# Improving the Quality of Bread Made from "Short" Gluten Flours Using a Fungal Pentosanase and L-Cysteine Combination

**ALEXANDRU STOICA[1*], ELENA BARASCU[1], ANA-MARIA HOSSU[2]**

[1] Valahia University of Târgovişte, Faculty of Environmental Engineering and Food Science, Department of Food Engineering, 18-24, Unirii Str., 130082, Târgovişte, Romania

[2] VALAHIA University of Târgovişte, Faculty of Sciences and Arts, Department of Chemistry, 18-24, Unirii Street, 130082, Târgovişte, Romania.

*The "short" gluten flours cause problems in breadmaking due to high elasticity and low extensibility of gluten, which negatively affect dough capacity to retain the gases released from fermentation. Until now, the technological solution used was dough supplementation with reducing agents, particularly L-cysteine . Also, the use of enzymes that have as a substrate the flour pentosans can improve bread quality. The aim of this study is to demonstrate that the interdependence between the action of a reducing agent (L-cysteine ) and a fungal pentosanase can cause a superior improvement of physical quality indices of bread - volume, porosity and elasticity. For this purpose the characteristics of used flour were determined (moisture, ash content, wet gluten content, gluten deformation index, gluten index, Alveograph characteristics, Falling Number) and were done baking tests. The resulting bread was analyzed and the results showed an increase of 26% for volume, 11% for porosity and 10% for elasticity compared to the control sample (unsupplemented bread) when using the combination of additives, higher values than for their singular use.*

*Keywords: pentosanase, L-cysteine, gluten network, "short" gluten flours.*

Some flours possess a "short" gluten which is characterized by a very low deformation index and dough made †from them are overly elastic and very little extensible. Gas retention capacity of dough is limited because $CO_2$ released from alcoholic fermentation encountered a strong resistance from the gluten network. The result is a reduced loaf volume and porosity [1].

In order to relax the gluten and increase its extensibility [2], in the breadmaking process these flours are supplemented with L-cysteine [3,4], amino acid from natural proteins [5] or with proteases. L-cysteine †is capable to reduce the disulfide bonds between the gluten proteins (gliadin and glutenin) and release the -SH groups [6]. By relaxing the proteic matrix, the dough becomes weaker and more extensible [7].

Wheat flours contain 2-3% hemicelluloses, the highest amount being localized in the aleurone and bran layers [8]. The higher the flours extraction is, the bigger the quantity of hemicelluloses becomes.

Hemicelluloses from wheat flours are represented by the pentosans, mainly arabinoxylans - AX (~ 85%) and arabinogalactan-peptides. Depending on their extractability in water, AX can be grouped into soluble water-extractable AX - WE-AX (with a positive effect on bread volume and porosity) and insoluble water-unextractable AX - WU-AX (with a negative effect in breadmaking) [9]. AX have a specific property - oxidative gelation [10], forming an additional strength structure in the dough. The most remarkable properties of cereal AX are their high capacity to retain water (especially WU-AX) and their ability to increase the viscosity of the dough [11].

To counteract or reduce the negative effect of WU-AX, in breadmaking there was tested the use of hemicellulases, pentosanases and xylanases from different origins [12], produced by microbial fermentation using different microorganisms as enzymes sources [13]. Some of them have proven their efficiency [14,15], being adopted as processing aids. These enzymes have the ability to catalyze the conversion of WU-AX to WE-AX that increase dough viscosity [16], with positive implications on increasing the quantity of fermentation gases retained in the system. On the other hand, because of their conversion into WE-AX, WU-AX no longer represents a factor of interrupting the continuity of dough proteic film [17]

The aim of the study is to verify the hypothesis according to which the use of associations between pentosanases and L-cysteine will lead to an improved quality of bread as a result of dough relaxation by various combined actions: reducing the number of disulphide bridges between gluten proteins (made by L-cysteine), the destruction of secondary pentosans structure formed from oxidative gelation and hydrolysis of insoluble arabinoxylans with a negative effect on bread (made by pentosanases).

## Experimental part

*Materials and methods*

*Flour* used in baking tests was purchased from S.C. Compan S.A. Targoviste. The quality indices, determined by analysis, are presented in table 1.

Values of quality indices show that flour is very good for breadmaking but possess a "short" gluten. For this reason, flour needs to be improved regarding the gluten extensibility.

*L-Cysteine* was purchased as a commercial preparation named Cisto'Pan from Beldem Food Ingredients. The preparation is a white powder and contains 10% L-cysteine .

The *enzyme preparation* used was a fungal pentosanase (trade name Pentopan 500 BG from Novozymes) which

* email: stoicaandi@yahoo.com

**Table 1**
CHARACTERIZATION OF "SHORT" GLUTEN FLOUR USED IN BAKING TESTS

| Quality index | Value |
|---|---|
| Moisture (%) | 13.53 |
| Ash content (% dry weight.) | 0.64 |
| Wet gluten content (%) | 27.60 |
| Gluten deformation index (mm) | 4 |
| Glutenic index | 48.02 |
| Alveograph characteristics | P =184mm$H_2O$ |
| | L = 21mm |
| | P/L = 8.76 |
| | W = $174\times10e^{-4}$J |
| Falling Number (sec.) | 251 |

showed superior efficacy than fungal hemicellulases or bacterial xylanases in the case of flour used as a substrate [18]. Purified preparation produced by submerged fermentation from a strain of *Humicola insolens* has an activity with an optimum *p*H between 5 and 6 and an optimum temperature of 40°C. Inside the baking oven, enzymes are completely inactivated.

Compressed yeast (Pakmaya) was acquired from SC ROMPAK LLC Paşcani.

Salt (sodium chloride) - presented the characteristics specified in STAS 1465-72.

Flour analysis

*Flour moisture* was measured indirectly using the *oven-dry method* (ICC Method No 110/1). The sample was maintained at 130 ± 2°C (rapid method) for an hour. Humidity is expressed as a percentage by dividing the loss weight to initial sample weight.

The *ash content* of the flour was determined by high temperature incineration (at 900-920°C). The result, expressed as a percentage, was obtained by dividing the weight of incombustible residue on sample dry weight.

*Wet gluten content* was determined by washing the dough resulted from flour with a 2% salt solution in order to remove the starch and other solubles from the sample. The residue remaining after washing is the wet gluten [19]. The results (expressed in percentage) was obtained by dividing the weight of wet gluten to initial sample weight.

*Gluten deformation index* was determined by keeping a wet gluten ball (5 g) for an hour at 30°C and calculating the difference between final and initial diameter of the ball.

*Gluten index* (after the method developed by ICA Bucharest) was calculated using the formula $Ig = G.U.\times(2-0.065\times Id)$, where G.U. = wet gluten content (%), Id = gluten deformation index (mm).

*α-amylase activity* of the flour was determined by the *Falling Number method* (ICC Method 106/1, AACC 56-81B). The method is based on the starch liquefying action of á-amylase and expresses this as the time (in seconds) required to stir and allow the stirrer to fall a measured distance through a heated aqueous flour gel that is undergoing liquefaction [20].

*The Alveograph test* to determine the rheological properties of dough (ICC Method No.121, AACC 54-30A, ISO No 5530/4) uses the Chopin Alveograph that inflates a thin sheets of dough, forming a bubble. The obtained parameters are:

- P – the overpressure; it is regarded as a measure of dough tenacity and it is representative for the elastic properties of dough;
- L - a measure of dough extensibility;
- P/L – the configuration ratio, approximates the alveogram shape combining dough tenacity (P) with its extensibility (L);
- W - the energy required to inflate the dough until breaking. The value of W is widely used as a measure of flours strenght and is often used as a key indicator of the dough behaviour in the breadmaking process [20].

Baking test

Baking test provides the most important information on the influence of different ingredients or additives on the bread quality. Dough was prepared using the straight dough method. The bread formula was consisted of flour - 300g, compressed yeast - 9g (3%), salt - 4.5 g (1.5%), different doses of additives and the optimum amount of water to obtain a maximum volume of control sample. Bread was obtained using the Moulinex bread machines where all parameters are automatically controlled. The baking test was triplicated and, after baking, loaves were cooled for 3 h at ambient temperature. Bread quality was determined by measuring weight, volume of loaf (after 3 h), porosity and elasticity (after 20 h).

Bread analysis

Bread volume was determined using the rapeseed displacement method and Fornet apparatus. *Porosity* and *elasticity* of bread crumb were determined in accordance with STAS 91-83. [21].

After compliting the baking test, the obtained values for physical characteristics of bread (volume, porosity and elasticity) were combined to form a bread score.

**Results and discussions**

From table 2 it can be seen that, by adding L-cysteine in flour, it results an increase of loaf volume reaching a maximum of 19.95% compared with the control sample. The same aspect can be found when using only fungal pentosanase, the maximum volume increase being 17.02%. The results are logical, since the used flour has a lower pentosans content and L-cysteine is the agent that acts directly on gluten network - the main responsible of dough strength.

Hypothesis that using a combination of fungal pentosanase and L-cysteine will lead to a substantial improvement in volume is therefore true, the maximum

**Table 2**
INFLUENCE OF FUNGAL PENTOSANASE - L-CYSTEINE COMBINATIONS ON BREAD VOLUME

| VOLUME ($cm^3$/100g bread) | | Fungal pentosanase (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 |
| L-Cysteine (ppm) | 0 | 312.53 | 343.32 | 365.75 | 360.46 |
| | 50 | 345.;58 | 353.19 | 381.54 | 378.32 |
| | 70 | 374.90 | 371.49 | 386.70 | 393.18 |
| | 90 | 364.36 | 374.01 | 386.73 | 384.13 |

| POROSITY (%) | | Fungal pentosanase (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 |
| L-Cysteine (ppm) | 0 | 69.31 | 71.58 | 72.98 | 71.51 |
| | 50 | 73.57 | 71.98 | 75.17 | 75.00 |
| | 70 | 74.42 | 72.84 | 75.17 | 76.87 |
| | 90 | 74.13 | 73.19 | 74.60 | 74.37 |

**Table 3**
INFLUENCE OF FUNGAL PENTOSANASE - L-CYSTEINE COMBINATIONS ON BREAD POROSITY

| ELASTICITY (%) | | Fungal pentosanase (ppm) | | | |
|---|---|---|---|---|---|
| | | 0 | 50 | 100 | 150 |
| L-Cysteine (ppm) | 0 | 85.80 | 90.14 | 93.07 | 90.79 |
| | 50 | 90.99 | 91.64 | 91.52 | 92.28 |
| | 70 | 92.35 | 91.76 | 93.74 | 94.52 |
| | 90 | 92.90 | 93.05 | 92.81 | 91.95 |

**Table 4**
INFLUENCE OF FUNGAL PENTOSANASE - L-CYSTEINE COMBINATIONS ON BREAD ELASTICITY

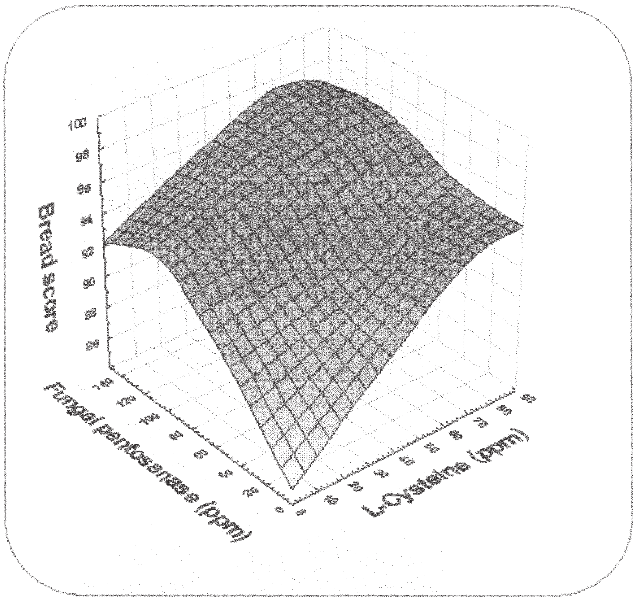

Fig. 1. Modification of bread score by adding different doses of pentosanase and L-cysteine

volume increase being 25.80% compared to the control sample.

Bread porosity values show a more pronounced increase (Table 3) when the two additives are used together (10.90% versus control sample porosity) compared to maximum increase promoted by fungal pentosanase (5.29%) or L-cysteine (7.37%). Also, in case of bread porosity the same trend as in case of volume can be observed: the increase promoted by dough supplementation with L-cysteine is superior compared to that made by pentosanase, as a result of direct action of the reducing agent on gluten-resistant matrix.

Both for the volume and the porosity, the explanation for obtaining higher values using the combination of the two additives refers to an interdependence between the action of L-cysteine and fungal pentosanase: reducing the disulfide bonds from gluten structure by reducing agent and, therefore, a more advanced protein unfolding facilitates the access of enzyme to the specific substrate while WU-AX hydrolysis, realised by pentosanase, makes that a greater quantity of water to become available for a completely hydration of gluten and resulting WE-AX increase the dough viscosity. The consequence of these combined actions is the increases of dough capacity to retain the gases resulted from alcoholic fermentation and, thereby, improve bread volume and porosity.

The two additives used in conjunction determines an increase in the bread elasticity (table 4) by 10.16% compared to 8.27% for the involvement of only L-cysteine or 8.47% when it is used only fungal pentosanase.

One may find that the maximum values of the physical characteristics of bread produced in the case of using fungal pentosanase-L-cysteine combination are obtained for a dose of pentosanase superior to that necessary to obtain the highest values for singular use of this. On the other hand, the unfolding made of L-cysteine increases proteins susceptibility of being attacked by endogenous proteases, thus increasing the probability that dough might contain a higher amino acids level. Also, the hydrolytic activity of pentosanase leads to the formation of small amounts of pentoses (xylose and arabinose). It creates, therefore, the conditions for improving the sensorial quality of bread, because amino acids and pentoses can interact by Maillard reactions, which is important for the bread taste or smell and the color of bread crust.

Regarding the score recorded for all physical properties of bread (important indicators of bread quality), it can be seen from figure 1 that, by increasing the quantities of additives concomitant to certain values , there is a clear improvement of bread score, obvious higher in comparison with that obtained by adding only pentosanase or L-cysteine.

It can be said that the use of fungal pentosanase-L-cysteine combinations significantly contributes to the improvement of the physical characteristics of the final product, the result recorded for the optimal dose combination of the two additives (98.63 points) being higher than scores that are obtained for singular use of fungal pentosanase (maximum 93.94 points) or L-cysteine (maximum 95.18 points).

## Conclusions

The results confirm that both L-cysteine and fungal pentosanase are good solutions to improve the quality of bread made of white flour with “short” gluten. When L-cysteine is used, the volume increase was maximum 19.95% compared with the control sample (17.02% for pentosanase), the maximum for porosity 7.37% (5.29% for pentosanase) and for the elasticity maximum 8.27% (8.47% for pentosanase). However, a high improvement of the physical quality of the bread is to use combinations of the reducing agent and fungal pentosanase, values obtained revealing an increase of ~ 26% for volume, ~ 11% for porosity and ~ 10% for elasticity. Obviously, the calculated score of these quality characteristics of bread lead to the same conclusion (maximum 98.63 points for bread supplemented with the combination of the two agents compared with maximum 93.94 points for fungal pentosanase and maximum 95.18 points for L-cysteine).

Taking into account the conditions of the baking tests, the combination of the two additives may be a solution to improve the quality of bread made from "short" gluten flours in the pan bread technology.

## References


1. BORDEI, D., Tehnologia modernă a panificaţiei, Ed. AGIR, Bucureşti, 2004, p.344.
2. ANGIOLONI , A., DALLA ROSA, M., J. Food Eng., **80**, 2007, p.22.
3. BORDEI, D., TEODORESCU, F., TOMA, M., Ştiinţa şi tehnologia panificaţiei, Ed. AGIR, Bucureşti, 2000, p.257.
4. JOYE, I.J., LAGRAIN, B., DELCOUR, J.A., J. Cereal Sci., **50**, 2009, p.4.
5. STAUFFER, C.E., Functional Additives for Bakery Foods. Van Nostrand Reinhold, New York, 1990, p.42.
6. BANU, C., STOICA, A., BĂRĂSCU, E., BUŢU, N., RESMERIŢĂ, D., VIZIREANU, C., LUNGU, C., IORDAN, M., Aplicaţii ale aditivilor şi ingredientelor in industria alimentară, Editura ASAB, Bucureşti, 2010, p. 563.
7. STOICA, A., BANU, C., Aditivi chimici si biochimici utilizaţi in panificaţie, Ed. Bibliotheca, Târgovişte, 2002, p.59.
8. HAMER, R.J., Enzymes in the baking industry – in Enzymes in food processing, Blackie Academic & Profesional, London, 1995, p.186.
9. COURTIN, C., DELCOUR, J.A., J. Cereal Sci., **35**, 2002, p.226.
10. CARVAJAL-MILLAN, E., GUILBERT, S., DOUBLIER, J.L., MICARD, V., Food Hydrocolloid, **20**, 2006, p.53.
11. AEHLE, W., Enzymes in Industry, WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim, 2007, p.106.
12. CABALLERO, P.A., GOMEZ, M., ROSELL, C.M., J. Food Eng., **81**, 2007, p.45.
13. WHITEHURST, R.J., VAN OORT, M., Enzymes in Food Technology. Second Edition. Blackwell Publishing Ltd., 2010, p. 117.
14. STEFFOLANI, M.E., RIBOTTA, P.D., PÉREZ, G.T., LEÓN, A.E., J. Cereal Sci., **51**, 2010, p.372.
15. DIACONESCU, D., Rev. Chim. (Bucharest), **62**, no.7, 2011, p.756.
16. VARDAKOU, M., KATAPODIS, P., TOPAKAS, E., KEKOS, D., MACRIS, B.J., CHRISTAKOPOULOS, P., Innov. Food Sci. Emerg., **5**, 2004, p.107.
17. COLLINS, T., HOYOUX, A., DUTRON, A., GEORIS, J., GENOT, B., DAUVRIN, T., ARNAUT, F., GERDAY, C., FELLER, G., J. Cereal Sci., **43**, 2006, p.83.
18. STOICA, A., Annals. Food Science and Technology, **13,** 2, 2012, p.148.
19. ***, Wheat and flour testing methods. A guide to understanding wheat and flour quality, Wheat Marketing Center, Inc., Portland, Oregon, 2004, p. 27.
20. BRAMBLE, T., A Guide to Understanding Flour Analysis, King Arthur Flour Company, 2005.
21. ***, Romanian Professional Standards Collection: Milling – Breadmaking.


Manuscript received: 15.04.2013