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Prediction of Protein Fractions Distribution in Wheat (*Triticum aestivum* L.) Mill Products

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The research was conducted to describe distribution of protein fractions in wheat (*Triticum aestivum* L.) mill products (semolina, flour and bran) and evaluate the possibility of prediction of protein fractions distribution from values of bread-making quality (protein and gluten content, Zeleny sedimentation volume) evaluated on wholemeal and specific flour. The content of protein fractions was determined by size-exclusion high performance liquid chromatography. Significantly highest glutenin content was found in flour (5.01%). The investigated mill products did not differ in gliadin content, the content of albumin/globulin fraction reached the highest values in bran (3.60%). The model of prediction of glutenin and gliadin content in mill products explained 31–62% and 83–92% of the original variability. The protein fractions distribution in wheat mill products could be satisfactorily predicted from known values of protein and gluten content evaluated on wholemeal and Zeleny sedimentation volume evaluated on specific flour.

Keywords: *Triticum aestivum* L., protein fractions, flour, semolina, bran

Introduction

Wheat milling is a process that consists of controlled grain breaking, purification and reduction. The breaking stage is performed by a series of pairs of corrugated rolls. Passing through the rolls the shear forces causes the endosperm fractures along cell walls in the presence of a higher proportion of small spherical starch granules into various size nodules of endosperm (Edwards et al. 2008). The forces are also re-directed towards the skins, causing their fragmentation into relatively small pieces. Some of the skins become powdered up in the flour and semolina. The aim of purification is to remove much of the loose skinny material. During reduction stage the size of nodules of starchy endosperm is reduced. The milling process traditionally leads to the separation of flour and semolina from the bran and germ. Flour is the primary product obtained of wheat milling while semolina is too coarse to be considered as flour. Bran is the co-product which consists of separated

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skins plus adhered endosperm (Ranken et al. 1997). The mill products differ in bread-making quality because of various quantity and quality of wheat proteins (Goesaert et al. 2005), and unique properties of wheat starch (Park et al. 2009). The wheat proteins can be divided into four major groups based on their solubility. Albumins are soluble in water, globulins are insoluble in pure water but soluble in dilute NaCl solutions, and insoluble in high NaCl concentrations, gliadins are soluble in 70–90% ethanol, and glutenins are soluble in dilute acid or alkali (Inglett 1974; Wieser 2007). Glutenins are considered to be the most important components of gluten with respect to baking quality. Glutenins are polymeric proteins that can be classified according to their molecular weights as high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (Belderok et al. 2000; Gianibelli et al. 2001). The HMW-GS are minor components in terms of quantity, but they are key factors in the process of bread-making (Dendy and Dobraszczyk 2001; Gianibelli et al. 2001). The HMW-GS form intrachain and interchain disulfide bonds in native gluten and contribute to the dough strength (Hamer and Hoseney 1998; Shewry 2009). The LMW-GS are able to form large aggregates that are related to the dough strength. Gliadins are smaller monomeric proteins (Dendy and Dobraszczyk 2001). Gliadins are considered to contribute to the viscosity and extensibility of gluten (Belderok et al. 2000). Glutenins and gliadins are found in the endosperm of the mature wheat. The albumin and globulin fractions are believed to be of no or only minor importance with respect to the baking quality and mainly occur in the outer layers of the wheat kernel with lower concentration in the endosperm (Goesaert et al. 2005).

Even if the contribution of wheat protein fractions to bread-making quality of mill products is known, the commonly used testing methods (protein content, Zeleny sedimentation volume, etc.) are not able to measure their content.

The aim of our work was to (i) verify compliance with the prerequisites for creating the prediction model of the protein fractions distribution, i.e. confirm significant differences in the distribution of protein fractions in mill products (semolina, flour, bran); evaluate the correlations between content of protein fractions in mill products and parameters of bread-making quality (ii) build the prediction model for the protein fractions distribution in semolina, flour and bran based on bread-making parameters evaluated on wheat wholemeal. The paper follows the previous research of the relationships between the content of protein fractions and the parameters of wheat bread-making quality (Burešová et al. 2012).

Material and Methods

Material

The research was performed on 24 varieties of winter wheat (*Triticum aestivum* L.) of different bread-making quality. The investigated varieties were cultivated in growing period 2008–2009 in locality Žabčice, Czech Republic. The harvested grain was subjected to analyses. Each grain sample was divided into three parts. Each of the parts was milled separately.

Methods

Grain milling

Three parts of each grain sample were tempered to 15% moisture content. One part was milled on laboratory mill Chopin CD 1; semolina, flour and bran fractions were collected and used for protein extraction and analyses. Other part was ground into wholemeal on a laboratory mill Falling number LM 3100. Wholemeal was used to evaluate parameters of bread-making quality. Last part was milled on a Brabender Sedimat Mill; the specific flour for Zeleny sedimentation volume was obtained.

Bread-making quality

The bread-making quality was evaluated by the parameters that are known to be affected by protein quality and quantity. The parameters were determined according to standard methods. The content of protein and gluten were evaluated in wholemeal; the special flour was prepared for evaluation of Zeleny sedimentation volume. The protein content in dry matter was assessed by the Dumas combustion method according to ICC Standard no. 167 (ICC 2000). The protein content was calculated as nitrogen content multiplied by 5.7. The gluten content in dry matter was assessed according to ICC standard no. 155 (ICC 1994). The dough was prepared from the wholemeal by adding a buffered sodium chloride solution. The wet gluten was isolated by washing this dough with sodium chloride solution. The sedimentation volume according to Zeleny was measured in agreement with ISO 5529 (ISO 2007).

Each laboratory test was performed on two test portions simultaneously or rapidly one after the other. The arithmetic mean of the two determinations was taken as the result if the conditions of repeatability set by the standards were satisfied. If the absolute difference between two independent single test results was outside standard limits, the two determinations were reduplicated.

Size-exclusion high performance liquid chromatography (SE-HPLC)

The proteins were extracted and analysed using the procedure of Dachkevitch and Autran (1989). The SE-HPLC apparatus was the Agilent 1100 Series; the BioSep™ SEC-S4000 (Phenomenex, California, USA) size-exclusion analytic column (300 × 7.8 mm) used with a guard column (7.5 × 75 mm). Absolute areas of the peaks (Area), relative areas of the peaks (Area%) and the peak heights (Height) were recorded. The content of glutenin N_{Glu} [%], gliadin N_{Gli} [%] and albumin/globulin $N_{Alb+Glo}$ [%] fractions were calculated from relative area of peak and total protein content in dry matter to reflect the absolute content of protein fraction in the mill products.

Statistical analyses

The differences in protein fractions content in the mill products were tested by analysis of variation (ANOVA). Pearson correlation coefficients were used to test the relationships

between parameters of bread-making quality and content of protein fractions in the mill products. The stepwise forward multiple regression method was used to build the prediction model. All statistical analyses were performed using Statistica 9 software (StatSoft, Inc.).

Results

The significant differences in distribution of protein fractions in mill products were confirmed. The content of glutenin fraction was higher in flour (5.01%) than in semolina (4.66%) but the increase was not significant (Table 1). Significantly lowest content of glutenin fraction was found in bran (3.77%). The content of gliadin fraction was decreasing in the following order flour-semolina-bran. The content of albumin/globulin fraction was significantly different in the investigated mill products. Bran, flour and semolina, resp., contained 3.60%, 2.03% and 2.47%, resp., of albumin/globulin fraction.

The values of correlation coefficients showed that content of glutenin and gliadin fractions in all of investigated mill products was significantly positively correlated with the protein content, Zeleny sedimentation volume and gluten content (Table 2).

Table 1. Means of protein fractions content in semolina, flour and bran [%][a]

	N_{Glu}	N_{Gli}	$N_{Alb+Glo}$
Semolina	4.66a	9.51a	2.47b
Flour	5.01a	9.63a	2.03c
Bran	3.77b	9.02b	3.60a

[a] The values presented are means of duplicate measurements. Different letters in the same column indicate a significant difference between means at the 5% level according to Fisher LSD test.

Table 2. Correlation coefficients between protein fractions content and bread-making parameters

	FN	PC	SEDI	GL	GI
Semolina					
N_{Glu}	–0.02	0.74*	0.56*	0.75*	–0.24
N_{Gli}	–0.04	0.95*	0.70*	0.74*	–0.24
$N_{Alb+Glo}$	–0.04	0.03	–0.06	0.03	0.12
Flour					
N_{Glu}	–0.17	0.72*	0.64*	0.71*	–0.18
N_{Gli}	–0.19	0.95*	0.69*	0.76*	–0.24
$N_{Alb+Glo}$	–0.30*	0.16	–0.19	0.13	–0.08
Bran					
N_{Glu}	–0.10	0.53*	0.34*	0.44*	–0.25
N_{Gli}	–0.28	0.90*	0.64*	0.75*	–0.19
$N_{Alb+Glo}$	–0.05	0.34*	0.35*	0.32*	0.16

* $p < 0.05$

FN – Hagberg falling number, PC – total protein content in dry matter, SEDI – sedimentation volume according to Zeleny, GL – gluten content in dry matter, GI – gluten index

N_{Glu} – content of glutenin fraction, N_{Gli} – content of gliadin fraction, $N_{Alb+Glo}$ – content of albumin/globulin fractions

Strong correlation between quality parameters and content of protein fractions confirmed the possibility of building the model for prediction of protein fractions distribution in mill products based on known values of quality parameters evaluated for grain and wholemeal. The degree of conformity between measured and calculated values show the coefficients of determination, i.e. the coefficients of determination were used as indicators of how well the model fit the data. The values of coefficient of determination were highest for content of gliadin in the mill products. The values (0.91, 0.92 and 0.83) indicated that the model explained 83–92% of the original variability and the conformity between predicted and observed values of gliadin content in semolina, flour and bran was very good (Table 3).

Table 3. Coefficients of determination (R^2) between observed and predicted values of protein fractions content in semolina, flour and bran

	N_{Glu}	N_{Gli}	$N_{Alb+Glo}$
Semolina	0.62*	0.91*	0.17
Flour	0.60*	0.92*	0.42*
Bran	0.31	0.83*	0.18

* $p < 0.05$

The model of gliadin distribution in semolina, flour and bran was based on known protein content; gluten content could be supplemental parameter (Table 4). For example the absolute contents of glutenin and gliadin fractions in flour can be calculated from parameters evaluated for wholemeal:

$$N_{fGlu} = 0.03 \cdot GL + 0.02 \cdot SEDI$$

where N_{fGlu} content of glutenin fraction in flour
GL gluten content in wholemeal
SEDI Zeleny sedimentation volume

$$N_{fGli} = -3.42 + 0.82 \cdot PC - 0.03 \cdot GL$$

where N_{fGli} content of gliadin fraction in flour
PC protein content in wholemeal
GL gluten content in wholemeal

The model of prediction glutenin content in semolina, flour and bran explained 31–62% of the original variability (Table 3) and the glutenin content in mill products could be satisfactorily predicted from known values of protein and gluten content and Zeleny sedimentation volume (Table 4).

Table 4. Values of intercepts and the regression coefficients (B coefficients) in prediction model

		N_{Glu}	N_{Gli}	$N_{Alb+Glo}$
Semolina	Intercept		–2.64	3.05
	NL	0.16	0.82	
	GL	0.03		
Flour	Intercept		–3.42	3.63
	NL		0.82	
	GL	0.03	–0.03	
	SEDI	0.02		
Bran	Intercept		–3.58	
	NL	0.27	0.73	

$p < 0.05$; non-significant values suppressed

Discussion

The significant differences in distribution of protein fractions in mill products and correlation between content of protein fractions and parameters of bread-making quality (Table 1) reached expected values. Semolina, flour and bran were expected to differ in content of protein fractions because they were separated from various anatomical parts of a wheat kernel with different chemical composition (Belitz et al. 2009). Simultaneously differences in chemical composition affected the adhesion forces between protein matrix and starch granules that are known to influence the way of endosperm breaks during milling (Greffeuille et al. 2006; Edwards et al. 2008) and the size of mill products nodules. The best baking properties were found for flour rich in gluten proteins while semolina had lower content of gluten proteins and lower bread-making quality. The content of gluten protein was lowest in bran collected from skins plus adhering endosperm, contrary some of the skins become powdered up in the flour and semolina and increased the content of albumin/globulin fraction in these mill products. The perfect separation of endosperm and skins is not possible because of variability in cell content; only whole cells are readily removed from skin (Edwards et al. 2008).

The detailed research has been done to describe the correlation between parameters of bread-making quality and content of protein fractions. The significant correlation between protein content, gluten content, Zeleny sedimentation volume and content of glutenin and gliadin in semolina and flour (Table 2) were in agreement with the conclusions published by many authors (Ciaffi et al. 1996; Dendy and Dobraszczyk 2001; Labuschagne et al. 2004). The content of albumin/globulin fraction was significantly correlated with a few bread-making parameters but the correlation was weak that was expected. These results confirmed the possibility of building the model for prediction of protein fraction distribution in mill products based on known values of quality parameters evaluated for grain and wholemeal. The model is based on known values of protein and gluten content in wholemeal (Table 4). The incorporation of Zeleny sedimentation volume into the model of glutenin content prediction in flour improved the conformity between measured and calculated values because values of Zeleny sedimentation volume are in close correlation

with the content of high molecular weight glutenin subunits (Dendy and Dobraszczyk 2001) that reached the highest values in flour (Table 1). The content of albumin/globulin in the mill products could not be satisfactorily predicted from known values of bread-making quality (Table 3).

The wide research has been performed to explain the effect of protein quantity and quality on bread-making quality of wheat grain. It is well known that proteins and other grain components are irregularly distributed in grain and as the various mill products are collected from different parts of grain, they differ in physical and chemical composition. Therefore the values of standard quality parameters evaluated on wholemeal or specific flour are not exactly valid for all mill products collected during milling process. Our results confirmed that the values of protein and gluten content evaluated for wholemeal, Zeleny sedimentation volume evaluated for specific flour, could be used for prediction of protein fractions distribution in the mill products. The conformity between measured and predicted values was the best for gliadin content in semolina and flour. The model of gliadin distribution in semolina and flour was based on known protein content; gluten content could be supplemental parameter. The gluten content in the mill products could be satisfactorily predicted from known values of protein and gluten content and Zeleny sedimentation volume evaluated for wholemeal and flour for testing Zeleny sedimentation volume. The content of gliadin in bran, albumin/globulin in the mill products could not be satisfactorily predicted from known values of quality parameters evaluated for the wholemeal and flour for testing Zeleny sedimentation volume.

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