Dedicated to the anniversary of Prof. Ing. Jiří Petr, DrSc., Dr.h.c.

STUDIES OF PROTEIN FRACTION IN GRAIN OF SPELT WHEAT (*TRITICUM SPELTA* L.) AND COMMON WHEAT (*TRITICUM AESTIVUM* L.)*

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In the years 1999 to 2000 on two localities of České Budějovice (ČB) and Humpolec (HUM) under low input conditions five spelt wheat varieties (*T. spelta* L.) – Altgold (CH), Lueg (B), Rouquin (B), Ostro (Germany) and Oberkulmer Schwarzer (Germany) were cultivated. Two Czech common wheat varieties Brea and Samara cultivated on the locality České Budějovice were chosen as reference varieties. TKW, crude protein content, and contents of three protein fractions (1 – albumins and globulins, 2 – gliadins, 3 – glutenins) obtained by the method after Osborn, measured by the standard method after Kjeldahl, were studied on hulled grain. Electrophoreogram of different fractions was done for three selected varieties: Altgold, Rouquin and common wheat Brea in PAGE conditions according to the international method ISTA-SDS and the percentage of different fractions was evaluated densitometrically. The results obtained confirmed a marked influence of medium as well as genetic conditionality on percentage of different protein fractions. The capacity of spelt wheat to accumulate crude proteins significantly and their fractions, even under conditions of low inputs, was confirmed. Gliadin fraction that will evidently limit its utilisation for gluten-free diet was proved. In view of quantitative comparison of extracted fractions found by the method after Kjeldahl and preliminary densitometric measurement on gel, mutual irreplaceableness of both methods follows. Nevertheless, densitometric and spectrophotometric evaluation, respectively, should be a suitable complement to evaluate both nutritional and technological qualities in view of evaluation of protein molecules only.

spelt wheat; common wheat; protein fractions; electrophoresis; gluten-free diet

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INTRODUCTION

Grain crops are an important source of energy and proteins in nutrition of population. In view of cover of need of proteins in food cereals are on the second place in the Czech Republic after meat with 30 per cent (Bojňanská, 1993). A low share of albumin fraction, above all in common wheat, leads generally to conclusion to evaluate a wheat protein as non-full-value one. Limiting amino acids in common wheat are as follows: lysine, treonine, tryptophane and sulphur amino acids. In this respect higher representation of nutritionally higher quality spelt wheat should be suitable (Grela, 1996).

Spelt wheat (*Triticum spelta* L.) means older form of hulled hexaploid wheat cultivars in its development with a genetic structure AA BB DD (Kling, 1988). Winzeler and Rügger (1990) report that initial independent form of hexaploid cultural wheats is spelt wheat, but not a hexaploid hulled form of *Triticum aestivum*.

Many varieties have been tested in the Czech Republic since 1990 and areas of cultivation on ecological farms reached about 600 hectares (Vlasák, 1995).

The crude protein content in spelt wheat is given in a relatively great range (12–20%) (Cubbada, Marconi, 1994). Grela (1996) in comparative experiments between spelt wheat, common wheat and triticale found higher average content of crude protein in spelt wheat (14.5%). Similar values were recorded by Jorgensen et al. (1996) under fertilisation 100 kg $N.ha^{-1}$. Cubbada, Marconi (1994) report average crude proteins content in spelt wheat grain over 20%. Grela (1996), Ranhotra et al. (1995), Hein (1997), Jorgensen et al. (1996) and other authors presented that crude protein content in spelt wheat grain shows a relatively great variability during the years under study.

Despite this variability some spelt wheat varieties (Ostro, Oberkulmer, Loge) predominated over other spelt wheat and common wheat varieties (Hein, 1997; Kontturi, 1997) statistically significantly by their crude protein content.

Except small modifications of extraction agents, an original Osborn's method of 1907 is still used for fractionation of proteins of cereal grain, based on the principle of their different solubility (Králová, 1991). Albumins and globulins are soluble in 0.1 M Na-phosphate buffer with 1 M NaCl. Gliadins can be solved in 70% ethanol and form some 35–45% of protein component of wheat grain. Glutenins are soluble in 0.02 M NaOH or 0.05 M of acetic acid and their quantitative percentage from the content of crude protein amounts to about 20–30% (Králová, 1991; Prugar, Hraška, 1986; Bojňanská, 1993).

Prugar and Hraška (1986) consider a year as a significant factor affecting representation of different fractions. Generally, original wheat varieties showed lower variability in the year compared to their hybrids. The percentage of gliadin component for utilisation of spelt wheat for food suitable for those affected by coeliac disease was studied most frequently in quantification of spelt wheat fractions. The contents of these fractions ranged between 5 and 7% of the total grain dry matter (Grela, 1996; Abdel-Aal, 1996). Storage proteins of wheat grain find their marked utilisation as markers of economically important traits also in legal protection of varieties. Their similar utilisation like in spelt wheat is presupposed (Šašek, Černý, 1997).

Though Osborn's classification of protection is well known and much used almost a century, a lot of studies prove that transitions between different fractions are not so sharp and are much dependent on temperature, time of extraction and even upon an intensity of shaking (Byers et al., 1983). Transition from heavy fraction of high-molecular weight glutenins into gliadins spectra has been often manifested, together with overlapping of lighter glutenin spectrum of light-molecular weight with alpha, beta and gamma gliadins in the medium SDS-PAGE (Abdel-Aal et al., 1996). Mosse and Baudet (1963) mention that at the temperature of 22 °C as much as 50% of grain protein passed into the first fraction. It follows from this that relatively marked portion of other fractions at this temperature was a part of albumins. In additions, the change of electrophoretic spectra can be affected by the effect of external factors of medium, such as cultivation of one of two identical varieties under the conditions of low content of sulphur in soil, eventually when reduction detergents (e.g. dithiotreitol during extraction) were used (Kim, Bushuk, 1995; Pomeranz et al., 1989).

MATERIAL AND METHOD

In the years 1999 to 2000 on two localities of České Budějovice (ČB) (380 m above sea level), soil – acid cambisol, gleyic, sandy-clay) (Table I) and at Humpolec (Table II) (HUM) (575 m above sea level) under low input conditions five spelt wheat (*T. spelta* L.) varieties – Altgold (CH), Lueg (B), Rouquin (B), Ostro (Germany) and Oberkulmer Schwarzer (Germany) were cultivated. Two Czech common wheat varieties Brea (E – elite baking quality) and Samara (C – unsuitable for baking utilisation) cultivated on the locality in České Budějovice. Legume-grain mixture was used in both cases as a forecrop.

TKW, crude protein content, and the contents of three protein fractions (1 – albumins and globulins, 2 – gliadins, 3 – glutenins), obtained by the

I. Soil and climatic conditions on the locality České Budějovice

	III	IV.	V.	VI.	VII.	VIII.	Average in vegetation	Year average
Average temperature (°C)								
1999	5.6	9.3	14.6	16.3	19.5	17.9	13.9	9.3
2000	4.9	12	15.6	18.6	16.5	19.3	14.5	9.9
30 years average (°C)	3.4	8.1	13	16.2	17.7	17.1	12.6	8.2
Sum of precipitation (mm)								
1999	24.1	22.9	75.1	43.5	72.9	68.2	306.7	505.1
2000	86.4	6.7	45.2	68.6	104	73.2	383.8	601.0
30 years average (mm)	32	46.5	70.1	93	77.8	78.8	398.2	582.8

Soil conditions						
Year	pH	P mg.kg^{-1}	K mg.kg^{-1}	Mg mg.kg^{-1}	Ca mg.kg^{-1}	N-tot %
1999	6.3	91	125	144	1364	0.1290
2000	6.1	106	131	125	1578	0.1307

II. Soil and climatic conditions on the locality Humpolec

	III.	IV.	V.	VI.	VII.	VIII.	Average in vegetation	Year average
Average temperature (°C)								
1999	4.3	7.9	14	15.3	18.9	17.8	13.0	8.3
2000	3.2	11.2	15.3	16.9	15.2	18.5	13.4	8.9
30 years average (°C)	1.7	6.4	11.4	14.5	15.9	15.5	10.9	6.6
Sum of precipitation (mm)								
1999	31.3	26.3	51.8	95.2	68	38	310.6	530.2
2000	128.8	23	64.9	58.7	112.7	42.7	430.8	715.2
30 years average (mm)	38.2	43.1	80.5	91.2	79.5	81.2	413.7	675.3

Soil conditions						
Year	pH	P mg.kg^{-1}	K mg.kg^{-1}	Mg mg.kg^{-1}	Ca mg.kg^{-1}	N-tot %
1999	5.25	130	380	84	1083	0.11
2000	5.3	246	146	111	1704	0.23

method according to Osborn of 1907, measured by the standard method according to Kjeldahl and using the protein converting factor 5.7, were finding in hulled grain. In both selected spelt wheat varieties – Altgold, Rouquin and common wheat Brea electrophoreogram of different fractions under PAGE conditions was made according to the international method ISTA-SDS (Linsekns, Jackson, 1992) and percentage of different fractions using the program Bio Profil was evaluated densitometrically.

Fractionation of protein from meal

Albumins and globulins were separated using 1 M NaCl in the medium Na-phosphate buffer at pH 7, overnight and at 4 °C. Separation itself occurred after 15-minute centrifugation at 6500 revolutions. The solution was analysed by the standard method according to Kjeldahl.

Gliadins were separated using 70% ethanol for 4 hours at the room temperature. The separation itself carried out in the same way like in the case of albumins and globulins. Extraction solution 25% 2-chlorethanol was used for evaluation of gliadins on electrophoreogram.

Glutenins were separated by 0.02 M NaOH at the temperature 4 °C for 4 hours. Further procedure is identical with the above procedure.

Protein fractions devoted for electrophoresis were prepared by the above procedure and lyophilised for 6 hours. Lyophilised material was processed by the standard method ISTA-SDS.

Statistical evaluation was done using the program Statistika 6.0 in two subsequent steps. In the first part spelt wheat varieties were evaluated separately on both localities and subsequently the locality in České Budějovice (ČB) was evaluated individually, including both reference varieties of common wheat.

RESULTS AND DISCUSSION

Spelt wheat showed markedly higher crude protein content during the period under study compared with both reference varieties. The highest contents were found in original obsolete spelt wheat varieties Altgold (ČB) – 18.53%, Ostro (HUM) – 18.07%, Oberkulmer Schwarzer (ČB) – 17.82% from the year 1999. On the contrary, the lowest values were found in both reference varieties Brea (10.54% or 11.87%, respectively) and Samara (11.9 or 13.04%, respectively) in both the years under study. High statistical significance of all studied factors follows from resulting statistical analyses (Table III and Fig. 1) both in the case of evaluation of spelt wheat on two localities and in evaluation of spelt wheat varieties with both reference varieties on the locality České Budějovice. Nevertheless, the highest per cent effect on the studied content of crude proteins had the year in both cases.

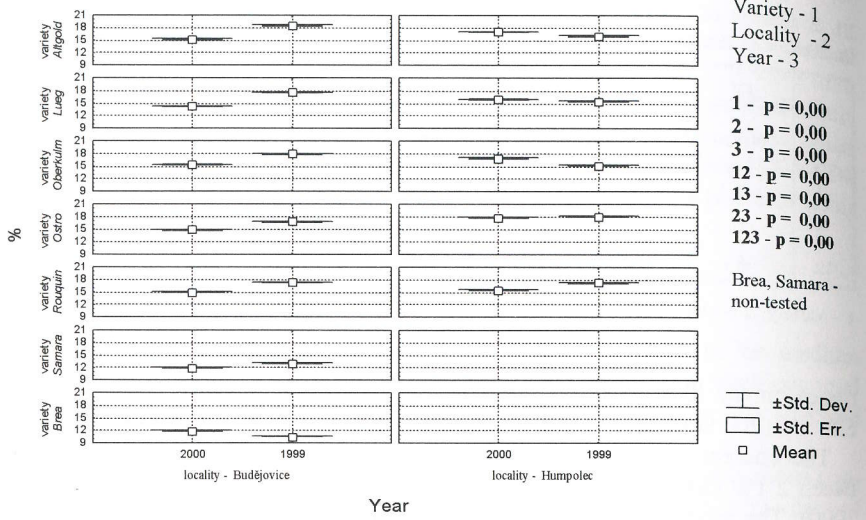
III. Evaluation of crude protein content in spelt and common wheat on the locality České Budějovice in the years 1999 and 2000

	Df	MS	df	MS			
	effect	effect	error	error	*F*	*P*-level	influence of factor (%)
1	6	11.33	10	0.01	852.88	0.00	35.49
2	1	19.56	10	0.01	1472.00	0.00	60.20
12	6	1.59	10	0.01	119.84	0.00	4.89

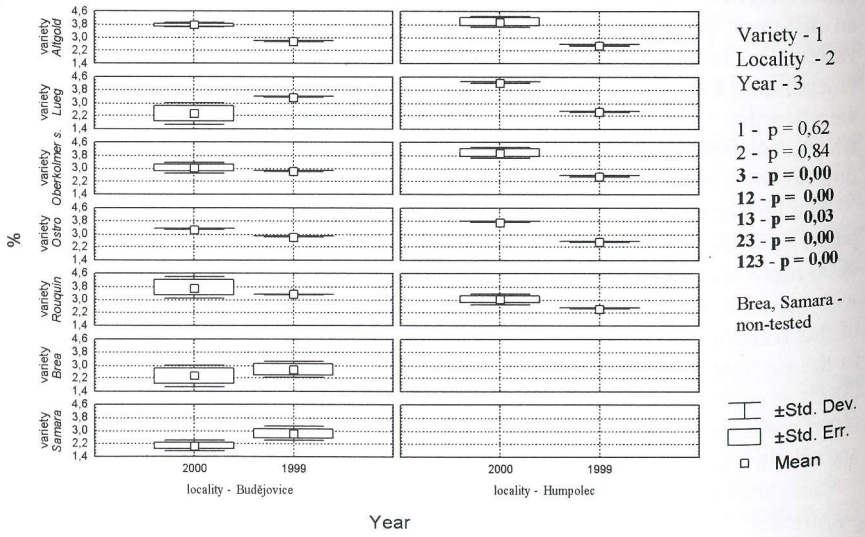
1 – variety, 2 – year

The contents of albumins and globulins in the studied years ranged between 2.1% (Samara, ČB, the year 2000) and 4.2% (Lueg, HUM, the year 2000). The growth of this fraction in evaluated spelt wheat varieties in 2000 can be seen in Fig. 2 compared with the year 1999. The variety Lueg in České Budějovice is the only exception that manifested falling trends together with both reference common wheat varieties. In evaluation of spelt wheat varieties on different localities (Fig. 2) only the year was significant, the effect of variety and locality was not confirmed. On the contrary, it follows from an independent evaluation on the locality České Budějovice with both reference varieties that the effect of variety and significance of the year was removed, what tells about statistically significant difference in the content of this fraction between spelt wheat and reference varieties.

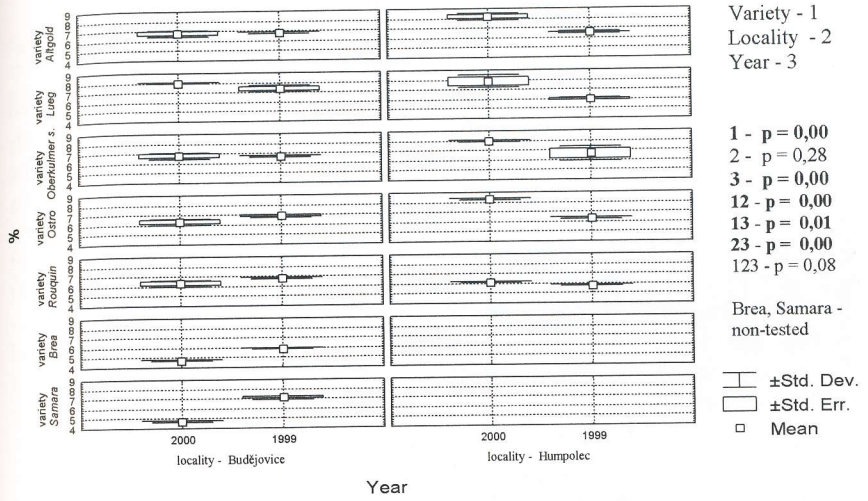
The content of gliadins was not so fluctuating in both studied years compared with the previous fraction. While on the locality České Budějovice in 2000 there was a general trend of the fall of the content of gliadins, the course was opposite on the locality Humpolec and in 2000 an increase in the content of this fraction was recorded in all studied varieties (Fig. 3). The highest value in the content of gliadins was found in the variety Altgold (HUM, the year 2000) – 8.18%, the lowest one in the content of gliadins was found in the reference variety Brea in 2000 – 4.67%. The effect of variety and year was statistically significant in view of statistical evaluation of all studied spelt wheat varieties. On the contrary, the effect of locality was not statistically significant. Highly statistically significant effect of both studied factors – variety and year – was proved from partial statistical evaluation on the locality České Budějovice and from evaluation of reference varieties. In both cases of evaluation the year was the most marked factor (per cent effect of factor – 33% or 42.5%, respectively). Nevertheless, neither the factor of variety was



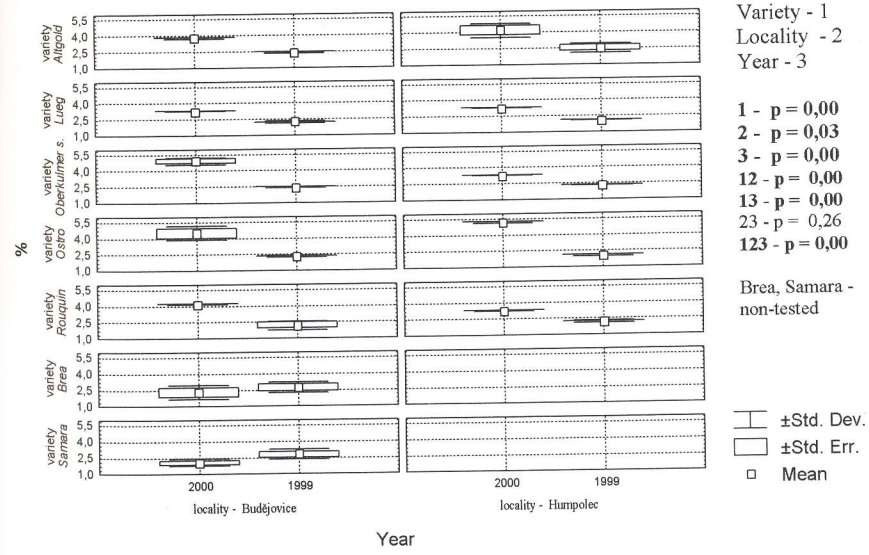
1. Content of proteins



2. Content of albumins and globulins



3. Content of gliadins



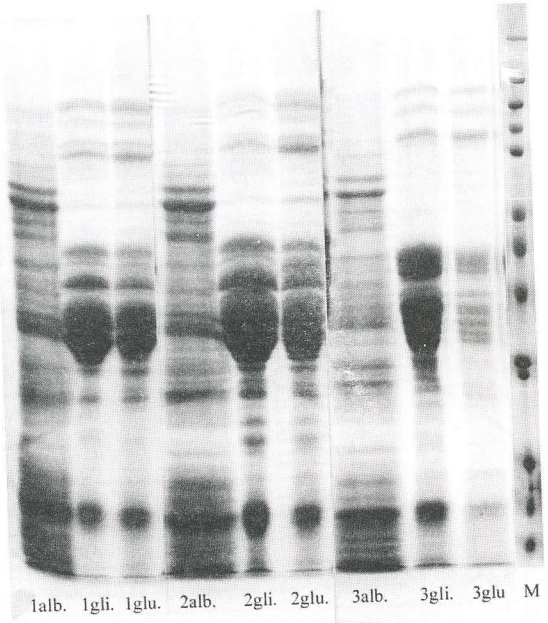
4. Content of glutenins

negligible (per cent effect of factor – 10.28% in case of independent evaluation of spelt wheat varieties).

The content of glutenins fluctuated in different years and within tested varieties as affected by the year. All evaluated spelt wheat varieties identically on both localities manifested in 2000 a considerable increase in the content of glutenins compared with the year 1999. On the contrary, in reference varieties of common wheat this effect was opposite and a slight decrease in the content of this fraction occurred in 2000 (Fig. 4). The highest content of glutenins was found in the variety Ostro (HUM, 2000) – 5.14%, the lowest contents were found in the reference variety Brea (1.67% or 1.87%, respectively – Tab. IV, Fig. 4). Highly statistically significant effect of variety and year follows from statistical evaluation, and in this case also the effect of locality was statistically significant. Unambiguously greatest effect of year (91%) on evaluated fraction follows from per cent share of different factors, while the share of the effect of variety and site amounted to 2.25% or 1.17%, respectively. Partial statistical evaluation on the locality České Budějovice with both reference varieties of common wheat was similar and the year was the most important factor affecting the content of a fraction.

Protein spectra of albumins and globulins (Fig. 5) among spelt wheat varieties or reference variety of common wheat, respectively, was very close and corresponded by its character to the finding of American authors in the spectrum of water-soluble proteins from two varieties of common wheat in non-reducing conditions (Kim, Bushuk, 1998). However, the finding in quality aspect of highly similar spectra of gliadins and glutenins inside the studied varieties and mutual quantitative ratio of all three fractions by densitometric measurement (Table V) is interesting. In congruency with Canadian authors (Abdel-Aal, 1996) high-molecular spectra of bands that were marked by Canadian authors as HMW-glutenin spectra, were found in gliadin fractions in the zone 80–115 kDa. Another significant zones in gliadin fraction of molecular weight 54–24 kDa and 16–3 kDa will be probably composed of LMW (light molecular weight) – fraction of glutenins and gliadins.

The greatest percentage of fraction of albumins and glutenins was found from mutual quantitative evaluation of fractions on gel in all three studied varieties and ranged between 42 and 52%. Glutenin fractions with percentage 13–25% showed the lowest values. Reasons for this disproportion, compared with quantitative measurements after the Kjeldahl method, should be searched for in the chemical composition itself. While Kjeldahl's method evaluates the content of total mineralizable nitrogen that is then in the case of wheat converted by coefficient 5.7 into the content of crude protein, densitometric evaluation is based on visualisation only of protein molecules by specific coloration of Coomassie Blue R 250. Another reason is a probably



5. Electrophoreogram of three spelt and common wheat protein fractions

alb – albumins and globulins
gli – gliadins
glu – glutenins
1. Altgold (*T. spelta* L.)
2. Rouquin (*T. spelta* L.)
3. Brea (*T. aestivum* L.)

different affinity of this colour to different protein fractions of spelt and common wheat fractions.

The fact of overlapping of LMW glutenins and gliadins or the possibility of the transition of HMW, respectively – and partially also LMW – fraction of glutenins into gliadins in the medium SDS-PAGE was published abundantly (Pomeranz et al., 1988; Abdel-Aal et al., 1996; Ovesná et al., 2000) and there is some historical development in this aspect. Miflin and Shewry (1979) propose the name gliadin for proteins of wheat grain endosperm, Field et al. (1982) classify proteins of endosperm in non-aggregating and aggregating gliadin (present glutenin). Other studies based on combination of more technologies (HPLC and SDS-PAGE electrophoresis), eventually two-dimensional electrophoresis, genetic studies with monosomic lines and studies of amino acids sequences of split fractions allowed to separate different protein components and to locate on genome (Pomeranz et al., 1988).

Relatively constant sum of extractive fractions in both reference varieties of common wheat was found in complex evaluation of protein fractions together with detection of higher average content of sum of extractive fractions in 2000 compared with the year 1999 in all evaluated spelt varieties caused above all by great increase particularly of glutenin fraction on both

IV. Tab. Average content of protein fractions, crude protein and TGW* in tested spelt and common wheat varieties

Variety	Locality	Year	Albumins + globulins (%)	Gliadins (%)	Glutenins (%)	Sum (%)	Crude protein (%)	Difference (%)	TGW (g)
Altgold	ČB	2000	3.77 ± 0.14	6.77 ± 0.33	3.74 ± 0.00	14.28 ± 1.57	15.18 ± 0.11	0.90	45.6
Altgold	ČB	1999	2.77 ± 0.04	6.81 ± 0.08	2.42 ± 0.05	11.99 ± 2.18	18.53 ± 0.13	6.54	48.0
Altgold	HUM	2000	3.97 ± 0.33	8.18 ± 0.33	4.21 ± 0.66	16.35 ± 2.14	17.02 ± 0.12	0.67	46.2
Altgold	HUM	1999	2.53 ± 0.03	6.54 ± 0.10	2.53 ± 0.41	11.60 ± 2.08	15.95 ± 0.11	4.35	53.0
Lueg	ČB	2000	2.34 ± 0.66	7.94 ± 0.00	3.27 ± 0.00	13.55 ± 2.70	14.24 ± 0.10	0.70	47.0
Lueg	ČB	1999	3.37 ± 0.01	7.37 ± 0.33	2.26 ± 0.12	12.99 ± 2.41	17.57 ± 0.12	4.58	40.6
Lueg	HUM	2000	4.20 ± 0.00	7.88 ± 0.66	3.27 ± 0.00	15.34 ± 2.20	15.91 ± 0.11	0.57	50.0
Lueg	HUM	1999	2.45 ± 0.00	6.01 ± 0.13	2.09 ± 0.01	10.55 ± 1.94	15.48 ± 0.11	4.93	43.6
Oberkulmer S.	ČB	2000	3.04 ± 0.33	6.77 ± 0.33	4.91 ± 0.33	14.71 ± 1.69	15.33 ± 0.11	0.62	47.6
Oberkulmer S.	ČB	1999	2.82 ± 0.00	6.70 ± 0.06	2.44 ± 0.01	11.96 ± 2.11	17.82 ± 0.13	5.86	48.4
Oberkulmer S.	HUM	2000	3.97 ± 0.33	7.94 ± 0.00	3.27 ± 0.00	15.18 ± 2.26	16.90 ± 0.12	1.72	51.0
Oberkulmer S.	HUM	1999	2.53 ± 0.04	6.66 ± 0.76	2.33 ± 0.01	11.52 ± 2.22	15.14 ± 0.11	3.62	57.0
Ostro	ČB	2000	3.27 ± 0.00	6.30 ± 0.33	4.54 ± 0.66	14.11 ± 1.40	14.74 ± 0.10	0.63	53.6
Ostro	ČB	1999	2.83 ± 0.01	6.91 ± 0.15	2.34 ± 0.08	12.07 ± 2.25	16.71 ± 0.12	4.64	42.6
Ostro	HUM	2000	3.74 ± 0.00	8.27 ± 0.00	5.14 ± 0.00	17.15 ± 2.07	17.76 ± 0.13	0.61	46.0
Ostro	HUM	1999	2.52 ± 0.01	6.36 ± 0.09	2.01 ± 0.01	10.88 ± 2.13	18.07 ± 0.13	7.19	56.0
Rouquin	ČB	2000	3.73 ± 0.66	6.30 ± 0.33	4.14 ± 0.00	14.18 ± 1.28	14.98 ± 0.11	0.80	50.0
Rouquin	ČB	1999	3.31 ± 0.03	6.76 ± 0.02	2.21 ± 0.42	12.27 ± 2.13	17.30 ± 0.12	5.03	45.0
Rouquin	HUM	2000	3.04 ± 0.33	6.07 ± 0.00	3.27 ± 0.00	12.38 ± 1.52	15.55 ± 0.11	3.18	54.0
Rouquin	HUM	1999	2.47 ± 0.03	5.74 ± 0.01	2.22 ± 0.08	10.43 ± 1.76	17.26 ± 0.12	6.83	56.0
Samara *T. aestivum*	ČB	2000	2.10 ± 0.33	4.67 ± 0.33	3.04 ± 0.33	9.81 ± 1.34	11.90 ± 0.08	2.09	48.2
Samara *T. aestivum*	ČB	1999	2.84 ± 0.44	7.11 ± 0.44	2.18 ± 0.44	12.13 ± 2.40	13.04 ± 0.09	0.91	45.0
Brea *T. aestivum*	ČB	2000	2.34 ± 0.66	4.67 ± 0.66	1.87 ± 0.66	8.88 ± 1.37	11.87 ± 0.08	2.99	45.2
Brea *T. aestivum*	ČB	1999	2.74 ± 0.48	5.89 ± 0.48	1.67 ± 0.48	10.29 ± 1.98	10.54 ± 0.07	0.25	44.0
Average *T. spelta*	ČB	2000	3.23 ± 0.65	6.82 ± 0.67	4.12 ± 0.65	14.17 ± 1.68	14.89 ± 0.41	0.72	48.8
Average *T. spelta*	ČB	1999	3.02 ± 0.28	6.91 ± 0.28	2.33 ± 0.18	12.26 ± 2.06	17.59 ± 0.64	5.33	44.9
Average *T. spelta*	HUM	2000	3.78 ± 0.46	7.67 ± 0.89	3.83 ± 0.81	15.28 ± 1.99	16.63 ± 0.85	1.35	49.4
Average *T. spelta*	HUM	1999	2.49 ± 0.04	6.26 ± 0.44	2.23 ± 0.24	10.98 ± 1.89	16.38 ± 1.17	5.40	53.1

TGW* – thousand grain weight

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V. Mutual per cent ratio of protein fractions obtained by densitometrical evaluation on electrophoreogram (Fig. 5)

Variety / Fraction	Albumins and globulins (%)	Gliadins (%)	Glutenins (%)
Altgold (*T. spelta*)	46.29	28.83	24.88
Rouquin (*T. spelta*)	41.79	34.06	24.15
Brea (*T. aestivum*)	51.65	35.16	13.19

VI. Evaluation of albumin and globulin fractions in spelt and common wheat on the locality České Budějovice in the years 1999 and 2000

	Df	MS	df	MS			
	effect	effect	error	error	*F*	*P*-level	influence of factor (%)
1	6	0.57	14	0.14	4.05	0.01	45.97
2	1	0.00	14	0.14	0.01	0.93	
12	6	0.53	14	0.14	3.81	0.02	42.74

1 – variety, 2 – year

VII. Evaluation of gliadin fractions in spelt and common wheat on the locality České Budějovice in the years 1999 and 2000

	Df	MS	df	MS			
	effect	effect	error	error	*F*	*P*-level	influence of factor (%)
1	6	2.23	14	0.04	52.19	0.00	39.33
2	1	2.41	14	0.04	56.22	0.00	42.50
12	6	0.99	14	0.04	23.03	0.00	17.46

1 – variety, 2 – year

localities. Great importance of the year was confirmed on representation of different fractions (Prugar, Hraška, 1986) and higher plasticity of spelt wheat to these conditions. Positive effect on higher percentage of protein fractions in 2000 should have higher average temperature by 2 °C in the period from April to June as well as good moisture reserve caused by higher precipitation in March compared with the previous year. More compact and balanced stand in 2000 and more favourable weather in August of 2000 (Tables I and II) have a positive impact on better ripening of the stand and

VIII. Evaluation of glutenins fractions in spelt and common wheat on the locality Č. Budějovice in the years 1999 and 2000

	Df	MS	df	MS			
	effect	effect	error	error	*F*	*P*-level	influence of factor (%)
1	6	1.91	14	0.07	29.24	0.00	13.01
2	1	11.74	14	0.07	179.76	0.00	79.97
12	6	0.96	14	0.07	14.69	0.00	6.54

1 – variety, 2 – year

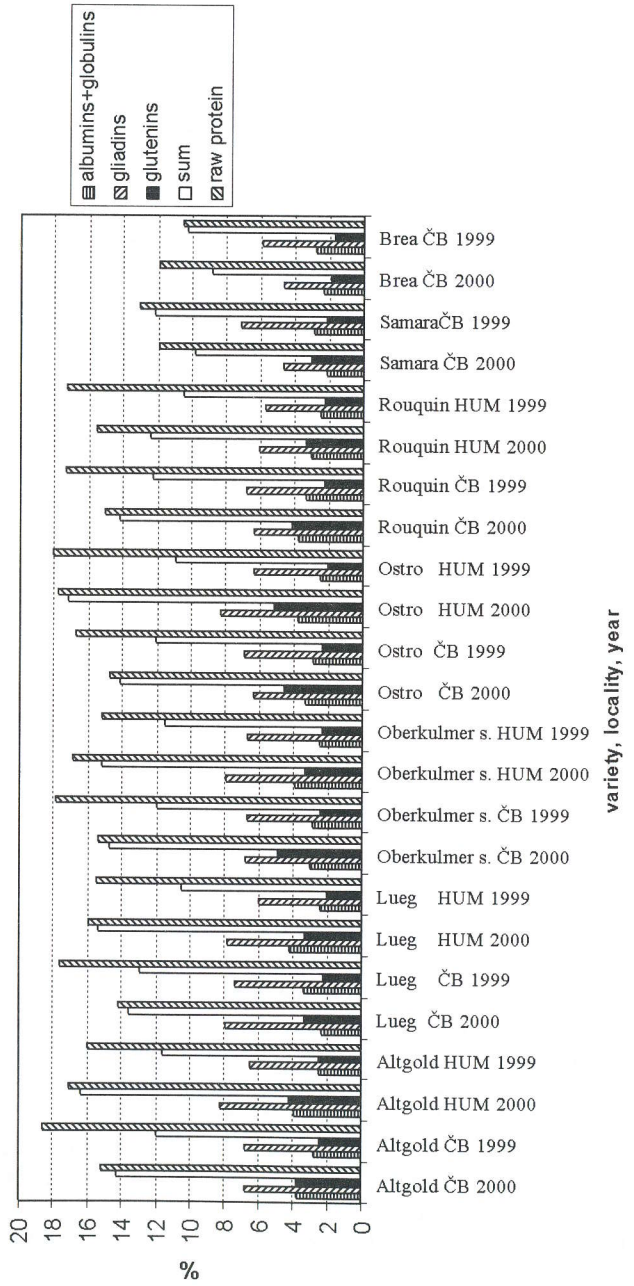
IX. Evaluation of factors influence in tested protein fractions in spelt wheat (on 2 localities in 2 years – see Figs. 1, 2, 3, 4)

Factor / Fraction	Albumins and globulins	Gliadins	Glutenins	Crude protein
	influence of factor (%)	influence of factor (%)	influence of factor (%)	influence of factor (%)
1		10.28	2.25	2.92
2			1.17	1.62
3	57.03	32.98	90.92	34.58
12	4.68	7.67	1.40	5.79
13	2.34	3.30	2.19	1.64
23	29.33	42.64		50.05
123	5.50		1.56	3.38

1 – variety, 2 – locality, 3 – year

probably had the greatest effect on higher percentage of extractable protein fractions compared with the year 1999. The content of crude protein was on the locality Humpolec similar in both years. On the contrary, on the locality České Budějovice was considerably lower. The content of crude protein corresponded by its range to the finding of Italian authors Cubbada and Marconi (1995). In this connection in further similar studies it will be necessary to investigate also seasonal fluctuations of nitrogen in soil.

High percentage of insoluble protein fraction in the year 1999 should be explained probably by lower degree of ripeness of spelt wheat during harvest and on the locality in České Budějovice also by lower stand density what led to formation of greater number of fertile tillers with uneven degree of ripeness. Unfinished process of ripeness thus should cause a great share of struc-



6. Content of protein fractions in spelt and common wheat

tural proteins with lower representation of storage proteins (Pelikán, Liška, 1978). Nevertheless, it is necessary to mention that according to Prugar and Hraška (1986) biosynthesis of storage proteins takes place by their proper way independent on protoplasmic proteins, which are not their predecessors.

CONCLUSION

The results obtained confirmed a considerable effect of medium together with genetic conditional character regarding different protein fractions. The ability of spelt wheat to cumulate significantly crude proteins and their fractions has been confirmed, even under conditions of low inputs. Gliadin fraction was proved in spelt wheat that will evidently limit its utilisation for gluten-free diet. The fact of 1999 is interesting when spelt wheat cumulated unusually high percentage of crude protein outside extractable protein fraction. The question is how great influence should be given to soil and climatic complex on one side, and to lower degree of spelt ripeness and difference caused by the possibility of mutual intermingling and transition of protein fractions on the other side.

In view of quantitative comparison of extracted fractions found by the classic method after Kjeldahl and preliminary densitometric measurement on gel, mutual irreplaceableness of both methods follows. Nevertheless, densitometric and spectrophotometric evaluation, respectively, should be a suitable complement to evaluate both nutritional and technological quality in view of evaluation of only protein molecules only.

The principle of Osborn's classification of protein fraction, based on firm boundaries of protein solubility in the given solvent, should be specified in the future very exactly by the used extraction agents and reaction conditions and view of nutritional and technological qualities we cannot eliminate direct protein analyses.

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V letech 1999 až 2000 bylo na dvou lokalitách v Českých Budějovicích (ČB) a Humpolci (HUM) pěstováno v podmínkách low input 5 odrůd pšenice špaldy (*T. spelta L.*) – Altgold (CH), Lueg (B), Rouquin (B), Ostro (SRN) a Oberkulmer Schwarzer (SRN). Jako referenční odrůdy byly zvoleny dvě české odrůdy pšenice seté – Brea a Samara – pěstované na lokalitě v Českých Budějovicích. Na vyloupaném zrnu byla zjišťována HTZ, obsah celkového dusíku a obsahy tří bílkovinných frakcí (1. albuminy a globuliny, 2. gliadiny, 3. gluteniny) získané metodou podle Osborna, měřené standardní metodou podle Kjeldahla. U třech vybraných odrůd Altgold, Rouquin a pšenice seté Brea byl vyhotoven elektroforeogram frakcí bílkovin v podmínkách PAGE podle mezinárodní metody ISTA-SDS a dále bylo uskutečněno denzitometrické hodnocení. Výsledky potvrdily genetickou podmíněnost i značný vliv prostředí na podíl bílkovinných frakcí. Byla potvrzena schopnost špaldy významně kumulovat dusíkaté látky, a to i za podmínek nízkých vstupů. V pšenici špaldě byla prokázána gliadinová frakce, která bude zřejmě limitovat její využití pro bezlepkovou dietu. Z kvantitativního porovnání extrahovaných frakcí zjištěných klasickou metodou podle Kjeldahla a orientačního denzitometrického měření na gelu vyplývá vzájemná nezastupitelnost obou metod, nicméně denzitometrická, resp. spektrofotometrická hodnocení mohou být vhodným doplňkem posuzování nutriční i technologické kvality.

pšenice špalda; pšenice setá; bílkovinné frakce; elektroforéza

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