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Prediction of rye dough behaviour and bread quality using response surface methodology

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Bread making is a hydro-thermal process; therefore, knowing the behaviour of the main constituents of the flour at different temperatures allows control of the quality of the end-product. Mixing and thermal characteristics were studied using the Mixolab system and response surface methodology was used to investigate the influence of particle size distribution of the flour, pH and the addition of hemicellulase enzyme on the thermo-mechanical behaviour of the whole rye flour and on bread quality. A central composite face-centered design, with two levels of fineness modulus (1.78 and 1.26), two levels of pH (6.5 and 3.8) and three levels of added enzyme (0, 50 and 100 mg/kg of flour), was used. The results indicated that thermo-mechanical variables – water absorption, development time, dough stability, protein weakening, starch gelatinization, starch gelling and cooling setback – as well as bread quality are influenced by the three factors investigated.

Significant correlations were found between water absorption and pH, enzyme level, fineness modulus and their interactions. Dough stability was significantly influenced by all the independent variables, as well as by the interaction between pH and fineness modulus. Starch gelling and cooling setback were influenced by the interaction between pH and enzyme level. Concerning bread quality, both porosity and specific volume were affected by enzyme level and pH, as well as by the interaction between fineness modulus and enzyme level.

Keywords: bread quality; enzyme; fineness modulus; Mixolab; pH; rye flour

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Introduction

Starch characteristics, α-amylase activity, and the concentration of arabinoxylans and the presence of the enzymes used for their hydrolysis (Weipert 1995; Repeckiene *et al*. 2001; Gomez *et al*. 2009), are the main quality factors influencing rheological properties of the rye dough.

Starch is the major contributor to the structure of rye bread, but the role of proteins is also important (Wannerberger *et al*. 1997). The dough structure is mainly built up by pentosans and proteins at temperatures of 30 °C, whereas starch plays a major role at temperatures above 45 °C (Grabner 1999). The role of starch in rye dough is affected by pH, dough composition and formulation, enzymatic activity, and the extent of starch degradation (Pomeranz *et al*. 1984). Rye starch gelatinizes at a lower temperature than wheat starch (Gudmundsson and Eliasson 1991), and also shows a lower retrogradation tendency (Fredriksson *et al*. 1998). The influence of acids and of variations in pH on the properties of dough, or its constituents, has been examined in several studies (Armero and Collar 1996; Clark *et al*. 2002).

Other components that play a key role in dough rheology and bread quality are the arabinoxylans. Water-extractable arabinoxylans have beneficial effects on the processability of the rye dough and the resulting bread as they increase the viscosity of the dough, improve bread volume and delay starch retrogradation (Vinkx and Delcour 1996; Rasmussen *et al*. 2001; Courtin and Delcour 2002; Primo-Martín and Martínez-Anaya 2003).

While rye flour contains only small concentrations of endogenous enzymes that degrade arabinoxylans, several commercially available enzyme preparations can be added in baking. Characteristics, such as substrate specificity for water extractable or unextractable arabinoxylans and the activity levels of individual enzymes, indicate the use of these preparations for specific applications. Endoxylanases with specificity for water unextractable arabinoxylans are usually used to reduce crumb firmness and increase the specific volume of bread, whereas endoxylanases with specificity for water extractable arabinoxylans or enzyme-solubilised arabinoxylans are used to reduce specific volume (Poutanen *et al*. 1995; Vinkx and Delcour 1996; Rasmussen *et al*. 2001; Courtin and Delcour 2002).

Rye proteins do not form a gluten network, but they seem to be important during the dough mixing step since they have some aggregation abilities (Field, Shewry and Miflin 1983) and are surface active (Wannerberger *et al*. 1997).

Bread making is a hydro-thermal process and therefore information on the temperature-dependent behaviour of the most important flour constituents is relevant for controlling the quality of the end product.

The objectives of the present study were to use response-surface methodology to study the influence of flour-particle size distribution, pH and enzyme addition on the thermo-mechanical behaviour of rye flour. The Mixolab system was used to measure the evolution of the bread during the entire technological process, from the dough making to starch retrogradation. Mixolab is a relatively new device used to describe the rheological behaviour of dough subjected to a dual mixing and temperature constraint, and enables the measurement of the real-time torque produced, by dough mixing, between two kneading arms (Haros, Ferrer and Rosell 2006; Collar, Bollain and Rosell 2007; Rosell, Collar and Haros 2007; Marco and Rosell 2008; Kahraman *et al*. 2008; Ozturk *et al*. 2008).

Materials and Methods

Materials

The rye grain used was purchased from a local market (Galati, Romania) and was used to prepare two types of flour for use in the study. The fineness modulus of these two flours was determined according to Godon and Willm (1994). The first flour was obtained from the rye that was ground in a laboratory mill with a disc and had a fineness modulus of 1.78. The particle size distribution of the whole rye flour was determined according to Asro (2008) using a Retsch sieve shaker with a set of standard sieves (500, 180 and 125 μm). This flour consisted of a 26.5% fraction with particle size >500 μm, a 45.0% fraction with a particle size between 500 and 180 μm, an 8.1% fraction with particle size between 180 and 125 μm, and a 20.4% fraction with particle size <125 μm.

In order to obtain the second flour, the fraction with particle size >500 μm was ground in a laboratory mill (Perten Mill 3100) to reduce the bran particle size but avoid damage to the starch granules. The resulting fractions were blended with the remaining fractions from the first milling to get a flour with fineness modulus of 1.26, consisting of a 0.5% fraction with particle size >500 μm, a 50.7% fraction with particle size between 500 and 180 μm, a 23.4% fraction with particle size ranging between 180 and 125 μm, and a 25.4% fraction with particle size <125 μm.

The enzyme preparation Pentopan BG 500 (Novozymes®, Denmark) was used to vary hemicellulase activity. This enzymatic product is obtained by submerged fermentation of *Humicola insolens* and has a declared pentosanase and hemicellulase activity of 2700 Farvet Xylan Units per gram.

The required pH values of the samples were adjusted using food grade lactic acid (80% lactic acid; Sigma-Aldrich, UK).

Methods

Thermo-mechanical behaviour: The whole rye flour properties were studied using the Mixolab device (Chopin, Tripette and Renaud, France). The operating parameters of the device during the tests are specified in Table 1, and a typical curve generated by the Mixolab device is shown in Figure 1. The variables recorded were: water absorption required to get a constant torque of 1.1 Nm (maximum torque during mixing; C1 in Figure 1), and torque values at key points indicated in Figure 1 as: C2 (the torque associated with protein weakening based on the mechanical work and temperature), C3 (reflects starch gelatinization), C4 (indicates the stability of the starch gel formed) and C5 (measures starch gelling during the cooling stage). Cooling setback was calculated as C5 – C4, and defines the amylase activity.

Baking test: This test was performed according to Repeckiene *et al.* (2001). The dough was prepared by mixing 0.25 kg flour, water (according to the water absorption indicated by the Mixolab, and equals the quantity of water that the flour can absorb to achieve the consistency of

Table 1. Settings of the Mixolab

Parameter	Value
Mixing rate (rpm)	80
Dough weight (g)	75
Tank temperature (°C)	30
Temperature of the first plateau (°C)	30
Duration of the first plateau (min)	8
Temperature of the second plateau (°C)	90
First temperature gradient (°C/min)	4
Duration of the second plateau (min)	7
Second temperature gradient (°C/min)	−4
Temperature of the third plateau (°C)	50
Duration of the third plateau (min)	5
Total analysis time (min)	45

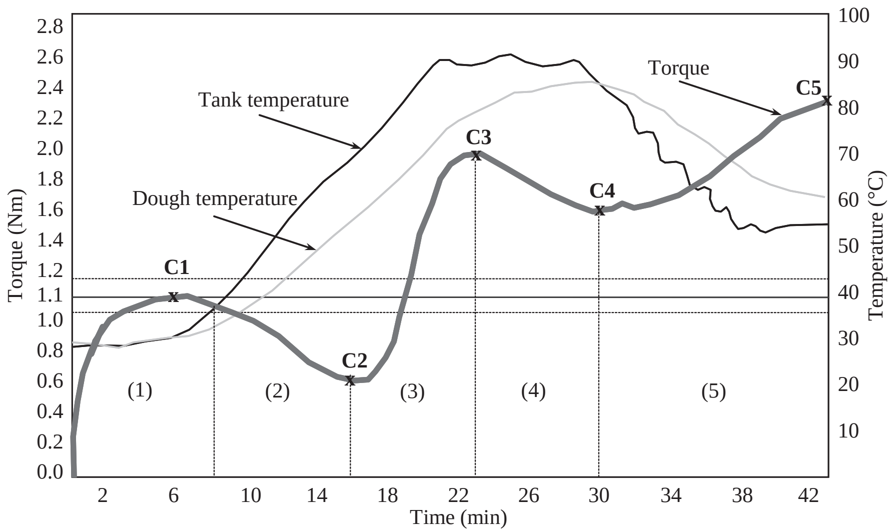

Figure 1. Typical curve recorded by the Mixolab. The stages in the process are: (1) = dough development (constant temperature 30 °C); (2) = thermal weakening of the proteins; (3) = starch gelatinization; (4) = enzymatic activity at constant heating rate; (5) = starch gelling. —— indicates the torque of 1.1 Nm based on which water absorption is established; – – – horizontal lines represent the tolerance limits required for acceptable consistency.

1.1 (± 0.05) Nm during the constant temperature (30 °C) phase), NaCl (15 g/kg flour) and yeast (30 g/kg flour), and kneading the mixture by hand for 3 min. The dough was then fermented in a laboratory leavener for 60 min at 28 °C. After floor time the dough was divided into 2 pieces (0.2 kg each). These pieces were moulded by hand and then proofed for 50 min at 30 °C in a pan (base area of pan was 6 cm × 6 cm, height was 7 cm, and top area 8 cm × 8 cm). The samples were then baked at 186 °C for 45 min, and cooled at room temperature for 75 min before measuring weight and specific volume (ASRO 2008). Bread specific volume was determined through the rapeseed displacement method using a bread volumeter (Fornet, Chopin, France). Image analysis was used to estimate bread porosity. Breads were cut into two halves vertically and the cut side of one of the halves was placed over the glass of a scanner (HP Scanjet 5470C, USA) having a resolution of 300 dpi. The scanned colour images were first converted to grey scale and analyzed using the Image J software (http://rsb.info.nih.gov/ij; Datta *et al*. 2007) that uses the contrast between the two phases (pores and solid part) in the image. Pixel values were converted into distance units using bars of known length. The largest possible rectangular cross-section of the bread halves was cropped and, after adjusting the threshold, the pore area as fraction of total area was determined using the software.

Experimental design

A central composite face-centered design was used to investigate the effects of particle size distribution, pH and enzyme addition, and their interactions, on the thermo-mechanical behaviour of the rye flour. Design factors tested were: two levels of particle size distribution/fineness modulus (X_1; +1 and −1 corresponding to fineness moduli of 1.26 and 1.78, respectively), two values of dough pH (X_2; +1 and −1 corresponding to pH values of 6.5 and 3.8, respectively), and three levels of enzyme addition (X_3; +1, 0 and −1 corresponding to additions of 0, 50 and 100 mg/kg of flour, respectively). The design resulted in 12 different treatment combinations. The parameters of the Mixolab curve (water absorption, development time, dough stability, protein weakening, starch gelatinization, starch gelling and cooling setback), specific volume and the bread porosity were taken as response variables.

The influence of independent variables on the response variables was estimated (using StatSoft STATISTICA 8.0.360) based on F test of the response terms in the third-order interaction model:

$$Y=\beta_0+\beta_1 X_1+\beta_2 X_2+\beta_3 X_3+\beta_{12} X_1 X_2+\beta_{13} X_1 X_3+\beta_{23} X_2 X_3+\beta_{123} X_1 X_2 X_3,$$

where Y represents the predicted response; β_0 is the intercept, $\beta_1, \beta_2, \beta_3$ are regression coefficients for the design factors and β_{12}, β_{13}, β_{23}, β_{123} are the coefficients for the cross-product terms. The significance of each coefficient was tested at a significance value of 0.05.

The model equation was used to build three-dimensional response surface plots for each dependent variable. The goodness of the fit was expressed as R^2.

Results and Discussion

The application of the response surface model yielded the regression equations set out in Table 2 for the empirical relations between the parameters of the Mixolab curve, or the bread quality estimators, and the independent variables.

Table 2. Equations for the empirical relations between parameters of the Mixolab curve or bread quality estimators and the independent variables†

Dependent variable	Regression equation	R^2
Water absorption	$75.067 - \mathbf{11.180}X_1 + \mathbf{0.221}X_2 - \mathbf{0.391}X_3 + 0.344\mathbf{X_1X_2} + \mathbf{0.221}X_1X_3 + 0.128\mathbf{X_2X_3} - \mathbf{0.078}X_1X_2X_3$	0.997
Development time	$-5.724 + \mathbf{5.012}X_1 + \mathbf{1.422}X_2 + 1.336X_3 + \mathbf{1.140}X_1X_2 + 1.056X_1X_3 - 0.213X_2X_3 + 0.168X_1X_2X_3$	0.996
Dough stability	$-10.911 + \mathbf{11.132}X_1 + \mathbf{1.444}X_2 + \mathbf{0.214}X_3 - 0.970X_1X_2 + \mathbf{0.166}X_1X_3 - 0.041X_2X_3 + 0.032X_1X_2X_3$	0.997
Protein reduction (C2)	$0.340 + \mathbf{0.540}X_1 - 0.033X_2 - \mathbf{0.120}X_3 + 0.042X_1X_2 + 0.004X_1X_3 - 0.002X_2X_3 + 0.002X_1X_2X_3$	0.994
Starch gelatinization (C3)	$2.396 - 0.153X_1 - \mathbf{0.850}X_2 + 0.078X_3 + 0.075X_1X_2 - 0.041X_1X_3 - 0.015X_2X_3 + 0.006X_1X_2X_3$	0.993
Starch gelling (C5)	$7.541 - 3.106X_1 - 1.084X_2 - \mathbf{0.398}X_3 + 0.647X_1X_2 + 0.305X_1X_3 + \mathbf{0.679}X_2X_3 - 0.059X_1X_2X_3$	0.953
Cooling setback (C5-C4)	$0.220 + 0.322X_1 - \mathbf{0.336}X_2 - \mathbf{0.063}X_3 - 0.012X_1X_2 - 0.054X_1X_3 + \mathbf{0.109}X_2X_3 - 0.011X_1X_2X_3$	0.976
Porosity	$1.629 - 0.732X_1 - \mathbf{0.181}X_2 - \mathbf{0.057}X_3 + 0.107X_1X_2 - \mathbf{0.066}X_1X_3 + 0.010X_2X_3 - 0.010X_1X_2X_3$	0.949
Specific volume	$\mathbf{1.711} - \mathbf{0.099}X_1 - \mathbf{0.027}X_2 + \mathbf{0.059}X_3 + 0.002X_1X_2 - \mathbf{0.061}X_1X_3 - 0.003X_2X_3 - 0.001X_1X_2X_3$	0.974

† X_1 = flour fineness, X_2 = dough pH, X_3 = level of enzyme addition. Regression coefficients shown in bold were statistically significant ($P < 0.05$).

The water absorption was significantly negatively influenced by the fineness modulus. The results obtained indicate a higher water absorption capacity for the sample with a fineness modulus of 1.26. The lower the particle size distribution of bran, implying a high fineness modulus, allowed greater water absorption, due to the larger surface area of particles.

On the other hand, the water absorption was positively influenced by pH and negatively by enzyme addition. Unlike wheat dough, where the water absorption depends mainly on the protein quantity and quality, in the case of rye dough water absorption and holding capacity are highly influenced by the concentration of pentosans. Moreover, the pentosans influence water distribution in the dough. In order to improve the water absorption capacity of the dough, enzyme preparations with both pentosanase and hemicellulase activity can be used. The present results indicate that enzyme addition at levels of 100 mg/kg of flour, generated polysaccharides with lower molecular weight and characterized by lower water absorption properties. On the other hand, when considering the interaction between enzyme level and fineness modulus, a positive effect on water absorption is evident (Figure 2a). Moreover, the mutual interaction between particle size distribution, pH value and enzyme addition significantly influenced water absorption capacity of the dough.

The development time was positively influenced by the particle size distribution and pH, as well as by the mutual interaction between these factors. At a pH of 3.8 samples with fineness modulus of 1.78 were characterised by a lower development time compared to that when pH was 6.5 (Figure 2b). The influence of the granularity of fractions on dough development time was noted previously by Haros *et al*. (2006), who showed that developing time was reduced for fractions with a granularity below 125 μm, while fractions with a granularity in the range 180 to 200 μm have higher development time.

Dough stability represents length of time, at a temperature of 30 °C, that the torque remains at 1.1 Nm. The dough stability was positively influenced by the fineness modulus, pH and enzyme level. Higher pH causes structural changes in the dough as a consequence of the influence on the physico-chemical properties of its main constituents (proteins, starch, and hemicelluloses), and the activity of protease and amylase. The influence of pH and ionic strength on mixing behaviour of dough was reported previously by Clarke, Schober and Arendt (2002) and Thiele *et al*. (2002). In addition, the dough stability was positively influenced by the interaction between fineness modulus and enzyme (Figure 2c), as a consequence of the greater access of enzymes to the substrate in samples with a higher degree of grinding.

When mixing is excessive, and the temperature increases, the torque decreases (stage (2) in Figure 1) and this could be related with the beginning of protein destabilization and unfolding (Rosell *et al*. 2007). In the case of rye, changes in dough behaviour during heating from 30 to 42 °C were related to enzymatic hydrolysis and redistribution of water in the dough. According to our results the minimum torque (C2) depended on the particle size distribution. Very rigid particles, such as those of protein, starch and arabinoxylans, are dispersed in the weak continuous phase. Moreover, the addition of the enzyme causes a reduction in the C2 torque. During baking, enzymatic hydrolysis and physical changes of the arabinoxylans in the continuous phases occurs and leads to dough softening (Autio *et al*. 1999).

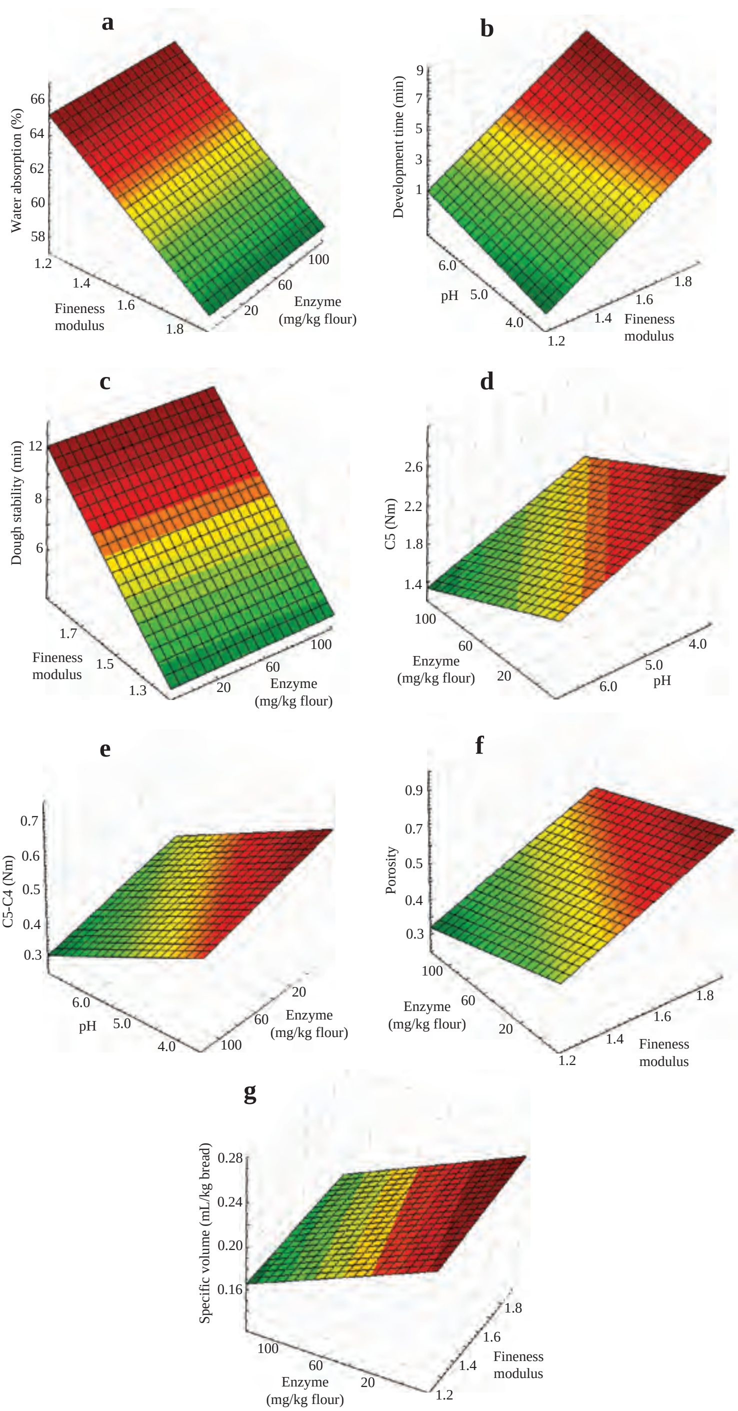

Figure 2. Response surface plots showing the interaction effects among the different design factors for the principal dependent variables.

As the temperature increases (stage (3) in Figure 1) starch granules absorb water and swell, causing the viscoelasticity (C3) to increase (Harros *et al*. 2006). The present results indicate that there is a significant negative correlation between pH and C3.

Mechanical shear stress and temperature bring about the physical breakdown of the granules and a reduction in the torque (minimum torque, C4, stage (4) in Figure 1). Further reduction in temperature causes the torque to increase again due to enhanced dough resistance as a consequence of starch gelling (maximum torque, C5, stage (5) in Figure 1). A negative correlation between enzyme addition and C5 was obtained, indicating that the resulting low molecular-weight pentosans positively influence bread quality in terms of crumb firmness. Moreover the interaction between enzyme level and pH had a significant positive influence on starch gelling (Figure 2d).

The cooling setback (C5 – C4) was negatively influenced by pH and enzyme level, whereas the interaction between these factors was positively correlated with C5 – C4 (Figure 2e).

Bread quality was estimated through porosity and specific volume. As for the cooling setback, the bread porosity was negatively influenced by the pH and enzyme level, and in addition, was negatively related with the interaction between enzyme level and fineness modulus (Figure 2f). Specific volume was negatively influenced by pH, fineness modulus and their interaction (Figure 2g), and positively by the level of enzyme addition. Concerning the influence of pH, the findings in the present study are in agreement with Clarke *et al*. (2002) who reported higher values of bread specific volume when lactic acid was added to the dough.

Conclusions

Due to the widespread consumption of rye bread in many countries of the world, the knowledge of the optimal parameters to be used for rye flour processing is very important.

The response surface methodology employed provided information on the effects of the fineness modulus of the rye flour, pH and enzyme addition, and the interactions among these factors, on the rheological properties of the rye dough and bread quality. These results allow optimization of the rye bread making process in order to get a better quality of the final product.

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