ISSN 1330-9862 *original scientific paper*
(FTB-3433)

Influence of Mixing Speed on Dough Microstructure and Rheology

Georgiana Gabriela Codină and Silvia Mironeasa*

Ştefan cel Mare University of Suceava, Faculty of Food Engineering, Universităţii 13, RO-720229 Suceava, Romania

Received: May 9, 2013
Accepted: September 20, 2013

Summary

The influence of mixing speed (80, 160 and 250 rpm) on dough microstructure and dough rheology has been investigated using epifluorescence light microscopy and Mixolab device. Principal component analysis was used to highlight the association between some analytical characteristics of wheat flour and Mixolab parameters during the initial kneading process (1st stage) at different mixing speeds. Significant correlations were found between the gluten deformation index and some Mixolab parameters (dough development time, work input at maximum consistency during stage 1) at all used mixing speeds. For each of the mixing speeds used, a mathematical model was developed using dough consistency and work input at different processing times (1, 2, 3, 4 and 5 min) as variables. The dough consistency and time point parameters give the best explanation of the variation of the work input, at the same mixing speeds and times.

Key words: Mixolab, work input, mixing speed, wheat flour, epifluorescence light microscopy (EFLM)

Introduction

The rheological properties of wheat flour dough are essential for the quality of the finished bakery products. Dough has a unique viscoelastic behaviour which is obtained by combining flour, water and mixing energy (*1*). The mixing conditions and mixing intensity, the amount of induced energy and mixing time significantly influence the dough rheological properties, leading to an optimum dough development, incomplete development or overmixed dough (*2*,*3*). The effect of mixing conditions on the rheological properties of wheat flour dough has been widely studied (*3–6*) and traditional instruments (*e.g.* farinograph, mixograph) (*2–4*,*7–11*) or empirical ones (*1*,*5*,*12–19*) have been used. Recent studies are very useful but limited, as they are focused only on the influence of mixing conditions on the dough rheological properties at a certain moment during bread making and they do not provide us with an overview. The launching on the market in 2005 of Mixolab, a new apparatus for determining the dough rheological properties, has led to new information regarding the rheological behaviour of dough during bread making as this device makes a complex analysis of rheological properties of the wheat flour dough (*20–24*). It allows in one single test to determine the hydration capacity of flour, dough behaviour during mixing (development time, stability or softening), heating-up behaviour at enzyme activity intensification, protein coagulation, and starch gelatinization and retrogradation during final cooling. Therefore, the possibility of changing the mixing speed of the Mixolab device allows an extremely complex evaluation of the changes of the dough rheological properties during mixing and its behaviour during bread making. To our knowledge, the application of Mixolab device has not been evaluated yet for wheat dough systems, targeting rheological changes caused by different mixing speeds.

The changes of the rheological behaviour of dough induced by the mixing speed have a very high influence

*Corresponding author; Phone: ++40 230 216 147; Fax: ++40 745 727; E-mail: codina@fia.usu.ro, codinageorgiana@yahoo.com
Both authors contributed equally to this research

on dough microstructure. The wheat flour dough network has been studied using light microscopy (LM) (*25,26*), atomic force microscopy (AFM) (*27*), scanning electron microscopy (SEM) (*28–30*), transmission electron microscopy (TEM) (*31,32*), confocal scanning laser microscopy (CSLM) (*33–39*) and epifluorescence light microscopy (EFLM) (*35*). EFLM is the newest method used for the evaluation of dough microstructure and to our knowledge, its application to evaluate the wheat dough has been done only by Peighambardoust *et al.* (*35*). The objective of the present study is to investigate the effect of various mixing speeds on dough microstructure at the same mixing time, effect that to our knowledge has not yet been evaluated by using epifluorescence light microscopy by any other authors.

Therefore, we consider that a study of the effect of different mixing speeds on wheat flour dough by epifluorescence light microscopy and rheological measurements using a Mixolab device is very useful for a better understanding of the changes that occur during the mixing of dough and its behaviour during bread making. In order to reduce the time of the bread making technological process, more and more bakers are using mechanical mixers with high mixing speeds. Usually the mixing speed of the common laboratory device used to analyse the dough rheological properties during mixing is lower than the mixing speed used on the industrial scale, which from the industrial point of view may create difficulties in managing the process of dough development. Earlier studies (*2–4,7–11*) have been based on the effect of dough mixing speed, using the farinograph or the mixograph, for example. In this report, we used the Mixolab at three different mixing speeds on a range of Romanian wheat flour samples, of which mean values were measured and then EFLM was applied to get a better understanding of dough microstructure during its processing.

Materials and Methods

Flour samples

The research was carried out on ten samples of different commercial wheat flour (harvest 2010) milled on an experimental Bühler mill (no. MDDO 1250) from S.C. MOPAN S.A. (Suceava, Romania). The chemical composition of the flour was determined according to the Romanian or international standard methods: moisture (ICC Standard Method No. 110/1, 1982) (*40*), protein content (ICC Standard Method No. 202) (*40*), wet gluten content (ICC Standard Method No. 106/1, 1984) (*40*), gluten deformation index (SR Romanian Standard Method No. 90, 2007) (*41*), ash content (ICC Standard Method No. 104/1, 1990) (*40*) and falling number index (ICC Standard Method No. 107/1, 1995) (*40*).

Protein and wet gluten content, gluten deformation index, water absorption and moisture content were in the range of 11.9–16.1 %, 23.6–30 %, 2–8 mm, 56.3–60 % and 12.8–14.9 % respectively. These parameters correspond to different types of flour which cover a wide range of bread making quality, from strong to good, in order to use multivariate analyses (principal component analysis, PCA, and multiple regression analysis) for evaluating the effect of some Mixolab parameters at different mixing speeds on dough properties. The wheat flour ash content varied from 0.63 to 0.67, with an average value of 0.65, which is a typical ash content of bread flour in Romania. The falling number index value was in the range of 200–518 s, which corresponded to the flour with the α-amylase content ranging from high to low (*42*).

Physical characteristics of dough measured by Mixolab

Mixing and pasting behaviour of wheat flour dough was studied using the Mixolab device (Chopin, Tripette et Renaud, Paris, France) according to the standard method (ICC Standard Method No. 173) (*40*). The instrument analyses the protein quality and strength, amylolytic activity and starch gelling. The settings used in the study were: mixing temperature 30 °C for 8 min, then heating until 90 °C at a rate of 4 °C/min, holding at 90 °C for 7 min, cooling down to 50 °C for 10 min and finally holding at 50 °C for 5 min. During a Mixolab sample analysis, five different stages can be noticed. First the standard dough behaviour during mixing at 30 °C is recorded. When the dough temperature increases up to approx. 60 °C, the protein structure begins to destabilize, causing weakening of the protein stability. Then, when the dough temperature exceeds 60 °C, starch gelatinization takes place, leading to an increase of dough consistency. In the fourth stage, temperature is held at 90 °C and dough viscosity decreases due to the physical breakdown of the starch granules. In the last stage (the fifth stage), starch gelling occurs during the dough cooling, leading to starch gelatinization and retrogradation.

The Mixolab curves were registered at 80, 160 and 250 rpm at constant hydration. The amount of water added to the dough was determined on the basis of a known moisture content of the sample in order to achieve a dough consistency of 1.1 N·m.

Flour samples were evaluated for the following Mixolab parameters: dough stability (ST), *i.e.* time until the loss of consistency is less than 11 % of the maximum consistency reached during mixing, expressed in min; dough development time (DT), *i.e.* the time required to reach the maximum consistency during stage 1 (min); dough temperature during stage 1 (T_C1), *i.e.* dough temperature at maximum consistency during stage 1 (C1), expressed in °C; work input when mixing the dough to C1 torque (WI), *i.e.* the energy required to develop the dough until it reaches the maximum consistency during stage 1 (C1), expressed in (W·h)/kg; work input at the end of stage 1 (WIF), *i.e.* the energy required to develop the dough until the end of stage 1, expressed in (W·h)/kg; maximum consistency during stage 1 (C1), *i.e.* the maximum torque during mixing, expressed in N·m; minimum consistency during stage 2 (C2), *i.e.* the minimum value of torque representing the protein weakening based on the mechanical work and the increasing temperature, expressed in N·m; maximum consistency during stage 3 (C3), *i.e.* the maximum torque produced during the heating stage expressing the starch gelatinization, in N·m; minimum consistency during stage 4 (C4), *i.e.* the stability of the starch gel formed, expressed in N·m; maximum consistency during stage 5 (C5), *i.e.* starch retrogradation during the cooling stage, expressed in N·m; difference between points C1 and C2 (C1-2), *i.e.* the protein network strength under increased heating, in N·m; difference between points C3 and C2 (C3-2), *i.e.* the gelatinization rate, expressed in N·m; and difference between points C5 and C4 (C5-4), *i.e.* the indicator of bread shelf life, expressed in N·m.

Epifluorescence light microscopy

Dough samples obtained at various speeds of mixing (80, 160 and 250 rpm) during 3 and 5 min were used for EFLM observations. When applied for 3 min, these mixing speeds resulted in mechanical energy inputs of 5.01, 10.78, 19.96 (W·h)/kg and when used for 5 min of 8.70, 18.73 and 34.81 (W·h)/kg, respectively. The maximum value of dough development time for the wheat flour used in this study was 5.52, 3.62 and 2.48 min obtained at 80, 160 and 250 rpm, respectively. The collected samples were prepared according to Peighambardoust *et al.* (*35*) by staining them with 1 % rhodamine B and 0.5 % fluorescein in 2-methoxyethanol for at least 1 h. The dough samples were observed with Motic® AE 31 (Motic, Optic Industrial Group Co. Ltd., Xiamen, PR China) inverted epifluorescence microscope operated by catadioptric objectives LWD PH 20× (N.A. 0.4). The externally mounted 6V/30W halogen Koehler illumination system provides optimum illumination at any magnification. Raw images were acquired using Moticam 2300 digital colour camera. Digital images were taken in JPG format using RGB mode (12 bits per each channel) with a resolution of 1280×1024 pixels (300 dpi).

Image analysis

All samples were studied at high magnifications (image size of 1×1 mm), allowing better characterization of the sample microstructure. To quantify EFLM images with respect to the amount of protein matrix within the dough samples mixed at different speeds, ImageJ (v. 1.45, National Institutes of Health, Bethesda, MD, USA) image processing program was used. EFLM images were loaded into the software and RGB (red–green–blue) colour split was made. In some cases, the images were not sharp as a result of unwanted deviations or variations in the background light. To decrease these drawbacks, the unsharp mask filter at the moderate range (Gaussian blur, radius of 5 μm and Mask weight of 0.5) was applied to all EFLM images. The image properties: brightness, contrast, level and colour were also adjusted.

Statistical analysis

All results obtained from three replications were analysed using the Statistical Package for Social Sciences (v. 16, SPSS Inc., Chicago, IL, USA). The correlation analyses were performed at the probability levels of 95 and 99 %. The data showing the variation in the mixing speed according to the Mixolab parameters were plotted using the STATISTICA® statistical package (v. 6.0, StatSoft, Tulsa, OK, USA).

The selected method to analyse the interdependence among all the variables is the principal component analysis (PCA). PCA is a multivariate analysis technique that is intended to reduce the number of variables by preserving as much as possible the variance of the original data, resulting in a smaller set of variables. In this way, the new variables are uncorrelated, namely principal components are determined, expressed as a linear combination of the original variables and characterized by a maximum variability. The loading plot shows how much each variable contributes to each PC and is used for identification of trends, relations between variables, exploration of similarities, while allowing the detection of possible groups of variables. Multivariate analysis takes into account different mixing speeds (80, 160 and 250 rpm) in order to show all the interactions between the parameters of the samples and to highlight their combined effects.

The multiple regression analysis, a multivariate procedure of predicting the values of criteria based on several predictors (*43*), was used in order to highlight the linear combination of the time points and torque parameters at different mixing speeds and thus evaluate the wheat flour dough consistency when correlated with the work input.

The 'simultaneous' method (which SPSS calls the Enter method) was used to determine whether the work input at different mixing speeds of 80 (WI_80), 160 (WI_160) and 250 rpm (WI_250) was functionally related to wheat flour dough consistency for each time point during mixing in the first stage of the Mixolab curve (1, 2, 3, 4 and 5 min).

For multiple regression, the following equation was used:

$$Y'=b_0+b_1 \cdot X_1+b_2 \cdot X_2 \qquad /1/$$

where Y' represents the estimated value for the criterion variable, b_0 is the constant value, b_1 and b_2 are regression coefficients of the predictive model, X_1 and X_2 are the values of the predictor variables: X_1 represents time points at 80, 160 or 250 rpm, and X_2 represents torque at 80, 160 or 250 rpm. Eq. 1 contains predictor values (time in minutes during mixing speed at 80, 160 and 250 rpm: T_80, T_160 and T_250, and dough consistency in N·m for each time point: C_80, C_160 and C_250) and the coefficient b_i (i=0, 1, 2), which are calculated based on the correlation coefficient between each predictor and criterion (WI_80, WI_160 and WI_250), their value expressing the contribution of each predictor in estimating the criterion.

All the regression models were well correlated with the measured data and significant at $p<0.01$. Student's *t*-test was used to determine the significance of each coefficient in the regression model. Fisher's test for the analysis of variance was performed on experimental data in order to evaluate the statistical significance of the predictive models.

Results and Discussion

The effect of mixing speed on the EFLM analysis of dough

The microstructure of wheat dough mixed for 3 and 5 min at different speeds is shown in Fig. 1. We can clearly see from the obtained images how the amount of the protein matrix increased with energy addition and therefore with mixing time and speed. At lower mixing

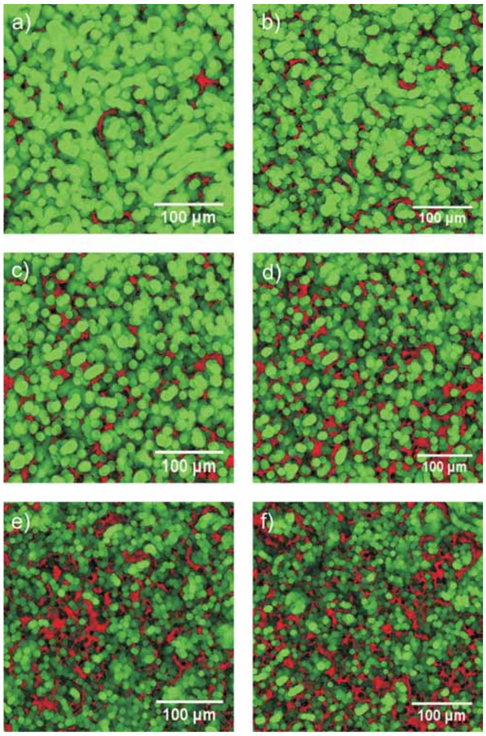

Fig. 1. Images of dough microstructure taken by EFLM: a) at 80 rpm for 3 min (5.01 (W·h)/kg), b) at 80 rpm for 5 min (8.70 (W·h)/kg), c) at 160 rpm for 3 min (10.78 (W·h)/kg), d) at 160 rpm for 5 min (18.73 (W·h)/kg), e) at 250 rpm for 3 min (19.96 (W·h)/kg), and f) at 250 rpm for 5 min (34.81 (W·h)/kg). Green are starch granules, while red is protein

time and speed, the swollen proteins just start to become interconnected. As dough is progressively developed by increasing mechanical energy input, the protein masses are stretched into a continuous network and surround most of the starch granules. Images show that the mixing of wheat flour dough at 80 (Figs. 1a and b) and 160 rpm (Fig. 1c) leads to the formation of distinct areas of starch granules which are the most visible, demonstrating structural inhomogeneity of the dough. In this image, discontinuous protein phases are distributed among starch granules, which predominate. With minimum mixing time and speed, even naked starch granules are seen, probably due to the incomplete hydration of the flour. The protein matrix formation is minimal in wheat flour dough mixed for 3 min at 80 rpm as compared to that in wheat flour dough mixed for 5 min at 80 rpm and wheat flour dough mixed for 3 min at 160 rpm. At 5.01 (W·h)/kg (Fig. 1a), it can be seen how gluten proteins are not yet distributed among the starch granules because the dough is partially developed. Various sizes of starch granules can also be seen covered with the protein network film, which increases with the addition of energy (*33*). Increasing the mixing time (5 min) and speed to 160 rpm and, therefore, dough mechanical energy input to 18.73 (W·h)/kg (Fig. 1d) transformed the discontinuous protein phase into a homogeneous one. The protein matrix became more and more evenly structured, being more evenly distributed around the starch granules. Mixing dough at higher speed (250 rpm) led to the formation of a very finely homogenous gluten structure with starch granules highly embedded in the protein matrix. The formed gluten fibres completely surrounded starch particles and other dough components, having extremely fine gluten strands and high resistance to breakage.

The effect of mixing speed on the dough physical characteristics obtained with a Mixolab

The results obtained for all the samples with the mean values and standard deviations are given in Table 1.

Table 1. Mixolab parameters of flour samples at different mixing speeds

Mixolab parameters	*v*(mixing)/rpm											
	80				160				250			
	Mean	Range min.	Range max.	S.D.	Mean	Range min.	Range max.	S.D.	Mean	Range min.	Range max.	S.D.
ST/min	8.3	4.2	9.6	1.1	5.1	3.0	8.5	1.7	3.4	1.9	4.7	1.0
DT/min	2.4	1.2	5.5	1.4	2.0	1.2	3.6	1.1	1.4	1.1	2.5	0.6
C1/(N·m)	1.1	1.1	1.1	0.01	1.4	1.1	1.9	0.2	1.6	1.2	2.0	0.2
T_C1/°C	30.3	28.5	31.8	1.1	31.7	29.2	33.6	1.4	33.0	29.9	34.8	1.6
C2/(N·m)	0.4	0.4	0.5	0.05	0.6	0.4	0.9	0.1	0.6	0.5	0.9	0.1
C1-2/(N·m)	0.7	0.6	0.8	0.05	0.9	0.6	1.1	0.1	1.0	0.7	1.3	0.1
C3/(N·m)	1.7	1.4	2.1	0.2	2.4	1.8	2.9	0.3	2.8	2.2	3.2	0.3
C3-2/(N·m)	1.2	1.0	1.5	0.2	1.8	1.4	2.1	0.2	2.2	1.7	2.6	0.2
C4/(N·m)	1.5	0.7	2.0	0.4	1.8	1.0	2.9	0.6	1.9	1.2	2.9	0.5
C5/(N·m)	2.2	0.9	3.2	0.7	2.6	1.4	4.7	1.0	3.7	1.8	5.4	1.2
C5-4/(N·m)	0.7	0.2	1.5	0.4	0.7	0.4	1.9	0.5	1.8	0.6	3.4	0.9
WI/((W·h)/kg)	2.1	1.4	2.8	0.5	14.5	12.1	19.6	2.3	16.7	13.4	20.0	2.3
WIf/((W·h)/kg)	13.4	7.2	18.1	4.2	24.0	16.0	32.6	5.2	28.9	25.4	33.5	2.7

min. and max. represent the mean values, minimum and maximum obtained for all the 10 flour samples analysed
Mixolab parameters: ST=stability; DT=development time; C1, C3, C5=maximum consistency during stages 1, 3 and 5; C2, C4=minimum consistency during stages 2 and 4; T_C1=temperature during stage 1; C1-2=difference between points C1 and C2; C3-2=difference between points C3 and C2; C5-4=difference between points C5 and C4; WI=work input when mixing the dough to C1 torque; WIf=work input at the end of the stage 1

A plot of good quality baking flour recorded by the Mixolab device at different mixing speeds (80, 160 and 250 rpm) is shown in Fig. 2.

The patterns obtained during mixing, pasting and gelling greatly varied with the mixing speeds used. Therefore, the variation in mixing speed modifies all the parameters registered on the curve by the Mixolab. It must be mentioned that the first stage of the Mixolab curve is the only one that provides information about the wheat flour dough rheological behaviour during the kneading stage comparable with those determined by other rheological devices like Brabender farinograph. The rest of the curve shows dough behaviour during heating and offers information about protein weakening, starch properties and flour enzyme activity, simulating its behaviour during baking process, thus reducing the labour requirements for testing. The correct level of α-amylase activity in wheat flour according to Codină *et al*. (*44*) can be established by Mixolab device. The stability of the starch gel (C4) and the difference between points C5 and C4 (C5-4) are the best indicators of falling number index assessment.

The graphical representation of the stability values in relation to the mixing speeds and the C1 values registered in the first stage of the Mixolab curve is shown in Fig. 3.

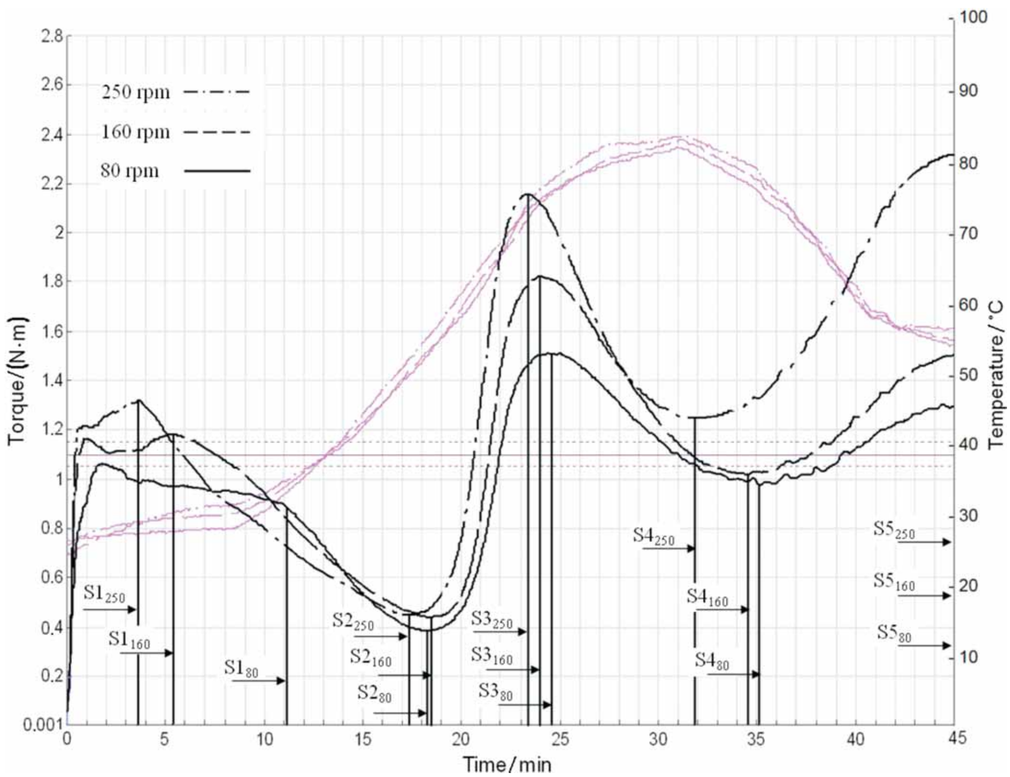

Fig. 2. Mixolab curves recorded at different mixing speeds (80, 160 and 250 rpm) for good quality baking flour
S1=initial mixing, S2=protein weakening, S3=starch gelatinization, S4=stability during baking, and S5=retrogradation are the stages of Mixolab curves; pink curves indicate dough temperature

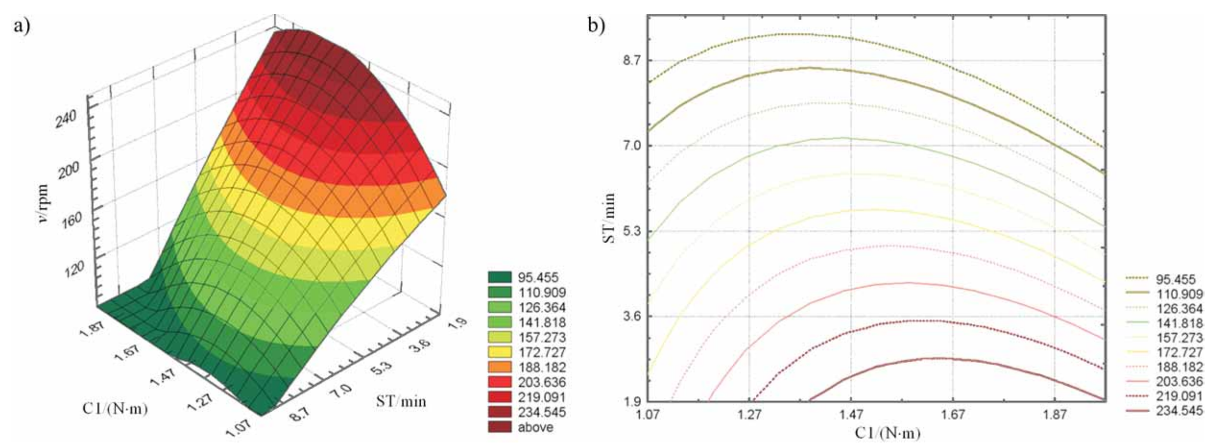

Fig. 3. Mixing speed (*v*) as a function of the torque (C1) and stability (ST): a) spatial representation, and b) representation by contour curves

The stability of the dough decreases (*6,9,45*) and C1 increases (*6,9,12*) simultaneously with the increase of the mixing speed. This can be attributed to the increase of the mixing speed, which leads to a higher depolymerisation of the gluten network, accompanied by a higher exposure to the surface of the reactive groups, capable of forming links with those of neighbouring molecules and, therefore, form a greater number of new interchain cross-links at new positions, including interchain disulphide bonds. The number of interchain disulphide bonds and the speed with which they are broken (from the disulphide (S-S) in sulphydril (S-H) bonds) also increase due to the energy input resulting from dough deformation (*46*).

Fig. 4 shows the values of the development time parameter according to the various mixing speeds and the temperature value in the first stage of the Mixolab curve. Increasing the mixing speed of the Mixolab had the effect of decreasing the dough development time (*8,9*) and increasing dough temperature. The temperature growth, depending on the mixing speed (*11*), is due to the conversion during kneading of a part of the mechanical energy into heat.

The second stage of the Mixolab curve corresponds to the initial heating of the dough. By increasing the dough temperature, its consistency decreases (*16*) to a minimum value (torque C2), which could be related to the beginning of the protein destabilization or weakening (*47*). Fig. 5 shows the variation of the difference between the points C1 and C2 (C1-2) according to the mixing speed change and the torque C2 value.

An increase in the value of C2 peak torque and the difference between the points C1 and C2 (C1-2) together with the increase of the mixing speed are due to the protein network structure. This is probably due to the fact that at low mixing speed, the protein network has a more compact structure as a consequence of a lower mechanical shear stress followed by an increase in dough temperature, involving the exposure to a low surface on which the proteolytic enzymes can act comparatively to a more developed structure, and so to a high surface exposure in the case of higher mixing intensity.

When dough is heated above 60 °C (third stage of the Mixolab curve), these wheat proteins are denatured, releasing some quantity of free water in the system. A higher mixing speed increases the amount of free water in the dough due to a more intense protein denaturation. This water, which the starch molecules take up and swell (*1,48*), leads to higher values of C3 torque and differences between the points C3 and C2 (C3-2) with the increase of the mixing intensity, as it is shown in Fig. 6.

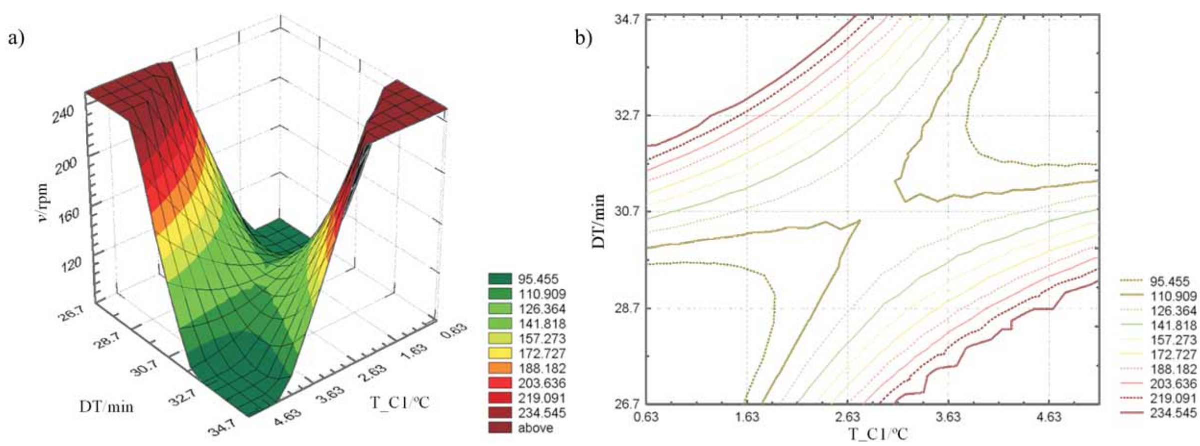

Fig. 4. Mixing speed (*v*) as a function of the temperature during dough development (T_C1) and development time (DT) in the first stage of the curve: a) spatial representation, and b) representation by contour curves

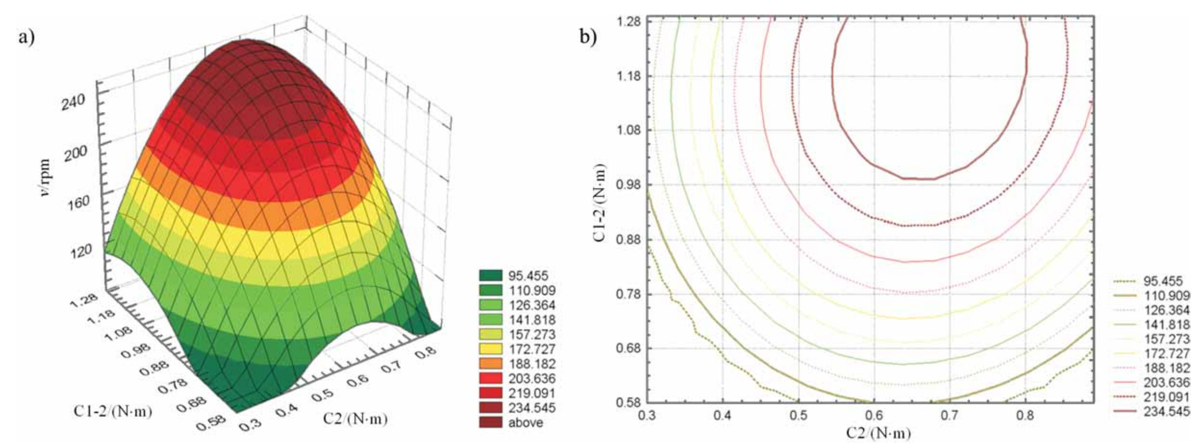

Fig. 5. Mixing speed (*v*) as a function of torque for the minimum consistency in the second stage of the curve (C2), and difference between points C1 and C2 (C1-2): a) spatial representation, and b) representation by contour curves

As the temperature increases, the role of proteins becomes secondary, the starch gelatinization being responsible for further torque variations (forth and fifth stage of the Mixolab curve) (*49*). With continued heating, starch granules become distorted, and soluble starch is released into the system. Soluble starch and the continued uptake of water (more available in the case of high mixing speed) by the remnants of the starch granules (*1*) are responsible for the increase in the C4 and C5 torque values and the difference between the points C5 and C4 (C5-4) with the increase of the mixing speed. In Figs. 7 and 8 the values of C4 and C5 parameters are reported as functions of the mixing speeds and the C5-4 value.

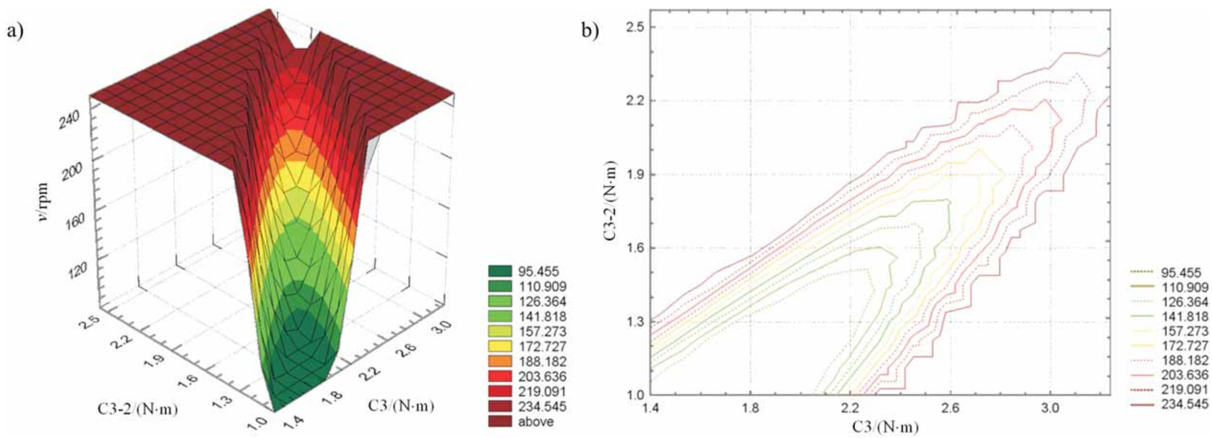

Fig. 6. Mixing speed (*v*) as a function of torque for the maximum consistency in the third stage of the curve (C3), and difference between points C3 and C2 (C3-2): a) spatial representation, and b) representation by contour curves

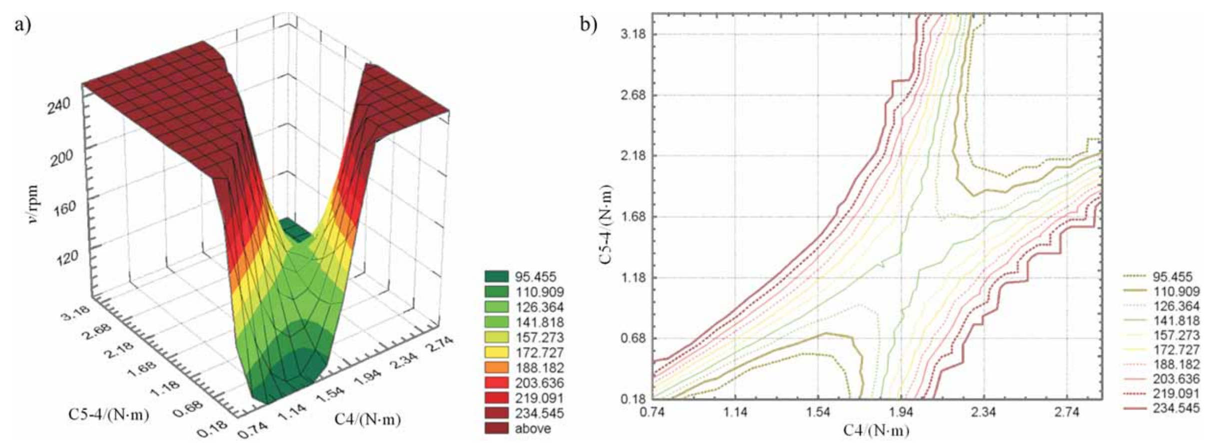

Fig. 7. Mixing speed (*v*) as a function of torque for the minimum consistency in the fourth stage of the curve (C4), and difference between points C5 and C4 (C5-4): a) spatial representation, and b) representation by contour curves

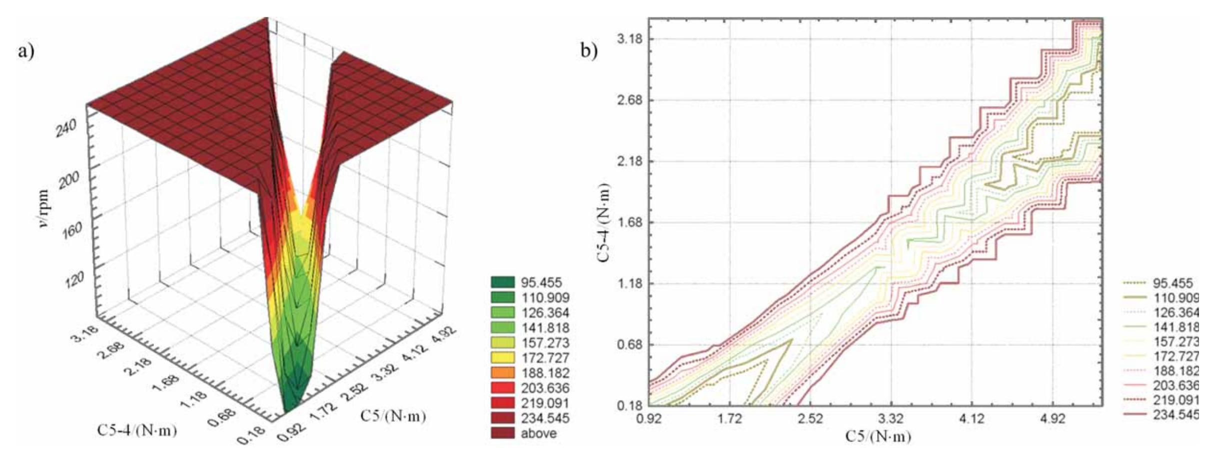

Fig. 8. Mixing speed (*v*) as a function of torque for the maximum consistency in the fifth stage of the curve (C5), and difference between points C5 and C4 (C5-4): a) spatial representation, and b) representation by contour curves

Relationship between the wheat flour and Mixolab curve parameters in the first stage at different mixing speeds

In order to identify the type of association between the flour parameters and its characteristics in the first stage of Mixolab curve at mixing speeds of 80, 160 and 250 rpm, the principal component analysis (PCA) was used. The obtained data showed that the first two principal components are responsible for 73.3 % of the variance, while the dispersion of the rest of the data could be considered as random. The first principal component (PC1) explained 55.5 % of the total variance and the second principal component (PC2) accounted for 18.9 % of the total sample variation (Fig. 9).

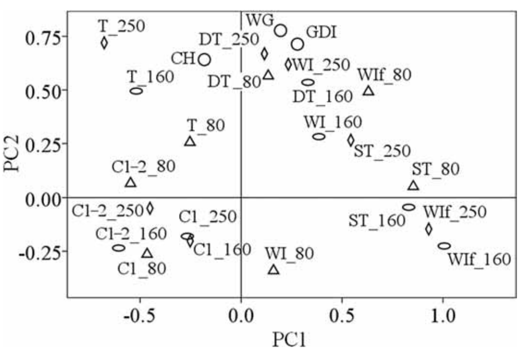

Fig. 9. PCA loadings of the Mixolab rheological parameters in the first segment of the curve at different mixing speeds and analytical characteristics of the wheat flour: C1_80, C1_160 and C1_250=maximum consistency during stage 1; C1-2_80, C1-2_160 and C1-2_250=difference between points C1 and C2; DT_80, DT_160 and DT_250=development time; ST_80, ST_160 and ST_250= dough stability; T_80, T_160 and T_250=time of mixing; WI_80, WI_160 and WI_250=work input when mixing the dough to C1 torque; WIf_80, WIf_160 and WIf_250=work input at the end of stage 1; where 80, 160 and 250 denote mixing speed in rpm; CH=water absorption and WG=wet gluten

The PC1 is mainly influenced by the dough stability and the work input shown at the end of the first stage (WIf) at various mixing speeds, whereas the PC2 is mainly influenced by the wheat gluten, gluten deformation index and dough development time at all the mixing speeds studied. The bi-plot of the loadings shows a very high influence of protein 'quality' (in our study given by wet gluten content and deformation index) on dough mixing properties and on its final consistency (*2,9–12,18,25, 50*). Work input at maximum consistency in the first stage of the curve (WI) and dough development time (DT) are higher as the flour is stronger (*18*). Along the PC2 loading component, DT_250 and WI_250 are closely associated with gluten deformation index, and inversely correlated with C1_250.

Therefore, between gluten deformation index (GDI) and work input at maximum consistency in the first stage of the curve (WI), higher positive correlations were obtained with the increase of the mixing speed. Significant direct correlations ($p<0.05$) were obtained between the GDI and the work input at 160 rpm (R=0.761) and between the GDI and the work input at 250 rpm (R=0.774). The dough development time was significantly positively correlated with gluten deformation index at all the mixing speeds used: at a level of $p<0.01$, between the dough development time at 80 rpm and GDI, R=0.878, and between development time at 160 rpm and GDI, R=0.825, at a level of $p<0.05$, between dough development time at 250 rpm and GDI, R=0.675. This fact is explained by dough consistency of higher viscosity, which leads to an increase in the dough resistance to mixing (*12*). Therefore, in less time it absorbs more energy than the dough of low consistency, which requires longer mixing time to consume the same amount of energy. So, positive correlations between the dough development time and work input at maximum consistency as can be seen in the first stage of the curve were obtained. These correlations improved dramatically when the speed of the Mixolab was increased to 160 or 250 rpm. The development time at 80 rpm had insignificant correlation with WI_80 at the probability level of 95 %, whereas DT_160 and DT_250 showed significant correlation with WI_160 (R=0.750) and WI_250 (R=0.711) respectively, at a level of 0.05. However, DT_80 is inversely correlated with C1_80 and closely correlated with GDI.

Along the PC1 component, the dough stability and work input parameters at the end of the first stage show a close association and are inversely correlated with C1 torque and the difference between points C1 and C2 (C1-2) at the used mixing speeds. Higher correlations were obtained between the stability at 80 rpm and work input at the end of the first stage at 80 rpm (R=0.711, p=0.05), and between the stability at 160 rpm and work input at the end of the first stage at 160 rpm (R=0.826, p=0.01). Regarding the dough stability at 250 rpm, this variable shows low correlation with work input at the end of the first stage at 250 rpm (R=0.254).

Significantly negative correlations were obtained between the stability at 80 rpm and the difference between points C1 and C2 at 80 rpm (R=–0.789, $p<0.05$), between the stability at 160 rpm and the difference between the points C1 and C2 at 160 rpm (R=–0.841, $p<0.01$). Regarding the correlations obtained between the work input at the end of the first stage and the difference between points C1 and C2, significant correlations were obtained only between the work input at the end of the first stage at 160 rpm and the difference between points C1 and C2 at 160 rpm (R=–0.678, $p<0.05$).

The second component (PC2) makes the distinction between the wet gluten and work input at 80 rpm, which are opposed. Wet gluten was heavily weighed on the second PC. Along the PC2 component, the parameters obtained at the speed of 160 rpm: maximum consistency during stage 1, difference between points C1 and C2, stability and work input at the end of stage 1 are inversely correlated with development time and work input when mixing the dough to C1 torque. Regarding PC2, the parameters: water absorption (CH), maximum consistency in the first stage of the curve (C1) and the difference between the points C1 and C2 at all the mixing speeds placed on the left of Fig. 9 show that they contribute to a larger extent to the evaluation of the association between some analytical wheat flour characteristics and the Mixolab parameters, at different mixing speeds, in comparison with the variables on the right. PC2 distinguishes C1 torque from the stability at all the mixing speeds used. Significant correlations were obtained between the stability at 160 rpm and C1 torque at 160 rpm (R=–0.766, p<0.05), and between the stability at 250 rpm and C1 torque at 250 rpm (R=–0.815, p<0.01). The dough stability decreases and C1 torque increases simultaneously with the mixing speed increase, which is in agreement with the results obtained by Zounis and Quail (*9*) and Hwang and Gunasekaran (*45*), on other rheological devices. From the point of view of C1 torque, significant direct correlations with the difference between the points C1 and C2 were obtained: C1_160 and C12_160 (R=0.804, p<0.01), and C1_250 and C12_250 (R=0.688, p<0.05).

The multivariate prediction models

In this study, the effect of mixing speed at 80, 160 and 250 rpm was analysed at comparable levels of work input. The mixing speed experiments were performed at different processing times (1, 2, 3, 4 and 5 min) leading to different dough consistency and work inputs into the dough. The variables inserted in the regression model were the dough consistency and work input at different mixing speeds (80, 160 and 250 rpm) for each time point (at 1, 2, 3, 4 and 5 min). The mean values and standard deviations for each variable included in the construction of the linear multivariate regression model are shown in Table 2.

The accuracy of each regression model was evaluated by the determination coefficient (R^2), by the value of the adjusted coefficient of determination and by the standard errors of the estimate (Table 3).

The value of the determination coefficient (R^2=0.87) for model 1 (at 80 rpm) shows that 87 % of the variance of the work input at 80 rpm is applied to the linear combination of dough consistency during kneading at 80 rpm and time points at 80 rpm variables. This model explains 86 % (R^2_{adj}=0.86) of the variation of the work input at the speed of 80 rpm parameter, the global effect having a high level (*50*). The value of the determination coefficient for model 2 (at 160 rpm; R^2=0.92) and 3 (at 250 rpm; R^2=0.91) shows that 92 % of the variance of the work input at 160 rpm and 91 % of the variance of the work input at 250 rpm is applied to the linear combination of the variables dough consistency and mixing time at the speed of 160 rpm, and dough consistency and mixing time at the speed of 250 rpm respectively. The values of the adjusted determination coefficients (R^2_{adj}= 0.92 and 0.90) show that model 2 explains 92 % of the

Table 2. The variables considered in the multiple regression analysis

	Regression model variables						
	WI_80 (W·h)/kg	WI_160 (W·h)/kg	WI_250 (W·h)/kg	C_80 N·m	C_160 N·m	C_250 N·m	T_80, T_160, T_250/min
Mean value±S.D.	4.8±2.8	14.5±7.6	23.9±1.2	1.04±0.04	1.4±0.2	1.4±0.2	3.0±1.4

Variables of the regression model: WI_80, WI_160 and WI_250=work input; C_80, C_160 and C_250=dough consistency for each time point; T_80, T_160 and T_250=time of mixing at the speed of 80, 160 and 250 rpm

Table 3. Significant coefficients (95 % confidence interval) of the commercial wheat flour of the 'simultaneous' regression fitting model for work input at different mixing speeds of Mixolab on wheat flour dough consistency for each time point using the 'Chopin S' protocol

Factors	Work input at different mixing speeds					
	WI_80/((W·h)/kg) Model 1		WI_160/((W·h)/kg) Model 2		WI_250/((W·h)/kg) Model 3	
	Value	SE	Value	SE	Value	SE
CTE	10.25	3.30	–17.40	2.21	–21.40	3.54
T_80/min	1.75	0.10	–	–	–	–
T_160/min	–	–	4.99	0.22	–	–
T_250/min	–	–	–	–	7.66	0.37
C_80/(N·m)	–10.23	3.12	–	–	–	–
C_160/(N·m)	–	–	12.40	1.46	–	–
C_250/(N·m)	–	–	–	–	15.43	2.11
R	0.93		0.96		0.95	
R^2	0.87		0.92		0.91	
R^2_{adj}	0.86		0.92		0.90	
SE of estimate	1.00		2.13		3.44	

Independent variables were time points at 80 rpm (T_80), 160 rpm (T_160) and 250 rpm (T_250), and dough consistency during kneading at the mixing speed of 80 rpm (C_80), 160 rpm (C_160) and 250 rpm (C_250); CTE=constant of the fitted equation, R=correlation coefficient, R^2=coefficient of determination, R^2_{adj}=adjusted square coefficient of the fitting model, SE=standard error variation of the work input at 160 rpm, and model 3 explains 90 % of the variation of the work input at 250 rpm, the global effect having a very high level (*51*). The ANOVA results showed the efficiency of each of the three regression models. Since the value of the statistical significance of F test is small ($p<0.0001$), it can be concluded that the models presented in Table 3 are significant, the dough consistency and time points (at 1, 2, 3, 4 and 5 min) at different mixing speeds (80, 160 and 250 rpm) give the best explanation of the variation of the work input, at the same mixing speed and time.

The values of the regression coefficients b_i of the regression equations for the three models and the corresponding standard errors are given in Table 3. All regression coefficients are statistically significant at the level of 0.01.

For all three models, at all mixing speeds used, the work input when mixing the dough increased with the increase of the mixing time. These results are in agreement with those of Cuq *et al.* (*3*) (measured by the farinograph). The increase of the mixing time and thus of the dough work input led to an exponential increase of the resistance of the dough consistency to deformation (*11*) until the optimal mixing time was achieved, corresponding to the maximum consistency in the first stage of the curve, followed by a decrease in these values, almost simultaneously with the Mixolab curves. This fact is due to the depolymerisation of the gluten network and it is characterized by a partial solubilisation of the insoluble gluten proteins (*52*). Maximal consistency point during the first stage was reached faster when the dough was mixed more intensively, which is in agreement with the results obtained in the studies by Zheng *et al.* (*5*) and Muchová and Žitný (*6*) on other rheological devices.

Conclusions

This paper highlights for the first time the effect of various mixing speeds on the dough microstructure during the same mixing time using a fluorescence technique. Regarding dough microstructure studied by epifluorescence light microscopy (EFLM), the images taken at various mixing speeds differed clearly. At 250 rpm, the protein network looked more compact and continuous than at 160 and 80 rpm. The amount of starch granules decreased to a certain extent in wheat flour dough with the increase of the mixing speed.

Varying the mixing speed of the Mixolab device allowed us to obtain information about the dough behaviour at different stages of bread making, which were more comparable than those used by the industrial mixers. Increasing the mixing speed of the Mixolab had a high impact on most of the tested rheological parameters. Therefore, the dough development time and its stability decreased, whereas the parameters related to protein weakening (C2 and C1-2), starch gelatinization (C3 and C3-2), amylolytic activity (C4) and starch gelling (C5 and C5-4) increased as a consequence of the increase of the mechanical shear stress of wheat flour dough induced by the increase of the mixing speed.

The principal component analysis of the data set shows a high correlation between gluten deformation index and development time. The dough stability was also positively correlated with the work input at the end of stage 1, but negatively associated with the C1 torque at all the mixing speeds used.

The obtained regression models indicate that the wheat flour dough consistency at each time point (1, 2, 3, 4 and 5 min) can be used to predict the dough work input at all the Mixolab mixing speeds of 80, 160 and 250 rpm, at the 0.01 level of significance.

By using the Mixolab, this study gave more insight into the dough rheology during mixing as well as its weakening under high-temperature regimes that mimic the baking conditions to some extent. Although this study was performed with ten flour samples with highly variable baking potential, more assays are needed with more samples having either high or low baking potential.

Acknowledgements

The authors thank to S.C. Enzymes & Derivates S.A., Romania, for technical support.

References

1. D.T. Campos, J.F. Steffe, P.K.W. Ng, Rheological behavior of undeveloped and developed wheat dough, *Cereal Chem. 74* (1997) 489–494.
2. R.S. Anderssen, P.W. Gras, F. MacRitchie, The rate-independence of the mixing of wheat flour dough to peak dough development, *J. Cereal Sci. 27* (1998) 167–177.
3. B. Cuq, E. Yildiz, J. Kokini, Influence of mixing conditions and rest time on capillary flow behaviour of wheat flour dough, *Cereal Chem. 79* (2002) 129–137.
4. R.H. Kilborn, K.H. Tipples, Factors affecting mechanical dough development. I. Effect of mixing intensity and work input, *Cereal Chem. 49* (1972) 48–53.
5. H. Zheng, M.P. Morgenstern, O.H. Campanella, N.G. Larsen, Rheological properties of dough during mechanical dough development, *J. Cereal Sci. 32* (2000) 293–306.
6. Z. Muchová, B. Žitný, New approach to the study of dough mixing processes, *Czech J. Food Sci. 28* (2010) 94–107.
7. Ž. Kurtanjek, D. Horvat, D. Magdić, G. Drezner, Factor analysis and modelling for rapid quality assessment of Croatian wheat cultivars with different gluten characteristics, *Food Technol. Biotechnol. 46* (2008) 270–277.
8. J.R. Oliver, H.M. Allen, The prediction of bread baking performance using the farinograph and extensograph, *J. Cereal Sci. 15* (1992) 79–89.
9. S. Zounis, K.J. Quail, Predicting test bakery requirements from laboratory mixing tests, *J. Cereal Sci. 25* (1997) 185–196.
10. A.R. Wooding, S. Kavale, F. MacRitchie, F.L. Stoddard, Link between mixing requirements and dough strength, *Cereal Chem. 76* (1999) 800–806.
11. A.J. Wilson, M.P. Morgenstern, S. Kavale, Mixing response of a variable speed 125 g laboratory scale mechanical dough development mixer, *J. Cereal Sci. 34* (2001) 151–158.
12. A.M. Janssen, T. van Vliet, J.M. Verijken, Rheological behavior of wheat gluten at small and large deformations. Comparison of two glutens differing in bread making potential, *J. Cereal Sci. 23* (1996) 19–31.
13. J.J. Kokelaar, T. van Vliet, A. Prins, Strain hardening properties and extensibility of flour and gluten doughs in relation to breadmaking performance, *J. Cereal Sci. 24* (1996) 199–214.

14. K.M. Lindborg, C. Tragardh, A.C. Eliasson, P. Dejmek, Time-resolved shear viscosity of wheat flour doughs – Effect of mixing, shear rate, and resting on the viscosity of doughs of different flours, *Cereal Chem. 74* (1997) 49–55.

15. M. Safari-Ardi, N. Phan-Thien, Stress relaxation and oscillatory tests to distinguish between doughs prepared from wheat flours at different varietals origin, *Cereal Chem. 75* (1998) 80–84.

16. V.K. Rao, S.J. Mulvaney, J.E. Dexter, Rheological characterisation of long- and short-mixing flours based on stress-relaxation, *J. Cereal Sci. 31* (2000) 159–171.

17. M.C. Puppo, A. Calvelo, M.C. Anon, Physicochemical and rheological characterization of wheat flour dough, *Cereal Chem. 82* (2005) 173–181.

18. R. Kuktaite, H. Larsson, S. Marttila, E. Johansson, Effect of mixing time on gluten recovered by ultracentrifugation studied by microscopy and rheological measurements, *Cereal Chem. 82* (2005) 375–384.

19. A. Angioloni, D.M. Rosa, Effects of cysteine and mixing conditions on white/whole dough rheological properties, *J. Food Eng. 80* (2007) 18–23.

20. C. Collar, C. Bollain, C.M. Rosell, Rheological behaviour of formulated bread doughs during mixing and heating, *Food Sci. Technol. Int. 13* (2007) 99–107.

21. K. Kahraman, O. Sakiyan, S. Ozturk, H. Koksel, G. Sumnu, A. Dubat, Utilization of Mixolab to predict the suitability of flours in terms of cake quality, *Eur. Food Res. Technol. 227* (2008) 565–570.

22. S. Ozturk, K. Kahrman, B. Tiftick, H. Koksel, Predicting the cookie quality of flours by using Mixolab, *Eur. Food Res. Technol. 227* (2008) 1549–1554.

23. S. Heo, S.M. Lee, I.Y. Bae, H.G. Park, H.G. Lee, S. Lee, Effect of *Lentinus edodes* β-glucan-enriched materials on the textural, rheological, and oil-resisting properties of instant fried noodles, *Food Bioprocess Technol. 6* (2011) 553–560.

24. R. Moreira, F. Chenlo, M.D. Torres, B. Rama, Influence of the chestnuts drying temperature on the rheological properties of their doughs, *Food Bioprocess Technol. 91* (2013) 7–13.

25. K. Autio, M. Salmenkallio-Marttila, Light microscopic investigations of cereal grains, doughs and breads, *LWT-Food Sci. Technol. 34* (2001) 18–22.

26. M.T. Holtzapple: Lignin: Structure and Analysis. In: *Encyclopedia of Food Science, Food Technology, and Nutrition*, R. Macrae, R.K. Robinson, M.J. Sadler (Eds.), Academic Press, London, UK (1993) pp. 2731–2738.

27. A.R. Kirby, A.P. Gunning, V.J. Morris, Atomic force microscopy in food research – A new technique comes of age, *Trends Food Sci. Technol. 6* (1995) 359–365.

28. D. Indrani, P. Prabhasankar, J. Rajiv, G.V. Rao, Scanning electron microscopy, rheological characteristics, and bread-baking performance of wheat-flour dough as affected by enzymes, *J. Food Sci. 68* (2003) 2804–2809.

29. A.D. Roman-Gutierrez, S. Guilbert, B. Cuq, Description of microstructural changes in wheat flour and flour components during hydration by using environmental scanning electron microscopy, *LWT-Food Sci. Technol. 35* (2002) 730–740.

30. I.C. Bache, A.M. Donald, The structure of the gluten network in dough: A study using environmental scanning electron microscopy, *J. Cereal Sci. 28* (1998) 127–133.

31. M.P. Lindsay, J.H. Skerritt, The glutenin macropolymer of wheat flour doughs: Structure-function perspectives, *Trends Food Sci. Technol. 10* (1999) 247–253.

32. V. Kontogiorgos, H.D. Goff, S. Kasapis, Effect of aging and ice-structuring proteins on the physical properties of frozen flour-water mixtures, *Food Hydrocoll. 22* (2008) 1135–1147.

33. L. Lee, P.K.W. Ng, J.H. Whallon, J.F. Steffe, Relationship between rheological properties and microstructural characteristics of nondeveloped, partially developed, and developed doughs, *Cereal Chem. 78* (2001) 447–452.

34. S.H. Peighambardoust, A.J. van der Goot, T. van Vliet, R.J. Hamer, R.M. Boom, Microstructure formation and rheological behaviour of dough under simple shear flow, *J. Cereal Sci. 43* (2006) 183–197.

35. S.H. Peighambardoust, M.R. Dadpour, M. Dokouhaki, Application of epifluorescence light microscopy (EFLM) to study the microstructure of wheat dough: A comparison with confocal scanning laser microscopy (CSLM) technique, *J. Cereal Sci. 51* (2010) 21–27.

36. D. Peressini, S.H. Peighambardoust, R.J. Hamer, A. Sensidoni, A.J. van der Goot, Effect of shear rate on microstructure and rheological properties of sheared wheat doughs, *J. Cereal Sci. 48* (2008) 426–438.

37. T.J. Schober, S.R. Bean, D.L. Boyle, S.H. Park, Improved viscoelastic zein-starch doughs for leavened gluten-free breads: Their rheology and microstructure, *J. Cereal Sci. 48* (2008) 755–767.

38. M. Jekle, T. Becker, Dough microstructure: Novel analysis by quantification using confocal laser scanning microscopy, *Food Res. Int. 44* (2011) 984–991.

39. J.C.G. Blonk, H. Vanaalst, Confocal scanning light-microscopy in food research, *Food Res. Int. 26* (1993) 297–311.

40. Standard Methods of the International Association for Cereal Chemistry, ICC Nos. 110/1, 106/1, 104/1, 107/1, 202, 173, International Association for Cereal Science and Technology, Vienna, Austria (2010).

41. Wheat flour, Analysis Method SR 90, Romanian Standards Association (ASRO), Bucharest, Romania (2007).

42. V. Nechita, Correction of flours with α-amylase deficit taking into account dough's damage, *Food Environ. Saf. 7* (2008) 8–10.

43. D.C. Howell: *Statistical Methods for Psychology*, Wadsworth-Cengage Learning, Belmont, CA, USA (2007) pp. 517.

44. G.G. Codină, S. Mironeasa, C. Mironeasa, Variability and relationship among Mixolab and falling number evaluation based on influence of fungal α-amylase addition, *J. Sci. Food Agric. 92* (2012) 2162–2170.

45. C.H. Hwang, S. Gunasekaran, Determining wheat dough mixing characteristics from power consumption profile of a conventional mixer, *Cereal Chem. 78* (2001) 88–92.

46. H. Mirsaeedghazi, Z. Emam-Djomeh, S.M.A. Mousavi, Rheometric measurement of dough rheological characteristics and factors affecting it, *Int. J. Agric. Biol. 10* (2008) 112–119.

47. C.M. Rosell, E. Santos, C. Collar, Physical characterization of fiber-enriched bread doughs by dual mixing and temperature constraint using the Mixolab, *Eur. Food Res. Technol. 23* (2010) 535–544.

48. A. Angioloni, D.M. Rosa, Dough thermo-mechanical properties: influence of sodium chloride, mixing time and equipment, *J. Cereal Sci. 41* (2005) 327–331.

49. C.M. Rosell, C. Collar, M. Haros, Assessment of hydrocolloid effects on the thermo-mechanical properties of wheat using the Mixolab, *Food Hydrocoll. 21* (2007) 452–462.

50. J. Lefebvre, N. Mahmoudi, The pattern of the linear viscoelastic behaviour of wheat flour dough as delineated from the effects of water content and high molecular weight glutenin subunits composition, *J. Cereal Sci. 45* (2007) 49–58.

51. J. Cohen: *Statistical Power Analysis for the Behavioral Sciences*, Lawrence Erlbaum Associates, Hillsdale, NJ, USA (1988) pp. 426–433.

52. F. Bèkès, P.W. Gras, R.B. Gupta, D. R. Hickman, A.S. Tatham, Effects of a high M_r glutenin subunit (1Bx20) on the dough mixing properties of wheat flour, *J. Cereal Sci. 19* (1994) 3–7.