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*Jelena M. Dodić, Dušanka J. Pejin, Stevan D. Popov, Siniša N. Dodić, Jasna S. Mastilović, Jovanka V. Popov-Raljić**

Faculty of Technology, Novi Sad
* Faculty of Agriculture, Belgrad

INFLUENCE OF ADDITION OF AMYLASE PREPARATION TO DOUGH ON FERMENTATIVE ACTIVITY OF BAKER'S YEAST

ABSTRACT: Dough samples with different content of amylases were investigated, immediately after mixing and after 7, 14 and 30 days of frozen storage. The obtained results show that the fermentation time is shorter, both in fresh and frozen samples, when amylase sample 1 was added, compared to dough without enzymes. The addition of amylase 2 to dough resulted in minimal decrease of "rising" time, both iz frozen and fresh dough samples. The rising time of fresh samples was shorter when amylase 3 was added to dough. The specific fermentative activity of fresh dough samples is increasing by about 10% compared to the control sample, for all amounts of amylase 1 and 2 added to the dough. The fermentative activity of yeast in frozen samples increased by 5—10%, after keeping of dough with the addition of amylase 1 for 14 days. The specific fermentative activity of fresh dough samples increased compared to the control, for all amounts of added amylase 3 to the dough. In frozen dough samples the fermentative activity of yeast decreased by 10% for all added amounts of amylase 3. Baked goods made of fresh and frozen dough, prepared with the addition of amylase 1, are better than the ones made of control dough sample, considering all evaluated parameters.

KEY WORDS: amylase, baker's yeast, fermentative activity

INTRODUCTION

The properties of yeast cells with improved freeze tolerance were investigated and the connection between freeze tolerance and cell composition (content of trechalose, amino-acids, lipids in cell membrane, level of thermoresistent proteins, respiratory cell capacity) was found (Tanghe, 2002). Sharadananant and Khan (2003a) have found that the addition of hydrophilic gumms (gum arabica and carboxy methyl cellulose) decreases the content of water in the dough that can be frozen, while the addition of κ-carrageenan affects negatively the frozen dough. The developed CO_2 content in dough depends on the content of fermentable sugars and amylose activity. Starch is

hydrolyzed in the presence of amylases and sugars are formed as the result (maltose and other). The amount of sugars depends on the content of amylolytic enzymes and state of starch in the dough (A u e r m a n L. J., 1988). The ability of flour water suspension to produce a certain amount of maltose at certain temperature and certain time is known as the amylolytic activity of flour. The ripe wheat grain contains practically only β-amylase, while germinated wheat contains both β- and active α-amylase. Temperature and pH affect the α- and β-amylase in different way. The optimal activity of α-amylase is at higher temperatures (but is also inactivated). At the same time, β-amylase is more stable at lower pH values. The lower the pH value, the lower the inactivation temperature of amylases, especially of α-amylase. The higher the water content and lower the starch concentration, the lower the temperature of optimal effect and inactivation of amylase. The inactivation temperature of amylases is affected by the rate and duration of heating of dough where amylolisys takes place. The optimal temperature of β-amylase is 62—64°C, in dough made of Type 850 flour with baker's yeast (pH 5,9), and of α-amylase 70—74°C. The complete inactivation of β-amylase and α-amylase was 82—84°C and 97—98°C, respectively. A u e r m a n (1988) reported that certain activity of α-amylase was found in the center of the crumb of bread made of such dough. The action of enzymes depends on dimensions of flour particles, dimensions of starch grains and degree of mechanical damage during grinding of grain, e.g. specific free surface of starch globules, where β-amylase can act. When the flour particles and starch grains are smaller and more damaged, the flour is more favourable for action of β-amylase (A u e r m a n L. J., 1988). The addition of β-amylase to wheat flour increases insignificantly the ability of maltose production, pointing to slight excess in flour. The addition of α-amylase in the same amounts, increase several times the ability of maltose production, proportionally to the added amount of α-amylase. Most probably, α-amylase splits starch to low-molecular dextrins which can be easily transformed to maltose, under the influence of excess amounts of β-amylase in the flour. The oxidation of sulphydril group (-SH), present both in α- and β-amylase, decreases signifficantly the activity of these enzymes. The activity of α-amylase depends, to a certain degree, on primary amino groups.

MATERIAL AND METHODS

Wheat flour, of average quality, usually found at the market, was used for dough production. No additives were used for control dough samples, while commercial amylase was mixed into the dough for some trials. The analyses were performed according to the Regulations on methods of physical and chemical analysis for quality control of cereals, milling and baked products, pasta and frozen products (Yugoslav Official Register 74/88): content of moisture, ash, proteins, acid value, wet gluten content (K a l u đ e r s k i G., F i l i p o v i ć N., 1998), farinographic, extensographic and amylographic characteristics. The fermentative activity of yeast was determined by modified method for Determination of baker's yeast activity (JUS E.M8.024, Official Register

SFRJ 56/87). The fermentation time of dough was determined according to standard method JUS E.M8.020. The differential method was used for experimental baking; chosen to obtain small bakery goods (100 g) of best quality under the given conditions. The following parameters of baked goods were investigated: mass and volume of baked products, height/diameter ratio, circumference. The penetrometric value was determined using the following method: Use of SUR penetrometer PNR 6 for the evaluation of physical characteristics of crumb of bread and baked goods. The Dallman number was also determined (K a l u d e r s k i , F i l i p o v i ć, 1988). The amount of yeast for mixing was 2%, calculated on flour, using fresh baker's yeast with 30% of dry matter. The amount of salt was 1,8%, compared to flour. The amount of added water depends on the water content of used yeast, and is calculated in such a way that the water content of dough is constant. Amylases were added to dough, amylase 1 (recommended for the improvement of volume and freshness of baked goods, especially in case of frozen doughs) in concentrations 0,008%, 0,014% and 0,02%; amylase 2 (recommended for the improvement of volume, color and flavor of baked goods), in concentrations 0,002%, 0,007% and 0,012% and amylase 3 (recommended for improvement of flour quality, e.g. quality of finished baked goods), in concentrations 0,002%, 0,0035% and 0,005%). All dough constitutents were tempered at 35°C, as regulated in the method used for the analysis of fermentative activity of yeast. The dough was prepared in the mixer, and after the mixing dough samples were frozen. The time and conditions of freezing and defrosting were chosen on the basis of the results obtained during investigations of freezing dynamics (T o p o l i ć, 2004).

RESULTS AND DISCUSSION

The biggest decrease of volume of small bakery goods was found after 7 days of keeping the dough in frozen state (Table 1).

Table 1. Evaluation of small bakery goods without additives

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	101,77	101,75	102,43	99,18
Volume of small bakery goods [ml]	336,67	248,00	228,00	252,00
Height/diameter ratio [1]	0,56	0,55	0,56	0,49
Circumference, O_1/O_2 [cm]	26,5/27,2	24,2/24,6	24/23,5	23,97/24,37
Penetrometric number	94,7	63,3	48,3	44,5
Dallman number	7—8	8	8	7—8

After 14 days of keeping, the volume of small bakery goods decreased further by 20 ml, e.g. 32,28% compared to the fresh sample. According to height/diameter ratio, the form of the small bakery goods was not changing significantly after 14 days of keeping in frozen state. However, after 30 days of keeping in frozen state and defrosting, the height/diameter ratio is decreased. The decrease of penetration value shows that the keeping in frozen state

affects the rheological characteristics of dough. The Dallman number, rather uniform in all samples, points to the fact that freezing, keeping of dough in frozen state and defrosting have no significant influence on shape and distribution of pores, e.g. appearance of crumb. The addition of amylase sample 1 to dough, results in an increase of specific fermentative yeast activity in fresh — nonfrozen samples. The biggest increase was in dough where 0,0014% of enzyme was added, while different amounts of enzymes did not result in different specific fermentative yeast activity (Figure 1).

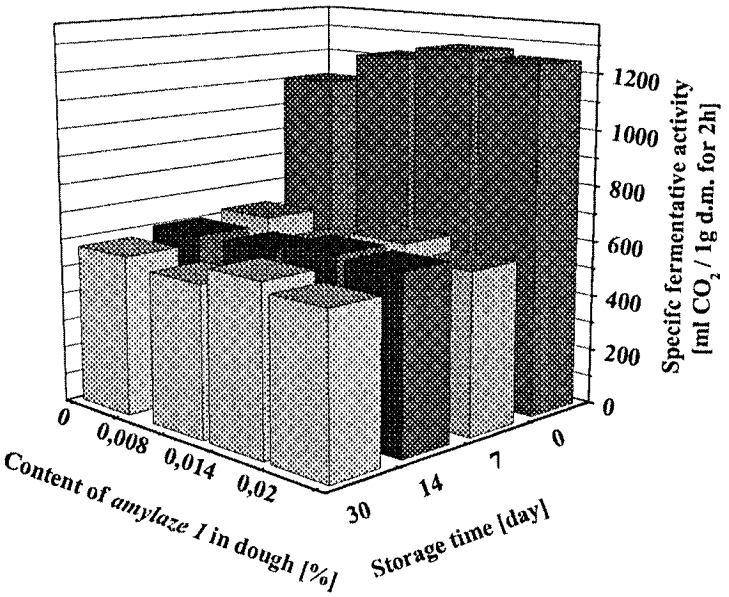

Figure 1. Influence of amylase 1 content in dough and storage time at −18±1°C on specific fermentative yeast activity

In samples prepared with 0,0014% of enzyme, kept at —18±1°C for different period of time, the increase of specific fermentative activity of yeast was minimal compared to dough samples without enzyme.

Table 2. Evaluation of small bakery goods obtained from dough with the addition of 0,008% of amylase 1

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	102,00	103,10	102,78	101,43
Volume of small bakery goods [ml]	410,00	268,00	228,00	260,00
Height/diameter ratio [1]	0,57	0,56	0,60	0,55
Circumference, O_1/O_2 [cm]	28,8/28,6	24,9/25,0	24,5/23,9	24,4/24,7
Penetrometric number	152,2	75,5	51,7	68,5
Dallman number	7	7—8	8	7—8

The volume of small bakery goods decreases during freezing, keeping at low temperatures and defrosting. The biggest decrease of volume was found after 7 days of frozen storage, while the smallest volume was determined in small bakery goods made of dough kept in frozen state for 14 days. According to height/diameter ratio, the shape of small bakery goods is not changing significantly with the exception of small bakery goods obtained from dough kept in frozen state for 14 days. The same can be concluded on the basis of the measured circumference. The freezing process affects significantly the penetrometric value, and it decreased almost by 50% in small bakery goods made of dough kept for 7 days in frozen state compared to small bakery goods made of fresh dough. Freezing, keeping of dough in frozen state and defrosting have no effect on shape and distribution of pores, e.g. appearance of crumb, as concluded on the basis of almost constant value of Dallman number.

Table 3. Evaluation of small bakery goods obtained from dough with the addition of 0,014% of amylase 1

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	101,00	102,48	99,93	100,83
Volume of small bakery goods [ml]	430,00	280,00	270,00	270,00
Height/diameter ratio [1]	0,58	0,54	0,56	0,56
Circumference, O_1/O_2 [cm]	28,4/29,0	25,3/25,4	24,7/25,3	24,7/25,5
Penetrometric number	168,5	85,5	57,8	87,3
Dallman number	7	7	7—8	7—8

The biggest decrease of volume, 34,88%, was registered in small bakery goods made of dough kept at –18°C for 7 days (Table 3). The time of keeping at low temperatures has no significant effect on the change of volume of baked small bakery goods. The biggest change of shape, e.g. smallest "rising" was registered in small bakery goods made of dough kept for 7 days in frozen state. Freezing, keeping of dough in frozen state and defrosting have no effect on shape and distribution of pores, e.g. appearance of crumb, as concluded on the basis of almost constant value of Dallman number.

Table 4. Evaluation of small bakery goods obtained from dough with the addition of 0,02% of amylase 1

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	100,85	103,25	99,48	101,93
Volume of small bakery goods [ml]	450,00	312,00	276,00	260,00
Height/diameter ratio [1]	0,54	0,52	0,52	0,54
Circumference, O_1/O_2 [cm]	28,5/29,0	26,1/26,3	25,1/25,4	24,7/25,2
Penetrometric number	155,8	85,3	67,7	72,7
Dallman number	7	7	7—8	7—8

Keeping of dough in frozen state for 14 and 30 days affects the volume of final bakery products and the registered decrease was 38,66% and 42,22%

respectively, compared to small bakery goods made of fresh dough (Table 4). The freezing process affects the decrease of circumference of small bakery goods, however, this parameter is not changing significantly with the time of keeping in frozen state. The addition of amylase 2 to dough (Fig. 2) affects the fermentative activity of yeast in the dough. The fermentative activity increased in fresh samples, however, the amount of amylase added to the activity of yeast in the dough. The specific fermentative activity decreased almost to half of the initial value, for all applied amounts of amylase 2, as the consequence of freezing process. It was also found that the time of keeping at low temperatures has no or very weak effect on fermentative activity of yeast.

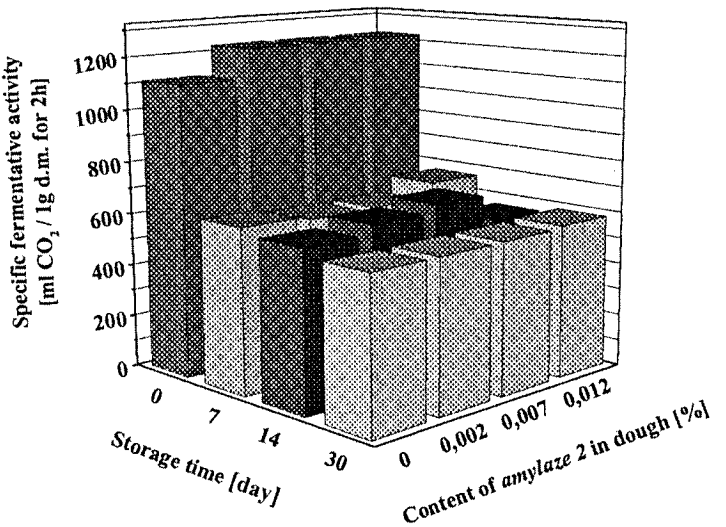

Figure 2. Influence of amylase 2 content in dough and keeping time at −18±1°C on specific fermentative yeast activity

Table 5. Evaluation of small bakery goods obtained from dough with the addition of 0,002% of amylase 2

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	101,75	103,45	104,90	103,93
Volume of small bakery goods [ml]	320,00	252,00	272,00	240,00
Height/diameter ratio [1]	0,57	0,58	0,57	0,53
Circumference, O_1/O_2 [cm]	27,5/27,2	24,5/24,5	24,2/24,6	24,4/23,5
Penetrometric number	126,8	69,0	72,2	58,7
Dallman number	7	7	7—8	7—8

The volume of small bakery goods made of dough kept for 7 days at low temperatures was by 21,25% smaller compared with the volume of small bakery goods obtained from fresh dough (Table 5). The volume decrease was smaller in small bakery goods made of dough kept for 14 days in frozen state compared to the control sample. The height/diameter ratio of small bakery go-

ods made of fresh and frozen dough (kept 7 and 14 days in frozen state) was rather uniform, confirming that the freezing process is not the cause of deformation of small bakery goods. The freezing process affects significantly the penetrometric value, and this value decreased by 50% in small bakery goods made of dough kept for 7 days in frozen state compared to small bakery goods made of fresh dough. Dallman number is uniform in all samples of baked small bakery goods.

Table 6. Evaluation of small bakery goods obtained from dough with the addition of 0,007% of amylase 2

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	102,02	102,98	103,93	103,18
Volume of small bakery goods [ml]	325,00	286,00	284,00	280,00
Height/diameter ratio [1]	0,61	0,48	0,55	0,46
Circumference, O_1/O_2 [cm]	26,2/26,6	25,3/25,8	24,7/25,0	25,0/25,7
Penetrometric number	114,7	88,2	79,5	73,2
Dallman number	7	7	7	7

The biggest decrease of volume was found in small bakery goods made of dough kept for 7 days after freezing, and it was 12% (Table 6). Longer keeping of dough in frozen state does not affect the volume of final baked products. According to the change of height/diameter ratio, the biggest deformation of shape — lowest "rising", was registered in small bakery goods made of dough kept for 7, e.g. 30 days in frozen state. The freezing of dough, keeping in frozen state and defrosting have no significant influence on shape and distribution of pores, e.g. form of crumb.

Table 7. Evaluation of small bakery goods obtained from dough with the addition of 0,012% of amylase 2

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	102,00	100,63	103,73	101,45
Volume of small bakery goods [ml]	350,00	272,00	252,00	250,00
Height/diameter ratio [1]	0,58	0,51	0,56	0,53
Circumference, O_1/O_2 [cm]	27,7/27,1	25,3/25,4	24,1/23,8	24,3/24,1
Penetrometric number	101,7	58,8	57,3	47,3
Dallman number	7	7—8	8	7—8

During longer keeping of dough in frozen state the volume of baked small bakery goods is decreasing by 0,07% e.g. 0,01% compared to previously analyzed samples (Table 7). The shape of small bakery goods is changing rather slightly, as showed by height/diameter ratio. Penetrometric value also significantly decreased in samples with the addition of 0,012% of amylase 2, as the result of freezing. Keeping of dough in frozen state has no significant effect on elasticity and compressibility of crumb.

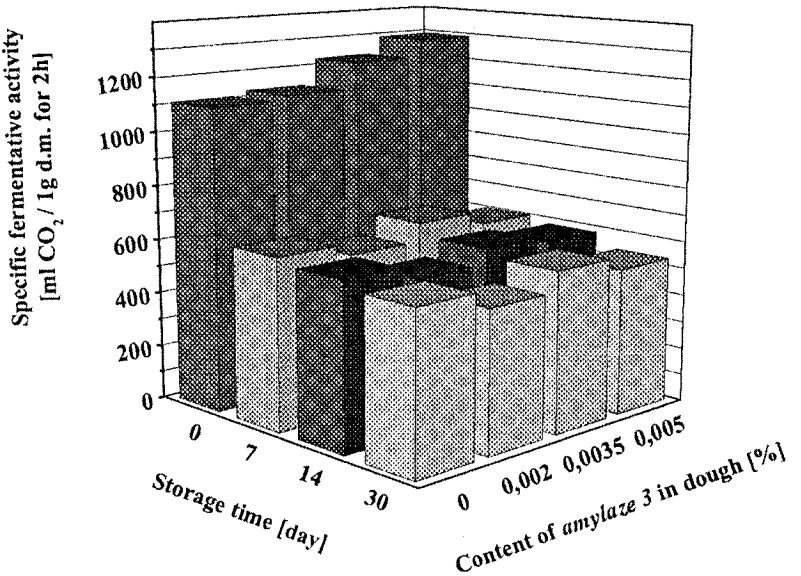

Figure 3. Influence of amylase 2 content in dough and keeping time at −18±1°C on specific fermentative yeast activity

The influence of amylase 3 content in dough and time of keeping at —18+1°C on specific fermentative activity of yeast is presented in Figure 3. The addition of amylase 3 to dough results in an increase of specific fermentative yeast activity in fresh samples and this increase is proportional to the increase of amylase amount. In samples with the addition of lowest and highest recommended concentration of amylase 3, the specific fermentative yeast activity in all frozen dough samples was lower than of the control sample (without enzymes), independently on keeping time.

Table 8. Evaluation of small bakery goods obtained from dough with the addition of 0,002% of amylase 3

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	102,00	100,63	103,73	101,45
Volume of small bakery goods [ml]	350,00	272,00	252,00	250,00
Height/diameter ratio [1]	0,58	0,51	0,54	0,53
Circumference, O_1/O_2 [cm]	21,1/21,1	25,3/25,4	24,1/23,8	24,3/24,1
Penetrometric number	101,7	58,8	57,3	51,3
Dallman number	7	7—8	8	7—8

The volume of small bakery goods made of dough kept for 7, e.g. 14 days after freezing at 18±1°C was by 22,29, e.g. 28,57% lower compared to the control sample (Table 8). The uniform values of height/diameter ratio of small bakery goods made of dough kept for 7, 14 and 30 days at low temperatures show that keeping at low temperatures does not result in deformation of

small bakery goods. However, the difference of height/diameter ratio of frozen and fresh samples is significant pointing to negative effect of freezing process. Longer time of keeping of dough in frozen state affects insignificantly the compressibility and elasticity of the crumb of finished bakery products, as presented by the minimal change of penetrometric value.

Table 9. Evaluation of small bakery goods obtained from dough with the addition of 0,0035% of amylase 3

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	101,65	104,30	104,58	101,08
Volume of small bakery goods [ml]	365,00	258,00	258,00	260,00
Height/diameter ratio [1]	0,60	0,53	0,55	0,52
Circumference, O_1/O_2 [cm]	27,2/27,9	24,4/24,6	24,17/24,6	24,9/25,0
Penetrometric number	114,3	60,2	59,3	57,4
Dallman number	7	7	8	7—8

After 7 days of keeping in frozen state, the volume of small bakery goods was by 29,31% lower compared to the control samples (Table 9). Longer keeping of dough has no negative influence on the change of volume. The biggest deformation, e.g. lowest "rising" determined on the basis of height/diameter ratio, was found in small bakery goods made of dough kept in frozen state for 7 e.g. 30 days. The penetrometric value is decreasing as the result of freezing, as presented by value measured in small bakery goods made of dough kept for 7 days in frozen state and compared to the control sample. The Dallman number has the same value in all samples.

Table 10. Evaluation of small bakery goods obtained from dough with the addition of 0,005% of amylase 3

Evaluated parameter	0. day	7. day	14. day	30. day
Mass of small bakery goods [g]	101,57	105,15	104,83	102,00
Volume of small bakery goods [ml]	380,00	264,00	256,00	256,00
Height/diameter ratio [1]	0,56	0,52	0,56	0,52
Circumference, O_1/O_2 [cm]	28,0/28,4	24,7/24,9	24,7/24,6	24,9/24,7
Penetrometric number	126,7	65,0	61,0	63,5
Dallman number	7	8	8	8

The biggest decrease of volume was in small bakery goods made of dough kept for 7 days in frozen state (Table 10). Longer keeping time had no significant effect on the change of this quality characteristic. The determined values of height/diameter ratio and circumference of baked small bakery goods show that freezing affects the change of form in a lesser degree. It was found that longer storage of dough has no negative influence on elasticity and compressibility of crumb. The Dallman number is uniform in all investigated samples.

CONCLUSION

The fermentation time of fresh and frozen samples was shorter when amylase 1, 2 and 3 were added to the dough. The specific fermentative yeast activity of fresh samples increased by about 10% compared to the control sample, for all added amounts of amylase samples 1, 2 and 3. In frozen samples, the specific fermentative yeast activity decreased by 10% for all added amounts of amylase 3. All evaluated parameters of baked goods made of fresh dough containing amylase 1, were better compared to samples made of the control dough. The characteristics of small bakery goods made of dough prepared with the addition of amylase 1 are better than of the control sample. Regarding all evaluated parameters of the baked goods made of dough with amylase 2, the ones containing 0,007% of enzyme are outstanding. Keeping time of frozen dough containing amylase 2 has no effect on quality of baked goods. The addition of amylase 3 to the dough does not result in significant improvement of quality of baked good made of both fresh and frozen dough.

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УТИЦАЈ ДОДАТКА ПРЕПАРАТА АМИЛАЗА ХЛЕБНОМ ТЕСТУ НА ФЕРМЕНТАТИВНУ АКТИВНОСТ ПЕКАРСКОГ КВАСЦА

Јелена М. Додић, Душанка Ј. Пејин, Стеван Д. Попов,
Синиша Н. Додић, Јасна С. Мастиловић, Јованка В. Попов-Раљић*
Технолошки факултет, Нови Сад
* Пољопривредни факултет, Београд

Резиме

Испитивани су узорци са различитим садржајем амилаза у тесту и то непосредно по замесу и након 7, 14 и 30 дана складиштења у замрзнутом стању. Резултати ових истраживања показују да се у незамрзаваним и замрзаваним узорцима време дизања теста скраћује додатком амилазе 1, у односу на тесто без додатка ензима. Код замрзаваних и незамрзаваних узорака теста, додатком амилазе 2 у тесто минимално се скраћује време дизања теста. Додатком амилазе 3, време дизања теста се, код незамрзаваних узорака, скраћује. Специфична ферментативна активност незамрзаваних узорака теста повећава се за око 10% у односу на контролни узорак, и то за све количине амилазе 1 и 2 додате у тесто. Додатком амилазе 2 у тесто, специфична ферментативна активност квасца у замрзаваним узорцима повећава се за 5—10%, након 14 дана чувања теста у замрзнутом стању. Специфична ферментативна активност незамрзаваних узорака теста повећава се у односу на контролни узорак, за све количине амилазе 3 додате у тесто. Специфична ферментативна активност квасца опада до 10% за све додате количине амилазе 3. Од незамрзаваног и замрзаваног теста, у које је додата амилаза 1, добија се пециво које је према свим оцењиваним показатељима боље од пецива добијеног од контролног узорка теста.