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INFLUENCE OF DOUGH FREEZING ON *SACCHAROMYCES CEREVISIAE* METABOLISM

ABSTRACT: The need to freeze dough is increasing in bakery production. Frozen dough can be stored for a long time without quality change. The capacity of bakery production can be increased in this way, and in the same time, the night shifts can be decreased. Yeast cells can be damaged by freezing process, resulting in poor technological quality of dough after defrostation (longer fermentation of dough).

The influence of frozen storage time of dough on survival percentage of *Saccharomyces cerevisiae* was investigated. Dough samples were taken after 1, 7, 14 and 28 days of frozen storage at –20°C. After defrosting, at room temperature, samples were taken from the surface and the middle part of dough (under aseptic conditions), and the percentage of living *S. cerevisiae* cells was determined. During frozen storage of dough, the number of living *S. cerevisiae* decreased. After 28 days of frozen storage, the percentage of live cells on the surface and inside the dough was 53,1% and 54,95%, respectively. The addition of k-carragenan to dough increased the percentage of living cells in the middle part of dough up to 64,63%. Pure cultures, isolated from survived *S. cerevisia* cells in frozen dough by agar plates method (Koch's method), were multiplied in optimal liquid medium for yeasts. The content of cytochromes in *S. cerevisiae* cells was determined by spectrophotometric method. The obtained results showed that the content of cytochromes in survived *S. cerevisiae* cells was not affected by dough freezing process. Growth rate and fermentative activity (Einchor's method) were determined in multiplied cells.

KEY WORDS: cytochromes, fermentative activity, freezing, κ-carragenan, survival, *Saccharomyces cerevisiae*

INTRODUCTION

Freezing and storage in the frozen state leads to decreased viability and fermentative activity of yeast cells (Berglund et al., 1991). Mazur (1963) reported that a number of factors affect damage, and it depends on whether ice is formed intracellularly (high freezing rates), or extracellularly (lower freezing rates). It was concluded that freezing regimes affect strongly the loss of cell viability. Cells are damaged during the freezing process, while the defrosting

regime affects insignificantly the survival of yeast cells. Yeast cell metabolism decreases drastically at low temperatures; therefore, long term storage of cells is possible.

High survival level can be achieved by appropriate freezing process: survival about 95% (Janković et al., 1999), or even 100% (Pejin, 1989). Freezing of yeast suspension in water affects decreased fermentative activity. The decrease of fermentative activity is lower when yeast is frozen in starch dough (Berglund, 1988).

Mayers and Attfield (1999) consider that the loss of yeast cell viability is affected by freezing of intracellular water, resulting in damage of cytoplasmatic membrane and increase of components in the cytoplasma. This can affect decrease of pH value, increase of dry matter content, and decrease of glycolytic enzymes activity in the cytoplasma.

According to Gao and Critser (2000), if the loss of water during freezing is higher than the critical moisture content, the proteins and cytoplasmatic membrane are irreversibly denaturated, and this can cause the death of cell. The denaturation of cell parts can be caused by: concentration increase of some ions, change of pH, and hydratation decrease of macromolecules in the cytoplasma. The fermentative activity of yeast in frozen dough is lower by 30—35%, compared to the control (Mikinčić-Pešut, 1989). Yeast resistance during freezing is affected by duration of dough fermentation before the freezing process, freezing and defrosting rate of dough, frozen storage time, and temperature oscillations during storage (Giannou et al., 2003).

Several ways of decreasing the effect of freezing and frozen storage on yeast survival and fermentative activity can be found in recently presented papers, even on improving characteristics and quality of frozen dough and obtained bakery products: addition of hydrocolloids, lower water content in prepared dough, higher amount of yeast, compared to the traditional production, and shorter dough fermentation before freezing (Giannou et al., 2003), use of instant yeast (Abd El-Hady et al., 1999), use of cryotolerant and/or cryoresistant strains of bakery yeast (Takano et al., 2002), use of modified yeast strains (Tanghe et al., 2000; Van Dijck et al., 2000; Tennissen et al., 2002).

The aim of the research presented in this work was to investigate the possibility of kapa-carragenan use to protect the yeast cells during freezing of dough. κ-carragenan-hydrocolloid — is biochemically inert, and can modify the dough structure, bind the free water and control water migration in the dough (Ribotta et al., 2004; Žeželj, 2005). Binding and immobilization of water decreases the ice crystal formation, and also the damage of glutene and yeast cells (Sharadanant and Khan, 2003b). This is significant from the standpoint of production of bakery products from frozen dough.

MATERIAL AND METHODS

Average quality commercial T-500 flour was used for the production of dough, which was frozen later. Quality characteristics were analyzed according

to the Regulations on methods of physical and chemical analyses for quality control of wheat, milling and bakery products, pasta and fast frozen dough (Yugoslav Official Register, N° 74/1988).

Dough intended for freezing was prepared according to the following procedure: flour + water + bakery yeast (2,5% calculated on flour), placed in the spin kneading machine with helical agitators, and mixed for 10 min at 85 rpm (control). Aiming to investigate the effect of hydrocolloids on yeast, during freezing of dough, κ-carragenan (Fluka AG, Buch, Switzerland) was added as a component into the dough prepared according to the described procedure.

The temperature of mixed dough was 20 ± 1°C (Kenny et al., 2001). The dough was divided into portions, formed without fermentation in mass (Barconas et al., 2003), frozen at –35 ± 1°C until –12 ± 1°C was reached in the centre of the sample (freezing chamber KOMA, the Netherlands), according to producer's recommendations. The frozen dough portions were packed in PVC bags, and stored at –20 ± 1°C (chamber KOMA), for 1, 7, 14 and 28 days. The samples were defrosted at +4 ± 1°C for 12 hrs, and for additional 1,5 hr at +20 ± 1°C. The number of living *Saccharomyces cerevisiae* cells was determined according to the method given in the Rulebook on methods of performing microbiological analyses and superanalyses of food products (Yugoslav Official Register N° 25, 1980). Pure cultures were transferred into the liquid nutritive medium for yeasts (Mihajlović, 1983). Specific growth rate, and fermentative activity according to Einhorn (Reiff et al., 1960), were determined in regular time intervals, during 24 hrs. The content of cytochromes was determined by method according to Oure and Suomalainen (1970), with the aim of determining the respiration intensity.

RESULTS AND DISCUSSION

Number and percentage of survived *S. cerevisiae* cells in frozen dough are presented in Table 1. During frozen storage of dough for 28 days, the percentage of living cells from dough surface is 53,11%, and from the centre 54,95%. Comparing these results, it is quite clear that the cells in the centre of the dough are protected from low temperature, and the number of survived cells is higher. In dough samples, prepared with addition of κ-carragenan, in concentrations 0,1, 0,3 and 0,5%, the number and percentage of survived cells increases both on the surface and in the centre. In dough with 0,5% a κ-carragenan, the percentage of survived cells on dough surface is 61,23%, by 8,12% higher compared to the control sample. In the centre 64,63% cells survived, about 10% more compared to the control.

Tab. 1 — Number and percentage of survived *Saccharomyces cerevisiae* cells in frozen dough samples with different content of κ-carragenan during storage

k-karagenan content (%)	Number of living cells x 10^4 in 1g of dough					Survirval percentage cells
	Days of keeping at –20 ± 1°C (dani)					
	0	1	7	14	28	
0,0 on surface	2350	2060	1562	1438	1248	53,11
0,0 in the middle	2475	2380	2123	1520	1360	54,95
0,1 on surface	2240	2090	1525	1498	1095	48,88
0,1 in the middle	2460	2110	1677	1570	1348	54,80
0,3 on surface	2320	1990	1572	1490	1345	57,97
0,3 in the middle	2450	2050	1648	1550	1525	62,33
0,5 on surface	2270	2100	1700	1425	1390	61,23
0,5 in the middle	2460	2030	1850	1630	1590	64,63

Tab. 2 — Specific growth rate of pure *Saccharomyces cerevisiae* cultures, isolated from frozen samples: the control dough and dough containing κ-carragenan

Days of keeping at –20°C	Place of cell sampling	μ-Specific growth rate (h^{-1})			
		Without κ-karagenan		With κ-karagenan	
		0—4	0—24	0—4	0—24
0	on surface	0.6074	0.1425	0.6575	0.1430
	in the middle	0.5678	0.1452	0.6441	0.1420
1	on surface	0.5756	0.1510	0.6471	0.1390
	in the middle	0.5269	0.1540	0.6427	0.1380
7	on surface	0.5175	0.1420	0.6307	0.1370
	in the middle	0.5100	0.1300	0.5832	0.1360
14	on surface	0.5165	0.1350	0.5833	0.1300
	in the middle	0.5099	0.1300	0.5175	0.1260
28	on surface	0.5170	0.1340	0.1813	0.1300
	in the middle	0.4946	0.1300	0.0965	0.1360

Specific growth rates were calculated according to the method by P e j i n (1989), using the data on content of dry matter determined during multiplication on *liquid nutritive medium*, under intensive aeration, during 24 h, at 30°C. Analyzing the results from Table 2, it can be seen that the growth of yeast cells is very intensive during the first four hours. It is also interesting to mention that the specific growth rate of cells, isolated from dough surface, is higher than of cells isolated from the centre of sample. The specific growth rate of pure *S. cerevisiae* culture decreases with longer storage of frozen dough. In fresh state, the specific growth rate of yeast cells was 0,6047 × h^{-1}, during the first 4 hours of cultivation, and after frozen storage of dough at –20°C, for 28 days, it was 0,517 × h^{-1}. The mean specific growth rate was calculated after 24 hrs of multiplication, and the obtained values showed that the specific growth rate of pure *S. cerevisiae* cultures decreases constantly with longer fro-

zen storage of dough. The mean specific growth rate decreases by about 10%, during 28 days of storage at –20°C.

The addition of hydrocolloid κ-carragenan affects positively the specific growth rate of pure cultures isolated from frozen dough samples with addition of 0,5% of κ-carragenan. In dough samples, stored for 14 days in frozen state, no significant decrease of specific growth rate, during the first four hours of cultivation was estimated. However, the specific growth rate of pure cultures isolated from dough, kept for 28 days at –20°C, was three times lower, compared to the isolates without addition of κ-carragenan. The "lag-phase" of these samples was considerably longer, causing decrease of specific growth rate in the first four hours of cultivation. It is interesting to mention that the mean specific growth rate, determined during 24 hours is higher for isolates from frozen dough samples prepared with κ-carragenan, than for solates from frozen doughs without κ-carragenan.

The results of fermentative activity of pure *S. cerevisiae* cultures isolated from frozen dough samples, with and without κ-carragenan, are presented in Table 3.

Tab. 3 — Fermentative activity of pure *Saccharomyces cerevisiae* cultures isolated from frozen dough samples, with and without κ-carragenan

Days of keeping at –20°C	Place of cell sampling	cm^3 CO_2/1g dry matter	
		Without κ-karagenan	With κ-karagenan
0	on surface	46.41	96.29
	in the middle	41.62	87.56
1	on surface	43.80	93.15
	in the middle	42.26	67.47
7	on surface	43.90	68.68
	in the middle	41.90	49.90
14	on surface	43.00	62.22
	in the middle	39.80	49.10
28	on surface	42.04	46.88
	in the middle	34.48	34.82

The fermentative activity of pure cultures isolated from frozen dough samples, containing κ-carragenan, was about 2 times higher compared to pure cultures isolated from dough without κ-carragenan (Table 3). This can be explained by the fact that this hydrocolloid binds the water around yeast cells, and protects them from freezing, preventing the formation of large ice crystals which can damage the cell wall and cytoplasmatic cell membrane.

It is interesting to mention that the fermentative activity of cells on dough surface is higher compared to the cells from the middle part. It leads to a conclusion that yeast cells, isolated from the dough surface, are less damaged by the freezing process, than the ones isolated from the middle.

The results of fermentative activity determination for cells isolated from frozen doughs after 28 days, show that the change of this characteristic is insignificant for dough samples without κ-carragenan.

Respiratory activity e.g. content of cytochromes after multiplication for 24 h, under aerobic conditions, was also determined in pure cultures, isolated from frozen dough samples, with and without addition of κ-carragenan (Tables 4 and 5).

Tab. 4 — Influence of storage time on cytochrome content in pure culture cells isolated from dough samples

Days of keeping at –20°C	Place of cell sampling	Cytochrome moles x 10^5/kg yeast with 25% dry matter					
		aa_3		b		c	
		605 nm	444 nm	560 nm	532 nm	520 nm	550 nm
0	on surface	6.45	4.76	6.98	6.28	4.39	5.68
	in the middle	5.32	3.72	5.76	5.76	3.92	4.76
1	on surface	5.24	4.33	6.68	5.46	3.34	5.27
	in the middle	2.62	3.17	4.97	4.82	2.47	3.22
7	on surface	0.88	2.66	4.81	4.79	2.39	2.93
	in the middle	0.87	2.10	4.70	4.72	2.35	2.89
14	on surface	0.65	2.70	5.76	5.73	2.85	7.00
	in the middle	0.57	2.70	5.48	4.82	2.40	5.98
28	on surface	0.42	2.94	5.04	3.45	1.51	6.70
	in the middle	0.37	2.58	4.99	3.00	1.45	5.70

The time of storage affects negatively the content of cytochromes aa_3, b and c (Table 4). The decrease of cytochromes aa_3 content is the highest, and of cytochromes b, the lowest. Cytochrome content decrease points to lower activity of respiratory enzymes, which provide energy for the growth of cells. Due to decreased enzyme activity, the yeast cells have no energy necessary for the growth, resulting in decrease of specific cell growth activity.

Tab. 5 — Influence of storage time on cytochrome content in pure isolate cells, isolated from frozen dough samples containing κ-carragenan

Days of keeping at –20°C	Place of cell sampling	Cytochrome moles x 10^5/kg yeast with 25% dry matter					
		aa_3		b		c	
		605 nm	444 nm	560 nm	532 nm	520 nm	550 nm
0	on surface	5.76	4.79	6.76	6.25	4.47	5.47
	in the middle	4.86	4.52	5.82	5.55	3.98	4.92
1	on surface	4.25	2.86	6.31	4.90	2.47	4.05
	in the middle	3.86	2.26	4.14	4.58	3.63	5.86
7	on surface	0.99	2.29	6.29	4.72	3.53	5.78
	in the middle	0.72	1.76	5.08	3.79	1.89	4.14
14	on surface	0.00	2.26	4.67	4.66	2.37	5.70
	in the middle	0.00	1.66	3.99	3.58	1.85	3.90
28	on surface	0.00	1.29	3.89	4.66	2.33	4.76
	in the middle	0.00	1.16	3.19	3.58	1.85	3.90

κ-carragenan has no positive effect on the content of cytochromes in yeast cells (Table 5). In yeast cells isolated after 14 days from frozen dough

storage, the content of cytochromes aa_3 is about 10 times lower, the contents of cytochromes b and c are also lower, compared to the samples from frozen dough without κ-carragenan.

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УТИЦАЈ ЗАМРЗАВАЊА КВАСНИХ ТЕСТА НА МЕТАБОЛИЗАМ *SACCHAROMYCES CEREVISIAE*

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Резиме

У пекарској производњи расте потреба за замрзавањем квасних теста. Замрзнута квасна теста могу се дуго чувати у замрзнутом стању без губитка квалитета. На овај начин може се повећати капацитет пекарске производње, а на тај начин смањити потреба за ноћним радом пекара. Процесом замрзавања квасних теста могу се оштетити квасне ћелије и њихов ензимски систем тако да ће након одмрзавања квасно тесто имати лошији технолошки квалитет (продужено време ферментације теста). У раду је испитивано како период чувања замрзнутог квасног теста утиче на проценат преживљавања *Saccharomyces cerevisiae.* Период чувања квасног теста је био 28 дана, на температури „–20°C". Током овог периода узимани су узорци за испитивање након 1, 7, 14. и 28. дана. У наведеним периодима узорци су одмрзавани на собној температури и са површине и из унутрашњости теста (под асептичним условима) узимани су узорци квасног теста и у њима је одређиван проценат преживелих ћелија *Saccharomyces cerevisiae.* Током чувања квасног теста у замрзнутом стању смањивао се постепено број преживелих ћелија *Saccharomyces cerevisiae*. Након 28. дана чувања квасног теста

у замрзнутом стању проценат преживелих ћелија на површини теста је био 53,11%; а у унутрашњости теста је био 54,95%. Додатак к-карагенана у тесто повећао је проценат преживелих ћелија у унутрашњости замрзнутог теста и до 64,63%. Од преживелих ћелија *S. cerevisiae* из замрзнутих теста методом агарних плоча (Коховом методом) изоловане су чисте културе. Чисте културе умножаване су, до потребних количина, у оптималној течној подлози за квасце. У ћелијама *S. cerevisiae* одређиван је садржај цитохрома спектрофотометријском методом. Добијени резултати садржаја цитохрома су показали да процес замрзавања квасног теста није утицао негативно на саржај цитохрома у преживелим ћелијама *S. cerevisiae*. Умноженим ћелијама одређивана је брзина раста и ферментативна активност Einchor-овом методом.