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Original scientific paper

THE INFLUENCE OF WHEAT GENOTYPE AND ENVIRONMENTAL FACTORS ON GLUTEN INDEX AND THE POSSIBILITY OF ITS USE AS BREAD QUALITY PREDICTOR

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Gluten index is an indicator of gluten strength as well as a parameter which simultaneously defines its quantity and quality. If compared to the farinographic and extensographic methods, gluten index determining is faster, less complicated and requires smaller amount of flour. The aim of this study has been to determine the significance of the influence of genotype and environmental factors on the value of gluten index and usability of these parameters as indicators and predictors of bread quality. Five local varieties of winter wheat (Simonida, NS 40S, Rapsodija, Pobeda, Zvezdana) were grown in a macro-experiment carried out in three locations (Novi Sad, Čačak, Sombor) during two seasons (2011/2012 and 2012/2013). Gluten index value determination was achieved by using ICC standard method 155 (1996), with a slight modification that included mechanical washing of gluten (*Theby*). Statistical analysis was performed by using IBM SPSS Statistics 20. Variance analysis revealed a statistically significant effect of genotype on gluten index value, whereas the influence of environmental factors, as well as the interaction of two factors, had no statistical significance. There was no correlation between the values of gluten index and meteorological factors such as average temperature and total precipitation during the phenophase of grain filling, and in the period from the beginning of flowering until the harvest. However, medium-strong negative dependence was found between gluten index and the index of heat stress, as well as between gluten index and the

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number of days with temperatures above 30°C. Weak negative dependence was recorded between gluten index and bread volume. Based on the value of gluten index, gluten varieties used in our study can be described as strong. Genotype proved to be the only cause of statistically significant variation of gluten index.

Key words: bread volume, environment, gluten, gluten index, genotype, wheat

INTRODUCTION

Among other factors, wheat quality is determined by the physical characteristics of the grain, the content and composition of proteins and starch content (DOWELL *et al.*, 2008). The protein content, as one of the most important parameters of wheat quality, is not sufficient in itself to explain the variation in bread quality (ZEČEVIĆ *et al*., 2007). What is essential for this explanation is the knowledge of the protein composition. The parameter that is both an indicator of the quality and quantity of protein is gluten index (GI) (GIL *et al*., 2011). Recently, GI has frequently been used as a parameter of technological quality, having in mind that it is determined faster and requires a smaller amount of flour when compared to farinographic and extensographic parameters (OIKONOMOU *et al*., 2015). However, in Israel it took precedence over other indicators of technological quality of wheat (GIL *et al*., 2011).

GI represents overall portions of gluten retained on the sieve after mechanical washing with 2% NaCl solution and subsequent centrifugation through a specially constructed sieve under standardized conditions, expressed as percentage (BONFIL and POSNER, 2012). GI is a measure of gluten strength. Weak gluten has a GI value of <30%, the normal GI = 30-80% and strong gluten GI >80% (OIKONOMOU *et al*., 2015), while ĆURIĆ *et al*. (2001) reported that the optimum value of gluten index is between 75% and 90%.

Based on the analysis of the results of 91 scientific works, OIKONOMOU *et al*. (2015) conclude that the greatest number of researchers found that the value of the GI parameter is affected by genotype and environmental factors, though the effect of genotype appears to be more significant. SHARMA *et al*. (2012) point out a significant impact of *Glu-D1* locus on GI value, while CESEVIČIENĖ *et al.* (2009) reported a significant positive impact of *Glu-1* score on the GI value. Temperature and precipitation, especially during the phenophase of grain filling, have a significant impact on the GI value (HADNAĐEV *et al*., 2013; ZEČEVIĆ *et al*., 2010; CESEVIČIENĖ *et al*., 2009). In addition to the above mentioned factors, GIL *et al*. (2011) and VIDA *et al*. (2013) emphasize a significant impact of the number of days with the maximum temperature above the 30°C. GIL *et al*. (2011) state equation:

$$\text{Stress index (SI)} = \Sigma[\text{Tmax} - 30],$$

where SI represents heat stress index, and Tmax stands for maximum daily temperatures of above 30°C.

The results of the investigation of the correlation between GI and bread volume as the main indicator of baking quality, are contradictory. BONFIL and POSNER (2012), POPA *et al*. (2014), DOWELL *et al*. (2008) and ĆURIĆ *et al.* (2001) did not determine the dependence of the two parameters, while DOBRASZCZYK and SALMANOWICZ (2008) reported contrasting results. Instead of gluten index parameter, POPA *et al*. (2014) proposed parameter gluten remained on the sieve (GRS), which has a very high correlation coefficient with bread volume. Mathematically, it can be expressed by the formula:

$$\text{GRS} = (\text{WG} * \text{GI}) / 100,$$

where WG represents wet gluten content.

The aim of this study has been to determine the significance of the influence of genotype and environmental factors on the GI value, the degree of dependence between this parameter and bread volume and usability of these parameters as indicators and predictors of bread quality.

MATERIALS AND METHODS

Five winter wheat varieties (Simonida, NS 40S, Rapsodija, Pobeda, Zvezdana) were grown in a macro-experiment carried out in three locations (Novi Sad, Čačak, Sombor) during two seasons (2011/2012 and 2012/2013). The harvest in the first experimental year took place on 21st June in Novi Sad and Sombor, and on 26th June in the locality of Čačak. In the second season the harvest was carried out on 2nd July in Novi Sad and Sombor, and the following day in the locality of Čačak. The object of the study has been the influence of genotype and environmental factors on technological quality parameter, gluten index, as well as the ability to predict the quality of the final product on the basis of gluten index value.

Determination of gluten index value was carried out by ICC *standard method* 155 (1996), with a slight modification that included mechanical washing of gluten (THEBY). Under standardized conditions washed gluten passed through a centrifugal sieve (2015, *Perten Instruments, Huddinge, Sweden*). The percentage representing the ratio of the mass of wet gluten retained on the sieve to the total mass of wet gluten is what gluten index stands for.

Bread volume was determined by calculation, with an assumption that a loaf consists of a truncated pyramid and a half-ellipsoid. The required dimensions were measured using a caliper.

Statistical analysis was performed by using IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

The highest average value of GI (96.5%) in all three sites and two experimental years had variety NS 40S, and the lowest (80.5%), variety Zvezdana. In the first experimental year, NS 40S had the highest value of the parameter (98%) while the variety Zvezdana had the lowest value of 80%. In the second experimental year, variety Zvezdana had the lowest value again (81%), while the variety NS 40S and Rapsodija had the highest value of 95%. The average value of the GI in the first experimental year was 87% and in the second 89.6%, while the total average value of the parameter was 88.3%. The least variation of the parameter was noticed in the variety NS 40S with a coefficient of variation of 2.52%, while the cultivar Simonida with a coefficient of variation of 14.39% showed the greatest variation of the parameter (Table 1).

Table 1. Average values of GI in the experimental years given for all cultivars, the total average value of gluten index and experimental years, gluten index varietal coefficient of variation (CV)

	Year			
Variety	2012	2013	Variety average	CV
Simonida	81.33	90.00	85.67	14.39
NS 40S	98.0	95.00	96.50	2.52
Rapsodija	91.67	95.00	93.33	4.96
Pobeda	84.00	87.00	85.50	7.85
Zvezdana	80.00	81.00	80.50	14.14
Year average	87.00	89.60		

Analysis of variance showed that the differences between the average values of the varieties are statistically significant, while the average value of the parameter differences between the two experimental years, as well as interactions, do not have statistical significance (Table 2).

Table 2. Analysis of variance for gluten index parameter

Causes of variation	DF	MS	F	P 0.05	P 0.01
Variety (V)	4	252.283	3.131**	10.807	14.747
Year (Y)	1	50.700	0.629	6.84	9.326
V x Y	4	26.783	0.332	15.286	20.851

The absence of statistically significant influence of environmental factors on the value of GI is in line with the results published by ŠIMIĆ *et al*., (2006), according to which only genotype had a statistically significant effect on the value of gluten index. ŞAHIN *et al*. (2012) did not identify a significant impact of environmental factors, but, in contrast to our study, found a significant effect of interaction. Contrary to the findings of our research, GIL *et al*. (2011), CLARKE *et al*. (2010) and VIDA *et al*. (2013) reported a significant impact of environmental factors, but emphasize that the effect of genotype is even more important, while ĆURIĆ *et al*. (2001) and CESEVIČIENĖ *et al*. (2009) reported the results which indicate that the impact of environmental factors is greater than the impact of the genotype. According to HRISTOV *et al*. (2013) varieties NS 40S, Rapsodija and Pobeda in the *Glu*-D1 locus contain 5 + 10 subunits of high molecular weight wheat glutenin, whereas Zvezdana and Simonida contain subunits 2 + 12. In our study, the average value of the GI of the varieties with subunits 5 + 10 is 91.78%, which is significantly higher compared to 83.09%, which is the average value of the parameter for the sorts with the subunits 2 + 12. Such results are consistent with those obtained by SHARMA *et al*. (2012), while TABIKI *et al*. (2006), established even more significant impact of *Glu-A1* locus on the gluten index value. Lower value of the GI of the varieties Zvezdana and Simonida is in line with the conclusions drawn by CESEVIČIENĖ *et al*. (2009) on the positive impact of *Glu* score on the value of GI, as *Glu* score of these varieties by HRISTOV *et al*. (2013) has the value 5. According to WIESER (2007) all glutenin subunits of the high molecular weights of the x-type, except for Dx5 subunit, contain four cysteine residues, three in the N-terminal and one in the C-terminal region. The cysteine residue from the S-terminal region and one of the three residues from the N-terminal region form interchain connections, while the other two residues of N-terminal region stabilize the subunit by forming intrachain connections. Dx5 subunit, unlike all the other x-type subunits, contains the additional fifth cysteine residue as well, which participates in the formation of a new interchain bond which increases the gluten strength and gluten index value, as confirmed in our study.

The values of the coefficient of variation (CV) of individual varieties shown in Table 1 clearly indicate that the varieties with lower GI value, i.e. varieties with weaker gluten, have higher coefficients of variation, which is in line with the results specified by VIDA *et al*. (2013). Evident negative dependence is confirmed by the correlation coefficient (r = -0.9) between GI and CV values of individual varieties.

Higher daily average temperatures and lower total amount of precipitation in the period from the beginning of phenophase of grain filling to the harvest stage, were recorded during the first experimental year (Table 3). The average precipitation in three localities in the phenophase of grain filling was 23.2 mm in the first experimental year and 93.2 mm in the second. At the same time, the average temperature of the three sites for the same period in the first year was 22.9°C, and in the second 20.2°C. The average values of the heat stress index were identified to be 26.33 in the first year, and 23.6 in the second. The total number of days with temperatures above 30°C during the phenophase of grain filling in all three sites was 28 in the first experimental year. In the second year, this number was lower (23), despite the fact that the harvest was done at a later time. Higher number of days with temperatures above 30°C, as well as a higher value of the heat stress index in the first experimental year are partly caused by later harvest in the locality of Čačak, since the maximum daily temperature for each of the five days of the harvest delay was above 30°C. The later harvest in the second trial year did not affect the value of the two parameters, since the maximum daily temperatures in all three locations during the additional period were below 30°C. Relatively cold and rainy weather during the last decade of June in the second experimental year actually caused the delayed harvest if compared to the first experimental year. Variations of recorded environmental factors are less significant if viewed in a broader time frame, that is, from the beginning of flowering until harvest. Number of days with temperatures above 32°C was 30 in the first, and 33 in the second trial year; the heat stress index was 27.23 in the first, and 28.2 in the second year, whereas the average temperature was 19.5 °C and then 18.5 °C; the average rainfall was 94.6 mm in the first, and 216.3 mm in the second trial year (Table 3). Statistically speaking, the average values of gluten index of the first and second experimental year were not that significant, despite these differences of meteorological parameters.

HADNAĐEV *et al*. (2013), did not determine statistically significant difference of gluten index in two vegetation years with substantially different amounts of precipitation in pre-harvest period, which is in accordance with our results. However, they determined a significant reduction of the parameter in the year with extremely hot pre-harvest period, while CESEVIČIENĖ *et al*. (2009) found a distinctive increase in the gluten index value in vegetation with extremely dry pre-harvest period. According to GIL *et al*. (2011), increased rainfall prolongs the phenophase of grain filling and lowers the gluten index value. Gluten index value decreases, since storage proteins with imbalanced relation between gliadin and glutenin accumulate in the later stages of phenophase of grain filling. Also, the prolonged time of phenophase of grain filling increases the possibility of exposing the plants to the heat stress. Temperatures above 30°C have a particularly negative impact on the gluten index value. According to TRIBOI *et al*. (2006), high temperatures affect the gluten index value by changing the length of certain phenophases and by influencing the formation of disulfide bonds of gluten proteins. High temperatures reduce the synthesis of gluten (ZEČEVIĆ *et al*., 2009). However reduction is less distinguished for gliadin than glutenin. Correlation coefficients determined in our study, and listed in Table 3, show a weak correlation between the two meteorological factors (average daily temperature and total rainfall) and the gluten index parameter. Yet, medium-strong negative dependence is found between the heat stress index factor, the number of days with temperatures above 30°C and gluten index. The values of gluten index of the varieties used in our study did not vary significantly under the effect of environmental factors, which is consistent with the conclusion reported by VIDA *et al*. (2013). They state that the varieties with strong gluten are more resistant to gluten index varying than varieties with weak gluten, when affected by environmental factors. The varieties used in our study had gluten strong enough to neutralize the effect of two distinctively different climatic years upon the gluten index value. Also, the number of days with temperatures above 30°C and the average value of the heat stress index (two out of four environmental factors in our study which showed a significant effect on the gluten index) were approximately the same in two successive years (especially if we consider the period from the beginning of flowering to harvest) and could not cause a statistically significant difference of the average annual values of gluten index.

Table 3. The values of gluten index in some localities and experimental years.

	environmental factor								
	Number of days with temperature above 30°C		Heat stress index		Average t (°c)		total precipitaiton amount (mm)		Gluten index (%)
local./year									
NS/12	7*	7**	16.9*	16.9*	19,7*	23**	79,7*	27,5**	95,8
ČA/12	15	14	45,2	44,7	19,2	23,1	123,3	17,8	81,4
SO/12	10	7	19,6	17,4	19,5	22,6	80,8	24,3	83,8
average	-	-	27,23	26,33	19,5	22,9	94,6	23,2	87
total	32	28	-	-	-	-	283,8	69,6	-
NS /13									
ČA /13	11	7	27,8	22,8	18,8	20	219,5	96,1	94,4
SO/13	12	9	30,9	25,9	18,2	20,4	185,5	57,8	88
average	-	-	28,2	23,6	18,5	20,2	216,3	93,2	89,6
total	33	23	-	-	-	-	648,8	279,6	
r	-0,68	-0,61	-0,56	-0,58	0,09	-0,26	0,1	0,24	

The values of environmental factors in the two periods (* period from the beginning of flowering until the harvest; ** period from the beginning of phenophase grain filling to harvest). The correlation coefficients (r) between the environmental factors in the two periods and the gluten index

The results of our study show a weak negative dependence between the value of GI - and bread volume and correlation coefficient r = -0.328. POPA *et al*. (2014), have found a weak but positive dependence between them and the correlation coefficient r = 0.18. ĆURIĆ *et al*. (2001) also found a weak dependence of gluten index and bread volume (r = 0.28), BONFIL and POSNER (2012) found no dependence, while in contrast to previous results, DOBRASZCZYK and SALMANOWICZ (2008) reported a significant correlation between the two parameters and the correlation coefficient r = 0.804. Low negative dependence of gluten index, determined in our study, is not in line with the conclusion stated by ĆURIĆ *et al*. (2001) that high GI value reduces bread volume. However, the same authors suggest that decrease in bread volume is evident only in the case of extremely strong gluten, which was not the case in our study. BONFIL and POSNER (2012) conclude that the GI can give some indication of gluten quality, but using GI as the principal quality parameter of bread (which is a trend in Israel in recent years) can be misleading. According to POPA *et al*. (2014), in order to efficiently predict bread volume, apart from using gluten index, other parameters can be used, e.g. gluten remaining on the sieve (GRS), which does not depend on the content of wet gluten and has a higher correlation coefficient (r = 0.79). In our study, the correlation coefficient value of GRS and bread volume was r = 0.769, almost identical to the one obtained in the study by POPA *et al*., (2014).

CONCLUSION

A statistically significant effect of genotype on the value of the GI parameter has been determined in our study, while the impact of the environment and the interaction has not shown a statistically important influence.

Varieties with subunit 2 + 12 had significantly lower parameter value in relation to varieties with subunit 5 + 10. Among the gluten index value and meteorological factors, average temperature and total precipitation during the phenophase of grain filling and in the period from the beginning of flowering until the harvest, no dependence has been established, while a medium-strong negative dependence has been found between gluten index and the heat stress index; and between gluten index and the number of days with temperatures above 30°C. Two of the four observed meteorological factors, which showed a significant effect on gluten index, had similar values during the two successive years and did not cause a statistically significant variation in average annual value of the parameter.

The established weak dependence of gluten index and bread volume suggests that the use of gluten index as a bread quality predictor is not completely reliable.

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EFEKAT GENOTIPA PŠENICE I FAKTORA SPOLJAŠNJE SREDINE NA GLUTEN INDEKS I MOGUĆNOST NJEGOVOG KORIŠĆENJA KAO PREDIKTORA KVALITETA HLEBA

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Izvod

Gluten indeks je indikator snage glutena i parametar koji istovremeno definiše njegov kvantitet i kvalitet. U odnosu na farinografske i ekstenzografske metode određivanje gluten indeksa je brže, jednostavnije i zahteva manju količinu brašna. Cilj ovog istraživanja bio je utvrđivanje značaja uticaja genotipa i faktora spoljašnje sredine na vrednost gluten indeksa, kao i upotrebljivost parametra kao indikatora i prediktora kvaliteta hleba. Pet domaćih sorti ozime pšenice (Simonida, NS 40S, Rapsodija, Pobeda, Zvezdana) uzgajano je u makro ogledu u tri lokaliteta (Novi Sad, Čačak, Sombor), tokom dve sezone (2011/2012 i 2012/2013. Određivanje vrednosti gluten indeksa vršeno je metodom ICC standard method 155 (1996), sa modifikacijom-mašinskim ispiranjem glutena (Theby). Statistička analiza rezultata izvršena je pomoću programa IBM SPSS Statistics 20. Analizom varijanse utvrđen je statistički značajan uticaj genotipa na vrednost gluten indeksa, dok su uticaji faktora spoljašnje sredine, kao i interakcija dva faktora bili bez statističkog značaja. Nije utvrđena zavisnost između vrednosti gluten indeksa i meteoroloških faktora srednje temperature i ukupne količine padavina tokom fenofaze nalivanja zrna i u periodu od početka cvetanja do žetve, dok je srednje jaka negativna zavisnost utvrđena između gluten indeksa i indeksa temperaturnog stresa i gluten indeksa i broja dana sa temperaturama iznad 30°C. Utvrđena je slaba negativna zavisnost gluten indeksa i zapremine hleba. Gluten sorti korišćenih u našem istraživanju može se, na osnovu vrednosti gluten indeksa, okarakterisati kao jak. Genotip se pokazao kao jedini uzrok statistički značajnog variranja gluten indeksa.

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