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Replacement of chemical oxidant with enzyme mixture

Ionela Niculita[a*], Camelia Arghire[a] , Iulian Izella[a], Vasilica Nechita[b]

[a] *"Enzymes & Derivates" Co., Conacul "Cantacuzino Pascanu", Costisa 617140, Neamt, Romania*
[b] *"Boromir" Co., Buzau, Romania*

Abstract

Thanks to changes that took place in the bakery, as well as increased demand for natural products, enzymes have won an important place in recipes for bread. The latest discoveries in biotechnology have resulted in obtaining new enzyme mixture for the bakery industry.

Oxidizing agents have a useful effect on the development and quality of bread, and then on volume, structure and texture of finished products. Therefore the replacement of chemical oxidant with enzyme mixtures may have useful effects in oxidation processes control. One of the enzymes used as oxidizing agents is glucoseoxidase (β-D-glucose-piranoz-dehydrogenase or β-D-glucose: oxygen-1 oxidoreductase), a dimer flavo-enzyme which contains 2 moles of flavin-adenin-dinucleotid (FAD).

Glucose oxidase is widely used for the determination of glucose in body fluids and in removing residual glucose and oxygen from beverages and foodstuffs. Glucose oxidase producing moulds such as Aspergillus and Penicillum Species are used for the biological production of gluconic acid.

Keywords: N enzyme mixture, oxidizing mixture, glucoseoxidase, replacement.

1. Introduction

This oxidizing agent is acting indirectly on gluten, inducing the formation of disulfide bridges and strengthening the protein network in the dough. Beta-glucosidase is a glucosidase enzyme which acts upon β1 bonds linking two glucose or glucose-substituted molecules (the disaccharide cellobiose). An exocellulase with specificity for a variety of beta-D-glycoside substrates. It catalyzes the hydrolysis of terminal non-reducing residues in beta-D-glucosides with release of glucose.

The enzyme consists of two identical polypeptide chain subunits (80,000 daltons) covalently linked by disulfide bonds. Each subunit contains one mole of Fe and one mole of FAD (flavin-adenine dinucleotide). Some research (Coulthard C.E., 1945) report the molecule to be approximately 74% protein, 16% neutral sugar and 2% amino sugars. They indicate that the FAD is replaceable with FHD (flavin-hypoxanthine dinucleotide) without loss of activity.

Addition of glucose oxidase increases oxygen consumption in dough and promote it's consistency growth. Achieving an optimal dough consistency is accelerated by increasing the quantity of enzyme added, the glucose and the peroxidase activity. A major use of glucose oxidase has been in the determination of free glucose in bodyfluids, food and agricultural products. However, it has been gaining increasing attention in the baking industry; its oxidizing effects result in a stronger dough. In some applications, it can be used to replace oxidants such as bromate and L-ascorbic acid. Other uses of glucose oxidase include the removal of oxygen from food packaging and removal of D-glucose from egg white to prevent browning.

The enzyme is highly specific for β-D-glucose. The α anomer is not acted upon. 2-deoxy-D-glucose, D-mannose and D-galactose exhibit low activities as substrate (Bentley 1966).

* Corresponding author: *e-mail address*: ionela.cretu@enzymes.ro

Stability: Dry preparations are stable for years when stored cold. Solutions are reasonably stable under a variety of conditions.

2. Materials and Method

All the determinations were made with a wheat flour type 650 with a very good quality (F1) and with a good quality (F2), both obtained by processing grain harvest from 2008. As reference in testing we used Belpan Gox 1500, provided by the company Enzymes & Derivates SA. This product is a glucoseoxidase itself with microbial origin, obtained from Aspergillus Niger. It's optimal enzymatic activity is at pH=5-7 and a temperature of 40-60˚ C.

Flour was analyzed in accordance with the methodology applied in the Romanian standards in force: STAS 90-88, STAS 6124-73, STAS 6283-83 and SR ISO 3093:1997. For those two types of flour the physico-chemical properties have been valued as follows:

Table 1. Flour parameters

Parameters	**Flour F_1**	**Flour F_2**
Ash , %	0.65	0.65
Moisture, %	14.1	14.2
Wet gluten, %	27	26
Gluten's deformation, mm	10	16
Falling Number, sec	272	265

Rheological behavior of dough was determined with the Chopin Alveograf according to SR ISO 5530-4:1998 and Farinograf Brabender according to SR ISO 5530-1:1998.

Using flour presented above, we have used different doses of Belpan GOX 1500, as sources of exogenous glucoseoxidase as follows:

- M – a witness sample without added exogenous enzyme;
- P_1 - sample with 5 g Belpan Gox 1500 / 100 kg flour;
- P_2 – sample with 7 g Belpan Gox 1500 / 100 kg flour;
- P_3 – sample with 10 g Belpan Gox 1500 / 100 kg flour;
- P_4 – sample with 13 g Belpan Gox 1500 / 100 kg flour;
- P_5 – sample with 15 g Belpan Gox 1500 / 100 kg flour;

3. Results and discussion

Rheological behavior of doughs, prepared according to the proposed schedule of work, was assessed on basis of determinations with Farinograph Brabender and Alveograph Chopin as indicated in next tables.

Table 2. Values obtained with Alveograph Chopin for F_1

Sample	**CH%**	**Development, min**	**Stability, min**	**Soaking, UB**
M (F1)	59.3	30''	1'40''	120
P1	58.2	50''	3'	100
P2	57.3	1'30''	3'20''	85
P3	57'1''	2'15''	5'	65
P4	56'8''	2'50''	5'40''	52
P5	56'5''	3'15''	6'30''	40

Table 3. Values obtained with Alveograph Chopin for F_2

Sample	**CH%**	**Development, min**	**Stability, min**	**Soaking, UB**
M (F2)	58.8	30''	50''	140
P1	57.6	50''	1'50''	125
P2	56.2	1'20''	3'15''	90
P3	55.8	2'	5'30''	85
P4	55.3	2'35''	5'45''	70
P5	54.6	2'40''	6'50''	50

If considering the rheological behavior of samples treated with Belpan Gox 1500 from the Alveograph (table 4), is evident an effect of strengthening the flour's gluten with a reduction of extensibility.

Table 4. Alveograph values (with Belpan Gox 1500)

Flour	Sample	Toughness P, mm H_2O	Extensibility L, mm	Surface of corrected curve, W	Configuration report, P/L
F_1	M	77	81	205	0.95
F_1	P1	79	77	221	1.02
F_1	P2	83	74	237	1.12
F_1	P3	89	71	242	1.25
F_1	P4	91	69	247	1.31
F_1	P5	93	67	253	1.38
F_2	M	65	82	165	0.79
F_2	P1	74	77	177	0.96
F_2	P2	80	75	187	1.06
F_2	P3	87	71	201	1.22
F_2	P4	92	67	220	1.37
F_2	P5	101	65	235	1.55

As can be seen from reading table above, the glucose oxidase added has resulted in increasing dough's toughness. Increasing dough's resistance is somewhat predictable, confirm the hypothesis that glucoseoxidase produces an effect of strengthening the network of dough's gluten.

Dough's extensibility, described through parameter L of Alveograph, shows a trend of reduction, caused by glucose oxidase, even if this change is not as drastic as in case of maximum pressure.

Both extensibility reducing and increasing the maximum pressure are even more obviously as the dose of enzyme used is higher. Also, adding more Belpan Gox 1500 in both flours F_1 and F_2 increasing the P/L report.

4. Conclusion

Glucose oxidase is an enzyme used for strengthening gluten's network. It's adding has like results a greater dough's resistance and a reducing of it's entensibility.

Improvement of dough's rheological properties is obviously, and that's why glucoseoxidase can be an viable solution for replacement chemical oxidants.

In conclusion, the addition of glucoseoxidase in dough leads to: increasing dough's strength and elasticity, increasing bread volume, improving texture and core, etc.

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