Effects of cropping system and genotype on variability in important phytonutrients content of the barley grain for direct food use

J. Ehrenbergerová[1], K. Vaculová[2], V. Psota[3], P. Havlová[3], V. Šerhantová[1]

[1]*Mendel University of Agriculture and Forestry in Brno, Czech Republic*
[2]*Agricultural Research Institute Kroměříž, Ltd., Czech Republic*
[3]*Research Institute of Brewing and Malting, PLC, Malting Institute Brno, Czech Republic*

ABSTRACT

In a four-year period (1997–2000), selected phytonutrients, of which the beta-glucan content is most important for human health, were studied in caryopses of different types of barley varieties and lines (standard, *waxy,* hulless) from two cropping systems. It was significantly highest in the *waxy* variety Washonubet (4.93%). The whole group of *waxy* types of barley showed a significantly higher mean content of beta-glucans (4.75%) than the varieties with standard starch composition (4.12%), the starch content, however, was significantly higher in standard varieties (61.98%) than in *waxy* types (60.30%). The hulless varieties, however, had a significantly higher mean starch content (61.73%) than the hulled forms (61.07%) and a significantly higher protein content (13.82% versus 13.00%). The hulless intensive varieties and lines had a significantly higher content of beta-glucans (4.34%) and protein (13.95%) than the hulled intensive varieties (4.07%, 12.65%). Chemical treatment and fertilization increased significantly only the mean content of protein in caryopses (13.77%) compared to the variants with the absence of treatment (13.13%), the content of beta-glucans and starch increased insignificantly (by 0.12% and 0.27%). Years and varieties participated most in the variability of starch content (31.67%, 28.08%), varieties in the content of beta-glucans and protein (per 22%), and (21%) the interaction of varieties and years in the variability of these two nutrients.

Keywords: spring barley; *waxy*; hulled; hulless; beta-glucan; protein; starch; cropping systems

Currently, those foods are preferred that are not only a source of nutrients but also have a genetically conditioned increased or different content of other nutritiously valuable substances with favourable effects on the consumers' health (Kerckhoffs et al. 2002). Foods with these effects are indicated as functional foodstuffs. The importance of direct consumption of barley grain adjusted for a diet of the prevention and treatment of common civilization diseases such as heart diseases and diseases of the circulation system, diabetes of the 2nd type, neoplasms, etc., is confirmed by a number of foreign research results and clinical tests (Newman et al. 1989, Lifschitz et al. 2002, and others).

In this connection, the principal health preventive and curative effect is ascribed to non-starch polysaccharides, especially to beta-glucans and arabinoxylans that form a significant portion of dietary fibre in a barley grain. The increased content of beta-glucans is connected with the presence of gene *nud* for a hulless type of grain, gene *lks2* for short awns and gene *waxy* for a different ratio of two main polysaccharides of starch – amylose and amylopectin (Swanston 1995). The locus *waxy*, confering for the content of amylase in starch (Ono and Suzuki 1957), is located on a short arm of chromosome 7H (Han et al. 1997). If the allele *waxy* is present in the endosperm, the ratio of amylase to amylopectin is roughly 25:75. In the presence of the homozygous recessive allele *waxy,* the content of amylose amounts to ca. 2–10% in the endosperm and of amylopectin to 90–98% (Washington et al. 2000), however, barley genotypes with nearly 100% amylopectin ratio are known too. These materials are indicated as *waxy* in the literature and besides the higher beta-glucan content they are also characterized by the whole range of different physical and chemical properties that are of decisive importance for the final utilization of a grain (Bhatty 1993, Swanston 1995).

The increased beta-glucan content is a characteristic feature of many varieties, first of all of hulless barleys that were registered in Canada, USA, Australia and Japan during the last 10–15 years. In the USA (Newman and Newman 1991) isogenous lines of barley based on the varieties Compana and Betzes were formed containing one or more genes that are connected with the increased beta-glucan content. Hulless barley forms for direct human nutrition are commercially important mainly in Canada where their acreage has already amounted to more than 250 000 ha. In Europe, though studied experimentally, they have not been registered yet.

The objective of this study was to assess the effect of growing and genetic factors on changes in the content of the basic nutritiously significant parameters of barley grain in the set of *waxy* varieties formed in the USA (Wa-

This study was supported by the projects of the Ministry of Education, Youth and Sports of the Czech Republic No. CEZ: J08/98: 43210001, and of the Ministry of Agriculture of the Czech Republic No. QD0057 and QD0183.

bet, Wanubet, Washonubet and Wapana) in comparison with selected Czech hulled varieties registered in the CR and also new breeding materials with a hulless type of grain.

MATERIAL AND METHODS

Field trials were conducted in 1997–2000 at an experimental station of MUAF in Žabčice that is located in the maize production area, subtype barley. Each year a block test was set up in four complete blocks and two cropping systems were applied:

1. Conventional system of growing – with seed treatment, application of mineral fertilizer (50 kg of N, 55 kg of P_2O_5 and 55 kg of K_2O in pure nutrients per 1 ha) in the form of a combined fertilizer in the whole dosage at pre-seeding treatment. During vegetation usual pesticides were applied.
2. Cropping without chemical treatment, i.e. without seed treatment, using mineral fertilizers and pesticides.

A list of varieties and lines included in the trial is presented in Table 1. The materials marked by KM were produced in Agricultural Research Institute Kroměříž, Ltd., as initial genetic sources of hulless barley for the final diversified utilization of a grain in the course of solution of research projects of NAZV. Of them, KM 1057 and KM 1771 were tested in field trials in 1994–1996 (Ehrenbergerová et al. 1997, 1999), the other materials are described in greater detail in some publications and final reports. The varieties Kompakt, Krona, Ladik belong to the collection of registered spring barley varieties, the variety Rubín belonged to the most important malting varieties in the Czech Republic in the past but it has already been discarded. The materials marked as Wabet, Wanubet, Washonubet and Wapana are experimental materials from the USA whose main characteristics are the presence of gene *waxy* and the increased beta-glucan content at the same time. These materials were obtained from the collection of professor C.W. Newman (University in Montana) and were handed over to Agricultural Research Institute Kroměříž for breeding utilization. For the consequential assessment, the studied varieties and lines were split into groups based on the type of grain (hulled, hulless), yield potential (intensive, extensive) and also on the starch composition (standard, i.e. with the ratio of amylose: amylopectin = ca. 25:75 and *waxy*, i.e. with the amylopectin ratio higher than 75%). A survey of classification is given in Table 1.

Table 1. Characteristics of the experimental material

Varieties	Type of grain	Yield potential	Composition of starch
KM 1057	hulless	extensive	standard
KM 1771	hulless	intensive	standard
KM 2062	hulless	intensive	standard
KM 2082	hulless	intensive	standard
KM 2092	hulless	intensive	standard
Kompakt	hulled	intensive	standard
Krona	hulled	intensive	standard
Ladik	hulled	intensive	standard
Rubin	hulled	intensive	standard
Wabet	hulled	extensive	*waxy*
Wanubet	hulless	extensive	*waxy*
Washonubet	hulless	extensive	*waxy*
Wapana	hulled	extensive	*waxy*

The detailed course of weather in 1997 to 2000 including the characteristics of individual periods is presented in Figure 1. Meteorological data were provided by the Institute of Landscape Ecology of MUAF in Brno.

Experiments were established after sugar beet, size of single plots was 10 m^2. The materials were treated in the course of vegetation according to the above-mentioned cropping systems, the harvest was carried out at full ripeness with small-plot harvester. After the harvest of experiments, samples were taken from the harvested grain, homogenized and used for chemical analyses realised by the RIBM in Brno. The following grain components were determined and evaluated (converted to grain dry matter): beta-glucan content (%), N-substances (NS = content of N × coefficient 6.25, %), and starch content (%). Beta-glucans were assessed by the FIA method. The principle of the method lies in forming the complex of a dyestuff Calcofluor White M2R, specific to beta-glucoside linkage with beta-glucans that is expressed by increasing the fluorescent intensity of the dyestuff recorded with spectrofluorimeter (Havlova 2001). Nitrogenous substances (NS) were assessed by Kjeldahl's method (EBC Analysis), starch by Ewers' method.

The identified data were processed by the statistical programmes UNISTAT and S-PLUS 4.5, namely multifactor analysis of variance, differences between years, manner of treatment and lines and varieties were assessed with the help of Tukey's test. The significance of the difference was evaluated on the 5% significance level. The programme Excel (Office 97) was used for graphical representation.

RESULTS

According to the table showing the analysis of variance (Table 2), the variability of beta-glucan content was significantly influenced by the experimental material and different cropping system and years of growing. Interactions of the evaluated factors showed a very high statistical ratio of significance level with the exception of the interactions of varieties and treatment where the level of significance was lower by an order. High singularity of the tested varieties and lines and also variance within the evaluated groups of genotypes are evident from a comparison of the mean values (Table 3). Unlike the other parameters, the lowest values of beta-glucan content

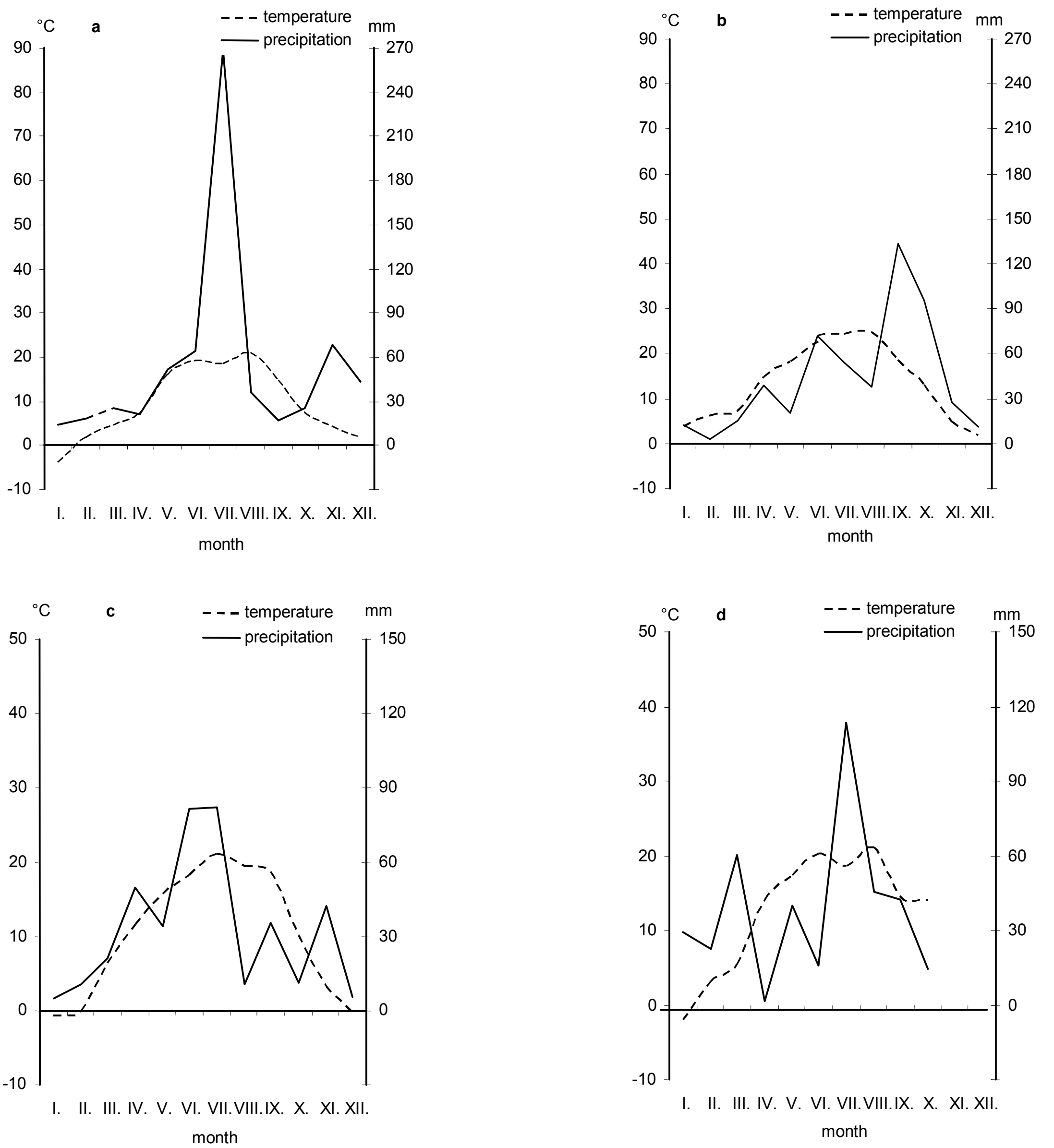

Figure 1. Mean monthly temperatures and total monthly precipitation for the years 1997 (a), 1998 (b), 1999 (c) and 2000 (d); based on the climate diagram according to Walter-Lieth

were measured in the line KM 1057 (3.52%), which was statistically significantly different in this parameter from all assessed materials. On the other hand, the *waxy* genotypes, first of all the variety Washonubet (4.93%), had the highest content of these polysaccharides although in hulled *waxy* types (Wabet and Wapana) no statistically significant difference from the Czech hulless lines, especially from KM 1771, KM 2062 and KM 2082, was found out. Hulled varieties Krona, Kompakt, Ladik and Rubín were significantly different from the line KM 1057 but with respect to the variability of the observed parameter it was not possible to determine their unambiguous difference in the content of beta-glucans from the other new hulless breeding materials. The variability of the values of beta-glucans in individual years was relatively high, all materials had the lowest mean content in 1997 (3.87%) while the highest mean content was determined in 1999 (4.62%). The years 1997–1999 differed statistically from each other, the year 2000, however, differed only when compared with the year 1997 (Figure 6). It is evident from Figure 2 that the beta-glucan content in chemically treated and untreated variants was practically the same and the mutual difference did not achieve a statistically significant value. This is also confirmed by the results presented in Figure 7; they indicate that of the total beta-glucan variability their content was least influenced by treatment (1.17%) but it was strongly influenced by the varieties and lines (22.08%), years (17.58%) and by the interactions of varieties and years (21.36%). The evaluation of differences between the genetically conditioned barley types (Figure 3) proved the highest content of beta-glucans in *waxy* types. Statistically sig-

Table 2. Analysis of variance of phytonutrients (1997–2000)

Sources of variability	Beta-glucans			Crude protein			Starch		
	d.f.	*SS*	*MS*	*d.f.*	*SS*	*MS*	*d.f.*	*SS*	*MS*
Varieties	12	58.15	4.85***	12	165.08	13.76***	12	604.53	50.38***
Treatment	1	3.07	3.07***	1	41.8	41.79***	1	2.51	2.51
Years	3	46.29	15.43***	3	39.78	13.26***	3	681.83	227.28***
Interaction varieties × treatment	12	6.81	0.57**	12	5.64	0.47	12	26.89	2.24
Interaction varieties × years	33	56.23	1.70***	33	150.92	4.57***	33	313.89	9.49***
Interaction treatment × years	3	21.69	7.23***	3	28.93	9.64***	3	131.53	43.85***
Error	337	74.54	0.22	397	257.63	0.65	252	498.38	1.98
Total	401	263.3	0.66	461	723.85	1.57	316	2153.06	6.81

*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

nificant difference of beta-glucans towards genotypes with the standard starch composition was 0.63% in the absolute value (i.e. by 13.3% more).

Similarly like the beta-glucan content, the content of nitrogenous substances (NS) was also significantly influenced by all three factors: cropping system, varieties and their interactions. Only the interaction of varieties with treatment was not statistically significant as a source of variability unlike most beta-glucans (Table 2). The mean values of NS content in individual varieties and lines were statistically significantly different (Table 3) in the whole set. But unlike the previous trait, the differences between the mean contents of NS were not so large and therefore the variability range was smaller. In the whole experiment the values of the content of NS fluctuated from 12.41% (variety Kompakt) to 14.35% (Wapana), in hulled varieties the mean content of NS did not exceed 13%. Though the data on hulless and *waxy* materials were similar, significantly different values were found there. The line KM 2082 and variety Wapana having the statistically highest content of NS (14.30 and 14.35%) of the whole set, were statistically significantly different from the lines KM 2062 and KM 2092, on the contrary, the varieties Wanubet and Washonubet had statistically less protein than KM 1057 and KM 1771 but insignificantly less than KM 2062 and KM 2092. Unlike beta-glucans the highest values of NS content (13.88%) were measured in 2000, the year 1997 differed statistically significantly from the mentioned year 2000 and also from the years 1998 and 1999, which were similar to each other (Figure 6). The mean content of NS in the treated (13.77%) and untreated (13.13%) variants of the test was significantly different (Figure 2), it means that after the application of mineral fertilizers and pesticides significantly higher values were detected. Similarly like in the assessment of beta-glucans, the main variability sourc-

Table 3. Means and differences of phytonutrients for varieties and lines (1997–2000)

Varieties	*n*	BG (% dry matter)		*n*	Protein (% dry matter)		*n*	Starch (% dry matter)	
		mean	difference		mean	difference		mean	difference
KM 1057	34	3.52	a	40	14.03	de	28	59.70	a
KM 1771	34	4.35	cde	40	14.03	de	28	61.74	bc
KM 2062	28	4.34	cde	34	13.77	cd	25	63.02	d
KM 2082	28	4.34	cde	34	14.30	e	25	62.57	bcd
KM 2092	28	4.34	cd	34	13.69	cd	25	62.67	cd
Kompakt	34	4.07	bc	40	12.41	a	28	61.87	bcd
Krona	34	3.94	b	40	12.83	ab	28	61.82	bcd
Ladik	28	4.08	bc	28	12.59	a	16	62.62	bcd
Rubin	28	4.22	bcd	28	12.80	ab	16	62.60	bcd
Wabet	34	4.71	efg	40	13.30	bc	28	58.94	a
Wanubet	32	4.82	fg	38	13.38	c	27	61.24	b
Wapana	26	4.49	def	26	14.35	e	15	58.86	a
Washonubet	34	4.93	g	40	13.54	c	28	61.48	bc

Means with different letters are statistically significant at $P \leq 0.05$

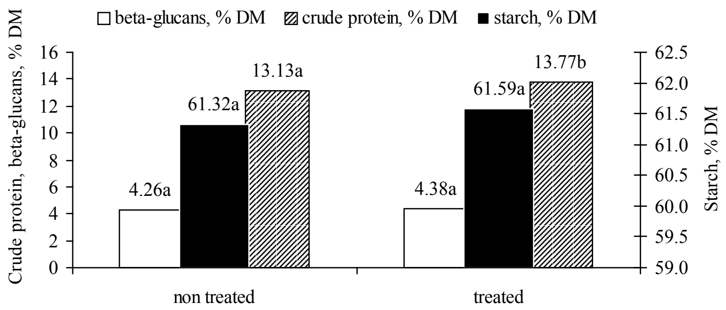

Figure 2. Means and differences of phytonutrients in different cropping systems. Means with different letters are statistically significant at $P \leq 0.05$

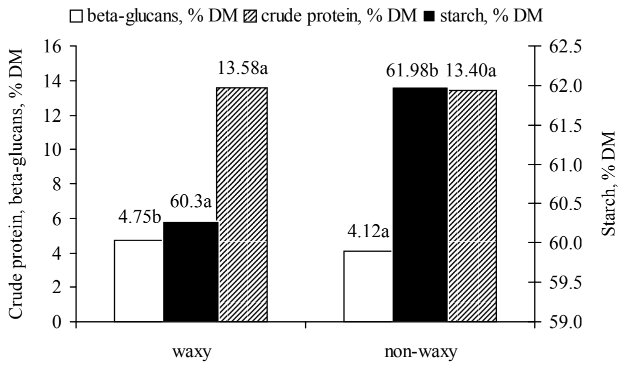

Figure 3. Means and differences of phytonutrients for genotypes with different starch compositions. Means with different letters are statistically significant at $P \leq 0.05$

es were experimental factors and interactions but the most powerful sources of variability were only the varieties and their interactions with the years (22.08 and 20.85% of the total variability – Figure 7).

The selected barley form was significantly reflected in the mean content of NS only if the hulled and hulless materials were compared (difference of 0.82% in favour of hulless – Figure 4). However, a significantly lower NS content was also determined for the hulled intensive, i.e. registered, varieties, which differed from the other groups by more than 1% of NS (Figure 5) based on the type of barley.

Varieties and experimental years affected the variability of starch content statistically significantly (Table 2). Unlike the two preceding parameters the effect of treatment did not prove significant nor the interactions of varieties were significant. As it follows from Table 3, the assessed materials, however, differed significantly from each another in the starch content too. The lowest content was determined in the line KM 1057 (59.7%) and hulled *waxy* varieties Wabet (58.94%) and Wapana (58.86%). On the contrary, the highest starch content was found in the hulless line KM 2062 (63.02%), which differed statistically significantly only from the line KM 1771 and two remaining hulless *waxy* varieties Wanubet (61.24%) and Washonubet (61.48%). The other materials, both the hulless new lines KM 2082, KM 2092 and hulled varieties (Kompakt, Krona, Ladik and Rubín) did not differ significantly from each other. Individual years were a mirror image of NS content (the year 1997 was an exception). The year 2000 had the lowest (59.92%) and the year 1998 the highest mean starch content (63.22%), the years 1998 and 1999 were different from 1997 and 2000 but not from each other (Figure 6). The system of cropping did not have a significant influence on starch accumulation in the endosperm in all assessed materials and years. Though the mean starch value was lower in the untreated variant (61.32%) compared to the treated one (61.59%), the mutual difference was not statistically significant (Figure 2). Out of all assessed parameters the total variability of this trait was affected most significantly by the operation of the main factors (59.87% – Figure 7), interactions accounted for 21.90% of total variability, and the year (31.67%) turned out to be the strongest source of variability and only then it was followed by the variety (28.08%). The intensive barley types (according to the harvest – see the methodology, Table 1), both hulled and hulless, had a statistically significantly higher starch content than the other compared types of barley, thus the hulled intensive varieties did not have more starch (62.12%) than the intensive hulless varieties (62.12%) – Figure 5. The hulled extensive genotypes, however, had statistically significantly less starch (58.9%) than the other compared groups of the materials.

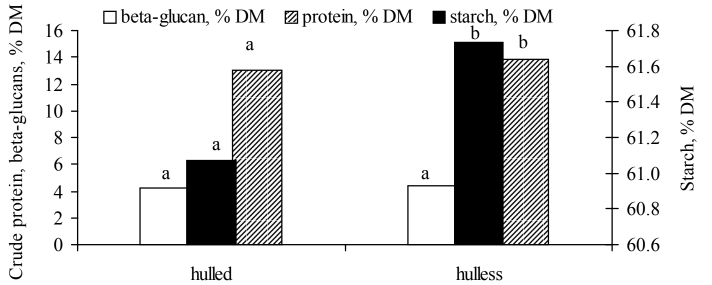

Figure 4. Means and differences of phytonutrients of genotypes with the different type of grain. Means with different letters are statistically significant at $P \leq 0.05$

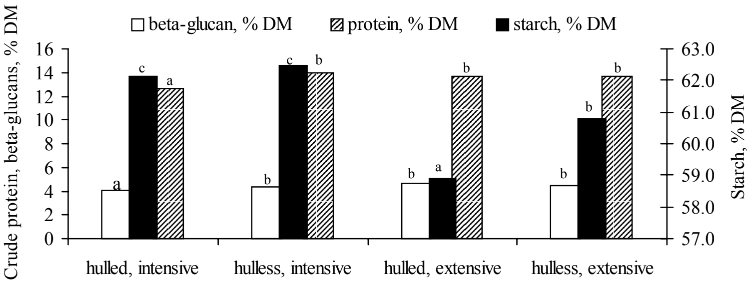

Figure 5. Means and differences of phytonutrients of genotypes with different yield potential. Means with different letters are statistically significant at $P \leq 0.05$

DISCUSSION

In this study, unlike the results achieved in the previous period but in the hulled malting type varieties (Ehrenbergerová et al. 1997, Persson 1998), all the factors (variety, year and way of cropping) proved to be significant ones influencing the content of beta-glucans and NS and starch. In accordance with the above-mentioned results, this study also determined the strongest effect of year on the changes in the content of starch and nitrogenous substances. The variability of starch content was affected by the year most strongly (31.67% of total variability). In our study, a certain portion of differences in the values of the studied parameters is influenced by a very wide spectrum of the mutually different genotypes.

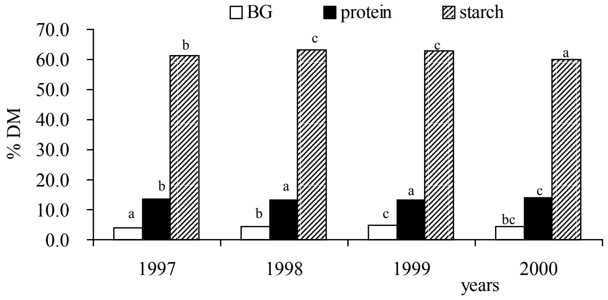

Figure 6. Means and differences of phytonutrients for particular years. Means with different letters are statistically significant at $P \leq 0.05$

It is also confirmed by a ratio of the varieties in the total variability of the parameters (28% in starch and 22% in beta-glucans and protein). The assortment of materials with a genetically determined higher content of beta-glucans was possibly reflected also in the increase of variability on average of the experimental years, which coincides with the results of the study of original barleys with *waxy* endosperm (Vaculová 1993). The foreign data on the beta-glucan content are not uniform either. Perez-Vendrell et al. (1996) found out that the variety and locality and year had a statistically significant effect on the content of beta-glucans in the caryopses of standard, spring and winter varieties. According to these authors (Fastnaught et al. 1996) a higher beta-glucan content was also in relation with the effect of year – heat and drought induced a higher content. This finding can be confirmed by our results as well because the lowest mean content of beta-glucans was measured in the extremely wet and cold weather in 1997, especially in the period of grain formation in the months of June and July (Figure 1a). Savin et al. (1997) examined the effect of high and higher temperatures on the content of NS, starch and BG in malting barleys in the phase of grain filling under the controlled conditions. In this study, the highest mean content of nitrogenous substances of the whole set was determined in 2000 when the lowest precipitation of all four years of observation (Figure 1d) and favourable temperatures of air were recorded in the month of June (but it was not different from the years 1998 and 1999). Oscarsson (1997) also confirmed the effect of locality on the content of NS,

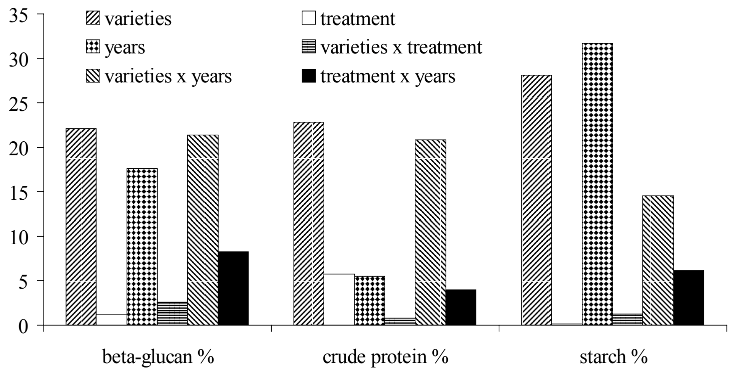

Figure 7. Ratio of individual variability sources on total variability of nutritiously parameters of barley

amylose, amylopectin and beta-glucans. She found out that the *waxy* barleys and also the materials with the increased ratio of amylose had more beta-glucans, thicker cell walls, and *waxy* barleys also more amylose in the subaleuron layer. Persson (1998) stated that the variety *Prowashonupana* had very thick cell walls and a high content of BG but a very low content of starch. In our case, however, it was not unambiguously true of the hulless *waxy* barleys – though on average they had a little lower starch content, the differences from the malting and some hulless materials were not significant. Fastnaught et al. (1996) reported that the *waxy* types of barley had the most beta-glucans (6.93% vs. 3.8% in standard varieties), which was also confirmed by our results. On average, the *waxy* varieties had 4.75% beta-glucans in grains and thus differed significantly from the varieties with standard starch (4.12%). Unlike these and other foreign data, however, the determined differences were not so large. Baidoo and Liu-YongGang (1998) as well as Boros et al. (1996) confirmed the higher beta-glucan content and at the same time the higher NS content in some hulless barley types. Some hulless forms (first of all lines KM 1771, KM 2082) also had an increased content of NS and at the same time more beta-glucans so they are ranked to the same significant group as the *waxy* varieties. Our study also assessed significantly more NS in the hulless barley forms versus hulled and in the *waxy* types versus the types of standard starch composition. In the malting type varieties, we also observed (Psota et al. 2002) a positive relation between the protein content in a caryopsis and beta-glucans but the concentration of the extract was in negative relation to the beta-glucan content in malt. Swanston (1995) observed the effect of the incorporation of one of the genes with additive effect on the BG content – of the genes *waxy*, *lk2* and *nud* in the series of isogenous lines derived from the variety Compana. He stated that the gene *waxy* had a worse effect on the modification of endosperm than gene *lk2* and that the combination of genes *waxy* and *nud* was of no additive effect in relation to the milling energy, extractivity or modification of cells. The higher beta-glucan content causes higher viscosity of extract, which is undesirable for the malting and feeding trend of grain utilization but on the contrary it is positively evaluated for direct consumption, for this reason the varieties considered in the USA as donors of higher beta-glucan content were also included in this study. As stated by Snehil-Kalra and Sudesh-Jood (1998), the higher content of beta-glucans is in negative relation to the NS content but it does not relate to the quality of NS. In our study a rather negative dependence between the starch content and NS was observed – that is a characteristic symptomatic of the standard malting varieties.

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Received on May 7, 2003

ABSTRAKT

Vliv systému pěstování a genotypu na variabilitu obsahu významných fytonutrientů v obilkách ječmene pro přímé potravinářské využití

Ve čtyřletém období (1997–2000) byly v obilkách odlišných typů odrůd a linií ječmene (standardní, *waxy,* bezpluché) ze dvou způsobů pěstování sledovány vybrané fytonutrienty, z nichž pro lidské zdraví je nejdůležitější obsah beta-glukanů, který byl signifikantně nejvyšší u *waxy* odrůdy Washonubet (4,93 %). Celá skupina *waxy* typů ječmene vykázala významně vyšší průměrný obsah beta-glukanů (4,75 %) oproti odrůdám se standardním složením škrobu (4,12 %), avšak obsah škrobu měly standardní odrůdy signifikantně vyšší (61,98 %) oproti *waxy* typům (60,30 %). Bezpluché formy měly významně vyšší průměrný obsah škrobu (61,73 %) oproti pluchatým formám (61,07 %) i významně vyšší obsah proteinu (13,82 % oproti 13,00 %). Bezpluché intenzivní odrůdy a linie měly významně vyšší obsah beta-glukanů (4,34 %) a proteinu (13,95 %) než pluchaté intenzivní odrůdy (4,07 %, 12,65 %). Chemické ošetření a hnojení signifikantně zvýšilo pouze průměrný obsah proteinu v obilkách (13,77 %) oproti variantám s absencí ošetření (13,13 %), obsah beta-glukanů a škrobu zvýšilo nevýznamně (o 0,12 % a 0,27 %). Ročníky a odrůdy se podílely nejvíce (31,67 %, 28,08 %) na variabilitě obsahu škrobu, odrůdy na obsahu beta-glukanů a proteinu (a 22 %), interakce odrůd s roky variabilitu těchto dvou nutrientů ovlivnily z 21 %.

Klíčová slova: jarní ječmen; *waxy*; pluchatý; bezpluchý; beta-glukany; bílkoviny; škrob; systémy pěstování

Corresponding author:

Doc. Ing. Jaroslava Ehrenbergerová, CSc., Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika
tel.: + 420 545 133 127, fax: + 420 545 133 302, e-mail: ehren@mendelu.cz