Evaluation of size distribution of starch granules in selected wheat varieties by the Low Angle Laser Light Scattering method

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ABSTRACT

The distribution of the size of wheat starch granules using the method LALLS (Low Angle Laser Light Scattering), followed by the evaluation of the effect of variety, experimental site and intensity of cultivation on the vol. % of the starch A (starch granules > 10 µm) was determined. The total starch content and crude protein content in dry matter of flour T530 in selected collection of five winter wheat varieties were determined. Vol. % of the starch A in evaluated collection of wheat varieties varied between 65.31 and 72.34%. The effect of a variety on the vol. % of starch A seemed to be more marked than the effect of site and intensity of cultivation. The highest vol. % of starch A reached evaluated varieties from the quality group C, i.e. varieties unsuitable for baking utilisation (except variety Contra with high total content of starch in dry matter of flour T530, but relatively low vol. % of starch A). A low vol. % of starch A was also found in the variety Hana (very good variety for baking utilisation). Certain variety differences followed from the evaluation of distribution of starch fractions of starch granules, forming starch A. In the case of varieties Hana, Contra and Siria higher representation of fractions up to 30 µm was recorded, while starch A in the varieties Estica and Versailles was formed in higher degree by size fractions of starch granules over 30 µm and particularly size fraction > 50 µm was greatest in these varieties of all evaluated samples. With increasing total starch content in dry matter of flour T530 the crude protein content decreased; the vol. % of starch A not always increased proportionally with increasing total starch content.

Keywords: winter wheat; varieties; starch granules; starch A; LALLS method

The starch can be considered as one of the strategic materials of the future. Its consumption is still increasing for food industry and non-food utilisation as well. As Lillford and Morrison reported (1997) the starch consumption is fast growing in industrial processing. The greatest utilisation is in paper, textile and chemical industries, where it serves for production of many products.

In the recent years, the interest in the utilisation of wheat as starch-making material has been increasing in Europe, particularly in the countries with soil-weather conditions comparable with those in the Czech Republic.

Though a great attention was paid to the quality of industrial potatoes for starch-making purposes, with respect to a long-term tradition, by Czech breeders, growers together with starch-making technologists, there are a lot of open issues in evaluation of the quality and suitability of wheat varieties or flours ground from them, respectively, given for the production of wheat starch.

At the present time wheat flours with good processing qualities for starch production, i.e. with good separating properties of starch from gluten and with a high yield of the starch of the category A, are required.

A part of our research was to determine the distribution of the size of wheat starch granules using the method LALLS (Low Angle Light Scattering), followed by the evaluation of the effect of a variety, experimental site and intensity of cultivation on the vol. % of the starch A, the total starch content and crude protein content in flour T 530 in selected collection of winter wheat varieties.

Wheat starch granules form two important size fractions. There are great granules, called *prima* or the starch A, of the particle size ranging from 10–15 µm to about 50 µm and small granules (*secunda* or starch B) of the particle size smaller than 10–15 µm. Small granules are firmly fixed to protein matrix, so they are hard to separate and decrease not only the quality of gluten, but also a yield of starch. Moreover, significantly higher amount of nitrogen was found in this small-particle fraction by the Kjeldahl method than in the starch A. This nitrogen, however, is not only of protein origin, but comes also from phospholipids that are firmly bound to starch granules (Cornell et al. 1994). Great starch granules have higher amylase concentration, they are easier degradable by activity of α-amylase, they gelatinise at lower temperatures than small starch granules (Raeker et al. 1998, Peng et al. 1999).

The size of starch granules is an important trait that affects the possibilities of utilisation and processing of wheat. The starch A has a decisive meaning for production of various products based on starch, the starch B is usually processed to ethanol.

A part of our research was conducted under the support of the grant from the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic No. 7222 *Wheat in marginal regions for starch production*.

Based on existing knowledge, the quality of starch flour is most affected by a wheat variety and way of grinding. If starch-making industry is concentrated on flours with high yield of starch A, in view of agriculture, suitable wheat varieties should be those with a low hardness of endosperm, low content of proteins and pentosans, and undamaged starch granules (high falling number and the values of amylographic maximum). The yield of starch B (starch granules < 10 μm) should be maximally 30% (Lindhauer and Zwingelberg 1997).

At the present, the fine flour is predominantly used for starch production. Coarser flours that generally manifest a lower amount of starch granules should be more suitable (Lindhauer and Zwingelberg 1997).

Many methods were developed to determine the distribution of starch particle size and many devices that allow more or less exact determination of fraction composition based on various principles. For example particle screen analysis, sedimentation, image analysis, laser light scattering, air separation belong to them. In total, all these methods form sufficient prerequisites for the possibility of exact determination of the particle size. Practically each of them is limited by certain way and is suitable only for certain type of material and for certain limit of the particle size.

For example, Cornell et al. (1994) studied and compared various methods of determination of the size of starch granules. In their study, they used the methods based on sedimentation of starch granules, laser light scattering and traditional microscopic methods. The method of measuring granules under microscope is time-consuming and hardly applicable to ordinary operational measurement. Sedimentation of starch granules was measured on the sedimentograph Shimadzu. Both the methods of sedimentation and the method of laser light scattering gave almost identical results for water suspension of native starches. In the case of starches that were subject to alkalisation to reduce the content of crude protein, subsequently they were neutralised and resuspended into demineralised water, the method of laser light scattering gave higher values than sedimentation.

Peng et al. (1999) used screen analysis and sedimentation analysis for evaluation of distribution of starch granules. The results of both the methods were not comparable, particularly at screen analysis it was not possible to separate completely the both types of starch granules A and B.

To evaluate the distribution of starch granules in wheat, Hareland (1994) used the method of laser light scattering, screen analysis and spectroscopic analysis using the NIR technique. The results obtained again were not identical.

Psota et al. (2000) used the method LALLS (low angle laser light scattering) to evaluate the size distribution of barley starch granules. The method LALLS after them should be used as an exact, reproducible and fast method to determine the size distribution of the starch granules.

MATERIAL AND METHODS

The samples of five winter wheat varieties (Hana – quality group A, Siria – quality group B and Contra, Estica and Versailles – quality group C) were used to determine the size distribution of the starch granules and to evaluate the effect of the site and intensity of cultivation on the vol. % of starch A, total starch content and crude protein content in flour T530. The samples were obtained from the experiments of variety testing stations of the Central Institute for Supervising and Testing in Agriculture Domanínek in the Czech-Moravian Highlands, Nechanice near Hradec Králové, Stachy in the Šumava Mountains, Chrastava near Liberec and Lípa near Havlíčkův Brod (brief characteristics of conditions of experimental sites are presented in Table 1).

The experiments were conducted under two intensities of cultivation:
- basic intensity: fertilisation N 90–110 kg N/ha, 60 kg P_2O_5/ha, 60 kg K_2O/ha, a herbicide was applied during the growing season
- increased intensity: identical rates of fertilisers, herbicide, fungicide and growth regulator were used during the growing season

Granule samples obtained from experiments were ground on the laboratory mill Bühler; fractions obtained were mixed to correspond to the ordinary baking flour T530. The starch content in flour dry matter (%) – usage method by Ewers, polamate A was used for determination of analyte – was determined in this flour, followed by crude protein content in flour dry matter (%) – usage method by Kjeldahl.

The LALLS method (low angle laser light scattering) was used to determine the distribution of the size of

Table 1. Characteristics of conditions (locations), of which originated evaluated wheat varieties

Location	Altitude (m)	Great soil group	Soil texture	Long-range annual average temperature (°C)	Long-range annual sum of precipitation (mm)
Domanínek	565	cambisol	sandy loam	6.4	602
Nechanice	235	luvisol	loam	8.1	582
Stachy	860	podzolic cambisol	loamy-sand	6.3	755
Chrastava	345	luvisol illimerized	loamy	7.1	798
Lípa	505	cambisol albic	sandy loam	7.7	632

Table 2. Distribution of the size of starch granules in evaluated wheat samples (the LALLS method)

Sample No.	Location	Variety	Intensity of cultivation	Vol. % of different size fractions of starch granules (µm)							Ratio of starch A and B
				< 10 (starch B)	10–15	10–25	10–30	10–50	> 50	> 10 (starch A)	
1	Domanínek	Hana	basic	34.13	12.22	40.62	49.90	62.87	3.00	65.87	1.93
2		Contra	basic	34.62	11.18	42.88	52.57	63.54	1.84	65.38	1.89
3		Siria	basic	31.89	10.80	39.04	48.87	63.89	4.22	68.11	2.14
4		Estica	basic	28.47	9.77	36.51	46.82	64.93	6.60	71.53	2.51
5		Versailles	basic	31.12	10.12	36.44	46.63	63.48	5.40	68.88	2.21
6		Hana	increased	33.94	12.41	41.08	50.05	62.55	3.51	66.06	1.95
7		Contra	increased	34.69	10.88	41.80	51.51	63.09	2.22	65.31	1.88
8		Siria	increased	32.46	10.90	38.52	48.22	63.10	4.44	67.54	2.08
9		Estica	increased	28.56	9.46	35.65	46.06	64.95	6.49	71.44	2.50
10		Versailles	increased	32.79	10.21	35.83	45.80	62.08	5.13	67.21	2.05
11	Nechanice	Hana	basic	32.84	12.29	42.18	51.48	63.99	3.17	67.16	2.05
12		Contra	basic	31.97	10.27	41.17	51.81	65.16	2.87	68.03	2.13
13		Siria	basic	32.89	11.42	39.22	48.89	63.20	3.91	67.11	2.04
14		Estica	basic	28.97	9.94	36.42	48.72	65.78	5.25	71.03	2.45
15		Versailles	basic	31.65	10.17	36.51	49.63	63.05	5.30	68.35	2.16
16		Hana	increased	33.64	11.97	40.25	49.54	62.90	3.46	66.36	1.97
17		Contra	increased	32.39	10.54	41.48	51.87	64.87	2.74	67.61	2.09
18		Siria	increased	33.64	11.39	39.66	49.28	62.99	3.37	66.36	1.97
19		Estica	increased	29.04	9.57	37.22	48.56	65.98	4.98	70.96	2.44
20		Versailles	increased	31.43	10.25	37.18	49.71	63.32	5.25	68.57	2.18
21	Stachy	Estica	basic	28.19	9.91	39.39	50.01	66.60	5.21	71.81	2.55
22		Versailles	basic	30.78	9.75	35.91	46.25	63.59	5.63	69.22	2.25
23	Chrastava	Estica	basic	27.66	9.43	39.08	50.49	67.64	4.70	72.34	2.62
24		Versailles	basic	29.32	10.23	40.21	51.18	66.71	3.97	70.68	2.41
25	Lípa	Estica	basic	30.89	10.43	38.02	48.33	65.36	3.75	69.11	2.24
26		Versailles	basic	30.85	10.61	36.67	46.86	63.91	5.24	69.15	2.24

starch granule on the apparatus Analysette 22 Fritsch (Fritsch GmbH: Instruction manual laser granule size Analysette 22).

For the statistical evaluation alone, the vol. % of starch A (starch granules > 10 µm) scattering analysis of variance of simple classification was used. Significance of differences in the vol. % of starch A among different samples was verified by the Scheffe test (homogenous samples are denoted by identical capital letters).

RESULTS AND DISCUSSION

The method LALLS was proved a very efficient and fast method, suitable for ordinary operational measurement. Its advantage consists in the fact that a small amount of a sample is needed and it can be used for a wide range of granule size. Owing to its speed, efficiency and reproducibility, it is the most frequent method used now by starch-making specialists to determine the size distribution of starch granules.

The results of evaluation of the size distribution of starch granules in evaluated wheat samples are presented in Table 2; the results of statistical evaluation are in Table 3.

The variety Estica reached the highest vol. % of starch A of the evaluated collection of samples (72.34%) under the basic intensity of cultivation at the site Chrastava. On the other hand, the lowest vol. % of starch A (65.31%) was found in the variety Contra under increased intensity of cultivation at the site Domanínek. Average value of vol. % of starch A in our collection of 26 wheat samples amounted to 68.51%. As Lindhauer and Zwingelberg (1997) reported, the vol. % of starch A in starch-making wheat varieties should be > 70%. Stoddard (1999) evaluated the vol. % of starch A and B not only in the wheat *T. aestivum*, but in related wheat species as well. The vol. % of starch A in Australian hexaploid wheat (*T. turgidum*) was ranging between 50 and 70%. Tetraploid wheats (*T. turgidum*) had the vol. % of starch A between 53 and 83%, diploid one-grained wheat (*T. monococcum*) between 38 and 77%.

Based on our results, the effect of a variety on the vol. % of starch A seems to be more marked than the effect of site and intensity of cultivation. The variety Estica of the quality group C was dominant in all evaluated locations by the

Table 3. Analysis of variance of simple classification and significance of differences in the vol. % of starch A among evaluated wheat samples (Scheffe, α = 0.05)

Sample No.	Location	Intensity of cultivation	Variety	Vol. % of starch A	*F*-test	Minimal significant difference	Statistical significance*
7	Domanínek	increased	Contra	65.31	1729.94	0.4617	A
2	Domanínek	basic	Contra	65.38			A
1	Domanínek	basic	Hana	65.87			B
6	Domanínek	increased	Hana	66.06			BC
16	Nechanice	increased	Hana	66.36			C
18	Nechanice	increased	Siria	66.36			C
13	Nechanice	basic	Siria	67.11			D
11	Nechanice	basic	Hana	67.16			DE
10	Domanínek	increased	Versailles	67.21			DE
8	Domanínek	increased	Siria	67.54			DEF
17	Nechanice	increased	Contra	67.61			EFG
12	Nechanice	basic	Contra	68.03			GH
3	Domanínek	basic	Siria	68.11			GH
15	Nechanice	basic	Versailles	68.35			HI
20	Nechanice	increased	Versailles	68.57			IJ
5	Domanínek	basic	Versailles	68.88			JK
25	Lípa	basic	Estica	69.11			K
26	Lípa	basic	Versailles	69.15			K
22	Stachy	basic	Versailles	69.22			K
24	Chrastava	basic	Versailles	70.68			L
19	Nechanice	increased	Estica	70.96			L
14	Nechanice	basic	Estica	71.03			LM
9	Domanínek	increased	Estica	71.44			MN
4	Domanínek	basic	Estica	71.53			N
21	Stachy	basic	Estica	71.81			N
23	Chrastava	basic	Estica	72.34			O

* homogenous samples are denoted by identical capital letters

highest vol. % of starch A (starch granules > 10 µm), i.e. a variety unsuitable for baking utilisation, followed by another variety from this quality group Versailles. On the other hand, the variety Contra (also the same quality group C) reached the lowest vol. % of starch A at the site Domanínek of the whole-evaluated collection. A low vol. % of starch A was also found in the variety Hana (quality group A).

As Lindhauer and Zwingelberg (1997) reported in their results, wheat varieties of the quality group C usually reach higher vol. % of starch A than high quality-grade or elite ones. These authors also found differences among evaluated wheat varieties in the starch yield A to 7%. This is almost identical with our results, because the differences in the vol. % of starch A ranged between 0.07 and 7.03% in wheat varieties evaluated by these authors.

Results of the variety Contra were manifested as somewhat surprising in the vol. % of starch A. It is a German variety, intended for biscuits and cookies purposes. Owing to a high total content of starch that is usually reached and a good separating properties of gluten, this variety is sometimes mentioned as a variety suitable for starch-making utilisation. Based on our previous results (Petr et al. 2001) regarding the total content of starch and wet gluten content, this variety seemed to be perspective for starch-making purposes.

Certain variety differences followed from the evaluation of distribution of size fractions of starch granules, forming starch A (Table 2). In the case of the varieties Hana, Contra and Siria higher representation of fractions up to 30 µm was recorded, while lower percentage remained for size fraction 30–50 µm and the greatest evaluated fraction > 50 µm was represented the least within the whole evaluated collection. On the other hand, starch A in the varieties Estica and Versailles was formed in higher degree by size fractions of starch granules over 30 µm and particularly size fraction > 50 µm was greatest in these varieties of all evaluated samples.

Raeker et al. (1998) who tested the size distribution of starch granules by the method LALLS in 34 samples of 12 wheat varieties cultivated in eastern part of the USA recorded marked variety differences.

Important variety differences in size of starch granules were also recorded with malting barley, even compared with the results from the sites of very different agro-ecological conditions (Goering et al. 1973). Psota et al. (2000)

Table 4. Vol. % of starch A, total starch content and crude protein content in dry matter of flour T530

Sample No.	Location	Variety	Intensity of cultivation	Vol. % of starch A	Starch content in dry matter of flour T530 (%)	Crude protein content in dry matter of flour T530 (%)
1	Domanínek	Hana	basic	65.87	78.92	10.48
2		Contra	basic	65.38	81.90	9.23
3		Siria	basic	68.11	81.00	8.55
4		Estica	basic	71.53	81.24	8.75
5		Versailles	basic	68.88	79.30	8.23
6		Hana	increased	66.06	77.11	11.57
7		Contra	increased	65.31	81.70	11.08
8		Siria	increased	67.54	78.40	10.84
9		Estica	increased	71.44	79.51	10.97
10		Versailles	increased	67.21	80.60	11.62
11	Nechanice	Hana	basic	67.16	75.62	12.66
12		Contra	basic	68.03	80.80	11.08
13		Siria	basic	67.11	78.30	11.46
14		Estica	basic	71.03	77.31	11.29
15		Versailles	basic	68.35	82.52	10.84
16		Hana	increased	66.36	76.70	11.21
17		Contra	increased	67.61	80.20	10.84
18		Siria	increased	66.36	78.64	10.13
19		Estica	increased	70.96	78.23	10.58
20		Versailles	increased	68.57	76.70	11.02
21	Stachy	Estica	basic	71.81	82.34	8.72
22		Versailles	basic	69.22	82.40	8.09
23	Chrastava	Estica	basic	72.34	80.10	9.27
24		Versailles	basic	70.68	78.65	8.94
25	Lípa	Estica	basic	69.11	81.64	9.21
26		Versailles	basic	69.15	82.20	9.19

found statistically significant differences among small and great starch granules between spring barley varieties Novum and Kompakt.

Moreover, it followed from Tables 2 and 3 that samples of wheat varieties from Domanínek (except the variety Estica that reached a high vol. % of starch A at this experimental station) and Nechanice were marked by slightly lower vol. % of starch A. On the other hand, the vol. % of starch A in the samples from Lípa, Stachy and Chrastava was higher.

When compared both intensities of cultivation that differed in the use of fungicide, insecticide and growth regulator, greater differences were not found in the vol. % of starch A.

Together with evaluation of the vol. % of starch A and the size distribution of starch granules in the studied collection of wheat variety samples we evaluated also the total content of starch and crude protein and crude protein in dry matter of flour (Table 4).

It is evident from Table 4 that marked differences were recorded within evaluated collection of samples in the content of crude protein among different varieties. The highest content of starch and the lowest content of crude protein reached the samples from Stachy and Lípa, the variety Contra from Domanínek reached a high content of starch under both intensities of cultivation, but also the varieties Siria, Estica and Contra under basic intensity of cultivation at Nechanice. On the other side, the lowest content of starch and simultaneously highest content of crude protein were found in the variety Hana at all sites where it was evaluated. These data are in congruency with Petr's et al. (2001) results who evaluated technological quality in a wide collection of winter wheat varieties – varieties of the quality group A (high quality), where the variety Hana is included, manifested higher content of crude protein and also lower starch content compared with the varieties of the quality group C (varieties unsuitable for baking utilisation) or B (bread, supplementary varieties).

In addition, a known fact followed from an evaluation of the content of crude protein and the total content of starch in dry matter of flour T530 that the content of crude protein is falling with increasing starch content. On the contrary, though not always confirmed the conclusion made by Kelfkens and Hamer (1991) according to whom 1% increase of crude protein in wheat grain represents the decrease of starch content by more than 2%.

If the total starch content in the flour T530 and the vol. % of starch A (Table 4) are compared, it is evident that the vol. % of starch A not always increased directly proportionally with increasing total starch content.

It can be well seen, e.g. in samples 2 and 7 (the variety Contra from Domanínek under basic and increased intensity of cultivation) that indeed they reached a high total starch content within the evaluated collection (almost 82%), but at the same time also the lowest vol. % of starch A of the evaluated collection (65.38 and 65.31%). It should be mentioned that comparison of samples 1 and 2 (Hana and Contra from Domanínek, basic intensity of cultivation), and samples 6 and 7 (the varieties Hana and Contra from Domanínek, increased intensity of cultivation) that reached comparable values of the vol. % of starch A, but great differences were found in the total starch content for the benefit of the variety Contra. Our results were different from the results of e.g. Zwingelberg and Lindhauer (1996) who evaluated the variety Contra and found in it both high total starch content and much higher vol. % of starch A compared with our results.

On the other hand, in the variety Estica that was marked by the highest vol. % of starch A at all evaluated sites, simultaneously with it, the total content of starch was relatively high.

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Received on June 25, 2002

ABSTRAKT

Hodnocení distribuce velikosti škrobových zrn u vybraných odrůd pšenice metodou Low Angle Laser Light Scattering

U vybraného souboru pěti odrůd ozimé pšenice (Hana, Contra, Siria, Estica, Versailles) z pěti pokusných lokalit a dvou intenzit pěstování byla hodnocena distribuce velikosti škrobových zrn metodou LALLS (Low Angle Laser Light Scattering) a dále sledován vliv odrůdy, pokusné lokality a intenzity pěstování na podíl škrobu A (škrobová zrna > 10 µm), celkový obsah škrobu a obsah N-látek v sušině mouky T530. Podíl škrobu A se u hodnoceného souboru odrůd pšenice pohyboval mezi 65,31 a 72,34 %. Vliv odrůdy na podíl škrobu A se na základě našich výsledků ukázal výraznější než vliv pokusné lokality a intenzity pěstování. Nejvyšším podílem škrobu A se vyznačovaly hodnocené odrůdy z jakostní skupiny C, tedy nevhodné pro pekárenské využití (kromě odrůdy Contra, která sice dosáhla vysokého celkového obsahu škrobu v sušině mouky T530, ale relativně nízkého podílu škrobu A). Jako nevhodné jsou pro škrobárenské účely odrůdy elitní, případně kvalitní, v našem případě odrůda Hana s velmi nízkým podílem škrobu A. Z hodnocení distribuce velikostních frakcí škrobových zrn, tvořících škrob A, rovněž vyplynuly odrůdové rozdíly. V případě odrůd Hana, Contra a Siria bylo zjištěno vyšší zastoupení frakcí do 30 µm, zatímco u odrůd Estica a Versailles byl škrob A tvořen ve vyšší míře velikostními frakcemi škrobových zrn nad 30 µm a zejména velikostní frakce > 50 µm byla u těchto odrůd ze všech hodnocených vzorků nejvyšší. Se zvyšujícím se celkovým obsahem škrobu v sušině mouky T530 klesal obsah N-látek, ne vždy však se zvyšujícím se celkovým obsahem škrobu se zvyšoval i podíl škrobu A.

Klíčová slova: ozimá pšenice; odrůdy; škrobová zrna; škrob A; metoda LALLS

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