

Multivariate Analysis of Wheat Flour Dough Sugars, Gas Production, and Dough Development at Different Fermentation Times

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Abstract

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A Principal Component Analysis method based on multivariate analysis was used to evaluate the correlation between the fermentable sugar content of dough and its behaviour during fermentation at different fermentation times of 60, 120, and 180 minutes. The concentration of fermentable sugars during dough fermentation (sucrose, glucose, maltose, and fructose) was determined using a High Performance Liquid Chromatography device. Also, the Chopin Rheofermentometer device was used for the analysis of gas production and dough development at the fermentation times mentioned above. From the aspect of the correlations established between the parameters obtained by the two devices, very good correlations were obtained. For example, the gas production showed a positive correlation with glucose content after 60 min of fermentation ($r = 0.846$) and a negative correlation with fructose content after 120 min of fermentation time ($r = -0.993$).

Keywords: rheofermentometer; yeast; fermentable sugars; Principal Component Analysis; HPLC

The main yeast substrates in wheat flour dough are fructose, glucose, sucrose, and maltose (SAHLSTROM *et al.* 2004). The content of these fermentable sugars is continuously modified throughout the technological process due to both their amylolytic production and yeast consumption during the fermentation process. Initially, the sugars consumed by yeast are the flour specific carbohydrates such as glucose, fructose, and sucrose and towards the end maltose which results from amylolysis during the technological process.

Various methods have been developed for measuring the content of these fermentable sugars in wheat flour, i.e. chemical, colorimetric, enzymatic, and chromatographic methods (MCCLEARY *et al.* 2006). Chromatographic methods are the most

efficient ones because they are capable of determining individual sugars from wheat flour and not only the total sugar or sucrose content, with little emphasis on the other individual sugars. Among the most frequently used chromatographic methods to measure the sugar content of wheat flour are the following ones: thin layer chromatography (SHIEH *et al.* 1973; HENRY & SAINI 1989), ion-exchange chromatography (ABOU-GUENDIA & D'APPOLONIA 1972), gas-liquid chromatography (LI *et al.* 1985) and high-performance liquid chromatography (POTUS *et al.* 1994; LANGEMEIER & ROGERS 1995; SAHLSTROM *et al.* 2004; CODINĂ & VOICA 2010; TSENG *et al.* 2010). Out of the above-mentioned chromatographic methods, high-performance liquid

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chromatography (HPLC) has become the preferred method to quantify simple sugars from cereal products due to the accuracy and precision of performed determinations (CODINĂ & VOICA 2010).

The evolution of the sugar content of wheat flour and dough during fermentation is extremely important in the bakery technological process and is determined in technological terms by the wheat flour dough gas production assessed by volumetric methods that measure the released gas (CO₂) volume and manometer methods that measure the gas production under pressure (SHUEY 1975; RUBENTHALES *et al.* 1980; SAHLSTROM *et al.* 2004). The most frequently used methods to assess wheat flour gas production are the volumetric methods using Fermentograph SJA (Nassjo, Sweden) and Rheofermentometer (Chopin, Villeneuve-La-Garenne, France) device (ŠVEC & HRUŠKOVÁ 2004). The rheofermentometer is one of the most modern devices used for the assessment of wheat flour technological quality (CZUCHAJOWSKA & POMERANZ 1993) and records the variations in a yeast rate of fermentation as a function of time (for example, CO₂ released per minute). It measures the development of dough during fermentation measured by a height sensor and gas development measured by a pressure sensor (KTENIOUDAKI *et al.* 2011).

The aim of this paper is to establish a series of correlations using the Principal Component Analysis (PCA) between the dough fermentable sugar content variation during the fermentation process by means of a HPLC device and wheat flour dough gas production using a Chopin Rheofermentometer and also a series of correlations between the quantity of gas production and the dough height increase at different fermentation times. To our knowledge, no report has yet stressed a series of correlations using a PCA method between the parameters measured by these two devices.

MATERIAL AND METHODS

Material. Wheat (2008 harvest) was milled on an experimental Buhler mill from S.C. Vel Pitar S.A. (Valcea, Romania). Flour quality tests which were conducted according to Romanian or international standard methods (ICC No. 104/1, 106/2, 105/2, 107/1, and SR 90:2007) indicated the following quality parameters: ash content 0.47%, wet gluten content 26.50%, protein content 11.40%, falling number 404 s, gluten deformation index 6.00 mm.

The concentrations of soluble mono- and disaccharides in flour are (in decreasing order) as follows: sucrose (2.16 mg/g), maltose (0.57 mg/g), fructose (0.53 mg/g), and glucose (0.45 mg/g). These values are in agreement with those reported by others (ABOU-GUENDIA & D'APPOLONIA 1972; POTUS *et al.* 1994; SAHLSTROM *et al.* 2004).

In this study four different commercial forms of bakery yeast *Saccharomyces cerevisiae* type provided by S.C. Rompak S.A Romania were used. Fructose, glucose, sucrose, and maltose (Merck KGaA, Darmstadt, Germany) were used without further purification.

Mixing conditions. The dough recipe comprised flour, water (accord. WA, %), 3% yeast (% flour basis), and 1.5% salt (% flour basis). The dough was kneaded for 15 min and afterwards, it was fermented for 180 min at a temperature of 30°C and 80% relative humidity. Analyses were performed before the insertion in the proofing device and after the following time periods: 60, 120, and 180 min of fermentation.

Sugar extraction. At the beginning, 10 g of the dough sample were boiled for 20 min in 100 ml of 60% ethanol. The sample was centrifuged for 15 min at a speed of 4000 rotations/min after the cooling phase. Afterwards, the supernatant was concentrated at the vacuum pump, and then it was filtered using Whatman 42 paper. Before the injection into the HPLC system, the filtered material was passed through a filtering membrane of 0.22 µm. Chromatographic determinations were done in a HPLC system equipped with Alltec refractive index detector. The working parameters of this system are the following ones: column – waters with aminopropyl-bonded phase – C18, 250 mm × 4.6 mm, 4 µm particle diameter; eluent – acetonitrile:water solution (with 0.125% w/v of sodium chloride added to minimise the interference from NaCl) = 75:25; flow rate 1 ml/min; temperature 30°C; injected volume 20 µl. The retention times in the column of the sugars were as follows: fructose – 5 min, glucose – 5.8 min, sucrose – 8.2 min, maltose – 9.8 minutes. Sugar concentration was calculated based on peak area measurements.

Measurement of gas production and dough development. The Chopin Rheofermentometer was used for analysing the fermentation behaviour of the wheat flour dough at different fermentation times of 60, 120, and 180 minutes. The volume of gas production (ml/g) and dough height (mm) under stress of 1.25 kg were automatically calculated from the curves obtained by the Chopin Rheofermentometer software (Chopin 1995).

Statistical analysis. All determinations were performed at least in triplicate. The values of the parameters are expressed as the mean \pm standard deviation at confidence interval of 95%. All data were analysed with the Statistical Package for the Social Science, Version 16.0 (SPSS Inc., Chicago, USA).

To verify the relation between variables, the following method of interpreting the Pearson correlation coefficients was used (KOTRLIK & WILLIAMS 2003): very strong association for a correlation of 0.7 or higher; substantial association for a correlation of 0.5 or higher; moderate association for a correlation of 0.3 or higher; low association for a correlation of 0.1 or higher.

A multivariate exploratory technique, Principal Component Analysis (PCA) was performed to highlight the correlations between fermentable sugars during the dough fermentation and its behaviour (gas production, dough height) at different fermentation times. This statistical multivariate analysis was used to observe similarities between fermentable sugars during the dough fermentation and its behaviour, reducing the dimension to two PCs, while keeping most of the original information found in the data. Only PCs with eigenvalues larger than one were retained for further analyses.

RESULTS AND DISCUSSION

Variation of the concentration of fermentable sugars during dough fermentation

The amount of fermentable sugars during the dough fermentation process using the bakery yeast *Saccharomyces cerevisiae* type is shown in Table 1.

Sucrose, which is present in wheat flour, has not been identified in the dough after kneading, for none of the analysed samples, which is in agreement with the results of POTUS *et al.* (1994) and LANGEMEIER and ROGERS (1995). This fact can be explained by the presence of the invertase enzyme in the yeast, which is a very active one that has rapidly broken down sucrose to glucose and fructose (SAHLSTROM *et al.* 2004; VERSTREPEN *et al.* 2004) in the yeast cell periplasmic space in the very first minutes of kneading. That is why at the beginning of the kneading process from the content of flour fermentable sugars only glucose, fructose, and maltose resulting by starch hydrolysis are only present in the wheat flour dough (SAHLSTROM *et al.* 2004). At the beginning of the fermentation process

Table 1. Variation in the concentration of fermentable sugars during dough fermentation

Fermentable sugars (mg/g dough)	Fermentation time (min)	Mean value \pm SD
Glucose	0	1.30 \pm 0.01
	60	1.50 \pm 0.02
	120	0.38 \pm 0.01
	180	0.21 \pm 0.01
Fructose	0	4.86 \pm 0.02
	60	3.20 \pm 0.02
	120	1.16 \pm 0.01
	180	0.61 \pm 0.01
Maltose	0	9.06 \pm 0.02
	60	15.89 \pm 0.03
	120	18.86 \pm 0.03
	180	9.47 \pm 0.02

SD – standard deviation

higher levels of glucose, fructose, and maltose were detected than those detected in the wheat flour.

During the dough fermentation process, their content increases especially in the first 60 min of fermentation because amylase, a starch which degrades enzymes in flour, is constantly generating new glucose and maltose. The fermentation of glucose and fructose occurs within the first 60 min to 120 min and the fermentation of fructose and maltose within the 120 to 180 minutes. Maltose fermentation is low until glucose and fructose are exhausted, according to Table 1. Maltose begins to ferment rapidly at 120 min of fermentation time.

Variation in gas production and dough height measurement during dough fermentation

The amount of gas production and dough height in the course of dough fermentation using the bakery yeast *Saccharomyces cerevisiae* type are shown in Table 2.

Gas production in wheat flour dough occurs after such a fermentation rate that indicates a minimum is reached at some time. This minimum corresponds to the moment of the exhaustion of fermentable sugars like glucose and fructose present in the dough which will force the bakery yeast to begin to utilise maltose (CODINĂ & VOICA 2010). Dough has a gas retention ability and therefore an increase in volume and height is limited. At first, the entire quantity of gas production is retained by the dough and its height increases rapidly. As

Table 2. The variation in gas production and dough height during dough fermentation (mean value \pm SD)

Fermentation time (min)	Gas production (ml/g)	Dough height (mm)
60	58.50 \pm 0.30	28.25 \pm 0.25
120	65.50 \pm 0.50	39.75 \pm 0.25
180	57.50 \pm 0.30	37.00 \pm 0.20

the fermentation process continues, the increase of dough height becomes slower until it reaches a maximum and the dough does not increase in height any longer. Even though the gas production continues in wheat flour dough during fermentation, only a part of the gas is retained, while the other part is lost. Due to the increase of pore volume resulting from the retention of gases and their pressure in pores, the pore walls are broken and a part of the gases previously retained is released. As a result, dough height decreases.

Correlation between sugar type and gas production measurement, dough height during dough fermentation

At the beginning of the fermentation time (until 60 min) the volume of gas production increases significantly due to the fact that in the first stage of fermentation, the fermentable sugars from the flour (glucose, fructose) content are used by the yeast leading to a gradual increase in CO₂ production. As a result, very high positive correlations were obtained between the gas production volume

within 60 min of fermentation (V_60) and glucose (G_60) ($r = 0.846$) and fructose present at this moment (F_60) ($r = 0.616$) in wheat flour dough.

The graphical representation of the value of gas production (V) in relation to the level of glucose (G), fructose (F), and fermentation time (T) is shown in Figures 1 and 2, respectively. These graphs show that the concentration of glucose and fructose decreases during fermentation from 60 min to 180 min, the dough gas production (V) increases up to 120 min of fermentation, and later on it decreases to 180 min of fermentation, reaching the gas production approximately equal to that of 60 min of fermentation. After 60 min of fermentation time, the increase in gas production is slowed down due to the exhaustion of flour sugar content by the bakery yeast and it becomes dependent on the fermentation rate of the dough maltose content (LANGEMEIER & ROGERS 1995), which will induce an increase of gas production. As a result, after 120 min of fermentation time we obtained a low correlation ($r = 0.128$) between the gas production (V_120) and the maltose concentration at that moment (M_120). We also obtained a moderate association ($r = 0.383$) between the gas production (V_120) and the glucose concentration (G_120) liberated from starch by amylases. The low values of correlation coefficients registered at 120 min of fermentation time are due to the flour used as raw material which shows a low α -amylase activity (NECHITA 2008) which does not allow maltose accumulation from flour starch in the environment leading to a delay in its metabolisation by the yeast cells. An indirect correlation ($r = -0.993$) was obtained between the gas production (V_120) at

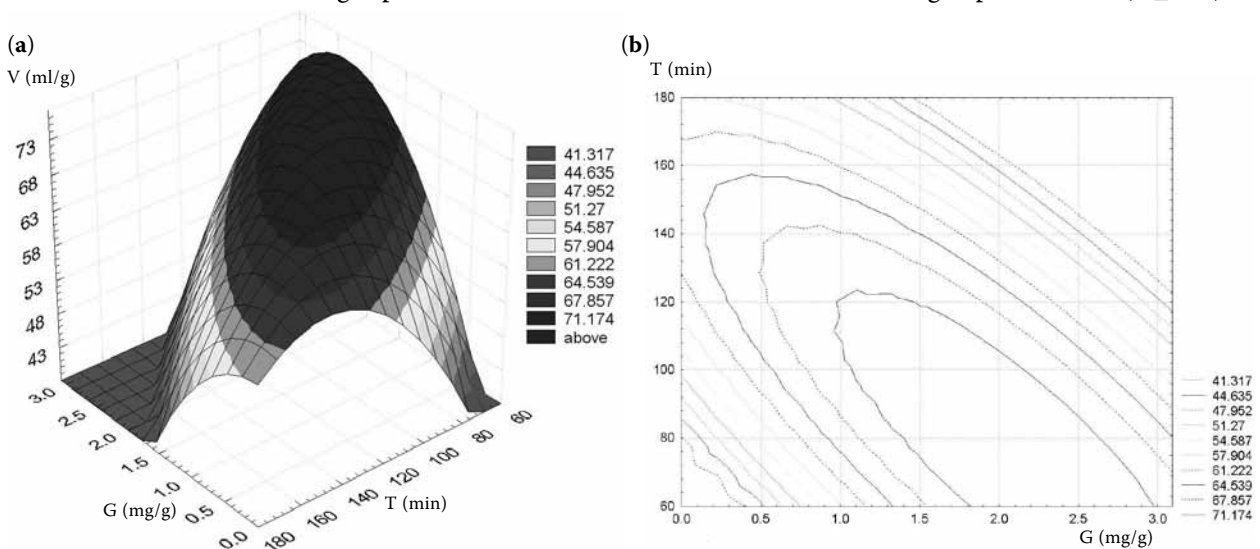


Figure 1. Variation of gas production (V) with glucose (G) and fermentation time (T): (a) spatial representation, (b) representation by contours curves

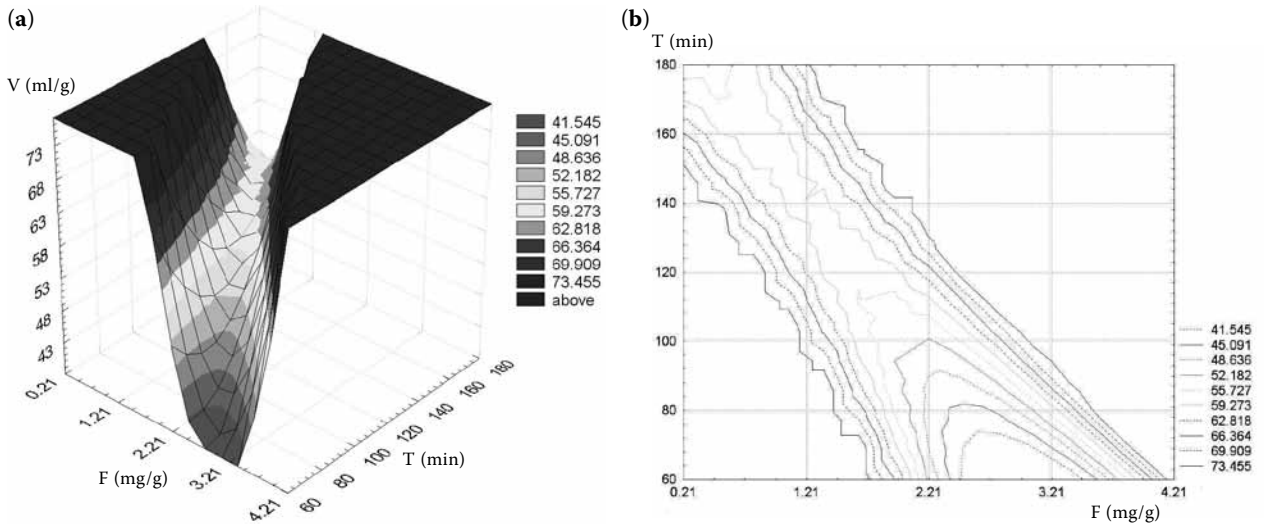


Figure 2. Variation of gas production (V) with fructose (F) and fermentation time (T): (a) spatial representation, (b) representation by contours curves

120 min of fermentation time and the fructose concentration (F₁₂₀) at that time because of fructose exhaustion from the dough system. After approximately 180 min of fermentation time, gas production (V₁₈₀) depends mostly on the amount of maltose (M₁₈₀) that was present in the dough at that moment. A good correlation ($r = 0.822$) was obtained between the gas production at 180 min (V₁₈₀) and the maltose content at that time (M₁₈₀), showing a relatively determinist relation. The graphical representation of the value of gas production (V) in relation to the level of maltose (M) and fermentation time (T) is shown in Figure 3. The variation of gas production depending on the fermentation time and maltose

concentration shows a maximum value at 120 min of fermentation.

As a result of gas retention in wheat dough, the dough volume increases its development that takes place in three directions: x , y and z . Along the x and y axes, dough development is limited by the fermentation vessel walls. The development along the z -axis depends on the quantity of gas retention in wheat dough, which, in turn, depends on the flour quality the dough was made of, constant from the quality aspect in this case for all the yeast used as raw material and the quantity of gas produced in the dough.

As far as this study is concerned, we can say that for flour of constant quality, the increase in

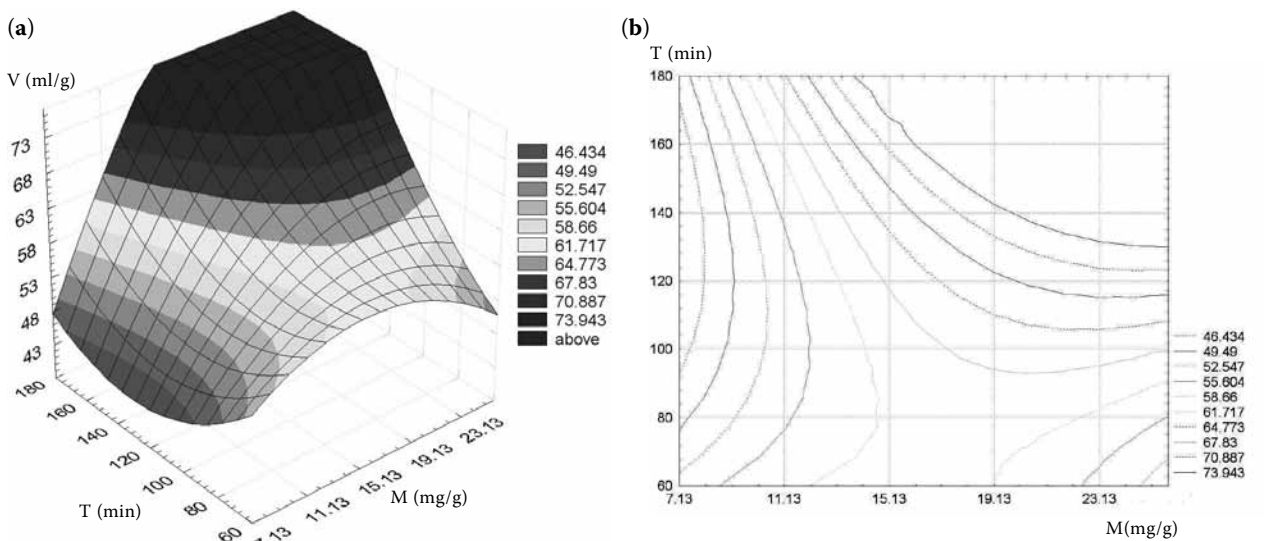


Figure 3. Variation of gas production (V) with maltose (M) and fermentation time (T): (a) spatial representation, (b) representation by contours curves

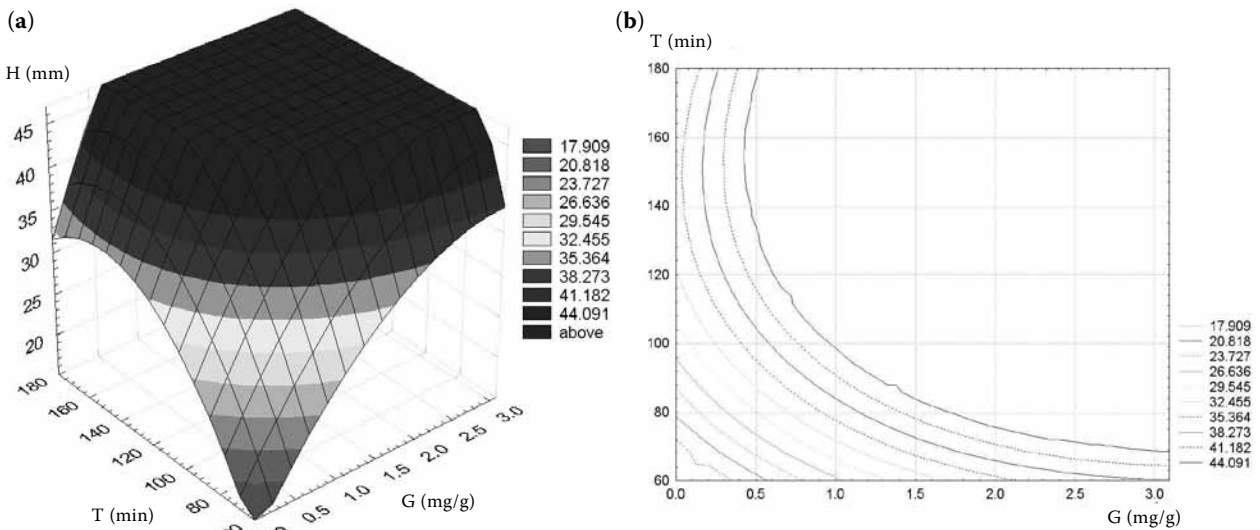


Figure 4. Variation of dough height (H) with glucose (G) and fermentation time (T): (a) spatial representation, (b) representation by contours curves

dough height is a measure of the amount of gas produced and therefore of the yeast fermentation capacity induced mainly by the amount of dough carbohydrates. Therefore, a very good correlation was obtained between the gas produced at 60 min of fermentation time (V_{60}) and dough height (H_{60}) ($r = 0.980$) and between the gas production at 120 min of fermentation (V_{120}) and dough height (H_{120}) ($r = 0.942$) at that moment. The graphical representation of the value of dough height (H) in relation to the level of glucose (G), fructose (F), and fermentation time (T) is shown in Figures 4 and 5, respectively. Depending on the maltose concentration and fermentation time, Figure 6 shows the variation of dough height. The dough height (H) shows the same trend as in the

case of gas production, with the distinction that at 180 min of fermentation, the decrease of dough height is much smaller than that of gas production.

A low negative correlation was obtained between the volume of gas production at 180 min of fermentation (V_{180}) and dough height (H_{180}) ($r = -0.264$) due to the gradual loss of gas retained in the dough, especially at the end of the dough fermentation process. At the same time, the values of correlation coefficients between the quantity of dough sugars and dough height at 60 and 120 min of fermentation follow the same trend as that between gas production and the quantity of sugars in wheat flour dough.

The correlations between the results of dough sucrose types during fermentation and the quantity of gas production, and the dough height at different

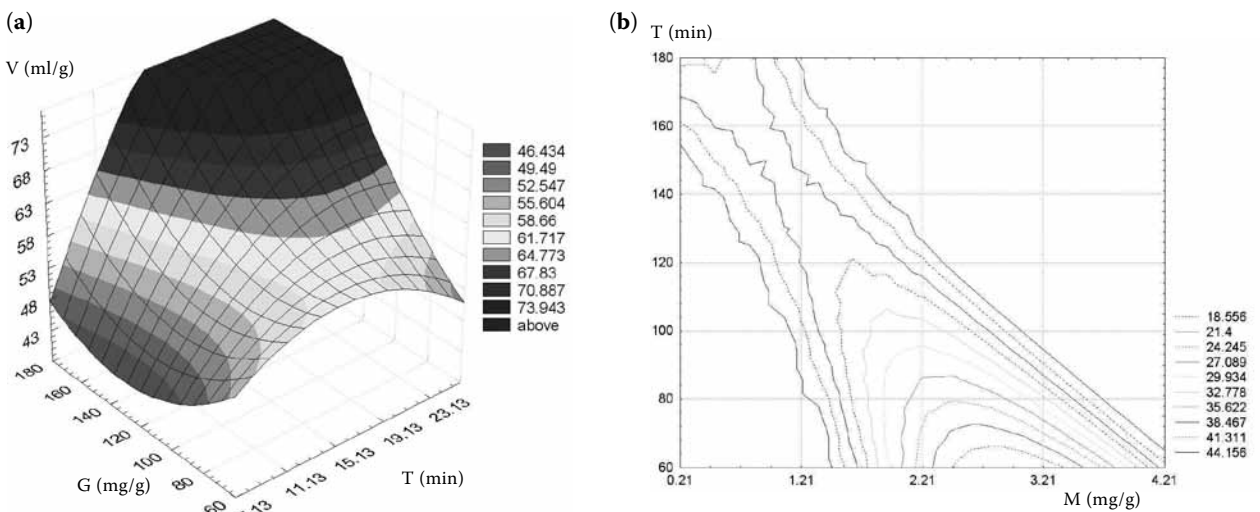


Figure 5. Variation of dough height (H) with fructose (F) and fermentation time (T): (a) spatial representation, (b) representation by contours curves

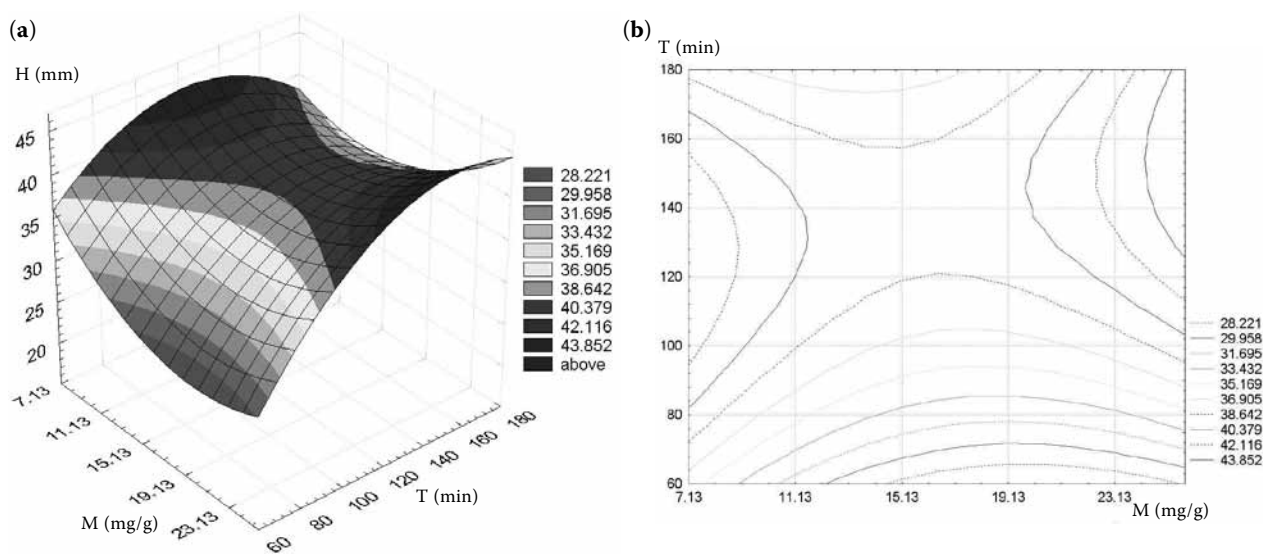


Figure 6. Variation of dough height (H) with maltose (M) and fermentation time (T): (a) spatial representation, (b) representation by contours curves

fermentation times are represented in Figure 7. The first two principal components explain 96.27% of the total variance (PC1 = 73.86% and PC2 = 22.41%).

In regard to the principal component PC1, we can notice a very good correlation between the glucose parameter (G₆₀) and dough volume (V₆₀) and dough height (H₆₀) at 60 min of fermentation. The position of these variables in relation to the first principal component shows that there is a very good correlation between them, which reflects the highest correlation coefficients, $r = 0.846$ and $r = 0.918$, respectively.

As to the second principal component PC2, we can notice the opposition between the fructose parameter at 120 min of fermentation (F₁₂₀) and the dough volume at that moment (V₁₂₀), as well as the opposition between the maltose parameter at 180 min of fermentation (M₁₈₀) and the dough

volume at that moment (V₁₈₀). Regarding the second principal component PC2, the parameters F₁₂₀ and M₁₂₀ are placed on the left of the graph, which shows that they contribute to the evaluation of dough behaviour at 120 min of fermentation time to a larger extent compared to the glucose parameter (G₁₂₀) at that moment. Using PCA, negative correlations were obtained between F₁₂₀ and V₁₂₀ ($r = -0.993$), H₁₂₀ ($r = -0.972$) on the one hand and positive correlations between M₁₈₀ and V₁₈₀ ($r = 0.822$) on the other hand.

CONCLUSIONS

The Principal Component Analysis of the data set shows a high association between some sugar types determined using an HPLC system and the

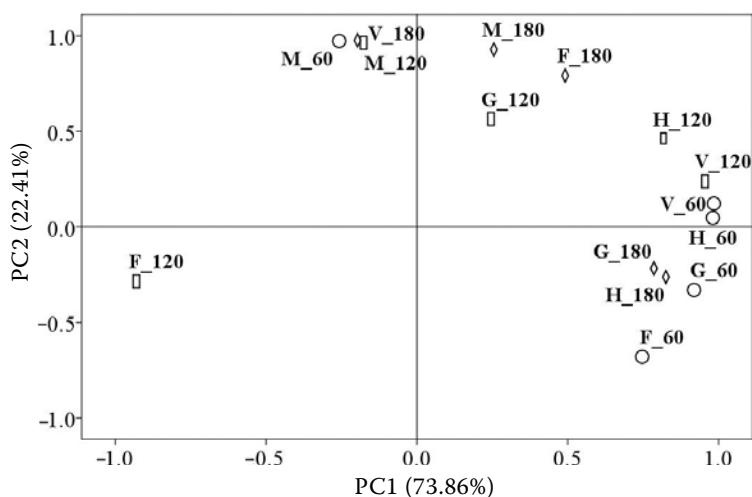


Figure 7. PC1 and PC2 score for the fermentable sugars types (F – fructose, M – maltose, G – glucose) during the fermentation, the gas production volume (V) and the dough height (H) at different fermentation times, respectively: to o – 60 min, □ – 120 min, ◇ – 180 min of fermentation

fermentation behaviour of the wheat flour dough at 60 and 120 min of fermentation time. The correlation established by PCA method between the parameters determined with the two devices highlighted the high positive correlation between the glucose parameter at 60 min of fermentation time (G_{60}) and gas production (V_{60}) ($r = 0.846$), and/or dough height (H_{60}) at this fermentation time ($r = 0.918$). Also, the fructose parameter at 120 min of fermentation time (F_{120}) was negatively correlated with gas production (V_{120}) ($r = -0.993$) and dough height (H_{120}) ($r = -0.972$) at that time.

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