Environnemental and genetical effects on protein composition measured by SE-HPLC and mixograph characteristics of the winter wheat grown in France and Hungary

S. Szilagyi, Eugène Triboi, Ri Z. Gy, Anne-Marie A.-M. Triboi, Gerard G. Branlard, M. Borbely

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Szilágyi, Sz.[1] - Triboi, E.[2] - Gy• ri, Z.[1] - Triboi, A. M.[2] - Branlard, G.[2] - Borbely, M.[1]

[1] University of Debrecen, Centre of Agricultural Sciences, Faculty of Agronomy, Department of Food Processing and Quality Testing, H-4032 Debrecen, Böszörményi str. 138., Hungary. Tel/fax: +36-52-417-572. szszilagyi@helios.date.hu; gyori@helios.date.hu; mborbely@helios.date.hu

[2] Institut National de la Recherche Agronomique Station d'Agronomie. 234, Avenue du Brézet, Site de Crouel, F-63039 Clermont-Ferrand cedex 02, Tel: +33-473624424, Fax: +33-473624457. France. Eugene.Triboi@clermont.inra.fr

SUMMARY

The protein content and composition are two major factors, which determine the quality of wheat flour. Recent research results show that the temperature and drought influence the accumulation of starch and the nitrogen fertilization influences the quantity of proteins in grain. That means that the variation of protein content could be influenced by the variation of the starch or protein accumulation.

We have analyzed 70 samples of different winter wheat varieties grown in various conditions (temperature, drought, and mineral fertilization). We have studied the quantitative and qualitative variations of wheat flour proteins in function of different conditions, and after that we correlated this parameters with the wheat flour technological properties measured by mixograph.

The protein content is determined by genotype and increases with the stress (high temperatures, drought) and with nitrogen supply, modifying starch and protein accumulation respectively. Furthermore, an increase in protein content preferentially is caused by increased storage protein content. The study of polymeric protein shows qualitative changes in extractible fraction.

The protein content and the PEb extractible fraction proportion are the most involved in the variations of the mixographic parameters.

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INTRODUCTION

The protein composition and technological properties of 70 different wheat samples were investigated. The wheat samples were cultivated in Hungary (29) and France (41) under different environmental conditions.

We have determined the moisture content, the thousand-kernel weight, protein content (N×5.7), 11 mixographic parameters, and protein composition. We studied the correlation between protein composition and mixographic parameters, and in the same time we tried to explain the variation of mixographic parameters in function of protein content using multiple regression step by step.

The distribution of monomeric and polymeric proteins as well as their solubility play important role in bread making quality of flour (Ciaffi *et al.*, 1996; Dachkevitch and Autran, 1989; Gupta *et al.*, 1993; Gupta *et al.*, 1996; Popineau *et al.*, 1994).

The soluble proteins extracted by phosphate or borate buffer containing SDS include monomeric proteins and smaller aggregates are mainly gliadins and salt-water soluble proteins (Dachkevitch and Autran, 1989; Jia *et al.*, 1996*a* and 1996*b*; Popineau *et al.*, 1994).

The ratio between soluble and insoluble proteins, which affects breadmaking quality, is a function of the protein composition that is genetically controlled. Environmental factors (temperature, water and nitrogen nutrition) also influence this ratio (Jia *et al.*, 1996*a* and 1996*b*; Stone and Nicolas, 1996)

MATERIAL AND METHODS

The plant samples

We have analyzed 70 winter wheat samples (29 Hungarian and 41 French), which were different varieties (GK Öthalom, Mv Magdaléna, Mv Matador, Fatima, GK Magvas, Mv 21, Thésée, Recital, Renan, Arche), and which were grown in different agro-ecological conditions (temperature stress, drought stress, different N-fertilizer doses).

The French wheat samples were grown in climate tunnels (Triboi *et al.*, 1996) in 2 m^2 containers, in soil under controlled water and nitrogen supply. For control and monitoring of temperature and gas exchange (CO_2 concentration), the containers were placed in closed transparent tunnels under natural light at 8 days after anthesis to study the interaction between temperature and water supply in grain

filling. The temperature inside the tunnels followed the track of outside temperature (O). Three thermal treatments were applied: 0-5ºC but higher than 5ºC, O and 0+5ºC but less than 35ºC. All treatments were irrigated with 30 mm water at anthesis. After anthesis each thermal treatment was irrigated (I) or water stressed (S). The irrigation (I) was near equal to the evapotranspiration measured in tunnels: 165 mm in O+5ºC I and OI treatments, and 212 mm in O+5ºC I. The drought conditions (S) the water supply was 13 mm at low temperature (O-5ºC) and 37 mm at high temperature (O+5ºC). The treatment O (= outside temperature) there were two levels of water deficit: no irrigation and 60 mm during the first half of the grain filling period. These treatments were applied 8 days after anthesis, so the canopy structure and especially the number of grains$\times m^{-2}$ was not modified. For biochemical analysis we chose only three treatments: O-5ºC/I, O+5ºC/I and 0-5ºC/S. With these treatments we compared the effect on the accumulation and distribution of proteins and the evaluation of breadbaking quality.

In Hungary there are a long-term field experiments in different regions of the country. We chose three experiment fields (Iregszemcse and Kompolt), and also we have analyzed the protein composition and technological properties of five wheat varieties cultivated at Látókép Experimental Station of the Center of Agricultural Sciences of Debrecen University. The applied NPK mineral fertilizer doses were different.

Flour we make by a Brabender Quadrumat Junior laboratory mill. The flour yield was 70 % approximately in each case. We have studied the same flour sample parameters (nitrogen content, extraction of different protein fractions, SE-HPLC, mixograph test.

The *nitrogen content* of dried wheat flour samples was measured by Kjeldahl method using a TECATOR Kjeltec Auto 1030 Analyser.

The mixograph test

Flours were tested by a 10 g-mixograph (National MFG Co., Lincoln Nebr.). The flour quantity was rectified to 14 % moisture content. The flour quantity necessary to measurement and the water volume added to flour were calculated with Martinant *et al.* (1998) formula.

$$Q = 8.66 \cdot \left(1 - \frac{FM}{100}\right)$$

Q = the quantity of flour necessary for the measurement
FM = the moisture content of flour determined by NIR.

$$V = 8.6 \cdot \left\{ W - \frac{\frac{FM}{100}}{1 - \frac{FM}{100}} \right\}$$

V = the water volume added to flour,
W = the water volume added to dried flour

$$W = 0{,}764 + 0{,}0069 \cdot P + 0{,}001025 \cdot H$$

P = protein content (Kjeldahl).
H = kernel hardness estimated on wholemeal by NIR Inframatic 8086.

We have determined 11 mixographic parameters according to Martinant *et al.* (1998). The evaluation of mixograms was performed in the INRA, using a Mixsmart® softvare. The parameters measured by mixograph were: MLV = Midline Left of Peak Value (%); MLW = Midline Left of Peak Width (%); MPTi = Midline Peak Time (min); MPV = Midline Peak Value (%); MPW = Midline Peak Width (%); MRV = Midline Right of Peak Value (%); MRW = Midline Right of Peak Width (%); MTxV = Midline Time X = 8 min Value (%); MTxW = Midline Time X = 8 min Width (%); MTxI = Midline Time X = 8 min Integral (%); and we calculated WS = Weakening Slope (%).

The analysis of proteins by SE-HPLC

The solubilization of wheat proteins was carried out according to „APAC" method Daniel *et al.* (2000). As first step we have obtained the so-called *extractible fraction* (PE), which is composed by small molecular weight proteins as albumins (ALB), globulins (GLOB) and gliadins (GLI). The *non-extractible fraction* (NE), which contains high molecular weight proteins, was dissolved after sonification (22.5 kHz, 30 s, 10 W).

The analysis by SE-HPLC of extractible proteins (PE) and non-extractible proteins (NE)
For analysis a Kontron HPLC equipment (Kontron Instruments, Milan, Italy) model 422 pump, 465 autosampler, 425 gradient former, 480 oven controller, DEG-104 degasing system and a Shimadzu SPD-6A UV spectrophotometric detector were used. The system was directed by Kontron software. A TSK G4000 SW (7.8 × 300 mm) silicagel column was used. The chromatographic conditions were: isocratical elution; eluent: 0.1 M-os Na-phosphate buffer + 0.1 % SDS (pH = 6.9); flow: 0.7 ml/min; temperature: 28°C; detector type: UV;wavelenght: 220 nm; injected volume: 75 • l. Before injection all samples were filtered through a (Lida, WI) membrane filter with 0.45 • m pore diameter. Typical chromatograms of PE and NE proteins are showed on **Figure 1.**
In PE fraction 6 peaks and in the NE fraction 5 peaks were definied. In the extractible fraction's 1st peak are mainly the protein aggregates; the 4th and 5th peaks are formed by monomers. In the PE and NE fractions we have defined different molecular weight intervals, showed the PE_{1-6} and NE_{1-5} in the **Table 1.**

Table 1. Derived protein fractions

Protein fractions	Definition
PEa (fraction extractible)	The 1-6 PE peaks, and the 4 and 5 NE peaks amount
NEa (fraction non-extractible)	A NE 1-3 peaks amount
PEb (fraction extractible)	(PEa) – PE 1 peak
NEb (fraction non-extractible)	(NEa) + PE 1 peak

The sequential extraction of flour proteins
We have used the Osborne's (1907) procedure modified by Marion *et al.* (1994) (**Table 2.**)

Table 2. Steps of extraction of wheat prolamins according to Marion *et al.* (1994) method

1. 833 mg flour + 26 ml 0.05 M Na-phosphate pH = 7.8 + 0.1 M NaCl buffer
2. Stirring at 4ºC during 1h
3. Centrifugation 30 min at 4ºC, 18000 min^{-1}
4. Supernatant = **albumins and globulins**
5. Pellets we stirred at 4ºC, during 1h in 26 ml 0.05 M Na-phosphate pH = 7.8 + 0.1 M NaCl buffer + 2 % (V/V) Triton X114 (Fluka, France)
6. Centrifugation 30 min at 4ºC, 18000 min^{-1}
7. Supernatant = **amphyphilic (lipid binding) proteins**
8. Pellets were stirred at 20ºC, during 1h in 20 ml 70 % (V/V) ethanol
9. Centrifugation 30 min at 20ºC, 18000 min^{-1}
10. Supernatant = **gliadins**
11. Pellet contains the **glutenins**

Determination the nitrogen content of monomer proteins containing solutions
The nitrogen content of monomer proteins (AG, AMP, GLI) containing solutions were determined using a ND 10 Nitrogen Detector type NCOT-meter equipped with a chemiluminescent NO detector. In the pellet after centrifugation are the glutenins (GLN): their nitrogen content we determined using Kjeldahl method.

RESULTS AND DISCUSSION

The mixographic results

68 flour samples were analyzed using a mixograph. The **Table 3.** contains the average, the standard deviation, the minimal and maximal values and the CV. The CV value in the case of MTxI was 14.4, and in case of MLW 39.3. That means that using a mixograph, the differences between technological parameters of wheat flour could characterize the differences between the flours. We could observe that in case of WS the CV is very high 112.4, in spite of the fact that was calculated in only one case WS=0.

Table 3. The mixographic parameters of flour samples

Parameter	Average	Std. deviation	Min-max	CV
MLV	29.8	4.527	20.1-39.5	**15.2**
MPV	31.7	5.565	20.6-44.5	**17.6**
MRV	29.4	4.315	20.4-39.9	**14.7**
MTxV	27.8	4.023	19.8-36.6	**14.5**
MPTi	4.26	1.486	1.75-8.64	**34.8**
MLW	14.5	5.700	8.2-31.5	**39.3**
MPW	12.1	3.512	7.5-21.1	**29.0**
MRW	8.83	2.339	4.6-14.5	**26.5**
MTxW	7.58	2.592	3.1-14	**34.2**
MTxI	219	31.647	156-297	**14.4**
WS = MPV-MRV	2.67	3.004	0-21.4	**112.4**
Kernel hardness	62.7	12.806	27.6-93	**20.4**

We have calculated the correlation between 11 mixographic parameters (**Table 4.**), and we found a very strong correlation between MLV, MPV, MRV and MTxI. That means that this parameters are independent. On the other hand between MLW and MPW, MRW and MTxW the correlation is strong (r = 0.85) in case of each pairs. This results the decrease of the number of independent parameters.
The MPTi is the parameter that showed weak correlation with the other 10 parameters, which was proved earlier by Martinant *et al.* (1998).

Table 4. Correlation between mixographic parameters (n = 68)

MLV	0.61	**0.94**	0.70	**0.97**	0.46	**0.92**	0.11	-0.14	**0.96**	0.49
	MLW	0.82	***0.85***	0.68	0.15	0.48	-0.26	-0.68	0.75	0.78
		MPV	0.82	**0.95**	0.32	0.82	-0.09	-0.38	**0.97**	0.70
			MPW	0.75	0.36	0.63	-0.03	-0.37	0.75	0.65
				MRV	0.56	**0.95**	0.18	-0.19	**0.97**	0.43
					MRW	0.67	***0.85***	0.28	0.42	-0.30
						MTxV	0.40	0.09	0.87	0.20
							MTxW	0.58	0.02	-0.65
								MPTi	-0.34	-0.63
									MTxI	0.58
										WS

The effect of temperature and drought on the kernels mass
The average of one kernel mass of the wheat cultivated in France was 26 mg for Récital, 39 mg for Thésée and 45 mg for Renan and in the case of Hungarian wheat grains mass were 34 mg for Mv 21, 39 mg for Mv Magvas, 43 mg for GK Öthalom, 45 mg for Fatima, 45 mg for Mv Magdaléna and 40 mg for Mv Matador.
Under optimal growing conditions (irrigation, low temperatures), the one kernel's mass of Renan is 55 mg, of Thésée is 50 mg and of Récital is 40 mg. This order can be observed under stress conditions grown samples too. The decrease of one kernel mass is correlated to increase of protein content: the wheat flour protein content increases if during the growth were a temperature stress or a drought stress.
According to Triboi and Triboi-Blondel (2001) the drought and high temperatures influences the starch accumulation in the grain, and in the same time the protein accumulation remains constant. In this case the protein content increase and the one kernel weight decrease.

The effect of nitrogen fertilization on the one kernel weight of different varieties
The Thésée variety was cultivated under eight different nitrogen supply. In case of Hungarian varieties we make also the same analysis with six series of 3 samples each. The first sample was in every case the control. The results show that:

- both in case of Thesée and Hungarian varieties, control kernels showed the minimal nitrogen content.
- Applying increasing nitrogen fertilizer levels, the protein content of grains increased in every cases, which is harmonious with Daniel and Triboi's (2000) results, that is to say the nitrogen fertilization – contrary with the effect of high temperatures and drought – increases the wheat protein content.

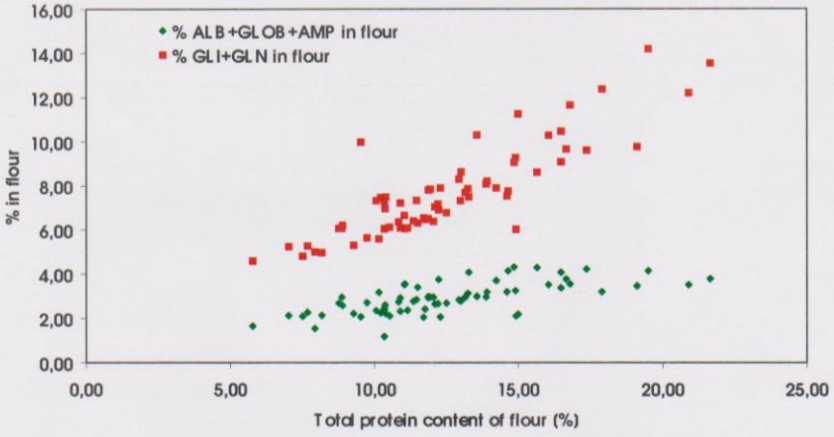
- In case of optimal nitrogen supply the protein content of the Thesée variety not exceed 11 %, but in case of high temperatures and drought, is nearly 18 %. The protein content is higher if the temperature is high than the nitrogen fertilizer level is high.
- The study of the interaction between the nitrogen supply before and after flowering shows that if the nitrogen supply after flowering is not limited, the kernel reach their protein potential, even in poor supply conditions.
- In case of Hungarian varieties, the increasing nitrogen supply shows an important increase of grains nitrogen content.

During the analysis of results we found an important variation of one kernel weight (from 12 mg to 57 mg) which can be explained with the variation of starch accumulation, which depends on variety, temperature and drought stress.
Protein content of flours shows an important fluctuation, which is caused by nitrogen supply and climatical stress.

The evaluation of protein quantity and quality
We can express the different protein fractions (AG, AMP, GLI, GLN) proportion in flour or in total protein content. In the first case we can see a quantitative change in the flour protein composition, and the second possibility seems the qualitative variation of proteins.

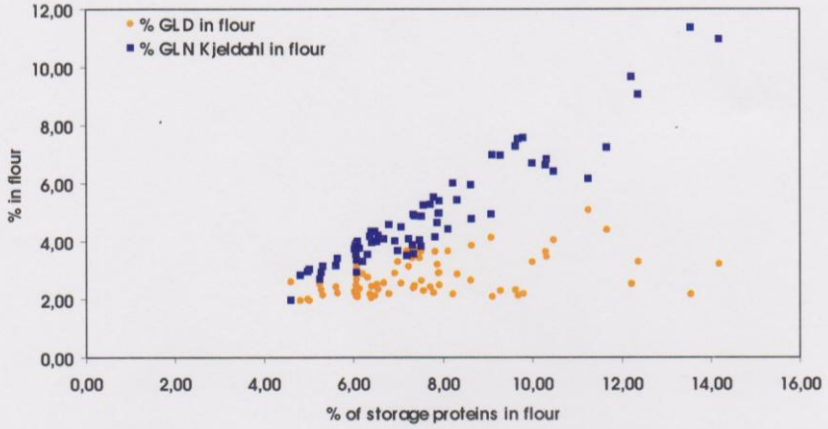
Study of different protein fractions of the flour
Daniel and Triboi (2000), Triboi and Triboi-Blondel (2001) shows that the increase of the protein content is caused by the increase of storage protein (gliadins and glutenins) content. Our results proved that. The metabolical proteins (albumins, globulins and lipid binding proteins) increased slightly (2-5 %) with the increase of protein content. The storage protein content (GLI, GLN) increased strongly (5-15 %) (**Figure 1.**). The contribution to the protein content increasing of storage proteins is not homogeneous, that is function of storage protein content (**Figure 2.**).

Figure. 1. The variation of metabolical proteins (ALB+GLOB+Amp) and storage proteins (GLI+GLN) in the flour in funtion of protein content



For the not-stressed (temperature or drought) wheat flour samples with storage protein content less than 10 %, the gliadin and glutenin content is near the same. In the case of this samples the protein content can be increased wit increasing nitrogen fertilizer levels. Samples which have the storage protein content over 10 %, the glutenin content increases much more that the gliadin content, which induce the change of the ratio of this protein groups (**Figure 2.**).
In the case of this samples, the high temperatures or drought stress resulted in an increase of the glutenin quantity, and the gliadins quantity remain relatively constant, which is 5 % in the flour.

Figure 2. The changes of GLI and GLN content of flour in function of storage protein content of flour

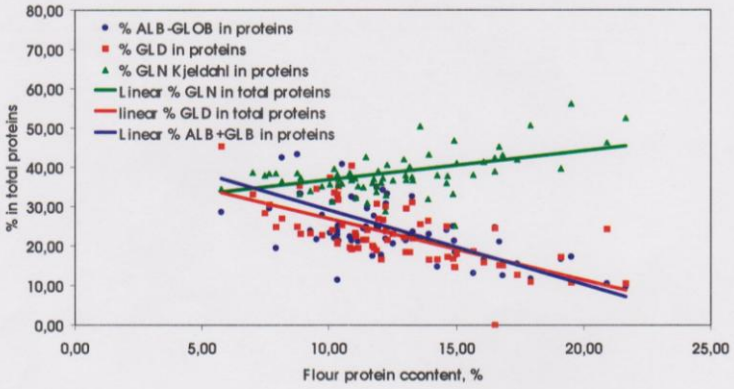


This result is contrary with the earlier findings of Triboi and Triboi-Blondel (2001): they found that the GLI/GLN ratio increase with the increasing protein content. It is also opposite to results of Daniel and Triboi (2000) because early they found that the high temperature was favorable to the synthesis of gliadins, not on the glutenins.

The variation of different protein fractions in proportion of total protein content

The qualitative change in proportion of total protein content of a protein fraction is presented in the **Figure 3.**

Figure 3. The variation of different types of proteins in total proteins in function of total protein content of flour



Our results show that when the protein content increases, only the glutenin proportion increases, and the gliadins, albumins+globulins and lipid-binding proteins proportion decrease.

Triboi and Triboi-Blondel (2001) found that with the increase of protein content of flour, the proportion of albumins+globulins decreased, which is harmonious with our results. But opposite to our presented results, with increasing protein content, the gliadins proportion in the total proteins increased. Gupta *et al.* (1992) found the same, respectively the gliadins proportion increased with an increase of total protein content of flour.

Investigations on the proteins using SE-HPLC

Contrary with the sequentially extraction when we separate monomeric proteins, in this case we can obtain an (easily) extractable fraction (so-called PE) and a relatively strongly aggregated non-extractible fraction (so-called NE).

Evolution of extractible and non-extractible fractions in function of the protein content of flour

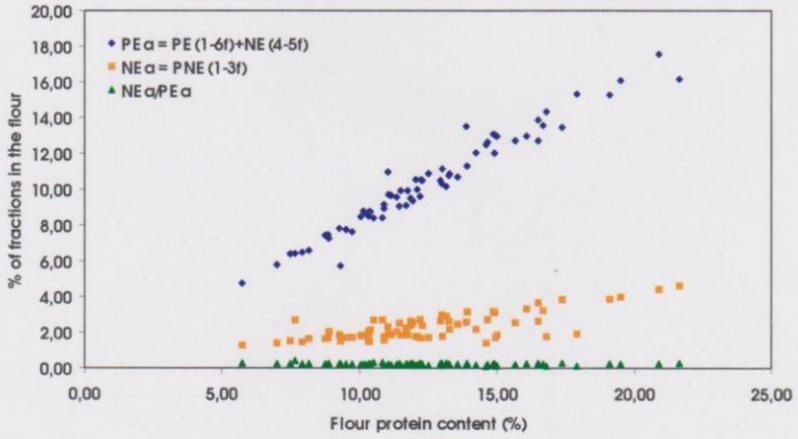
The used techniques define two classes of proteins:

– easily soluble proteins: PEa = (peaks 1-6 of soluble)+(peaks 4-5 of non-soluble)

– difficultly soluble proteins: NEa = peaks 1-3 of non soluble

The PEa is present in an important quantity (4-17 % in flour) than the NEa (2-5 % in flour) (**Figure 4.**). These two classes of proteins represent the 80 % of total proteins. The quantity of PEa increases linearly with the increase of total protein content. The NEa increase also, and the PEa/NEa ratio rest relatively constant, not depending by protein content.

Figure 4. The extractible fraction (PEa) and non-extractible fraction (NEa) in function of flour protein content



Study of the correlation between mixograph parameters and protein content, respectively protein composition

To study the effect of changes of protein composition on the different mixographic parameters, we made a regression analysis step by step with and without free term (**Table 5.**). With the determination of different independent variables coefficient we can estimate the effect of this variables.

Early we calculated the correlation coefficients between different mixographic parameters, to determine the dependent and independent variables.

The strongly correlated parameters (MLV, MPV, MRV and MTxI) which characterize the diagram's mid-line, are also strongly correlated to total protein content, which influence the free-term or whiteout free-term regression. In case of regression whiteout free-term, a second parameter, the extractible proteins proportion in the total proteins can explain better.

On the width of mixogram (W), the PE1 fraction has an important effect in the formation of the protein structure.

The quality of proteins (the proportion of non-extractible and extractible proteins, the proportion of non-extractible in total proteins) has effect on the second part of the mixogram (MRW and MTxW).

For this two mixographic parameters, like in the case of the other independent parameters (MPTi, MTxV and WS) the protein content is not a very important variable, because explains less than the quantitative variables like the proportion of peak 1 or 2 in flour (PE1 and PE2), or than the qualitative variables like the ratio non-extractible and extractible (for MPTi whitout free-term and for WS with free-term).

Table 5. Multiple regression step by step: the effect of composition of flour on the mixographic parameters

	MLV	MPV	MRV	MTxI	MLW	MPW	MRW	MTxW	MPTi	MTxV	WS	Hardness
	Regression step-by-step with free-term											
Free term	16.3	13.1	15.7	117	35.6	1.05	13.3	12.5	6.09	ns	-11.3	96.8
Protein content	1.1		1.11	5.58	-1.75	0.9						
PE1					15.3				-6.76			
PE2				16.6	4.91				2.06			8.82
PE3-5							-1.94		-0.49		2.32	
PEb		1.92										
NEb							6				-3.91	
NEb/PEb								26.5			30	
NEb/PT							-44.8					-65.8
PEb/PT					-43.6			-15.9			-6.3	
F1/F2											11.4	
R^2	57	72	61	60	80	63	49	39	42		79	19
	Regression step-by-step without free-term											
PT	1.16	1.53	2.65	5.74		0.98			-2.3			
PE1					14.5		-5.42			-4.2		
PE2				18.7			3.44			16.7		15.3
PE3-5					3.8						1.32	
PEb			-1.99		-1.42							
NEb											-1.84	
NEb/PEb							52.1	68.5	11.4			110
NEb/PT								-53.5				
PEb/PT	19.7	16.4	20.3	140			-46.5		2.94			
F1/F2										61.5		
R^2	99	99	99	99	97	97	97	94	93	99	87	96

This results show in one respect the primary role of protein content in the formation of the mid-line of mixogram, and for the other hand, the role of qualitative parameters (the quantity of different protein fractions in flour, the extractible proteins and total protein content ratio, the non-extractible and extractible proteins ratio) opposite to quantitative parameters.

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