International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:11, No:5, 2017

Characteristics of the Storage Stability for Different *Saccharomyces cerevisiae* Strains

Gomaa N. Abdel-Rahman, Nadia R. A. Nassar, Yehia A. Heikal, Mahmoud A. M. Abou-Donia, Mohamed B. M. Ahmed, Mohamed Fadel

***Abstract*—**Storage stability is the important factor of baker's yeast quality. Effect of the storage period (fifteen days) on storage sugars and cell viability of baker's yeast, produced from three *S. cerevisiae* strains (FC-620, FH-620, and FAT-12) as comparison with baker's yeast produced by *S. cerevisae* F-707 (original strain of baker's yeast factory) were investigated. Studied trehalose and glycogen content ranged from 10.19 to 14.79 % and from 10.05 to 10.69 % (d.w.), respectively before storage. The trehalose and glycogen content of all strains was decreased by increasing the storage period with no significant differences between the reduction rates of trehalose. Meanwhile, reduction rates of glycogen had significant differences between different strains, where the FH-620 and FC-620 strains had lowest rates as 18.12 and 20.70 %, respectively. Also, total viable cells and gassing power of all strains were decreased by increasing the storage period. FH-620 and FC-620 strains had the lowest values of reduction rates as an indicator of storage resistant. Where the reduction rates in total viable cells of FH-620 and FC-620 strains were 22.05 and 24.70%, respectively, while the reduction rates of gassing power were 20.90 and 24.30%, in the same order. On other hand, FAT-12 strain was more sensitive to storage as compared to original strain, where the reduction rates were 35.60 and 35.75%, respectively for total viable cells and gassing power.

***Keywords*—**Baker's yeast, trehalose, glycogen, gassing power.

I. Introduction

BAKER'S yeast is a mass of viable cells of *Saccharomyces* cerevisiae strain under aerobic conditions. *S. cerevisiae* grow with a rapid rate of reproduction [1]. Dough is inoculated with baker's yeast and incubated at the required temperature and time. The CO_2 produced during the incubation period of the baking process defined as gassing power that is the primary leavening agent in bread products [2], [3].

The important quality parameter is storage stability. In this respect, Jorgensen et al. [4] studied the effect of storage on trehalose and glycogen content of baker's yeast. During storage for 1 month at 5°C, both trehalose and glycogen were decreased. Plourde-Owobi et al. [5] reported that the trehalose accumulates up to 15% of the cell dry mass. In addition, Mahmud et al. [6] noticed that the trehalose is one of the reserve carbohydrates in the yeast *S. cerevisiae* and can constitute up to 25% of the dry mass of a cell.

Cahill et al. [7] reported that the glycogen is a polymer of glucose and serves as an intracellular store of carbohydrate in yeast cells for energy and metabolic intermediates. In addition, Wilson et al. [8] reported that the glycogen is a glucose polymer in branched form as a reserve source of carbon and energy for yeast cells. Moreover, Torija et al. [9] stated that glycogen is a polymer of glucose units linked by a-1, 4- bonds with a-1, 6-branches. It can accumulate to account for up to 10–15% of the cell dry mass in yeast. Glycogen converts to glucose during glycolysis pathway, then to pyruvate during gluconeogenesis pathway as useful source of carbon, energy (ATP) and reducing equivalents (NADH) for yeast cells [10].

Ismail [11] studied the gassing power of different baker's yeast strains and noticed that gassing power of *S. cerevisiae* (L20), *S. cerevisiae* (5.X), *S. cerevisiae* (SH), *S. cerevisiae* (B.56), *S. cerevisiae* (HX), *S. cerevisiae* (A12) and *S. cerevisiae* (YH) was 650, 725, 675, 650, 595, 625 and 675 cm^3 CO_2/h, in succession. Moreover, Ahmed [12] studied the quality parameters of eighteen *S. cerevisiae* strains. Ahmed reported that *S. cerevisiae* (5X) contained the highest trehalose 9.6%, while, *S. cerevisiae* (FD-16) contained the lowest trehalose 5.8%. At the same time, the count of total viable cells ranged between 10.9×10^9 and 13.1×10^9 CFU/g for *S. cerevisiae* (Alx) and *S. cerevisiae* (5X), respectively. Ahmed added that the highest fermentation power was 750 cm^3 CO_2/h for *S. cerevisiae* (5X), *S. cerevisiae* (A1), *S. cerevisiae* (FG) and *S. cerevisiae* (F.125), followed by *S. cerevisiae* (B.T. 700), *S. cerevisiae* (NF-10) and *S. cerevisiae* (Sal) as 725, 725 and 700 cm^3 CO_2/h, respectively. The fermentation power of other yeast strains was lower than that of the above-mentioned strains.

Aranda et al. [13] reported that the trehalose is a non-reducing disaccharide composed of two molecules of glucose linked by a α-(1,1)-glycosidic bond. The biosynthesis of trehalose occurs entirely in the cytosol. Trehalose has important metabolic roles as reserve carbohydrate. They added that the intracellular trehalose can accumulate up to 13% of biomass dry weight (0.13 g trehalose g^{-1} biomass).

Ismail[14] studied the quality parameters of different baker's yeast strains and reported that the percentages of trehalose content ranged from 4.20 to 8.10 %. Also, the gassing power of *S. cerevisiae* (HW), *S. cerevisiae* (Angel), *S. cerevisiae* (SH), *S. cerevisiae* (PA), *S. cerevisiae* (NIT), *S. cerevisiae* (BLG), *S. cerevisiae* (ALL), *S. cerevisiae* (OZ), *S.*

G. N. Abdel-Rahman is at the Dept. of Food Toxicology and Contaminants, National Res. Centre, Dokki, Giza, Egypt (e-mail: Gomma1985@yahoo.com).

N. R. A. Nassar and Y. A. Heikal are at the Food Science Dept., Faculty of Agric., Ain Shams University, Cairo, Egypt.

M. A. M. Abou-Donia and Mohamed B. M. Ahmed are at the Dept. of Food Toxicology and Contaminants, National Res. Centre, Dokki, Giza, Egypt.

Mohamed Fadel is a head of Microbial Chemistry Dept., National Res. Centre, Dokki, Giza, Egypt.

cerevisiae (SAF) and *S. cerevisiae* (PAK) was 550, 575, 675, 650, 875, 800, 775, 700, 800 and 850 cm^3 CO_2/h, consecutively.

Kus-Liskiewicz et al. [15] revealed that the trehalose is an essential factor to storage stability of baker's yeast. In addition, Yoshiyama et al. [16] noticed that the trehalose is a non-reducing disaccharide of glucose, is considered one of the most important molecules protecting against environmental stresses in *S. cerevisiae*. The aim of this study is investigation of storage stability of baker's yeast produced from three *S. cerevisiae* strains as comparison with baker's yeast produced by original strain of baker's yeast factory.

II. Materials and Methods

A. Activation of Yeast Strains

The source of yeast strains (*S. cerevisiae* FC-620, *S. cerevisiae* FH-620 and *S. cerevisiae* FAT-12) is department of Microbial Chemistry at National Research Centre, Egypt. Meanwhile, *S. cerevisae* F-707 strain were obtained from baker's yeast factory. The yeast strains were transferred to a fresh YMP agar slants (3 g yeast extract, 3 g malt extract, 5 g peptone and 20 g agar per liter of distilled water) and incubated at 30°C for 24 h.

B. Cultivation of Yeast Strains

Fifty ml of YMPS broth medium (3 g yeast extract, 3 g malt extract, 5 g peptone and 20 g sucrose per liter of distilled water) were placed in 250 ml Erlenmeyer flasks, then autoclaved for 30 min at 121°C. The sterile medium in each flask was inoculated by a loopful of an active yeast culture slant, incubated for 24 h at 30°C under shaking condition (150 rpm), then used as a stock inoculum [17]. At the end of incubation period, the yeast cells were recovered from the growth medium by centrifugation at 4500 rpm for 5 min and used for determination of storage stability.

C. Determination of Storage Stability

Baker's yeast was stored at 4 °C in refrigerator for 15 days and then the samples were taken every 3 days. Gassing power, total viable cells as well as glycogen and trehalose content were determined.

D. Determination of Gassing Power

SJA-Fermentograph NASSJO-Sweden was used for gassing power estimation of baker's yeast produced from different *S. cerevisiae* strains. The aim of analysis is to determine the total carbon dioxide produced per one hour through fermentation of baker's yeast in prepared dough (160 ml water, 10 g baker's yeast, 4g NaC1 and 280 g wheat flour, 72%). The prepared dough was mixed at 35°C for 5 min using a Diosna D-4500 mixer. The mixed dough was transferred to a plate and placed in Fermentograph at 35°C. The recorder of Fermentograph was allowed to draw the curve of produced CO_2 during 1 h. The gassing power was described as volume of produced CO_2 from prepared dough after 1 h. [18], [19].

E. Total Viable Yeast Cells Count

To prepare the yeast suspension, the yeast sample was added to the saline solution (1:10) then serially diluted and plated on medium of acidic dextrose agar using pour plate technique according to Egyptian Standard [19]. The inoculated plates were incubated at 30°C for 2 days. The developing colonies were counted and the total viable yeast counts were expressed as colony forming unit (CFU) per gram of yeast.

F. Glycogen and Trehalose Content

The sample of baker's yeast (about 3 g) was suspended in 25 ml of 0.25 M Na_2CO_3 using screw-top tubes and incubated at 95°C for 4 h. The mixture was adjusted to pH 5.2. One-half of the suspension was incubated over night with trehalase (0.05 U/ml) at 37°C and the second half with amyloglucosidase (1.2 U/ml) at 57°C. The suspensions were centrifuged for 3 min at 4500 rpm [20]. The glucose of supernatants was determined by dinitrosalicylic acid (DNS) method according to Gusakov et al. [21].

G. Statistical Analysis

The statistical analysis of data was achieved by one-way analysis of variance (ANOVA) using SAS program [22]. The experimental results were the average of three replicates (probability ≤ 0.05).

III. Results and Discussion

Baker's yeast produced by three *S. cerevisiae* strains (*S. cerevisiae* FC-620, *S. cerevisiae* FH-620 and *S. cerevisiae* FAT-12) as well as original strain (S. *cerevisae* F-707) were stored at 4°C in refrigerator to estimation the storage sugars content, total viable cells and gassing power as indicators for storage stability. Baker's yeast samples were taken up to 15 days with intervals of 3 days and the obtained results are presented in Tables I and II).

A. Effect of Storage Period on Trehalose and Glycogen Content

The results presented in Table I and illustrated by Fig. 1 showed that the *S. cerevisiae* FH-620 strain had the highest values of trehalose at all storage periods, followed by *S. cerevisiae* FC-620 strain as compared to original strain. Meanwhile, *S. cerevisiae* FAT-12 strain had the minimum values of trehalose with significant differences between the obtained values for all strains.

In addition, data in Table, I shows clearly that the trehalose content ranged from 10.19 to 14.79 % (d.w.) before storage. Meanwhile, the trehalose content of all *S. cerevisiae* strains was decreased by increasing the storage period. No significant differences were found between the tested strains with respect to the reduction rates of trehalose, this may be return to the studied *S. cerevisiae* strains have the equal activity of trehalase [23].

TABLE I
EFFECT OF STORAGE PERIOD ON TREHALOSE AND GLYCOGEN CONTENT OF *S. CEREVISIAE* STRAINS

Season	Strain	Storage period (days)						Rate of reduction (%)
		0	3	6	9	12	15	
Trehalose*	*S. cerevisiae* FC-620	13.52 ^{b} ± 0.29	13.03 ^{b} ± 0.24	12.32 ^{b} ± 0.29	11.74 ^{b} ± 0.25	10.77 ^{b} ± 0.19	9.25 ^{b} ± 0.14	31.58 ^{a} ± 0.44
	S. cerevisae F-707	12.63 ^{b} ± 0.21	12.12 ^{c} ± 0.24	11.52 ^{b} ± 0.27	10.95 ^{b} ± 0.33	9.95 ^{b} ± 0.37	8.51 ^{b} ± 0.36	32.65 ^{a} ± 1.73
	S. cerevisiae FH-620	14.79 ^{a} ± 0.27	14.24 ^{a} ± 0.22	13.54 ^{a} ± 0.28	12.99 ^{a} ± 0.26	11.95 ^{a} ± 0.26	10.30 ^{a} ± 0.19	30.36 ^{a} ± 0.01
	S. cerevisiae FAT-12	10.19 ^{c} ± 0.14	9.75 ^{d} ± 0.17	9.09 ^{c} ± 0.22	8.59 ^{c} ± 0.17	7.78 ^{c} ± 0.13	6.74 ^{c} ± 0.14	33.87 ^{a} ± 0.47
	LSD	0.92	0.86	1.05	1.02	1.00	0.89	3.62
Glycogen*	*S. cerevisiae* FC-620	10.69 ^{a} ± 0.24	10.35 ^{a} ± 0.21	10.10 ^{a} ± 0.12	9.46 ^{a} ± 0.08	9.01 ^{a} ± 0.05	8.47 ^{a} ± 0.03	20.70 ^{b} ± 1.47
	S. cerevisae F-707	10.05 ^{a} ± 0.17	9.71 ^{a} ± 0.19	9.17 ^{b} ± 0.12	8.52 ^{b} ± 0.14	7.97 ^{b} ± 0.16	7.33 ^{b} ± 0.19	27.08 ^{a} ± 0.66
	S. cerevisiae FH-620	10.46 ^{a} ± 0.23	10.23 ^{a} ± 0.16	9.98 ^{a} ± 0.16	9.54 ^{a} ± 0.08	9.10 ^{a} ± 0.10	8.56 ^{a} ± 0.14	18.12 ^{b} ± 0.43
	S. cerevisiae FAT-12	10.16 ^{a} ± 0.21	9.84 ^{a} ± 0.22	9.21 ^{b} ± 0.14	8.57 ^{b} ± 0.17	7.93 ^{b} ± 0.21	7.28 ^{b} ± 0.25	28.37 ^{a} ± 0.98
	LSD	0.83	0.77	0.53	0.49	0.56	0.68	3.78

* Determined on dry weight basis. Means followed by different superscripts within column are significantly different at the 5%level.

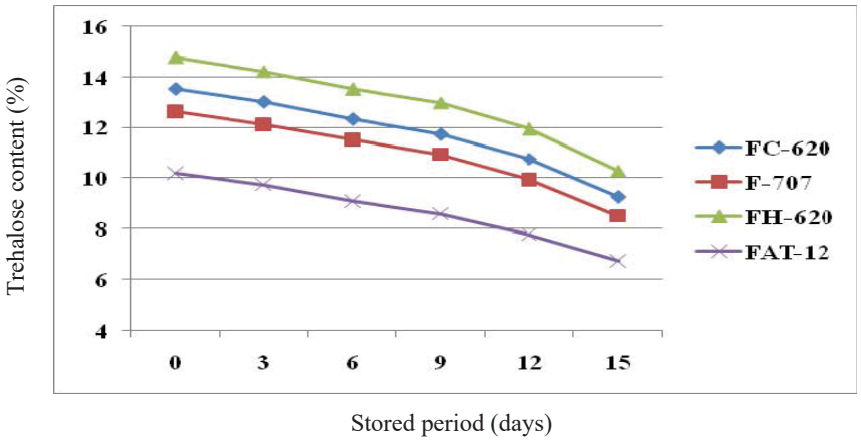

Fig. 1 Effect of storage period on trehalose content of *S. cerevisiae* strains

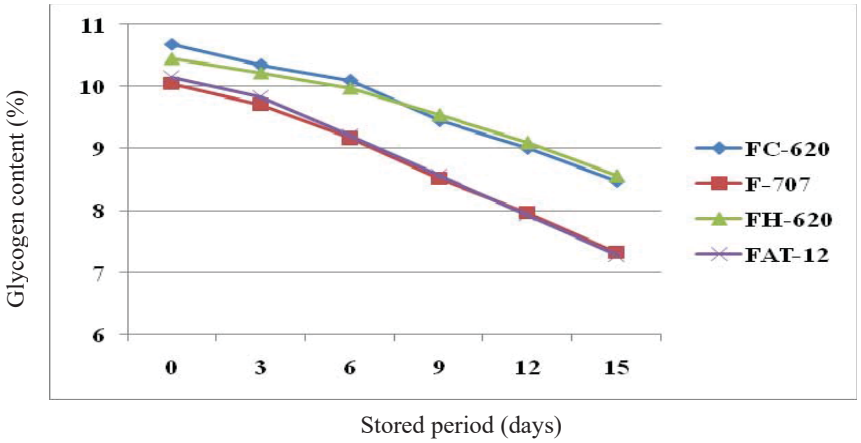

Fig. 2 Effect of storage period on glycogen content of *S. cerevisiae* strains

These results are in accordance with those obtained by Plourde-Owobi et al. [5], who stated that the trehalose accumulates up to 15% of the cell dry mass. Also, Aranda et al. [13] reported the yeast can accumulate trehalose up to 13% of biomass dry weight. Furthermore, Ahmed [12] noticed that the trehalose content of different *S. cerevisiae* strains ranged between 5.8 and 14.9%. Moreover, Ismail [14] stated that the trehalose content of *S. cerevisiae* NIT, *S. cerevisiae* BLG and *S. cerevisiae* NG strains were 12.2, 14.5 and 14.0%, respectively. The trehalose content of *S. cerevisiae* strains was decreased by increasing the storage period.

Regarding the storage effect on glycogen content of *S. cerevisiae* strains, data presented in Table I and illustrated by Fig. 2 show that the glycogen content ranged from 10.05 to 10.69 % (d.w.) before storage. Meanwhile, the glycogen content of all *S. cerevisiae* strains was decreased by increasing the storage period. Significant differences were observed between reduction rates of glycogen of different strains. This may be attributed to variation of enzymatic hydrolysis of glycogen by amyloglucosidase during storage period [4].

Also, it was found from the obtained results (Table I) that, no significant differences were observed between glycogen content of *S. cerevisiae* strains during storage at zero time and three days. On the other hand, *S. cerevisiae* FH-620 strain and *S. cerevisiae* FC-620 strain had the maximum values of glycogen at higher storage periods (from 6 to 15 days). Meanwhile, *S. cerevisiae* FAT-12 strain had the minimum values of glycogen at the same storage periods as compared to original strain, with significant differences between the obtained values.

These results are in agreement with those obtained by Jorgensen et al. [4] who studied the effect of storage on trehalose and glycogen content of baker's yeast. During storage for 1 month at 5°C, both trehalose and glycogen were decreased. Moreover, Torija et al. [9] stated that glycogen is a polymer of glucose units. It can accumulate to account for up to 10–15% of the cell dry mass in yeast.

The decrease in trehalose and glycogen content during storage of baker's yeast might be attributed to the interconversion of glycogen and trehalose to glucose and then to pyruvate through the glycolysis and the gluconeogenesis pathways, provide cells with useful source of carbon, energy (ATP) and reducing equivalents (NADH) [10].

B. Effect of Storage Period on Total Viable Cells and Gassing Power

With regard to the effect of storage on total viable cells and gassing power of different *S. cerevisiae* strains, the results presented in Table II and illustrated by Figs. 3 and 4 revealed that total viable cells and gassing power of all *S. cerevisiae* strains were decreased by increasing the storage period. In addition, *S. cerevisiae* FH-620 and *S. cerevisiae* FC-620 strains were more resistant to storage, where these strains had lowest values of reduction rates; being 22.05 and 24.70%, respectively for total viable cells and 20.90 and 24.30%, respectively for gassing power.

TABLE II
EFFECT OF STORAGE PERIOD ON TOTAL VIABLE CELLS AND GASSING POWER OF *S. CEREVISIAE* STRAINS

Season	Strain	Storage period (days)						Rate of reduction (%)
		0	3	6	9	12	15	
Total viable cells	S. *cerevisiae* FC-620	19.72 ^{b} ± 0.15	18.95 ^{bc} ± 0.18	18.21 ^{bc} ± 0.18	17.49 ^{b} ± 0.21	16.70 ^{b} ± 0.21	14.84 ^{b} ± 0.19	24.70 ^{c} ± 0.40
	S. *cerevisae* F-707	20.50 ^{a} ± 0.11	19.56 ^{ab} ± 0.28	18.88 ^{ab} ± 0.26	17.32 ^{b} ± 0.23	16.31 ^{b} ± 0.24	14.15 ^{b} ± 0.17	31.00 ^{b} ± 1.20
	S. *cerevisiae* FH-620	20.47 ^{a} ± 0.15	20.00 ^{a} ± 0.22	19.65 ^{a} ± 0.22	18.54 ^{a} ± 0.24	17.78 ^{a} ± 0.26	15.95 ^{a} ± 0.15	22.05 ^{c} ± 0.15
	S. *cerevisiae* FAT-12	20.12 ^{ab} ± 0.11	18.51 ^{c} ± 0.30	17.52 ^{c} ± 0.16	15.71 ^{c} ± 0.15	14.20 ^{c} ± 0.19	12.96 ^{c} ± 0.25	35.60 ^{a} ± 0.90
	LSD	0.52	0.98	0.82	0.83	0.89	0.76	3.06
Gassing power	S. *cerevisiae* FC-620	1338 ^{a} ± 12.5	1275 ^{a} ± 25.0	1238 ^{a} ± 12.5	1175 ^{ab} ± 25.0	1125 ^{a} ± 25.0	1013 ^{b} ± 12.5	24.30 ^{c} ± 0.20
	S. *cerevisae* F-707	1388 ^{a} ± 12.5	1313 ^{a} ± 37.5	1250 ^{a} ± 50.0	1163 ^{ab} ± 37.5	1100 ^{a} ± 25.0	963 ^{b} ± 12.5	30.65 ^{b} ± 0.25
	S. *cerevisiae* FH-620	1375 ^{a} ± 25.0	1325 ^{a} ± 50.0	1288 ^{a} ± 62.5	1225 ^{a} ± 50.0	1188 ^{a} ± 37.5	1088 ^{a} ± 12.5	20.90 ^{d} ± 0.50
	S. *cerevisiae* FAT-12	1363 ^{a} ± 12.5	1250 ^{a} ± 25.0	1150 ^{a} ± 50.0	1038 ^{b} ± 37.5	950 ^{b} ± 25.0	875 ^{c} ± 25.0	35.75 ^{a} ± 1.25
	LSD	64.9	141.0	186.9	151.3	112.5	64.9	2.72

Means followed by different superscripts within column are significantly different at the 5%level.

On other hand, *S. cerevisiae* FAT-12 strain were more sensitive to storage as compared to original strain, where these strains had highest values of reduction rates; being 35.60% and 35.75%, respectively for total viable cells and gassing power.

The variation in reduction rates of total viable cells and gassing power as an indicator of *S. cerevisiae* resistance to storage may be return to variation in trehalose content of *S. cerevisiae* strains (Table I), where *S. cerevisiae* FH-620 strain had the highest values of trehalose and was more resistant to storage.

These results are in agreement with those of Yoshiyama et al. [16], who noticed that the trehalose is considered one of the most important molecules protecting yeast cells against

environmental stresses in *S. cerevisiae*. Moreover, Ismail [14] reported that the gassing power of different *S. cerevisiae* strains was decreased by increasing the storage period. Also, Salem [24] reported that the total viable cells of baker's yeast were decreased by increasing the storage period.

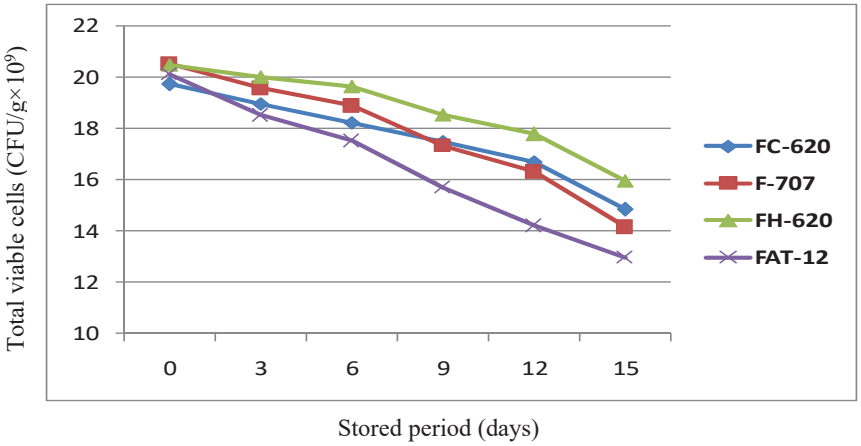

Fig. 3 Effect of storage period on total viable cells of *S. cerevisiae* strains

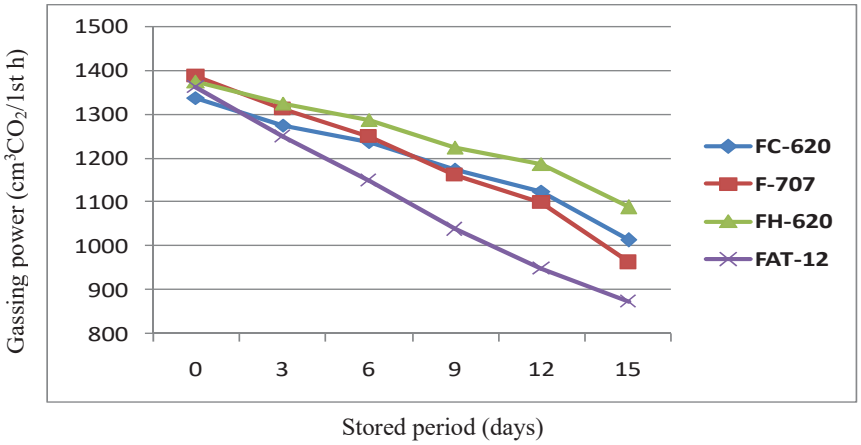

Fig. 4 Effect of storage period on gassing power of *S. cerevisiae* strains

Acknowledgment

This work was financially supported by the National Research Center, Dokki, Cairo, Egypt. The authors would like to thank Dr. Essam Abdel-Fattah Saleh and Dr. Mohamed Ramadan El-Gohary for their kind help and support.

References

[1] M. A. Eskarous. The Effect of Activators on the Production and Chemical Constitution of Yeast. PhD Thesis, Fac. Agric., Cairo Univ., Egypt, 1979, 176 p.
[2] W. Damtew. Studies on the development of baker's yeast using cane molasses. M.Sc. Thesis, Fac. Technol., Addis Ababa Univ., Addis Ababa, 2008, 189 p.
[3] M. N. Rezaei, E. Dornez, P. Jacobs, A. Parsi, K. J. Verstrepen and C. M. Courtin. Harvesting yeast (*Saccharomyces cerevisiae*) at different physiological phases significantly affects its functionality in bread dough fermentation. Food Microbiology, 2014, vol. 39 pp. 108-115.
[4] H. Jorgensen, L. Olsson, B. Ronnow and E. A. Palmqvist. Fed-batch cultivation of baker's yeast followed by nitrogen or carbon starvation: effects on fermentative capacity and content of trehalose and glycogen. Appl. Microbiol. Biotechnol., 2002, vol. 59 pp. 310-317.
[5] L. Plourde-Owobi, S. Durner, G. Goma and J. Francois. Trehalose reserve in *Saccharomyces cerevisiae*: phenomenon of transport, accumulation and role in cell viability. International Journal of Food Microbiology, 2000, vol. 55 pp. 33-40.
[6] S. A. Mahmud, T. Hirasawa and H. Shimizu. Differential importance of trehalose accumulation in *Saccharomyces cerevisiae* in response to various environmental stresses. Journal of Bioscience and Bioengineering, 2010, vol. 109 pp. 262-266.
[7] G. Cahill, P. K. Walsh and D. Donnelly. Determination of yeast glycogen content by individual cell spectroscopy using image analysis. Biotechnology and Bioengineering, 2000, vol. 69 pp. 312-322.
[8] W. A. Wilson, W. E. Hughes, W. Tomamichel and P. J. Roach. Increased glycogen storage in yeast results in less branched glycogen. Biochemical and Biophysical Research Communications, 2004, vol. 320 pp.416-423.
[9] M. Torija, M. Novo, A. Lemassu, W. Wilson, P. J. Roach, J. Francois and J. Parrou. Glycogen synthesis in the absence of glycogenin in the yeast *Saccharomyces cerevisiae*. FEBS Letters, 2005, vol. 579 pp. 3999-4004.
[10] A. B. Lanham, A. R. Ricardo, M. Coma, J. Fradinho, M. Carvalheira, A. Oehmen, G. Carvalho and M. A. Reis. Optimization of glycogen quantification in mixed microbial cultures. Bioresource Technology, 2012, vol. 118 pp. 518-525.

[11] A. M. Ismail. Chemical and Technological Studies on Baker's Yeast. MS Thesis, Fac. Agric., Al-Azhar Univ., Egypt, 2003.
[12] S. A. Ahmed. Microbiological and Chemical Studies on S. *cerevisiae* Resistant to High Concentrations of Sugar and Alcohol. PhD Thesis, Fac. Agric., Al-Azhar Univ., Egypt, 2003.
[13] J. S. Aranda, E. Salgado and P. Taillandier. Trehalose accumulation in *Saccharomyces cerevisiae* cells: experimental data and structured modeling. Biochemical Engineering Journal, 2004, vol. 17 pp. 129-140.
[14] A. M. Ismail. Comparison Study on Some Baker's Yeast Strains. PhD Thesis, Fac. Agric., Al-Azhar Univ., Egypt, 2006.
[15] M. Kus-Liskiewicz, A. Gorka and M. Gonchar. Simple assay of trehalose in industrial yeast. Food Chemistry, 2014, vol. 158 pp. 335-339.
[16] Y. Yoshiyama, K. Tanaka, K. Yoshiyama, M. Hibi, J. Ogawa and J. Shima. Trehalose accumulation enhances tolerance of *Saccharomyces cerevisiae* to acetic acid. Journal of Bioscience and Bioengineering, 2015, vol. 119 pp. 172-175.
[17] E. V. Soares, K. Hebbelinck and H. M. V. M. Soares. Toxic effects caused by heavy metals in the yeast *Saccharomyces cerevisiae*: a comparative study. Can J. Microbiol., 2003, vol. 49 pp. 336-343.
[18] M. Suihko and V. Mfikinen. Candida krusei in baker's yeast production. European J. Appl. Microbiol. Biotechnol., 1981, vol. 13 pp. 113-116.
[19] Egyptian Standard. Egyptian Standard of yeast Part 2: methods of analysis and testing for yeast. Egyptian Organization for Standardization and Quality Control, E.S. 191/2000.
[20] J. L. Parrou and J. Francois. A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells. Analytical Biochemistry, 1997, vol. 248 pp. 186-188.
[21] A. V. Gusakov, E. G. Kondratyeva and A. P. Sinitsyn. Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. International Journal of Analytical Chemistry, 2011, vol. 2011 pp. 1-4.
[22] SAS: Statistical Analysis System, SAS / STAT User's Guide. Release 6.03 Ed. SAS Institute, Cary, NC, 1028 PP., 1999.
[23] J. F. Mesquita, V. M. F. Paschoalin and A. D. Panek. Modulation of trehalase activity in *Saccharomyces cerevisiae* by an intrinsic protein. Biochimica et Biophysica Acta, 1997, vol. 1334 pp. 233-239.
[24] S. H. Salem. Applying HACCP system on dried yeast production line. MS Thesis, Botany and Microbiology Dept., Fac. of Science., Al-Azhar Univ., Egypt, 2010.