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Gluten Index for Wheat Products: Main Variables in Affecting the Value and Nonlinear Regression Model

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A gluten index test was recently introduced as a quicker method to measure wheat processing quality in comparison with the classical instrumental methods, such as a mixograph and farinograph. It is also a criterion defining whether the gluten quality is weak, normal, or strong. The gluten index test has gained wide acceptance as a method of determining gluten strength and is used in international trade specifications. The innovation of this work was to: (a) collect published data regarding the values of gluten index and form a database; (b) investigate the impact of variables, such as genotype, glutenin, and gliadins subunits, the level of nitrogen fertilization, irrigation, the protein content of flour, damages on crop and addition of enzymes, on the value of the gluten index aiming the comprehension of their interaction; (c) establish a relationship between gluten index and the two predictor variables, nitrogen fertilization level and protein content. The results suggested that the above variables have a contrary effect on the value of the gluten index, even for the same treatment of definite wheat genotype, which leads us to the conclusion that additional variables must account for defining the gluten index value. Out of all interactions between the experimental variables and gluten index, only nitrogen fertilization level and protein content were found to be significant, having a power law nonlinear relationship.

Keywords: Database, Model, Glutenin subunits, Wheat genotype, PCA

INTRODUCTION

Wheat (*Triticum* spp.) is one of world's major food crops with an annual world production for 2009 of about 681.9 million metric tons.[1] Wheat products are used for human consumption in many forms, such as bread, pasta, couscous, and other baked foods. Wheat gluten is used as a thickener in sauces, soups, and sweets. Durum wheat (*Triticum turgidum* L. subsp. *durum*) is the commodity of choice for production of high-quality pasta and couscous, while the common (*Triticum aestivum* L. subsp. *aestivum*) wheat is used mostly for bakery products.[2] The process of wheat milling

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(grooved steel rollers) separates or flakes off the starchy endosperm from the bran and the embryo while the starchy endosperm is cracked.[3] The separation should ideally occur at the level of the endosperm/aleurone layer interface if aleurone, which is high in ash content, is to be excluded from the semolina. The semolina is then ground into flour.[4] In some cases, the milling process includes the entire wheat kernel (whole meal).

Wheat contains a complex mixture of proteins that have a unique property of being able to form a viscoelastic dough when flour is mixed with water. When the starch and the water soluble proteins are washed out of a dough, the residual viscoelastic mass that remains contains mainly water insoluble protein fractions called "gluten." The composition of the "gluten" fractions of the wheat grain is essential for industrial quality. In practice, the term "gluten" refers to the proteins, because they play a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesivity, viscosity, and elasticity on dough. Gluten index (GI) is a criterion defining whether the gluten quality is weak (GI $<$ 30%), normal (GI $=$ 30–80%), or strong (GI $>$ 80%).[5] Wheat with similar protein contents can be classified according to GI values. In other words, GI has been correlated with protein strength variables.[6,7] The main method applied in the measurement of GI is the AACC Int. 38-12A or ICC Standard method 137-1.[8] In this procedure, 10.0 g $\pm$ 0.01 g of flour or whole meal is weighed and put into the Glutomatic wash chamber with an 88 micron polyester sieve (84 micron for wheat meal). After dispersing and mixing with salt, the mixture is washed to remove the starch and other solubles from the sample. The residue remaining after washing is the wet gluten. In the next step during centrifugation, the gluten is forced through a sieve. The percentage of gluten remaining on the sieve is defined as the GI, which is an indication of gluten strength.

The aim of the present study is to (a) compile and organize published data, regarding the values of GI and form a database; (b) explore the effect of experimental variables, such as genotype, glutenin, and gliadins subunits, the level of nitrogen fertilization, irrigation, damage on crop, and addition of enzymes on retrieved GI values; and (c) propose a model that indicates a simplified correlation between GI and the level of crop N-fertilization and protein content of flour or semolina.

MATERIALS AND METHODS

Data

An extensive bibliography search was made of the most popular food engineering and food science journals during recent years. The selected data for GI were compiled into a database developed in Excel®. In particular, this database containing the existing experimental values of GI and associated variables, is of great importance for food industries and cereal research teams, since it can be used as an effective tool to perform quick estimations, approximate assessments, low risk modifications, and comparison of final product properties concerning foods, based on flour or semolina. The main experimental variables can be organized into two groups. The first group, which is associated with the wheat crop or agricultural practices, includes variables, such as location of wheat crop, cultivars or varieties of wheat, nitrogen or sulfur fertilization level, irrigation level in wheat crop, and bug or insect damage in wheat crop. The second group, which is associated with the gluten, includes variables, such as allelic variations of high or low molecular weight glutenin subunits, the presence of specific γ-gliadins, the glutenin/gliadin ratio, whole meal or dehulled flour, addition of enzymes in gluten, and storage time of flour and flour blends of various genotypes.

Principal Component Analysis

Due to the fact that a large number of related continuous variables have an effect on GI, the statistical technique, which reduces this number to a smaller number of principal component analyses (PCA),

TABLE 1
Data compiled from the eight studies used in the statistical analysis

Country	*Year*	*Nr**	*Nr used***	*Reference*
Estonia	2004–2007	15	12	[9]
Poland	2003	72	28	[10]
Poland	2002–2004	4	4	[11]
Poland	1995	16	16	[12]
Poland	1993–1995	30	21	[13]
Spain	2000–2002	19	12	[14]
Spain	2000–2002	61	20	[15]

*Number of reported experimental values.
**Number of data used in regression analysis.

was used. This method has two main steps: (1) principle component or factor extraction determining the smallest number of components that can be used to best represent the interrelations among the set of variables and (2) component rotation and interpretation. Usually Oblimin rotation provides information about the degree of correlation between the components.

Data Fitting Procedure

A preliminary effort to establish a relationship between GI and any experimental variable showed that only N-fertilization level (kgN/ha) and protein content (%) have a significant statistical effect and a strong relationship. A set of about 113 observations (Table 1) was appropriate in order to examine the degree at which GI is related with the N-fertilization level (NF) and protein content (PC), while no other experimental conditions have been applied.

The proposed model function, which relates the GI with NF and PC of flour, can be described from the following equation:

$$\mathrm{GI} = \theta_1 \left(\frac{\mathrm{NF}}{\mathrm{A}}\right)^{\theta_2} \left(\frac{\mathrm{PC}}{\mathrm{B}}\right)^{\theta_3} + \mathrm{e}, \tag{1}$$

where $A = 100$ (kg N/ha) is a reference average value for *NF;* $B = 12.5\%$ is a reference average value for *PC*; θ_1, θ_2, and θ_3 are the adjustment parameters of the model; and *e* is independent centered random error. The above model considers power law dependency on the two independent variables. In a regression procedure, it is necessary to keep changing the values of the parameter estimates until the sum of the squared residuals reach a minimum:

$$\mathrm{SS_R} = \sum\nolimits_{\mathrm{i}=1}^{\mathrm{n_u}} \sum\nolimits_{\mathrm{j}=1}^{\mathrm{n_i}} (\mathrm{y_{ij}} - \hat{\mathrm{y}}_\mathrm{i})^2,$$

where n_u denotes the number of unique combinations of NF and PC levels, n_i is the number of replicated observations at the *i*th combination of NF and PC levels, y_{ij} is the experimental value of GI indexed by their NF and PC levels respectively, $\hat{y}_i$ is the estimated GI at the *i*th combination of NF and PC levels respectively. The more widely used methods of computing nonlinear least squares estimators are Hartley's modified Gauss-Newton method and the Levenberg-Marquardt algorithm.[16] In this study, we preferred the Excel Solver® with GRG nonlinear algorithm and convergence value equal to 10^{-5}. In order to start the nonlinear estimation algorithm, it is necessary to get good starting approximations for the parameters–values from which convergence is quickly obtained. For this

purpose, a system of equations was solved based on Eq. (1) using the data points and the obtained approximations were used for the actual oncoming analysis. Due to the fact of repeated experimental measurements, the tests of lack of fit are used as regression diagnostics. The sum of squared residuals (SS_R) can be split into two independent estimators: lack of fit and pure error. The first estimator is a pooled standard deviation between experimental and calculated values and depends on the functional part of the model:

$$\sigma_{\mathrm{LF}} = \sqrt{\frac{1}{\mathrm{n_u} - \mathrm{p}} \sum\nolimits_{\mathrm{i}=1}^{\mathrm{n_u}} \mathrm{n_i}(\bar{\mathrm{y}}_{\mathrm{i}} - \hat{\mathrm{y}}_{\mathrm{i}})^2},$$

where p denotes the number of adjustment parameters in the model, $\bar{y}_i$ is the mean of the experiment GI at the ith combination of NF and PC levels. The other estimator is the standard experimental error based on the variation observed in each set of replicated measurements and depends only on the data and not on the functional part of the model:

$$\sigma_{\mathrm{PE}} = \sqrt{\frac{1}{\mathrm{n} - \mathrm{n_u}} \sum\nolimits_{\mathrm{i}=1}^{\mathrm{n_u}} \sum\nolimits_{\mathrm{j}=1}^{\mathrm{n_i}} (\mathrm{y_{ij}} - \bar{\mathrm{y}}_{\mathrm{i}})^2},$$

where n denotes the total data set used to fit the model. The model considered acceptable regarding not one important independent variable is missing or misspecified in the model if the standard deviation between experimental and calculated values σ_{LF} is close to the standard experimental error σ_{PE}. In the case where σ_{LF} is greater than σ_{PE}, it is necessary to understand how much greater the value might typically be when the model does fit the data. Then the hypothesis can be rejected only when σ_{LF} is significantly greater than σ_{PE}. Rejecting the hypothesis that the model is adequate only when $\mathrm{Fcal} = {\sigma_{LF}}^2/{\sigma_{PE}}^2$ is greater than an upper-tail cut-off value from the F distribution with a user-specified probability (usually $p < 0.05$) of wrongly rejecting the hypothesis gives us a precise, objective, probabilistic definition when σ_{LF} is significantly greater than σ_{PE}.[16,17]

RESULTS AND DISCUSSION

Variables and Relationships

GI is directly linked to wheat's physicochemical characteristics and, therefore, to its quality. A total of 91 articles were retrieved from science documents of recent years, which reported experimental values of GI. The selected data were compiled and organized into a database developed in Excel® (not shown), which contains about 2000 values of different experimental conditions about GI.

The moisture content (db) of wheat seeds ranged from about 120 g kg^{-1} to 160 g kg^{-1} for examined wheat seeds. Figure 1 demonstrates the minimum, maximum, and average values of GI for genotypes (including cultivars, varieties, market classes, landraces, breeding lines), which have been reported more than four times. The above data are the control values of each experiment. In conclusion, discarding "Hercules" genotype the rest of genotypes show a small spread of GI values. The small spread of GI values indicates that there is no strong relationship between genotype and region of experiment. The statistical results of three treatments are presented in Fig. 2. As we can see in the case of undamaged versus damaged wheat crops from bugs or fungi, the average GI value is decreased probably as a result of the damage on the genes that control the synthesis of prolamins. However, when a fungicide or insecticide is applied on the crop, there is no difference between GI values. Similar results on GI values are also observed when enzymes are added in wheat flours.

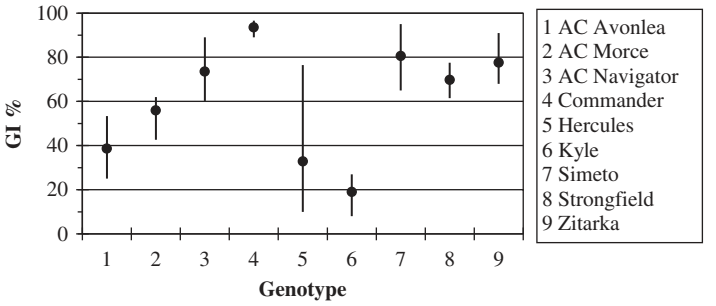

FIGURE 1 The minimum, maximum, and average values of GI for the most reported genotypes.

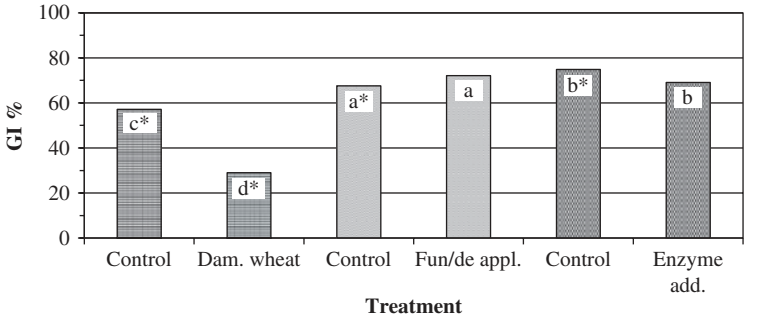

FIGURE 2 Statistical effects of three main variables versus control on GI value. *The same letter denotes a not statistically significant difference ($p < 0.05$). Dam. Wheat: Bug or insect damaged wheat; Fun/de appl.: fungicide application; Enzyme add.: Eenzyme addition.

Table 2 shows the effects of the main variables from the retrieved data as a result of their statistical analysis. It can be seen in this table that most workers found a statistical significance between the genotype, year, and environment with the GI. No statistically significant difference was found for the overall flour protein content level and the value of GI. It was found that there was statistical significance between glutenin subunits and GI, whereas in some cases differences were found between gliadins subunits and GI.

Preliminary Statistical Analysis

The underlying structure of the GI and the interrelationships among the variables (Table 3) was explored using exploratory factor analysis (EFA). EFA was performed on the data (2000 values) using PASW Statistics® v. 18, after first confirming that the data was suitable for factor analysis. Principal component analysis (PCA) was used to extract the factors followed by oblique rotation of factors using Oblimin rotation. The number of factors to be retained was guided by two decision rules: (1) Kaiser's criterion eigenvalues greater than 1 and (2) use of Horn's parallel analysis. Parallel analysis is one of the most accurate approaches to estimating the number of components. The size of eigenvalues obtained from PCA are compared with those obtained from a randomly generated data set of the same size. Only factors with eigenvalues exceeding the values obtained from the corresponding random data set are retained for further investigation. Parallel analysis was

TABLE 2
The statistical effects of the main variables on the value of GI as reported by authors

Genotype	*Year*	*Environment*	*N-Fertilization*	*Protein level*	*Irrigation*	*HMW-GS*	*GLI. S.*	*Reference*
**	**							[18]
				*				[5]
				ns				[19]
**		ns						[20]
**								[21]
*								[22]
				*		**	ns	[23]
	*							[24]
				*				[7]
**	**	**			**			[25]
**								[26]
			*					[27]
	***	***	ns					[15]
**								[28]
*								[29]
	***	***	ns					[14]
*								[30]
						***		[31]
	*		*	*				[32]
*						**	ns	[33]
				ns				[34]
*								[35]
				*				[36]
*	ns							[37]
***	*		**					[38]
**								[11]
				*				[39]
***								[6]
**								[40]
*								[41]
	ns					**		[42]
*								[43]
	**							[44]
***	***							[45]
Ns								[46]
					*	**	ns	[47]

ns: not significant; HMW-GS: high molecular weight-glutenin subunits; Gli.S: gliadins subunits.
*,**,***Statistically significant at level $\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.001$, respectively.

conducted using the software named Monte Carlo PA.[48] PCA revealed two eigenvalues exceeding 1, explaining 45.8% and 11.7% of the total variance, respectively. Furthermore, only these first two factors exceeded the criterion value obtained from parallel analysis. Following Oblimin rotation, the two factors showed a moderate intercorrelation ($r = 0.36$). Inspection of the pattern matrix (Table 4) showed a relatively clear two-factor. The highest loadings on component 1 are items NF, PC, A1-2, and G-45. All of these components have a positive effect on GI value. The main items on component 2, BD and G-42, are negative effect items. The loading plot for the principal components for GI (Fig. 3) shows that all the variables, which are located at right angles to each other, are independent of each other. NF is located almost perpendicular to FF, A1-1, and A1-2, indicating that NF and HMW-GS are independent. Afterwards, the prediction of GI based on the two most highly correlated parameters, NF and PC, was conducted.

TABLE 3
Variables used for PCA

Variable	*Abbreviation*	*Unit*
Year	Y	—
N-fertilization	NF	Kg N/ha
S-fertilization	SF	Kg S/ha
Irrigation	I	mm
Addition of enzymes	AE	%
Bug or insect damage on wheat crop	BD	%
Fermentation of flour	FF	%
Storage time of flour	TF	Days
Protein content of flour	PC	%
Glu-A1 (0)	A1-0	—
Glu-A1 (1)	A1-1	—
Glu-A1 (2*)	A1-2	—
Glu-B1 (20)	B1-20	—
Glu-B1 (14+15)	B1-14	—
Glu-B1 (6+8)	B1-6	—
Glu-B1 (7+8)	B1-7	—
Glu-B1 (7+9)	B1-7a	—
Glu-D1 (2+12)	D1-2	—
Glu-B3 (2)	B3-2	—
Glu-D3 (12)	D3-12	—
γ-gli (42)	G-42	—
γ-gli (45)	G-45	—
β-gli (52, 56)	B-52	—

Reliability of Model

The parameters (Table 5) were estimated with respect to the main criterion of minimizing the sum of squared residuals utilizing the Solver function of MS Excel®, which is ideally suited to fitting data with non-linear functions via an iterative algorithm. The final form of Eq. (1), taking into account the values of parameters, is:

$$\mathrm{GI} = 72.84 \left(\frac{\mathrm{FL}}{100}\right)^{0.44} \left(\frac{\mathrm{PC}}{12.5}\right)^{0.45}. \tag{2}$$

The standard experimental error $\sigma_{PE} = 4.98$ is smaller than the standard deviation between experimental and calculated values $\sigma_{LF} = 6.29$ and they are different by 26.3%. The F test (MS_{LF}/MS_{PE}) is equal to $1.60 < F_{crit(0.05,78,31)} = 1.64$ (P value equals 0.0724), which indicates that the difference is considered to be not quite statistically significant. Therefore, Eq. (2) is not missing or misspecified any significant terms and the regression model is satisfactory in predicting the GI value of flours with respect of NF and PC. Figure 4 illustrates the experimental values of PC versus the calculated values of PC. Most values lay near the diagonal, which indicates that the model fits in experimental data adequately. The effects of fertilization level and protein content on GI are show in Fig. 5. At low protein content levels, the increase in fertilization level resulted in a slight increase in gluten index value, while at high protein content levels, the increase in fertilization level resulted in a large increase in gluten index value.

TABLE 4
Pattern matrix of PCA

	Component	
	1	*2*
NF	.828	
PC	.785	
A1-2	.761	
G-45	.754	
Y	.720	
B1-6	.712	
SF	.708	
A1-0	.695	
B1-7a	.684	
A1-1	.628	
I	.612	
B1-7	.592	
B1-14	.586	
B-52	.547	
AE	.525	
FF	.485	
B3-2	.423	
BD		.726
G-42		.718
B1-20		.618
D3-12		.597
D1-2		.547
TF		.406

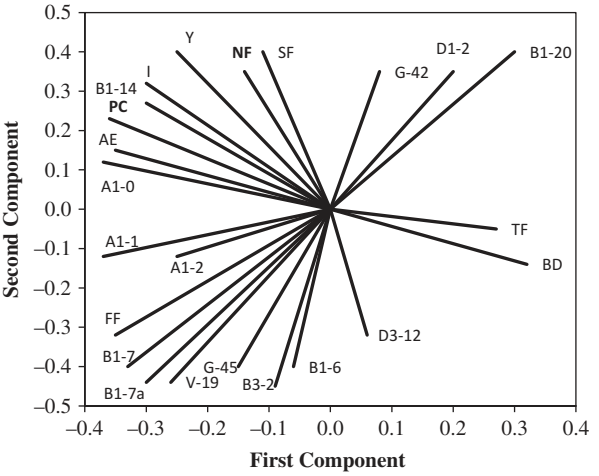

FIGURE 3 Principal components loading plot for GI.

CONCLUSIONS

GI is an important index of gluten quality, and is suggested to be an indicator of the status of the protein and often used to specify its technological usefulness. A database was compiled and organized and presented including GI values of wheat gluten. A large number of variables were found

TABLE 5
Parameter estimates

Parameter	*Estimate*	*Stand. error*	*Summary statistics*
θ_1	87.84	0.347	$N = 113$
θ_2	0.44	0.156	Standard error of estimate = 5.95
θ_3	0.45	0.194	Mean absolute error = 9%

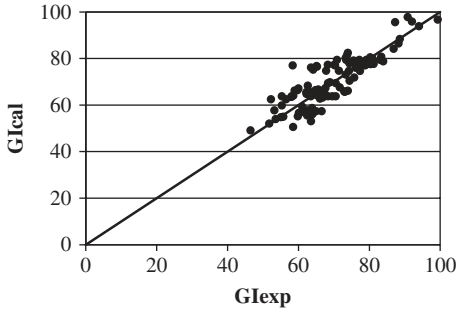

FIGURE 4 Plot of GIexp versus GIcal.

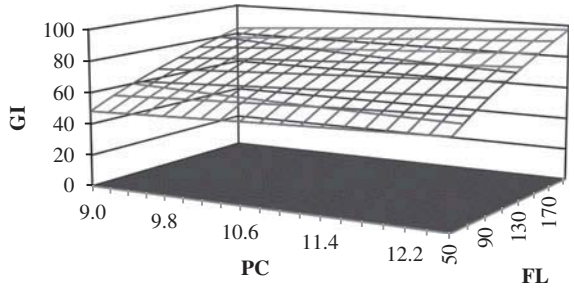

FIGURE 5 Response surface with contour plot showing the effect of NF and PC on the response of GI.

to affect the value of GI, which resulted in the large spread of values of GI. A statistical model was constructed, including the fertilization level and protein content variables, which shows a strong relationship between them and GI. Finally, a generic model, which considers power law dependency of explanatory variables' fertilization level and protein content, was established in order to predict the value of response variable GI. A more accurate model, which takes into consideration additional variables, such as the genotype of wheat, the presence of specific glutenin and gliadins subunits, etc., can be obtained in the future.

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