Modelisation of the Oxygreen® wheat ozonation process to improve bread dough quality and protein content: a response surface methodology study

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ABSTRACT

The Oxygreen® process is an ozonation process for wheat grain designed to remove micro-organisms and fungi, mycotoxins and pesticides, which is already authorized in France and Belgium. This technology is also very efficient to modify technological properties of wheat flour. A central composite experimental plan (3 levels; 3 factors) and response surfaces were generated using NemrodW®, to evaluate the influence of operational conditions on technological properties and total protein rate of wheat flour. A 5 G/kg ozone treatment was applied. The effect of humidification rate (HR) due to pre-treatment of wheat (2, 3 and 4% added water on grain weight), ozone pressure (OP) in the reactor (0.25, 0.45 and 0.65 bars) and ozone concentration (OC) in the inlet flow (80 - 90 and 100 G/m³ TPN) have been investigated. Dough alveographic data (P/L, W) and protein content (% P) have been followed on classical bread wheat (French miller's brand). Results were compared with a control.
In the studied field, P/L ranged from 1.02 (HR=2%, OP=0.25 bars, OC=80 G/m³ TPN) to 1.74 (HR=2.8%; OP=0.65 bars; OC=96 G/m³ TPN). W was between 282.8 10^{-4} J (HR=3.2%; OP=0.25 bars; OC= 80 G/m³ TPN) and 226.7.10^{-4} J (HR=4 %; OP=0.57 bars; OC =100 G/m³ TPN). We also noticed a change in the total protein rate which ranged from 10.8% (HR=2%; OP=0.65 bars; OC= 80 G/m³ TPN) to 12.23% (HR=4%; OP=0.33 bars; OC=93 G/m³ TPN).
Our study shows that the Oxygreen® wheat ozone treatment, depending on conditions of moisture, pressure and ozone concentration, leads to flours with very different technological properties and total protein rate. The phenomena involved in this ozonation process will be further investigated.

INTRODUCTION

Ozone is a gas used since many years for water treatment. This powerful oxidant makes it possible to destroy many micro-organisms and organic molecules. Recognized GRAS in 1997, this reagent is more and more used for food treatment **[1]** as grain **[2]**. The Oxygreen® process was developed for grain decontamination. It showed its performances in the destruction of bacteria, mycotoxins and insect larvae. In the same time, conditions applied using the Oxygreen® process lead to modifications of flour technological parameters **[3]**.
Aim of this works was to control these modifications. A central composite experimental plan (3 levels; 3 factors) gives us a model of several technological data. Alveographic data (W and P/L) and total protein rate are presented in this document.

MATERIALS AND METHODS

Wheat samples used in this study come from a batch of classical bread wheat (French miller's brand). Humidification rate of each experiment were controlled.
About 10 kilograms of wheat were treated in the Oxygreen® process (**Figure 1**). This reactor was supplied with ozone produced by an ozone generator. During the treatment the grain was agitated using an endless screw. Temperature was controlled by cooling water in a jacket. A 5 G/kg ozone treatment was applied for each experiment. Ozone pressure and concentration, and grain humidification rate were controlled according to a central composite experimental plan (3 levels; 3 factors) (**Table 1**).
Total protein content of each sample was measured on 30 mg freeze-dried and crushed wheat by elementary nitrogen analyzes. Used method was based on the method DUMAS (AOAC 7024) using a NA 2000 analyzer (Fisons instruments, Italy).
Each grain batch was milled. Baking tests of these flours were realized according to BIPEA protocol: Norme française NF V 03-716 de Février 2002 "Farines de blé tendre. Essai de panification de type pain courant français".
Statistical treatment was executed on excel and response surfaces were drawn with NemrodW®.

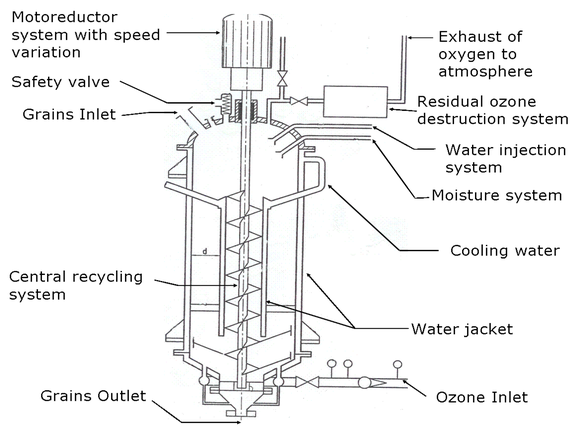

Figure 1: Oxygreen® reactor

Experiment	Humidification rate (% H_2O) X_1		Reactor pressure (Bars) X_2		[O_3] (g. O_3/m^3 TPN) X_3	
	Real variable	Coded variable	Real variable	Coded variable	Real variable	Coded variable
1	2	-1	0,25	-1	80	-1
2	4	1	0,25	-1	80	-1
3	3	0	0,45	0	80	-1
4	2	-1	0,65	1	80	-1
5	4	1	0,65	1	80	-1
6	3	0	0,25	-1	90	0
7	2	-1	0,45	0	90	0
8	3	0	0,45	0	90	0
9	3	0	0,45	0	90	0
10	4	1	0,45	0	90	0
11	3	0	0,65	1	90	0
12	2	-1	0,25	-1	100	1
13	4	1	0,25	-1	100	1
14	3	0	0,45	0	100	1
15	2	-1	0,65	1	100	1
16	4	1	0,65	1	100	1

Table 1: Experimental matrix

RESULTS AND DISCUSSION

Generally, the Oxygreen® treatment improves baking qualities of flour (results not presented here). Therefore, it would be interesting to make a correlation between baking and the technical parameters presented here. Those studies are being investigated.
First, it is interesting to note that ozone treatments led, in the majority of the treatments, to an increase of W, P/L and total protein content of all the samples. Indeed, W reaches a value of 289 10^{-4} J (experiment 8). P/L can be doubled (experiment 1) and the total protein content can increase to a value of 12.66% (experiment 10).
Second, these experiments allowed, by statistical analysis, to determine the predictive equations for the 3 answers studied in the experimental field. Using coded values (**Table 1**), effects solving the prediction equation (**Equation 1**) are then calculated (**Table 3**).
Table 3 analysis gives us a lot of information. Starting from untreated wheat with a W ranging between 200.10^{-4} J and 230.10^{-4} J, ozone treatment increases W to an average 260.21 10^{-4} J value: W was between 282.8 10^{-4} J (HR=3.2%; OP=0.25 bars; OC= 80 G/m³ TPN) and 226.7.10^{-4} J (HR=4 %; OP=0.57 bars; OC =100 G/m³ TPN). However, in the studied experimental field, the 3 factors had a negative effect on W. The humidification rate was the factor having the most important effect.
Average P/L value was equal to 1.21. Humidification rate was still the most important factor. P/L ranged from 1.02 (HR=2%, OP=0.25 bars, OC=80 G/m³ TPN) to 1.74 (HR=2.8%; OP=0.65 bars; OC=96 G/m³ TPN).

$$R = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2$$

Equation 1: quadratic equation determinated

Experiment	W ($10E^{-4}$J)	P/L	Protein content (%)
1	253	1,86	11,14
2	273	1,41	11,54
3	261	1,38	11,27
4	258	1,34	10,90
5	231	1,61	11,24
6	270	1,18	12,00
7	234	1,54	11,39
8	289	1,01	10,77
9	251	1,19	12,32
10	237	1,51	12,66
11	256	1,17	11,78
12	260	1,32	11,57
13	243	1,53	11,90
14	254	1,32	11,99
15	262	1,23	11,44
16	232	1,29	11,80

Table 2: W, P/L and protein content obtained

Effects	W ($10E^{-4}$J)	P/L	Protein content (%)
β_0	260,21	1,21	11,82
β_1	-5,10	0,01	0,27
β_2	-6,00	-0,07	-0,10
β_3	-2,50	-0,09	0,26
β_{12}	-7,50	0,07	0,00
β_{13}	-5,00	0,06	-0,01
β_{23}	3,50	0,00	0,04
β_{11}	-19,81	0,26	0,06
β_{22}	7,69	-0,09	-0,07
β_{33}	2,19	0,08	-0,33

Table 3: Coefficients values of the quadratic equation for each studied answer

Finally, the average total content of protein was equal to 11.82. We also noticed a change in the total protein rate which ranged from 10.8% (HR=2%; OP=0.65 bars; OC= 80 G/m³ TPN) to 12.23% (HR=4%; OP=0.33 bars; OC=93 G/m³ TPN). Humidification rate and concentration had positive effects on this answer.

Figures 2, 3 and 4 represent these observations.

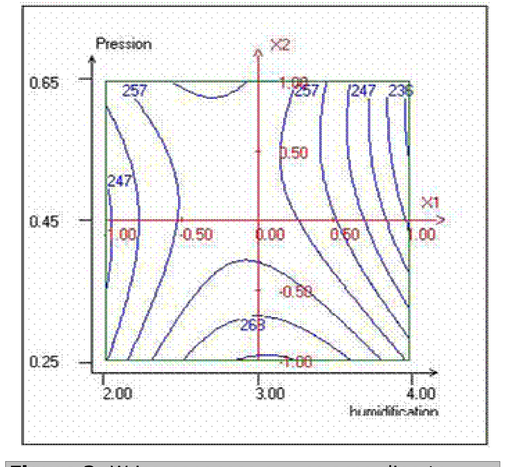

Figure 2: W isoresponses curves according to humidification rate and ozone pressure for a 90 g/m³ ozone concentration

Figure 2 shows an important W decreases at high ozone pressure and humidification rate. To maintain important W, for an average ozone concentration (90 G/m³), low humidification rate and ozone pressure will be preferred. W average rate indicates that ozone treatment increases W. **Figure 3** highlights the importance of humidification rate on the P/L ratio. To obtain a weak P/L, a 3 % humidification rate will be preferred. 2 and 4% humidification rates lead to high P/L ratio values.
Figure 4 indicates that a treatment with high moisture level and ozone concentration was necessary to obtain strong protein content wheat. Water presence facilitates the action of ozone.

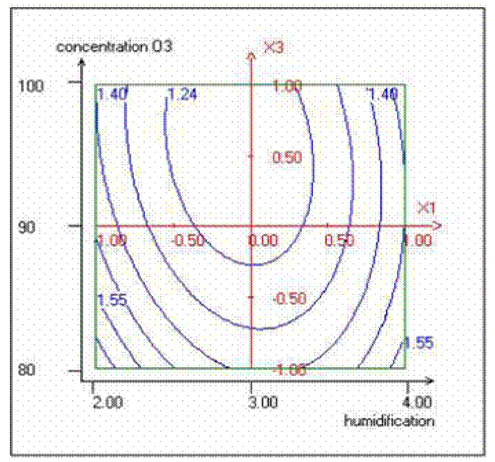

Figure 3: P/L isoresponses curves according to humidification rate and ozone concentration for a 0.45 bars ozone pressure

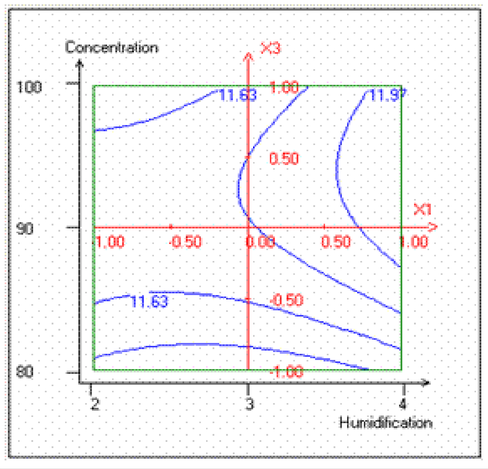

Figure 4: Total protein content isoresponses curves according to humidification rate and ozone concentration for a 0.45 bars ozone pressure

Oxidation of wheat grain components (starch, proteins, …) is the main explanation of these observations. Cross linking bonds (S-S, O-O, …) formation in the grain could increase W and P/L. Nevertheless, too high ozone treatment leads to a destructive oxidation. Grain peeling, during ozone treatment, can partially explain the protein concentration. Grain oxidation leading to a modification of elementary analysis sensibility could be present.

CONCLUSION

This work permits a better comprehension of the Oxygreen® process effects on proteins. These tests showed that under high ozonation conditions, starting from standard bread quality wheat, positive effects on W, P/L (ratio) and proteins content are observed. These 3 answers evolve differently according to the 3 factors studied. Furthermore established model equations permit the prediction of these 3 answers. This tool offers the ability to obtain extremely different flour qualities starting from only one single wheat.
Works in progress on different protein fractions analysis will allow us to improve our knowledge on the phenomena occurring during this treatment.

1 - Guzel-Seydim, Z.B., Greene, A.K. & Seydim, A.C. (2004). *Lebensmittel-Wissenschaft und-Technologie, 37*(4), 453-460.

2 - Mendez, F., Maier, D.E., Mason, L.J. & Woloshuk, C.P. (2003). *Journal of Stored Products Research 39*(1), 33-44.

3 - Coste. C., Bailli. A. & Dubois. M. (2004). *World Patent WO2004028695*