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Original research paper

EVALUATION OF TECHNOLOGICAL QUALITY OF WHEAT USING LAB–ON–A–CHIP ELECTROPHORESIS

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ABSTRACT: Technological quality of wheat flour is determined by various chemical, physical and rheological tests where the crucial factor is content and structure of proteins. In this paper, samples of four winter wheat experimental lines were examined. The results of qualitative and quantitative characterization of protein electrophoretic profile of gluten using a Lab–on–a–chip (LoaC) electrophoresis are shown. Rheological properties of the tested lines were determined by standard flour rheological methods (amylograph, farinograph, extensograph). The values of rheological parameters in conjunction with the electrophoretic results show that the best technological quality have Lines 1 and 3. This conclusion support the assumption that, in terms of quantity and structure, glutenins and gliadins have equal impact on the quality of wheat.

Key words: *wheat, electrophoresis, technological quality*

INTRODUCTION

Wheat is a widely spread culture whose one of many advantages is that it is adaptable to a wide range of climatic conditions and soil types. Of all the cereals, only wheat flour has the ability to create dough that can retain the gases generated during fermentation and baking, and to form a spongy, porous middle of bread (Shewry et al., 2001, Flæte *et al.*, 2005).

Technological quality of wheat flour is determined by the sum of different flour properties which influence the properties of the dough and its behaviour during processing, and ultimately the final product. It is determined by various chemical, physical and rheological tests where the most commonly used are empirical methods as a type of the most dominant, rheological, tests (farinograph, extensorgraph, amilograph and other) (Živančev et al., 2009). The protein content and structure are the most important factors determining the quality of the flour where higher protein content causes higher quality of the final product (Unbehend et al., 2003, Hayta et al., 2001).

Gluten proteins make up 80-85% of the total flour protein and function as wheat storage proteins. They are located in the endosperm of mature grains where they form a continuous matrix in which starch granules are embedded (Goesaert et al., 2005). In contact with water, gluten swells and, as a result, a fine mesh structure is formed due to protein interactions. Consequently, dough has features such as extensibility, flexibility, ability to retain gas and others (Žeželj, 2005).

Proteins of gluten complex consist of two fractions, gliadins, soluble in alcohol, and glutenin, insoluble in alcohol but soluble in dilute acids or alkali. Gliadins are monomeric proteins that are further classified into four groups α–, β–, γ–and ω–gliadins.

Gliadins are generally considered to contribute to the viscosity and elasticity of gluten. Although some authors have associated specific gliadin alleles with the quality of the final product (bread), it has been agreed that these proteins do not have a direct impact on the wheat quality in terms of dough strength. It is assumed that this is a result of close genetic linkage between low molecular weight glutenin subunits (LMW–GS) and gliadins (Gianibelli et al., 2001). Glutenins are polymeric proteins. They consist of two types of molecules: high molecular weight glutenin subunits (HMW–GS) and low molecular weight glutenin subunits (LMW–GS) (Song et al., 2007) which are interconnected with disulphide bonds and possess elastic properties. Glutenin subunits of large molecular weight are quantitatively minor components, but are crucial in the process of breadmaking since they determine the elasticity of gluten. LMW–GS compose about one–third of the total protein content of grain, and approximately 60% of the total glutenin. Despite their large quantity, they have been given less attention in research than the HMW–GS. This is mainly a consequence of the difficulties that arise in their identification of the one–dimensional SDS–PAGE gels (Gianibelli et al., 2001) and newer techniques, such as automated capillary chip electrophoresis allow improved characterization of all the glutenin complex subunits.

Previous research has expressed mixed opinions regarding the impact of gliadin on the technological flour quality. Namely, the majority of authors believe that this influence is conditioned by the quantity and quality of glutenin, whereas a smaller number prioritises gliadins, and some other authors consider the quantitative relationship between them by correlating it with the volume of bread (Unbehend et al., 2003).

The aim of this paper is to present results of testing qualitative characterization and quantitative gluten protein electrophoretic profiles of 4 domestic wheat production lines from 2008, and to correlate them with their technological quality.

MATERIAL AND METHODS

We have analysed samples of four winter wheat experimental lines formed in the selection centre of the Institute of Field and Vegetable Crops in Novi Sad:

1. Line 1
2. Line 2
3. Line 3
4. Line 4

The wheat samples come from the production year of 2007/2008 from the Novi Sad site. The criteria for their selection were the results of standard tests of technological quality, so that in the wheat lines tested there are those which exhibited different levels of technological quality.

Rheological tests were performed by using Brabender farinograph, extensograph and amilograph, according to the Regulation of methods of physical and chemical analysis for quality control of grain, milling and bakery products, pasta and quickly frozen dough (Official Gazette no. 74/1988).

Characterization of gluten proteins, with prior fractionation to gliadin and glutenin fraction, was performed using an automatic chip electrophoresis using the Agilent 2100 Bioanalyzer apparatus (Agilent Technologies, Santa Clara, CA) using the Protein 230 Plus Lab Chip kit according to the method specified by the manufacturer.

RESULTS AND DISCUSSION

Rheological tests performed on four domestic lines of wheat in order to assess their technological quality have shown that there are some differences in the values of specified parameters (Table 1).

Comparing the values of farinograph indicators, a sample of flour taken from the Line 1 showed the best quality which was confirmed by the value of the quality number, therefore belonging to the A2 quality group. Lines 2 and 4 showed extremely high water absorption, but also higher values of softening degree, which is an indication of inadequate quality of proteins which are unable to retain absorbed water inside the dough.

Tabela 1.
Value indicators of farinograph, extensograph and amilograph tested flour samples of four wheat lines

	Line 1	Line 2	Line 3	Line 4
FARINOGRAPH				
Water absorption (%)	61	63,3	59,8	63,4
Dough development (min)	2,4	1,9	2	1,8
Dough stability (min)	1,7	1,4	1,1	1,3
Dough softness (BU)	39	83	58	73
Quality number	72,6	59	64	60,8
Quality class	A2	B1	B1	B1
AMYLOGRAPH				
Viscosity peak values (BU)	499	592	785	273
EXTENSOGRAPH				
Energy (cm^2)	77	57	97	71
Resistance (BU)	293	240	371	277
Extensibility (mm)	146	144	145	147
Ratio(resistance/exstensibility)	2,05	1,72	2,62	1,93

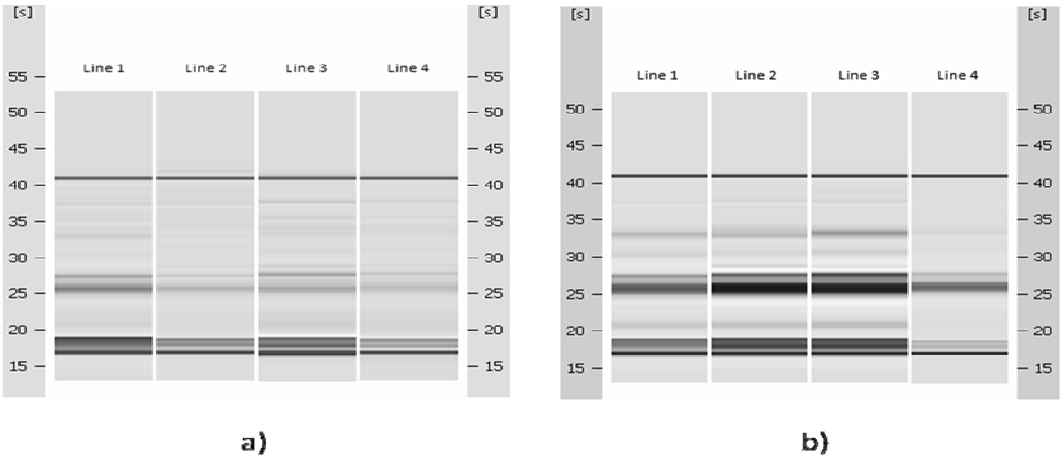

Figure 1. Gel image of the electrophoretically separated flour protein fractions (glutenin and gliadin) of the four wheat lines

In terms of extensograph indicator value, differences between samples of flour were the least in terms of dough extensibility, which is basically on the same level in the case of all four examined samples of flour. As a result, all samples, except for the flour sample taken from the Line 3, are characterized by the value of the relative number which lies within the optimum, while the sample taken from the Line 3 due to the highest value of dough resistance has the highest energy value measured by extensograph.

Maximum viscosity valuesmeasured by the amilograph show that samples of flour taken from Lines 1 and 2 have values within the optimum needed for the purposes of bakery industry, while flour from the Line 3 has great maximum viscosity value and the sample taken from the Line 4 has a small one – both inappropriate for treatment in bakery. Figures 1a and 1b show the gel images of electrophoretically separated protein fractions of flour samples originating from four domestic lines of wheat. On the one hand we can see the similarity of separated fractions of gluten complex protein found in the flour samples, in a qualitative sense, which is reflected in a similar distribution of protein bands in the bars as well as glutenin and gliadin. At the same time there is a visible difference in the intensity of the band colour on the gels, which represents different quantitative profiles of gluten fractions,

that is, different amounts of separated fractions of glutenin and gliadin.

Gel image of glutenin fractions (Figure 1a) shows that the glutenin fractions are in the range of molecular masses of 14 to 210 kDa. All the samples have the highest amount of protein in the glutenin fractions examined in the range of molecular masses of 35 and 60 kDa, where the sample of flour taken from Line 1 is significantly different in the above mentioned range when compared to the others. The results of electrophoretic testing of gliadin fraction (Figure 1b) show that a larger number of protein bands are also located in the area of molecular mass of 40 and 60 kDa. Also it has been noticed that there is a difference in the quantity of total gliadins between the samples, where samples of flour from Lines 2 and 3 stand out by their increased content.

Looking at the quantitative ratio of gliadin to glutenin in gluten complex of wheat lines tested samples (Figure 2) it is noticeable that this ratio is different in the case of all samples. According to the literature data, the optimal ratio of glutenin and gliadin is around 1:1 (Fido *et al.*, 1997; Peña, 2002; Radovanovic *et al.*, 2002; Goesaert *et al.*, 2005). However, by using different techniques of electrophoresis, different relationships are obtained in the same samples. Looking at the overall technological quality of the wheat lines tested, it can be concluded that the best technological quality was found in Lines 1 and 3. Bearing in mind that most authors agree that the technological quality of flour depends to a great extent on the glutenin fraction, by performing characterization of proteins of studied lines, it was found that the amount of gliadin also had an impact on the formation of technological quality.

Electrophoretic examination (Figures 3 and 4) showed that both glutenin and gliadin profiles are quantitatively different in each specific area of molecular masses. In the case of glutenin, that are peaks of molecular masses of 44 and 59 kDa, and in the case of gliadin that are peaks of molecular masses of 44 kDa and 121.5 kDa.

Samples of wheat Lines 1 and 3 have the largest amounts of glutenins with these molecular weights, while samples of wheat Lines 2 and 3 have the largest amounts of gliadin (44 kDa and 121.5 kDa) .

Taking into consideration the rheological parameters, the sample of Line 1 has the best technological quality and the total quantity of glutenin, which is noticeable on electropherogram (Figure 2). On the other side, the sample of Line 3, which has the highest total content of gliadin, has a higher degree of softening and significantly more energy compared to the sample line 1. Estimating the total technological quality, which was found to be better in sample Lines 1 and 3, it can be concluded that it was correlated with an increased amount of glutenin and gliadin with mentioned molecular masses.

This conclusion support the assumption that, in terms of quantity and structure, glutenins and gliadins have equal impact on the quality of wheat.

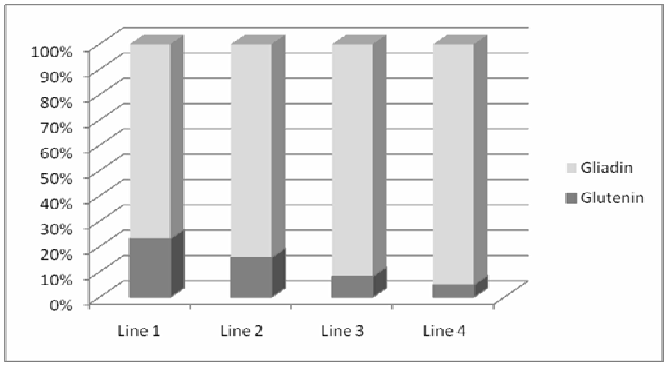

Figure 2. The quantitative ratio of gliadin to glutenin in gluten complex of flour samples of four wheat lines

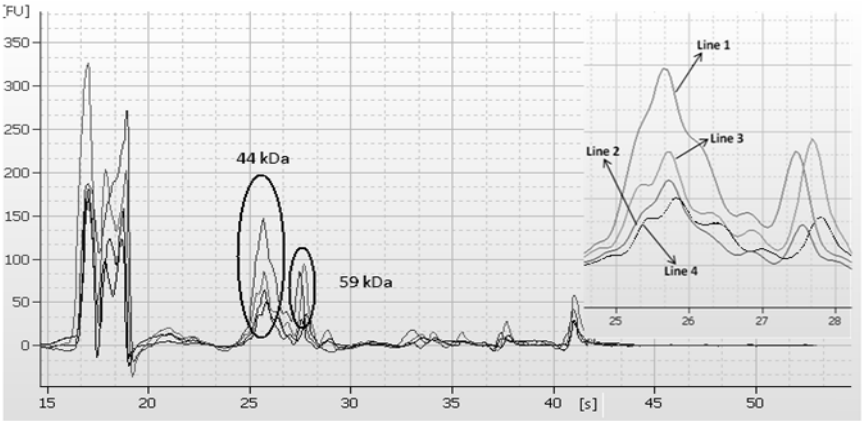

Figure 3. LoaC profiles of glutenin of flour samples of four wheat lines

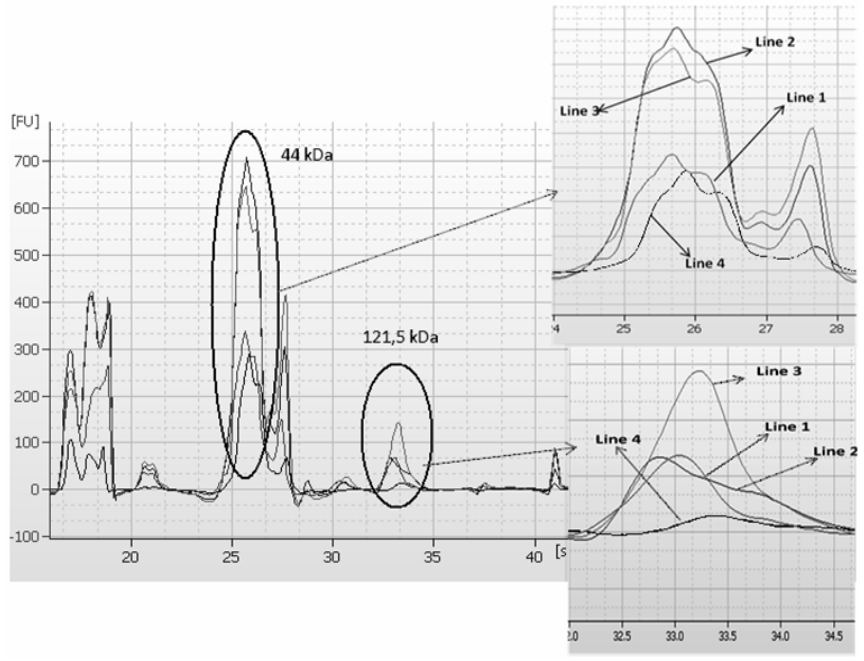

Figure 4. LoaC profiles of gliadin of flour samples of four wheat lines

CONCLUSIONS

Characterization of gluten proteins revealed that all investigated samples of wheat lines have a different quantitative ratio of gliadin to glutenin. Regarding the amount of glutenin fractions, the sample of Line 1 is distinguished by an increased content of this fraction in comparison to other samples. There is an evident difference in the total amount of gliadin, with the samples of flour from wheat Lines 3 and 4 distinguished by their increased content of this protein. Observing the total technological quality of tested wheat lines and taking into account the results of rheological tests, it can be concluded that the best technological quality have Lines 1 and 3. Bringing in connection examination of electrophoretic studies with the rheological properties of flour samples using standard methods, it can be concluded that amounts and structures of both glutenin and gliadin are equal indicators of technological quality of wheat.

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ПРОЦЕНА ТЕХНОЛОШКОГ КВАЛИТЕТА ПШЕНИЦЕ ПРИМЕНОМ LAB–ON–A–CHIP ЕЛЕКТРОФОРЕЗЕ

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Сажетак: Технолошки квалитет пшеничног брашна се утврђује различитим хемијским, физичким и реолошким тестовима, при чему је пресудан фактор садржај протеина и њихова структура. У овом раду испитивани су узорци четири експерименталне линије озиме пшенице. Приказани су резултати квалитативне карактеризације и квантитативних електрофоретских профила протеина глутена одређених помоћу Lab–on–a-–chip (LoaC) електрофорезе. Реолошке особине испитиваних линија брашна одређене су стандардним реолошким методама (амилографх, фаринограф, ехтенсограф). Вредности реолошких показатеља у спрези са електрофоретским резултатима показују да најбољи технолошки квалитет имају Линије 1 и 3. Ова констатација говори у прилог претпоставци да глутенини и глијадини и у погледу количине и структуре имају равноправан утицај на испољени квалитет пшенице.

Кључне речи: *пшеница, електрофореза, технолошки квалитет*

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