THE INFLUENCE OF ASCORBIC ACID AND L-CYSTEINE COMBINATION ON BREAD QUALITY

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Abstract
Ascorbic acid (AA) is recognized as the main agent for improving the quality of flours (mainly of the weak flours), without sanitary restrictions. AA is an oxidizing agent for dough, but only after its conversion to dehydroascorbic acid. Reducing agent L-cysteine is often used to improve strong flours, characterized by an elastic and resistant gluten, with low extensibility.
The aim of this study was to verify to what extent the concomitant supplementation of the dough with the two additives influences some quality indices of bread. The motivation for initiating this study was based on the possibility that the combined action of the two additives causes a redistribution in the dough of the main links that provide the gluten network functionality - covalent disulfide bonds. This redistribution may change the dough ability to retain the fermentation gases.
The characterization of the used flour was made by determination of moisture, wet gluten content, gluten deformation index and gluten index calculation.
For the resulting bread, the quality analyzed indices were loaf volume and crumb porosity. It was shown that the use of a combination of ascorbic acid and L-cysteine had a higher effect of improving bread quality, compared with the addition of a single additive, leading to an increase of loaf volume by 36% and 8% for porosity.

Keywords: ascorbic acid, L-cysteine, dough, disulfide bonds, dehydroascorbic acid.

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1. INTRODUCTION

Ascorbic acid (AA) is the most important agent to improve the quality of bread and is not subject to health restrictions. L-ascorbic acid is a reducing agent and acts as a dough and bread quality improver only if it is oxidized to dehydroascorbic acid - DHAA - the real oxidizing agent (*Every et al., 1999*). The decrease in the content of -SH groups in dough during kneading and fermentation in the presence of DHAA, demonstrate their oxidation by DHAA. The formation of disulfide bonds between protein molecules increases the elasticity of the dough and improves the baking properties (*Lu and Seib, 1998*). The process is carried out at high speed in the beginning and then decreases, probably due to the depletion of reactive-SH groups. The reactions which are carried out to improve the rheological characteristics of dough can be catalyzed by metal ions (Cu and Fe existing in the dough) or by enzymes present in the flour, or both.

Ascorbic acid requires oxygen to exert its effect and is a redox cycling agent that is not consumed during the reactions in the dough (*Joye et al., 2009*). There is a competition between AA and yeasts for oxygen incorporated into dough.

It was suggested a mechanism that involving two enzymes, ascorbic acid oxidase and glutathione dehydrogenase (*Stauffer, 1990*). *Nakamura and Kurata (1997)* indicates that the improvements are due not only DHAA and its oxidant action and, additionally, to superoxide radical ($O_2^{-}\cdot$) – an intermediate non-enzymatic product of AA oxidation to DHAA (*Bordei et al.*). The improved action of AA is due to rapid oxidation of reduced glutathione in the flour. In this way, there are minimized -S-S-/-SH interchange reactions by disulphide bridges of gluten structure (these reactions can

depolymerize proteins gluten and thin dough) (*Grosch and Wieser, 1999*).
AA acts slowly throughout the technological process (*Junqueira et al., 2007*) and it does not give an overoxidation effect (*Bordei et al., 2000*). The flour is weaker, the required dose of AA is higher. AA is introduced into preferment or dough in dissolved state (*Banu et al., 2010*). Normal doses of AA does not affect the acidity and pH of the dough.
AA can be used successfully in frozen doughs technology, alone or in combination with other oxidizing agents or oxidoreductases (*Faccio et al., 2012*).
Reducing agents are used in breadmaking to reduce the duration of kneading and fermentation (*Hui, 2006*) or when strong flours with resistant, elastic and low extensibility of gluten are processed (*Cauvain and Young, 2007*).
The mechanism by which reducing agents acting in the dough is to reduce intramolecular disulfide bridges in the gluten proteins structure (*Hui, 2006*). As a result, gluten is formed faster but has fewer intermolecular disulphide bridges, the dough thus becoming less elastic and more extensible. This is desirable when strong flours are processed. Also, reducing agent acts on the proteolytic enzymes of the flour, which passes from the oxidized form, inactive, in reduced form, active.
L-cysteine and its derivative – cysteine hydrochloride are the most reducing agents used in breadmaking. L-cysteine has an reducing action upon the proteins only during the dough kneading and not during the fermentation.
Simultaneous action of the two enhancers, ascorbic acid and L-cysteine, appears not recommended due to their antagonistic action in the dough, but it was proposed for French flours (*Grandvoinnet and Berger, 1979*).

2. MATERIALS AND METHODS

Materials

Flour. In the experimental studies a white flour was used. Its quality characteristics are presented in Table 1.
According to the values of the deformation index and gluten index, the used flour is very good but with "short" gluten, which theoretically should be less susceptible to obvious enhancements.
Ascorbic acid was purchased from SC Enzymes & Derivates Romania S.A.. The used preparation is a white crystalline powder having a concentration of 99.5-100% ascorbic acid.
The *L-cysteine* preparation was a white powder with the characteristic sulfur taste and smell, containing 10% L-cysteine, and was purchased from the Company Beldem Food Ingredients.

Methods

The flour quality was evaluated by determination of moisture content, wet gluten content, gluten deformation index and gluten index was calculated with the formula proposed by the Institute of Food Chemistry, Bucharest.
To determine the moisture content it was used the drying oven method (ICC Method No 110/1) and for evaluation of the wet gluten index and its deformation were applied methods provided by the Romanian standards - washing the dough with 2% NaCl solution and, respectively, the maintaining of a wet gluten sphere (having 5g weight) at 30°C for one hour and measuring its deformation.
The baking tests, in triplicate, were performed using automated machines Moulinex, and the basic dough was prepared with the following ingredients: flour - 300g, yeast - 9g, salt - 4.5 g, water - quantity used to obtain the highest volume of the control bread. The basic dough was supplemented with ascorbic acid and L-cysteine in different doses.

Table 1. Quality characteristics of the used flour

Moisture (%)	Wet gluten content (%)	Gluten deformation index (mm)	Indice glutenic
14,26	26,2	2	49

Weight and volume of the bread was determined after 2 hours from the end of baking and porosity after 20 hours. To evaluate the bread volume was used rapeseed displacement method with Fornet apparatus and the bread porosity was determined using the STAS 91-83 (*Romanian Professional Standards Collection: Milling - Breadmaking*).

3. RESULTS AND DISCUSSION

The used flour is a strong flour with "short" gluten, characterized by a high elasticity and low extensibility, which requires the addition of reducing agents to improve dough rheological properties.

From Table 2 it is found that dough supplementation with L-cysteine (without involving ascorbic acid) leads to an improvement in loaf volume which can reach 17.17% (for a 40ppm dose of L-cysteine) compared with control bread. This increase in volume is the effect of improving the capacity of the dough to retain the fermentation gasses, result of the reduction by L-cysteine of a number of disulfide bonds in the too strong gluten structure. The dough relaxation makes it to retain a greater volume of gas, having the effect of increasing bread volume.

Interesting is that by further increasing the amount of amino acid added to the dough (80ppm, 100ppm), together with the simultaneous addition of ascorbic acid in a dose of 25ppm and 50ppm, it obtain a higher volume increase, utmost result being achieved by the combination 80 ppm L-cysteine - 25 ppm ascorbic acid (36.15%). This can be explained by the following actions:

- the most advanced gluten proteins unfolding made by higher doses of L-cysteine (by reducing a larger number of disulfide bonds early in the process), accompanied by the appearance of an increased number of -SH groups likely to be oxidized;
- oxidation of -SH groups newly appeared in dough by ascorbic acid (continuous action) with reforming a part of the number of disulfide linkages.

Through these actions, a redistribution of disulfide bonds in the protein matrix occurs. Their disappearance from certain areas of the dough is accompanied by recurrence in other points, the effect being favorable in terms of dough rheological properties.

The same effect is found in the case of crumb porosity (table 3). The effect of the crumb raising is greater for the simultaneous use of the two enhancers. It is found that the most significant improvement for porosity (8%) is obtained for the same doses of additives used in combination. This improvement is explained by the retention to a greater amount of CO_2 produced by the yeast fermentation which causes a superior loosening of the dough with a positive impact on bread digestibility.

Table 2. Loaf volume variation for samples supplemented with ascorbic acid and L-cysteine.

Variants	Doses		Volume (cm^3/100g)	Percentual variation of volume (%)
	L-Cysteine	Ascorbic Acid		
V_1	0	0	264,86	0
V_2	10	100	280,10	+5,75
V_3	20	0	286,11	+8,23
V_4	40	0	310,36	+17,17
V_5	60	0	291,57	+10,08
V_6	60	25	303,06	+14,42
V_7	60	50	301,09	+13,67
V_8	80	0	284,78	+8,02
V_9	80	25	359,70	+36,15
V_{10}	80	50	351,62	+32,75
V_{11}	100	25	351,10	+32,56
V_{12}	100	50	355,48	+34,21

Table 3. Crumb porosity variation for samples supplemented with ascorbic acid and L-cysteine.

Variants	Doses		Porosity (%)	Percentual variation of porosity (%)
	L-Cysteine	Ascorbic Acid		
V_1	0	0	69,37	0
V_2	10	100	68,61	-1,09
V_3	20	0	69,91	+1,07
V_4	40	0	73,76	+5,89
V_5	60	0	71,62	+3,24
V_6	60	25	73,18	+5,49
V_7	60	50	72,17	+4,03
V_8	80	0	70,27	+1,29
V_9	80	25	74,58	+8,07
V_{10}	80	50	73,94	+7,02
V_{11}	100	25	73,82	+6,41
V_{12}	100	50	74,01	+7,08

4. CONCLUSIONS

Addition of L-cysteine (reducing agent) in the dough obtained from a white flour with good baking qualities but elastic, resistant and very little extensible gluten led to an improvement in the quality of bread, demonstrated by increasing the volume of 17.17% and porosity of 5.89%.

A combination ascorbic acid-L-cysteine has superior effects of quality improvement, leading to an increase in volume by 36% and 8% porosity. The favorable effect exerted by the use of combination can be attributed to the redistribution of disulfide bonds in gluten network, which leads to improved dough rheology and, mainly, to increase the system capacity to retain fermentation gases.

It is possible that, in the context of using weaker flours, to decrease the ratio L-cysteine-ascorbic acid which will give the best results.

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