspended in 0.5M NaCl and ground in a Waring blender. The slurry was filtered through muslin (approximate pore size 160 um) and centrifuged. The

Factorial ANOVA for the factors: Grand Mean=0.204 Grand Sum =3.670 Total Count =18 Rep. (Var 3: r) with values from 1 to 3. Factor A (Var 1: a) with values from 1 to 3. Factor B (Var 2: b) with values from 1 to 2

Table 1: Mean of absorbance at 620nm of starch

3	1	2	4	Total
1	*	*	0.201	1.204
2	*	*	0.197	1.180
3	*	*	0.214	1.286
*	1	*	0.206	1.235
*	2	*	0.233	1.397
*	3	*	0.173	1.038
*	*	1	0.204	1.837
*	*	2	0.204	1.833
*	1	1	0.209	0.626
*	1	2	0.203	0.609
*	2	1	0.234	0.703
*	2	2	0.231	0.694
*	3	1	0.169	0.508
*	3	2	0.177	0.530

Table 2: Summary analysis of variance for amylose absorbance at 620nm following different extraction of starch

S.O.V	df	MS	F	
Rep.	2	0.515	1.66	n.s
A	2	5.387	17.32	**
B	1	0.0009	0. 0029	n.s
AB	2	0.071	0.54	n.s
Error	10	0.311		
Total	17			

C V : 0. 86%

Table 3: Mean of amylose absorption wavelength at 620 nm resulted from working with different treatment of starch washing and two lines of naked barley

Nm	Treatment
0.206	First method
0.233	Second method
0.173	Third method
0.204	First cultivar
0.204	Second cultivar
0.209	First method x First cultivar
0.203	First method x Second cultivar
0.234	Second method x First cultivar
0.231	Second method x Second cultivar
0.169	Third method x First cultivar
0.177	Third method x Second cultivar

pellet was resuspended and washed in a sequence of 0.1M acetic acid, distilled water, 95% ethanol, distilled water and acetone and dried in a desiccator over silica gel overnight.

Method 2: Two or three grains were soaked overnight 0.2M ammonia solution, drained resuspended in 0.5M NaCl and ground. The slurry was filtered and centrifuged (as above). The pellet was resuspended and washed in a sequence of 4M NaCl, 5% sodium dodecyl sulphate (SDS), distilled water, 95% ethanol and acetone and dried overnight.

Method 3: Two or three grains were soaked overnight 0.5M NaCl and ground. The slurry was filtered and centrifuged (as above). The pellet was resuspended and washed in a sequence of 4M NaCl, 6M NaCl/50% sucrose (105 g NaCl + 300 g sucrose + 300 ml distilled water), distilled water, 2% SDS, distilled water and acetone and dried.

The apparent amylose content were determined with the same standard method which recently was modified (Mohammadkhani *et al.*, 1998) from 100 mg rice starch procedure (Juliano, 1971), therefore 5 mg of starch was weighed to the nearest 0.1 mg into a 25 ml beaker. The starch was dispersed in 1 ml of ethanol and then 2.7 ml of 1M NaOH was added with swirling to improve dispersion. The contents were heated to boiling on a sand bath at 175'C for 15 min during which the starch completely gelatinized. The beaker was cooled and the starch washed 2 to 3 times with distilled water into 25-ml volumetric flask. The flask vortex mixed and then duplicate 2.5-ml samples were taken into separate test tubes, neutralized with 2 ml 0.3N citric acid and 1 ml of fresh iodine solution was added. 14.5 ml distilled water were added and the sample was refrigerated for 20 minutes, then the tubes were vortex mixed and duplicate subsamples were read in the spectrophotometric at 620 nm. The samples covered 145 accession of naked barley was from Iran and the other Countries.

Results

The analysis of variance showed that the main effects of starch extraction (washing method) were significant (Table 2). Among three methods of washing, method 2 showed a statistically better result than the others. When the method 2 of washing with the 5-mg procedure of starch analysis applied to the naked barley considerable variation revealed (Fig. 1). In present work apparent amylose content ranged from 18 to 34% with the highest frequency, including more than about one-seventh of the collection, at 25% (Table 4). The distribution among the lines was approximately normal (Fig. 1). Ten lines of the lowest and ten highest amylose content from working with 145 lines of naked barley with Country of origin were demonstrated in Table 5.

Combination of different concentration of amylose from standard starch with related absorbance wave length were shown (Table 6).

Table 4: Range of amylose% in starch of 145 naked barley lines from different Country

Amylose%	No. line	Country	Amylose%	No. line	Country
19.3-28.8	9	China	23.4-32.6		England
25.4	1	Germany	28.5	1	Egypt
18.8-29.2	16	Pakistan	21.2-31.5	3	Japan
21.3-28.8	17	India	19.2-27.8	8	Iran
20.3-28.8	9	Ethiopia	20.6-33.4	30	Russia
21.1-32.7	20	Unknown	21.1-32.7	14	USA
30.3	1	Sweden	33.9	1	Turkmenstan
19.5-28.9	9	Afghanistan	20.1	1	Australia
			32.7-33.1	2	Moroco

Table 5: Ten lines of Min. and Max. of amylose% from 145 lines of naked barley collection with certificate no. and Country of origin

Amylose%	Line No.	Country	Amylose%	Line No.	Country
31.5	2080	USA	18.3	4062	Pakistan
31.5	5899	Japan	19.2	80173	Iran
31.7	5624	USA	19.3	4206-2	China
31.9	5204	Russia	19.5	4150-2	Afghanistan
32.1	5701	USA	19.8	4078	Pakistan
32.6	587	England	20.1	6022	Australia
32.7	5675	USA	20.3	6049-5	Ethiopia
32.7	5698	USA	20.5	3909-1	Ethiopia
33.4	3978-3	Russia	20.6	3995-1	Russia
33.9	743	Turkmenstan	20.8	7554	Russia

Table 6: Combination of different concentration of amylose from standard starch with related absorbance wave length

No.	ml am.27%	ml am.0%	Amylose%	Abs at 620nm
1	2.5	0	0	0.041
2	2	0.5	5.4	0.08
3	1.5	1	10.8	0.127
4	1	1.5	16.2	0.173
5	0.5	2	21.6	0.224
6	0	2.5	27	0.268
no.	ml am.95%	ml am.0%	Amylose%	Abs at 620nm
7	2	0.5	19	0.18
8	1.5	1	38	0.324
9	1	1.5	57	0.462
10	0.5	2	76	0.626
11	0	2.5	95	0.769
no.	ml am.95%	ml am.27%	Amylose%	Abs at 620nm
12	2	0.5	40.5	0.364
13	1.5	1	54.2	0.463
14	1	1.5	67.8	0.565
15	0.5	2	81.4	0.662
16	0	2.5	95	0.77

Amylose% = 131.21 *Abs-5.952

Discussion

A starch extraction method was adapted for use with small (2-3 grain) samples of naked barley. The superiority of the new cleaning method (extraction method 2) was demonstrated in the 13% and 34% higher absorbance values achieved for the similar materials compare with method 1 and 3 respectively.

The ammonia replaced with a weak NaCl left the proteins in a suitable condition to be worked into a ball of gluten during of grounding and sieving stage of starch extraction. The earlier sequence of reagents for washing the starch (method 1), namely acetic acid and ethanol, was similar to the Osborne series (Osbome, 1907) for dissolving protein from wheat, but SDS is well known to be an effective solvent for a wide range of proteins. South and Morrison (South *et al*., 1990).

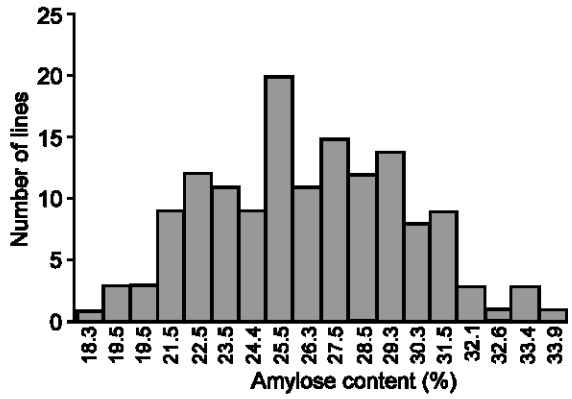

Fig. 1: Frequency distribution of amylose%in naked barley

demonstrated a considerable reduction in the amount of protein bound to starch when it has been washed with SDS. They also showed that washing with SDS and ethanol (as were used in method 2) was an effective way of removing some of the lipids bound to the surface of the starch, although it did not reach the lipids bound within the starch granules.

Fig.1 demonstrated that between the lowest and the highest amylose content of 145 lines of naked barley it was found 89% differences. The distribution among the lines was approximately normal.

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