THE INFLUENCE OF GLUCOSE OXIDASE AND PEROXIDASE ON RHEOLOGICAL PROPERTIES OF DOUGHS OBTAINED FROM WEAK FLOURS

Alexandru Stoica*
VALAHIA University of Târgovişte, Faculty of Environmental Engineering and Food Science,
Departament of Food Products Engineering, Unirii Bd., 18-24, 130082, Târgovişte, Romania
*E-mail: stoicaandi@yahoo.com

Abstract
Weak flours possess extremely deformable gluten, a characteristic that is transmissible to dough and which determines poor quality products, with low volume and porosity. In traditional technology, correcting these flours implies the use of chemical oxidants. The tendency to give up using these chemical additives, led to new solutions - the use of natural agents, enzymes from Oxidoreductases class.
The scope of the present scientific study was to test the manner in which the addition of exogenous oxidoreductases influences the rheological properties of dough varieties obtained from weak flours. With that end in view, two enzymatic preparations were used, namely, glucose oxidase and peroxidase, both of fungal origin. In order to characterize the tested flours, some features such as moisture, ash content, wet gluten content, gluten deformation index and Falling Number were determined. As for determining the rheological properties of dough, the alveographical method was used. This method use justification lies in the singleness of the method approach, this method being the only one able to achieve a biaxial extension of the tested piece of dough.
The results proved that supplementing the dough varieties obtained from weak flours with peroxidase and glucose oxidase has as an effect the decrease of extensibility and the increase of elasticity. These changings influence the increase of dough capacity to retain the gasses resulted from fermentation, with positive consequences on the resulted bread volume and porosity.

Keywords: weak flours, dough, rheological properties, glucose oxidase, peroxidase.

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1. INTRODUCTION

Rheology deals with the determining of flow and elastico-plastic properties of the systems (*Dobraszczyk and Morgenstern, 2003*), such a system being dough obtained from wheat flours (*Janssen et al., 1996*). The dough is a complex matrix which consists mainly of gluten, starch and water, being a typical viscoelastic system. The rheological tests were successfully applied on the wheat flour doughs as indicators of the gluten and starch molecular structure (*Dobraszczyk and Morgenstern, 2003*). It was noticed that there is a direct connection between the structure, the rheological properties of dough and the quality of the product (*Angioloni and Collar, 2008*).
The quality of bakery products is extensively determined by the quality and quantity of flour proteins. During dough kneading, the flour is hydrated and the glutenic proteins interact and make a proteic cohesive network – the gluten (*Caballero et al., 2007*), which is able to retain the gasses resulted from the process of alcoholic fermentation. The gliadins give viscosity and extensibility to the dough while the glutenins determines its elasticity (*Junqueira et al., 2007*).
One considers that the main role in gluten structure forming belongs to the intermolecular disulfidic bonds and the existing ratio between the free sulfhidril groups and the disulfidic bonds is conclusive for the rheological properties of dough (*Bordei, 2004*).
In breadmaking, there is a tendency of eliminating the chemical oxidants (potassium bromate, potassium iodate, azodicarbonamide, calcium peroxide, benzoyl peroxide etc). Their role may be taken by the enzymes and, because the improvement of performances in the breadmaking process of weak flours promoted by chemical oxidants is due to oxidation induced reactions on the dough compounds, the enzymes belonging to the Oxidoreductases class are concerned.

The oxidative enzymes have a strong impact on the sulfhidril/disulfide ratio and, consequently, on the dough properties (*Goesaert et al., 2005*). Glucose oxidase (E.C. 1.1.3.4) is, at present, an alternative solution to the use of chemical oxidant agents in breadmaking. The hydrogen peroxide resulted from the glucose oxidase catalyzed reaction promotes the disulphidic bonds forming between the glutenic proteins and oxidative gelation of water-soluble pentosans (*Caballero et al., 2007*). Another effect is that the hydrogen peroxide activates the flour catalase and peroxidase (*Michon ş.a., 2004*). Other researchers state that the enzyme addition to the dough leads to the occurance of transversal bonds between wheat globulines and albumines, while the glutenins are less involved (*Xu, 2005*). The calalyzed reaction of glucose oxidase occurs during dough kneading.
Peroxidase represents another interesting option to ameliorate the quality of weak flours (*Nicolas and Potus, 1999*). Peroxidase, along lipoxigenase and catalase, is naturally found in wheat flours (*Nicolas and Potus, 1999*). The addition of exogenous oxidoreductases induces not only the total enzyme activity increase in doughs, but also a protection effect of the endogenous enzymes during kneading. The main beneficiary of this effect is wheat extractable peroxidase which, during kneading, shows a remarcable stability (*Rakotozafy et al., 1999*). Peroxidase may cause the oxidative gelation of pentosans and/or protein polymerization (*Nicolas şi Potus, 1999*).

2. MATERIALS AND METHODS

Materials

Flours. For the performing experiments, FA1 and FA2 flours were used; these are white flours whose determined characteristics are briefly presented in Table 1.
According to the gluten deformation index and gluten index values, FA1 flour is *unsatisfactory*, while FA2 flour is *satisfactory* for breadmaking.
Fungal glucose oxidase (trade name Oxygo – produced by *Genencor International*). The preparation derived from *Aspergillus niger* is in the form of a yellow-green powder, having a density of 500g/l and a humidity of 5-8%. Besides the glucose oxidase activity, the preparation has a catalasic activity as well.
Fungal peroxidase (trade name *Belpan POX* – produced by *S.C. Enzymes & Derivates România S.A.*). The preparation derived from *Aspergillus niger* is in the form of a white-yellow powder, having a humidity less than 10% and an ash content less than 1,4% (dry weight basis).

Methods

The flour quality indexes were determined by the use of the following methods: humidity – the oven drying method; ash content – the calcination method at 900-920^{0}C; wet gluten content – the dough washing method with NaCl 2% solution; gluten deformation index - the method of maintaining a gluten sphere at 30^{0}C for one hour; α-amylase activity in flours – the method using the Falling Number device.

Table 1. Quality attributes of wheat flour

Flour Code	Moisture (%)	Ash content (% dry weight basis)	Wet gluten content (%)	Gluten deformation index (mm)	Glutenic index	Falling Number (sec.)
FA1	13,30	0,51	27,68	24	12,18	298
FA2	13,42	0,61	27,74	18	23,02	355

The rheological properties of dough were evaluated by *alveographical method* (*ICC Method No.121, AACC 54-30A, ISO No 5530/4).* The method implies the swelling of a dough disk in the form of a bubble up to breaking; it allows an analysis unique approach. During swelling, the piece of dough is stretched in two directions: along the parallel and along the meridian of the bubble. This type of deformation is called biaxial extension and is unique among dough rheological tests. Practically, the biaxial extension simulates the deformation type which occurs during the dough fermenting and proofing (*Bramble, 2005*).

The usual parameters obtained from an alveogram are as follows:

- P – the overpressure (mmH_2O); usually, the P value is seen as a *value of dough tenacity and resistance to deformation,* this one being related to the maximum pressure reached during swelling, and being representative for the *elastic properties* of dough;
- L – the length of the curve (mm), from the dough swelling point to the breaking point. It is a *value of the dough extensibility*.
- P/L – the configuration ratio; it is an alveogram shape approximation, by combining the dough tenacity (P) with its extensibility (L).
- G – swelling index; the conducted studies interrelate the value of G with the dough extensibility; there is a direct proportionality between L and G, given by the relation $G = 2{,}226\sqrt{L}$.
- W – it represents the energy needed to swell the dough till breaking. The value of W is a value of flours quality, often being used as basic indicator of the dough behavior in technological process.

Baking tests were conducted by means of automatic Moulinex machines, the basic recipe being: flour – 300g, yeast – 9g, salt – 4,5g, water – the quantity used when obtained the highest value of the control bread volume.

The control dough was supplemented with enzymes preparations.

The weight and volume of the resulted bread were determined 2 hours later since the end of baking, and the porosity was determined 20 hours later.

3. RESULTS AND DISCUSSION

The influence of glucose oxidase addition upon rheological properties of dough.

In order to determine the glucose oxidase effects upon the rheological properties of dough, described by alveogram parameters, both the flour FA1 and FA2 were supplemented with enzyme.

Out of the data given in Table 2, it can be observed that the glucose oxidase addition - whose catalytic action carried out with the participation of oxygen and glucose - leads to formation of hydrogen peroxide which activates endogenic peroxidase and catalase - results in the increase of maximum pressure P in case of dough varieties resulted from both flours compared to the control samples. The increase of dough resistance thanks to indirect intervention of the enzyme is predictable, thus confirming the hypothesis that glucose oxidase produces a strengthening effect of the gluten network from dough.

Table 2. Alveogram parameters for doughs made from FA1 and FA2 flours, unsupplemented (control samples) or supplemented with different doses of glucose oxidase

SAMPLE	Alveogram parameters				
	P [mmH_2O]	L [mm]	G	P/L	W [$10e^{-4}$J]
Control sample FA1 (CS FA1)	86	48	15,4	1,79	144
CS FA1 + 3ppm GOX*	93	46	15,1	2,02	138
Control sample FA2 (CS FA2)	101	34	13,0	2,97	133
CS FA2 + 5ppm GOX	182	30	12,2	6,06	215

*Glucose oxidase

This effect will be, for sure, of a smaller proportion in case of dough used in baking tests, where the oxygen content available to develop the catalytic activity of the enzyme is diminished because of the yeast (the dough prepared by alveographical method does not contain yeast).

The dough extensibility, described by parameter L of alveogram, shows a reduction tendency by glucose oxidase intervention, even if the changing is not as drastic as in case of maximum pressure. Also, the glucose oxidase addition to dough varieties obtained from the two types of flour leads to the increase of P/L ratio.

Although the changings resulted from the action of enzyme upon the parameter W may seem contradictory, they find their explanation in the different characteristics and conditions of the dough alveographically analyzed, compared to the one obtained when the baking test was conducted. Also, the particularities of technological regime adopted to conduct these tests must be taken into account especially that the dough is kept in a pan all baking long and the shape keeping task is partially taken over by the pan.

From Table 3 one can notice that by adding glucose oxidase to FA1 flour leads to an increase of 5% both in the volume and the porosity of the obtained bread. In case of flour FA2, the volume increase remains the same and the porosity gets to 3%.

The influence of peroxidase addition upon rheological properties of dough.

A comparison between the control sample alveogram parameters obtained from FA1 flour and those ones obtained after its being supplemented with fungal peroxidase was done.

Contrary to those opinions asserting that the alveographical method cannot render the changings determined by the peroxidase addition on the dough rheology, because the latter one might not supply the enzyme with the hydrogene peroxide needed to point out the catalytic activity, the data comprised in Table 4 stand out the contrary, thus confirming the decrease of extensibility expressed by parameter L of alveogram. This is due to the covalent reticulation induced by the enzyme among a series of dough components (mainly soluble arabinoxylans). The hydrogen peroxide needed by exogenous peroxidases to act is the result of the metabolic activity of lactic bacteria in flour and, more likely, it is not to be found in dough in a large enough quantity so that, one shouldn't notice an increase of dough resistance described by parameter P of alveogram.

This stagnation at parameter P level can be determined by a slower adaptability of fungal enzyme to the dough characteristics.

The increase in volume and porosity (Table 5) is the result of the dough capacity improvement to retain fermentation gasses, by adding peroxidase in dough. Although in case of this flour the quantity of pentosans is not very high, peroxidase, taking advantage of the endogenous hydrogen peroxide and also of the one produced by yeasts (*Liao et al., 1998*) or by the lactic bacteria from the dough (*Banu, 2000; Dan, 2000*), may produce the oxidative gelation of soluble pentosans, a mechanism by which a secondary network occurs in dough, having a supporting role to the unstable gluten matrix in dough.

Table 3. Quality indices of bread made from FA1 and FA2 flours, unsupplemented (control samples) or supplemented with different doses of glucose oxidase

SAMPLE	Bread quality indices	
	Volume (cm^3/100g)	Porosity (%)
Control sample FA1 (CS FA1)	355,29	71,18
CS FA1 + 3ppm GOX	374,03	74,72
Control sample FA2 (CS FA2)	333,9	71,60
CS FA2 + 5ppm GOX	350,20	73,76

Table 4. Alveogram parameters for doughs made from FA1 flour, unsupplemented (control sample) or supplemented with peroxidase

SAMPLE	Alveogram parameters				
	P [mmH_2O]	L [mm]	G	P/L	W [$10e^{-4}$J]
Control sample FA1 (CS FA1)	86	48	15,4	1,79	144
CS FA1 + 2,5 ppm POX*	86	44	14,8	1,95	137

* Peroxidase

Table 5. Quality indices of bread made from FA1 flour, unsupplemented (control sample) or supplemented with peroxidase

SAMPLE	Bread quality indices	
	Volume (cm^3/100g)	Porosity (%)
Control sample FA1 (CS FA1)	355,29	71,18
CS FA1 + 2,5 ppm POX	376,28	74,01

Although the results obtained by using alveographic method reveal a preeminence of glucose oxidase action compared to the one induced by peroxidase, it must be taken into account the fact that the dough prepared according to the method presents superior conditions of exhibation of the glucose oxidase activity and tougher conditions for a full activity of peroxidase due to the lack of yeast, which might contribute to the increase of hydrogene peroxide quantity in the system, strictly necessary for an intense activity of the enzyme.

On the other hand, the yeast presence in the dough used during the baking tests, supplies the peroxidase with a higher quantity of hydrogen peroxide which, at its turn, determines an intensification of the oxidative processes in the system. That is why the dough will have rheological characteristics different from those of the dough alveographically tested, probably closer to those recorded when adding glucose oxidase.

4. CONCLUSIONS

By supplementing the white flours with oxidoreductases has as a result the improvement of rheological properties of dough. The glucose oxidase addition increases the resistance and decreases the extensibility, at the same time with the increase of flours strenght. Under the working conditions of the alveographic method (yeast free dough), peroxidase determines the decrease of extensibility of high deformability doughs. In both cases, exogenous oxidoreductases addition led to the improvement of bread volume and porosity.

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