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INFLUENCE OF L-CYSTEINE AND A FUNGAL PROTEASE COMBINATION ON THE PHYSICAL PROPERTIES OF BREAD MADE FROM SHORT GLUTEN FLOURS

STOICA ALEXANDRU, IORDAN MARIA, POPESCU ELENA-CORINA,
BĂRĂSCU ELENA, HOSSU ANA-MARIA

Valahia University of Târgovişte, Bd. Unirii, 18-24, 130082, Târgovişte, Romania
stoicaandi@yahoo.com

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The processing of strong flours, with short gluten, creates problems due to the high resistant proteic network formed in dough. To improve the strong flours performance, the use of L-cysteine or proteases (fungal proteases especially) is recommended in literature. The authors of this work have studied the effect of a new combination of those additives on the physical properties of bread made from short gluten flours. That new combination was used taking into account that a synergistic effect could be obtained by the joint action of L-cysteine and proteases, effect reflected on the obvious improvement of bread quality indexes such as volume, porosity and elasticity.

Keywords: L-cysteine, fungal protease, disulfide bonds, gluten.

1. Introduction

Romanian flours are characterized by a very good protein content which frequently exceeds 13% and presents a deformation index of gluten predominantly having lower values than the domain between 5 to 15 mm (Grains Quality - 2005) in which flours are very good/good for bread production. The low deformation index most often (and especially if procedures for obtaining the bread are of short duration of fermentation) do not allow doughs to develop the optimum capacity to retain the fermentation gases. Therefore, it is necessary to supplement dough with additives which may decrease the high resistance of gluten. For this purpose, a series of chemical agents is currently used in bread-making, mainly L-cysteine which, through the reduction of gluten proteins disulfide bonds, decreases the dough tenacity and elasticity. Similar results can be reached using proteases of different origins, although their mechanism of action in the dough is different compared to L-cysteine (Stauffer, 1990; Bordei, 2004).

The combination of L-cysteine with proteases may not seem to be a solution for the improvement of breads from flours with strong gluten because the two additives use the same substrate - gluten network which is "the resistance structure" of the dough - and it is considered that a more apparent improvement can be achieved by increasing the used dose of one of these two additives. But, the hypothesis of a successful use of this combination becomes possible if it takes into account the fact that the action of the two additives on gluten is different: L-cysteine is involved in the interchangeable sulfhydryl-disulfide reactions with accessible crosslinked intermolecular disulfide bonds from the proteic structure of the dough, while proteases catalyze the cleavage of the polypeptidic chains (Stoica, 2007).

2. Materials and methods

2.1. Materials

Flours. In experiments, strong flour, with short gluten from SC COMPAN S.A. Targoviste (FA3) was used. The flour's determined characteristics are summarized in Table 1. The flour's quality indexes refer to the protein content (expressed by wet gluten content), moisture, the elastico-plastic characteristics of dough, purity and content of non-starch polysaccharides (judged by the ash content) and α-amylase activity.

Table 1. Characteristics of flour used in experiments

Flour code	Humidity (%)	Ash (% dry weight basis)	Wet gluten (%)	Gluten deformation index (mm)	Glutenic index	Alveogram parameters	Falling Number (s)
FA3	13.53	0.64	27.60	4	48.02	P =184mmH_2O	251
						L = 21mm	
						P/L = 8.76	
						W = 174×10e^{-4}J	

L-cysteine (trade name Cisto'Pan - provided by Beldem Food Ingredients Company) is presented in the form of white powder with taste and flavor of sulfur, with a content of L-cysteine - 10% and ash - maximum 1%.

Fungal protease (provided by *Genencor International*) derived from *Aspergillus oryzae* contains enzymes that are typically used in neutral and acid *p*H applications. On gluten, they are slow acting relative to other proteases. The activity of enzyme preparation is 500.000 HUT/g.

Compressed yeast. In baking tests, compressed baking yeast from Pakmaya (SC Rompak Paşcani LLC) has been used.

Salt (sodium chloride) - having the characteristics in accordance with STAS 1465-72.

2.2. Methods

Determination of flour moisture using drying method (ICC Method No 110/1). The setting of humidity was done by the indirect method, by drying. Analyzed flour was maintained at a certain temperature (classical method - at 105^{o}C for 4 hours; rapid method - at 130± 2^{o}C for one hour) until all the free water evaporates and other secondary effects that alter the chemical components not longer take place.

Determination of flour ash content using the burning method at 900-920^{o}C. Ash is defined (ICC Standard No. 104/1) as the quantity of mineral materials which remains, after applying the burning methods, as incombustible residue of the analyzed sample. The result is expressed as a percentage by reporting the mass of the residue at the dry matter of the analyzed sample.

Determination of the flour wet gluten content. The method is based on separation of gluten by washing the dough made from flour with a solution of NaCl, concentration of 2%. The result is expressed as a percentage gained by relating the weight of the wet gluten to the weight of meal flour taken into consideration.

Determination of deformation gluten index. The method involves the maintaining of a wet gluten sphere (5g) at a temperature of 30^{o}C, for one hour and the determination of the deformation by measuring two medium horizontal diameters (in mm) - before and after the rest period - and calculating the difference between them.

Determination of α-amylase activity in flours by the "Falling Number" (ICC Method 106/1, AACC 56-81B). The method, developed by *Hagberg-Perten*, is based on a rapid gelification of an aqueous suspension of flour in boiling water and measurement of liquefaction produced by α-amylase to starch gel obtained from sample of flour. The Falling Number is defined as the time (expressed in seconds) needed to an agitator to fall into gel flour heated, in a viscosimeter.

Alveographic method for determining the rheological properties of dough (ICC Method No.121, AACC 54-30A, ISO No 5530/4). Produced by Chopin, the Alveograph is an instrument that gives valuable information about the rheological properties of dough sample by measuring the pressures attained during the inflation of dough into a bubble. The alveogram characteristics are: P - known as the overpressure, P is the maximum pressure (mmH_2O), measured as the maximum height (h) in mm on the alveogram and multiplied by a factor of 1.1, P value being usually used as an indicator of dough tenacity and resistance to deformation; L - is the average length (mm) of the curve from the point where the dough bubble starts to inflate to the point where the bubble bursts and the pressure drops

suddenly, L being commonly used as a measure of dough extensibility; P/L – configuration curve ratio is thought to indicate general gluten performance; W - represents the energy required to inflate the dough bubble until rupture and generally indicates the baking strength of the sample (Manol, 2002; Bramble, 2005).

The baking test. In experiments, baking bread Moulinex machines have been used, which carry out all the process operations - mixing-kneading, re-kneading, fermentation, final proof, baking - in the same room in which operations parameters (temperature, time) are strictly controlled relying on the program, offering the possibility to correctly compare the obtained results. The dough was prepared using the direct method and the recipe (expressed for 100g flour):100g flour, yeast-3g (3%), salt-1,5g (1.5%), water – 60g (60%), additives - different doses related to flour weight.

Determination of bread volume by the method on the Fornet apparatus. The principle of this method is measuring the volume of rape seeds replaced by the bread using the Fornet apparatus, the results being expressed for 100g product.

Determination of bread porosity - STAS 91-83 method. The method consists in determination of the total volume of pores of a known volume of crumb, knowing its mass and density. To obtain an average of porosity, bread was cross-sectioned, removing the crust and crumb-shaped in three cylinders, from three different areas, which were subjected to measurement method.

Determination of bread elasticity - STAS 91-83 method. The method consists of pressing a piece of crumb cylinder for one minute and measure its return to the original position, after removing the force and after a rest for one minute. To achieve the analysis, crumb cylinders from the porosity test were used.

After completion of the baking test and determination of the physical properties of finished products (volume, porosity and elasticity), the authors proceed to establish a score based on the values obtained for these characteristics.

3. Results and discussions

Loaf volume, crumb porosity and elasticity are the most important physical characteristics of bread quality.

An effective and efficient improving of bread quality due to conjugation of the positive effects of fungal protease and L-cysteine (used together) in the strong flours processing is true (Table 2). Comparing loaf volumes of bread (measured by rape seed displacement method) it was concluded that the loaf volumes increase up to 401.93 cm^3/100 g when combination of the two additives is used and up to 379.97 cm^3/100 g (for fungal protease) or up to 374.90cm^3/100 g (for L-cysteine) when the additives added alone to the formulation. The loaf volumes increase up to a certain additive concentration; on further rise of the additive level the loaf volumes decreased.

Table 2. The influence of L-cysteine and fungal protease combination on volume of bread obtained from FA3 flour

Volume (cm^3/100g bread)		Fungal protease (ppm)			
		0	100	200	300
L-Cysteine (ppm)	0	312.53	350.78	379.97	374.96
	10	315.09	367.93	381.68	373.97
	30	333.05	368.11	396.08	392.64
	50	345.58	375.62	401.93	392.98
	70	374.90	382.47	396.57	396.49
	90	364.36	385.59	396.22	396.01
	110	360.14	389.40	395.48	393.05

The maximum increase of bread volume was nearly 30% when combination of the two additives were used compared with the improvements made separately by the two additives (19.95% by L-cysteine and 21.57% by fungal protease). The presented results show that, by the simultaneous participation on

gluten matrix relaxation, protease and L-cysteine support each other: the disappearance of intra- or intermolecular disulfide bonds from certain points of the protein network makes the substrate more sensitive for protease intervention, while breaking the protein chains by the fungal protease, offers to L-cysteine the possibility to "identify" new disulfide bridges for interaction. These interrelated actions finally lead to a more complete and balanced relaxation of tenacious gluten from FA3 flour, accompanied by a significant increase of the dough retention gas capacity which is a further increase in the volume of finished products.

Table 3 shows that the crumb porosity was improved when L-cysteine-fungal protease combination was used even if the improved achievement (11.39% compared to the value obtained for the reference) was not much higher than that promoted by the singular use of protease (10.60%).

Table 3. The influence of L-cysteine and fungal protease combination on porosity of bread obtained from FA3 flour

Porosity (%)		Fungal protease (ppm)			
		0	100	200	300
L-Cysteine (ppm)	0	69.31	73.00	76.66	74.98
	10	69.53	72.82	74.86	75.93
	30	71.17	73.78	76.32	75.99
	50	73.57	74.05	76.93	76.18
	70	74.42	74.81	76.78	77.21
	90	74.13	75.71	76.01	75.99
	110	71.85	75.59	75.61	75.17

For elasticity (Table 4), the maximum increase produced, under the same conditions is 11.50%, above those associated with singular use of protease (9.52%) or L-cysteine (8.27%).

Figure 1 gives a rationale graphic image for the significant positive effect achived by the L-cysteine and fungal protease combination on all the physical properties of bread made from the dough supplemented with these additives. The score obtained for the optimal combination levels was 99.52 points compared with maximum 97.06 points for fungal protease and maximum 95.18 points for L-cysteine.

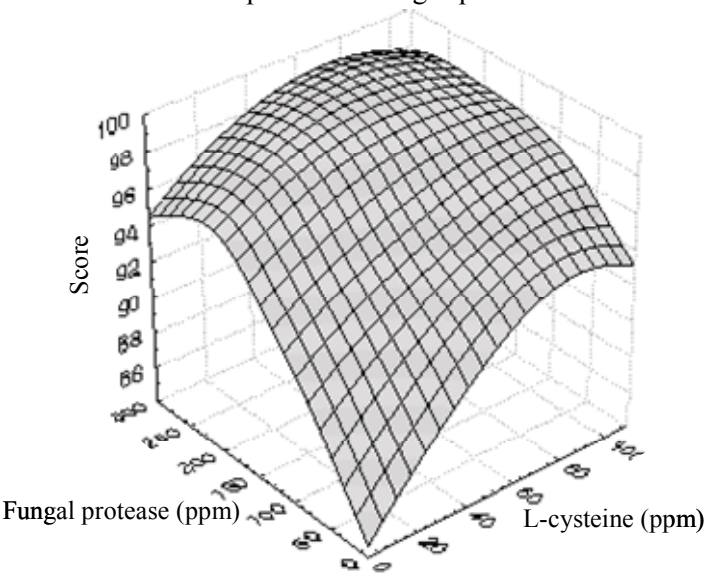

Figure 1. Changes in the bread physical properties score obtained by supplementation of dough from FA3 flour with different doses of L-cysteine and fungal protease.

It is noted that increasing the doses of the additive mixture, the score of the physical characteristics of bread also increases to a certain level, after which the trend is towards its decrease upon increasing the level of additivation.

Table 4. The influence of L-cysteine and fungal protease combination on elasticity of bread obtained from FA3 flour

Elasticity (%)		Fungal protease (ppm)			
		0	100	200	300
L-Cysteine (ppm)	0	85.80	88.29	93.97	89.72
	10	88.23	91.34	92.48	90.87
	30	88.38	90.71	93.86	93.13
	50	90.99	91.15	94.50	93.14
	70	92.35	92.94	94.28	95.67
	90	92.90	93.79	93.97	94.23
	110	92.47	92.66	93.38	93.99

4. Conclusions

The work advances a new approach for strong flour processing by using L-cysteine and fungal protease combination instead of singular utilization of one of these two additives (solution currently practised in bread-making). Through interdependent actions in dough (the disappearance of intra- or intermolecular disulfide bonds from certain points of the proteic network makes the substrate more sensitive for protease intervention, while breaking the protein chains by the fungal protease offers to L-cysteine the possibility to "identify" new disulfide bridges for interaction), L-cysteine and fungal protease promote a more complete and balanced relaxation of tenacious gluten, accompanied by a significant increase of the dough gas retention capacity. The joint action effect is to obtain a substantial improvement in terms of several bread quality indices (volume increase by ~ 30%, porosity over 11% and elasticity by 11.5%), values clearly superior to that promoted by the single use of L-cysteine or fungal protease.

The proposed solution creates the necessary preconditions for reducing energy kneading dough. It can be assimilated into modern methods of pan bread making (because of specific conditions, which have been made by baking tests) which use reduced fermentation and processing times, a shorter technological process also having positive effects on the economic efficiency.

References

Bordei, D. 2004. *Tehnologia modernă a panificației* (*Modern bread-making technology*). Editura AGIR, Bucureşti.

Bramble, T. 2005. *A Guide to Understanding Flour Analysi*s. King Arthur Flour Company.

Manol, P.W. 2002. *Measuring wheat protein or gluten quality*. Standards and Procedures Branch, Washington D.C..

Stauffer, C.E. 1990. *Functional additives for bakery foods*. Van Nostrand Reinhold, New York.

Stoica, A. 2007. *Teză de doctorat - Folosirea aditivilor biochimici pentru îmbunătățirea calității pâinii (Doctoral Thesis - The use of biochemical additives to improve the bread quality)*, Universitatea *Dunărea de Jos* Galați.

* * *. 2005. *Grains Quality - 2005*. Institutul de Bioresurse Alimentare. Ministerul Agriculturii, Pădurilor şi Dezvoltării Rurale.

* * *. 1988. *Colecție de standarde pentru industria de morărit şi panificație-vol.I. (Standards Collection for Milling and Bakery Industry- 1st Vol.)*. Ministerul Industriei Alimentare.

* * *. 1999. *AACC Method 54-30A*. Alveograph Method for Soft and Hard Wheat Flour.