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The influence of different forms of backery yeast *Saccharomyces cerevisie* type strain on the concentration of individual sugars and their utilization during fermentation

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Abstract

The research was performed on the fermentation dynamics of carbohydrates in dough by three commercial forms of Saccharomyces cerevisiae type of yeasts produced by S.C. Rompak S.A. Pascani, Romania (compressed yeast, instant active dry yeast and active dry yeast) at 0, 60, 120 and 180 minutes of dough fermentation. Experiments were performed using high-performance liquid chromatography for the analysis of sugars during dough fermentation. A Chopin rheofermentometer was also used to analyze gas production and dough height. Using HPLC, a variation of sugars quantity was observed on the entire duration of the fermentation, depending on the form of the used yeast, in the following ascending order: dry instant active yeast > compressed yeast > dry active yeast. From the point of view of the results obtained by Chopin rheofermentometer device, it was observed that the dough fermented with the compressed yeast has the highest released quantities of carbon dioxide.

Keywords: *yeast, dough fermentation, HPLC, Chopin rheofermentometer*

Introduction

The yeast used for bread manufacture is *Saccharomyces cerevisiae*, often referred to as simply baker's yeast. It converts the fermentable sugars present in the dough into carbon dioxide and ethanol as the main products. The fermentation intensity depends on the form of the yeast and the availability of fermentable sugars in the flour, including maltose produced by starch hydrolysis. **[1].** The total amount of mono- and disaccharides in flour varied about a mean of 4 mg/g of flour. Sucrose is the most abundant, accounting for more than 50% of the total soluble sugars **[2, 3, 4, 5].**

Henry R.J. and Saini H.S. (1989) consider that the most important carbohydrates from flour influencing the loaf volume are glucose, fructose and sucrose. The order in which these different carbohydrates are fermented by *Saccharomyces cerevisiae* is not at random, but rather is based on a specific hierarchy, glucose being the preferred sugar. It seems that glucose slows down the uptake of fructose because both sugars are imported by the same carriers, which have a greater affinity for glucose than for fructose **[6].**

Of the above mentioned carbohydrates, sucrose is converted almost immediately to glucose and fructose, due to the potent invertase of yeast **[4, 5, 7].** When the concentration of glucose and fructose is high enough, the maltose concentration in dough is increasing due to amylase, a starch degrading enzyme in flour, which is constantly generating new glucose and maltose in flour starch. When glucose and fructose are finished, the maltose concentration begins to decrease, making difficult for yeast cells to hydrolyze since they do not have the necessary enzymatic equipment.

In time, working methods and techniques such as thin layer chromatography **[8, 9, 10]**, ion-exchange chromatography **[2]** and latter-day HPLC **[4, 5, 7]** applied in the study of carbohydrate variation have been improved to increase the accuracy and precision of performed determinations.

To our knowledge, no report has emphasized so far the importance of fermentation time at different forms of yeast *Saccharomyces cerevisiae* strain on the sugar content in dough, determined by using the HPLC system.

The purpose of this study is to investigate how different forms of bakery yeast *Saccharomyces cerevisiae* type and the fermentation time, can affect the mono- and disaccharide concentration determined by HPLC system and gas production, using Chopin rheofermentometer device.

Material and methods

Materials. Commercial wheat flour (harvest 2008) was milled by an Buhler mill (no. MDDO 1250) from S.C. Sapte Spice S.A. Valcea.

Flour parameters selected for this study are presented in table 1. On the basis of physical-chemical and rheological data, the sample has an average quality for bread making.

Analytical characteristics		**Rheological characteristics**	
Parameter	Value	Parameter	Value
Ash content (%)	0.47±0.002	Water absorption (%)	56.9±0.10
Moisture content (%)	14.10±0.02	Development time (min)	2.3±0.20
Wet gluten (%)	26.50±0.1	Stability (min)	5.8±0.20
Gluten deformation (mm)	6.00±0.2	Weakening (B.U.)	84.0±1.0
Protein content (%)	11.40±0.01	Tenacity (mm H_2O)	91.0±2.0
Sucrose (mg/g)	2.47±0.02	Extensibility (mm)	45.0±2.0
Maltose (mg/g)	0.49±0.01	Swelling index	14.9±0.01
Fructose (mg/g)	0.42±0.02	Baking strength (10^{-4} J)	156.0±2.0
Glucose (mg/g)	0.33±0.02	Configuration ratio	2.0±0.02
Falling number (sec.)	404.00±2.0	-	-

Methods. Flour quality tests were accomplished according to Romanian or international standard methods: ash content (AACC Standard No. 08-21), moisture content (ICC Standard No. 202), wet gluten content (ICC Standard No. 155), gluten deformation (SR 90/2007), protein content (ICC Standard No. 202), falling number (ICC Standard No. 107/1) and the viscoelastic parameters, specific for the farinograph method (SR ISO 5530-1/1999) and for the alveographic method (AACC Standard No. 54-30A).

The yeast used in this study was *Saccharomyces cerevisiae* type made by S.C. Rompak S.A Romania in three different commercial forms: compressed yeast, instant active dry yeast and active dry yeast. Some characteristics (supplied by S.C. Rompak S.A Romania) of these yeasts are given in table 2. Fructose, glucose, sucrose, and maltose (Merck) were used without further purification.

Table 2. Characteristics of yeast samples

Characteristics	**Compressed yeast**	**Instant active dry yeast**	**Active dry yeast**
Moisture %	67.48	4.5	6.5
Protein content %	40	42	42
Measured volumes of liberated CO_2 during 1^h, 2^{hrs} and total of dough fermentation	740/1190/1930	1000	1000

Mixing conditions. Dough recipe consists of flour, water (accord. WA %), 3% yeast (% flour basis) and 1.5% salt (% flour basis). Instant and active dry yeasts are rehydrated before mixing. The dough was kneaded for 15 minutes and afterwards fermented for 180 minutes at 30˚C temperature and 80% relative humidity. During the fermentation period, two re-kneading were done at 60 minutes of 30 second each. Analyses were performed before the insertion in the proofing device after one hour of fermentation, two hours of fermentation and three hours of fermentation.

Sugar extraction. Firstly 10 g of dough sample were boiled for 20 min in 100 mL of 60% ethanol. After the cooling phase, the sample is centrifuged for 15 minutes at a speed of 4000 rotations / minute. Afterwards, the supernatant was concentrated in a vacuum pump and filtered by using a Whatman 42 paper. Additionally, the filtered material was passed through a filtering membrane of 0.22 µm before the injection into the HPLC system. Chromatographic determinations were carried out by using the HPLC system with the following operating conditions: column –Waters with aminopropyl-bonded phase -C18, 250 mm x 4.6 mm, 4 µm particle diameter; eluent – acetonitrile: water solution (with sodium chloride 0.125%w/v to minimize the interference from NaCl) = 75 : 25; flow rate 1 mL/min; temperature 30°C; injected volume 20 µl. An Alltec refractive index detector was used. Sugar concentration was calculated based on peak area measurements. The retention times of sugars are: fructose – 5 min; glucose – 5.8 min; sucrose – 8.2 min; maltose – 9.8 min.

Measurement of gas production and dough development. The fermentation behavior of flours is analyzed at different fermentation times: 30, 60, 90, 120, 150, 180 min, by using the Chopin rheofermentometer (Tripette&Renaud). The total volume of gas production (mL/g) and dough height (mm) under stress of 1.25 kg were automatically determined.

Expression of results. All determinations were performed at least in triplicate. Values of parameters were expressed as the mean ± standard deviation to a confidence interval for mean of 95%.

Results and discussion

Effect of yeast and fermentation time on dough sugar content. The amount of fermentable sugars during of the course of the dough fermentation, by three different commercial forms of baker's yeast, is shown in table 3.

Table 3. The concentration variation (mean ± standard deviation) of fermentable sugars during dough fermentation

The analyzed sample	**Fermentation time (min)**	**Sucrose** mg/g dough	**Glucose** mg/g dough	**Fructose** mg/g dough	**Maltose** mg/g dough
Dough produced with compressed yeast	0	-	1.56±0.02	5.54±0.01	8.43±0.02
	60	-	3.09±0.01	4.21±0.01	12.32±0.02
	120	-	0.14±0.01	0.92±0.01	15.82±0.02
	180	-	0.31±0.01	0.48±0.01	8.86±0.02
Dough produced with instant dry yeast	0	-	0.86±0.01	5.03±0.02	9.64±0.02
	60	-	1.63±0.01	3.86±0.02	15.08±0.03
	120	-	0.74±0.01	1.02±0.01	17.52±0.03
	180	-	0.56±0.01	0.88±0.01	9.56±0.02
Dough produced with active dry yeast	0	-	1.64±0.01	5.32±0.01	7.21±0.02
	60	-	0.31±0.01	2.75±0.01	14.05±0.02
	120	-	0.20±0.01	1.64±0.01	16.34±0.03
	180	-	-	0.21±0.01	7.13±0.02

It can be seen that sucrose has not been identified in the dough after kneading, in all of the analyzed samples. This is due to the presence of a very active invertase in yeast, which inverts the sucrose from flour into glucose and fructose during the kneading phase. Therefore, in almost all cases, at the beginning of the fermentation phase there have been detected higher levels for these carbohydrates, than the ones from flour. While the concentration of glucose and fructose is high enough, the concentration of maltose in dough increases (with a higher rate in the first hour of fermentation and a lower one in the second hour of fermentation) and only when the other sugars are exhausted the concentration of maltose begins to decrease. In the first hour of fermentation, the production of sugars by amilolitic means overcomes the consumption in the fermentation process, therefore the content of sugars increases. In the following hour, the content of reducing sugars decreases, because of their intense consumption by the yeast. The quantity of sugars throughout the entire dough fermentation varies with the yeast forms used in the following ascending order: instant active dry yeast > compressed yeast > active dry yeast. Thus, at the end of the fermentation process, there has been registered with 31.4% more sugars for the compressed yeast and with 49.8% sugars for the instant dry yeast in dough, over the active dry yeast. Likewise, after 180 minutes of fermentation, the dough prepared using active dry yeast is the only one which no longer contains glucose, fact which leads to the conclusion that this yeast has a high fermentation rate.

Effect of yeast and fermentation time on gas production measurement and dough development. The fermentation activity of the yeast expressed through the emission of carbon dioxide (figure 1), shows that this activity is more intense for the dough prepared with the compressed yeast, which registered a volume of gas with 79.09% higher than the one for the dough prepared by using instant dry yeast and with 19.04% higher for the dough prepared by using active dry yeast. Likewise, the value obtained for the maximum height of the dough (figure 2) is correlated with the quantity of carbon dioxide that is released; the highest value obtained was also registered for the compressed yeast.

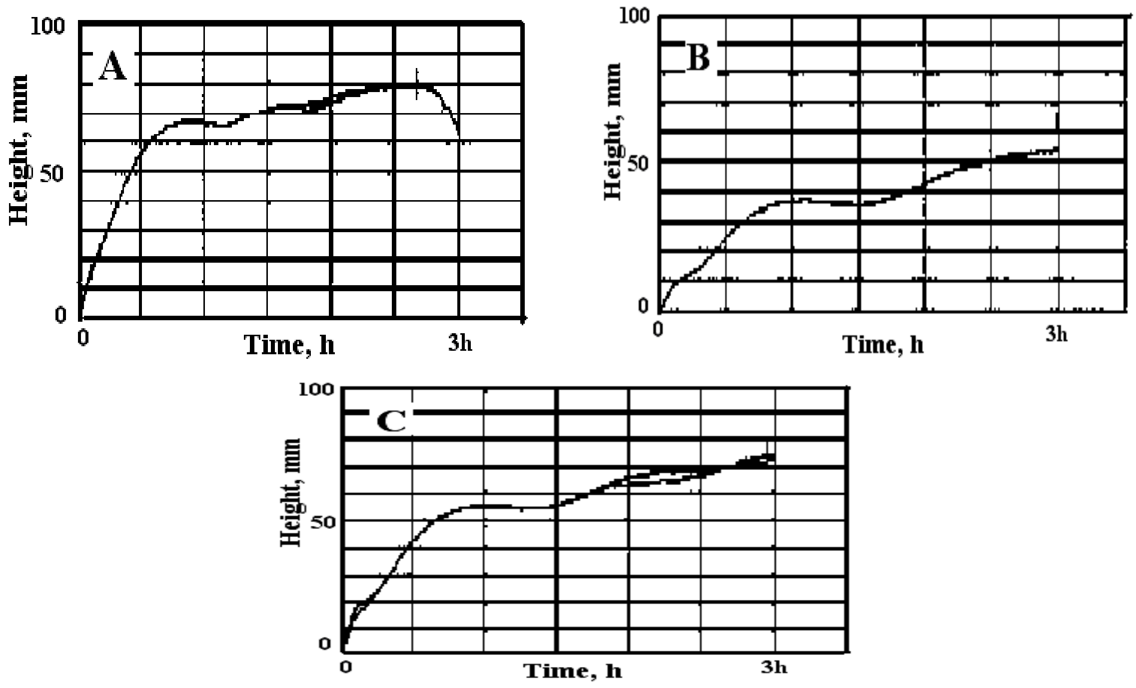

Figure 1. Gas release determined with the Chopin rheofermentometer for: A-compressed yeast, B-instant dry yeast, C-active dry yeast

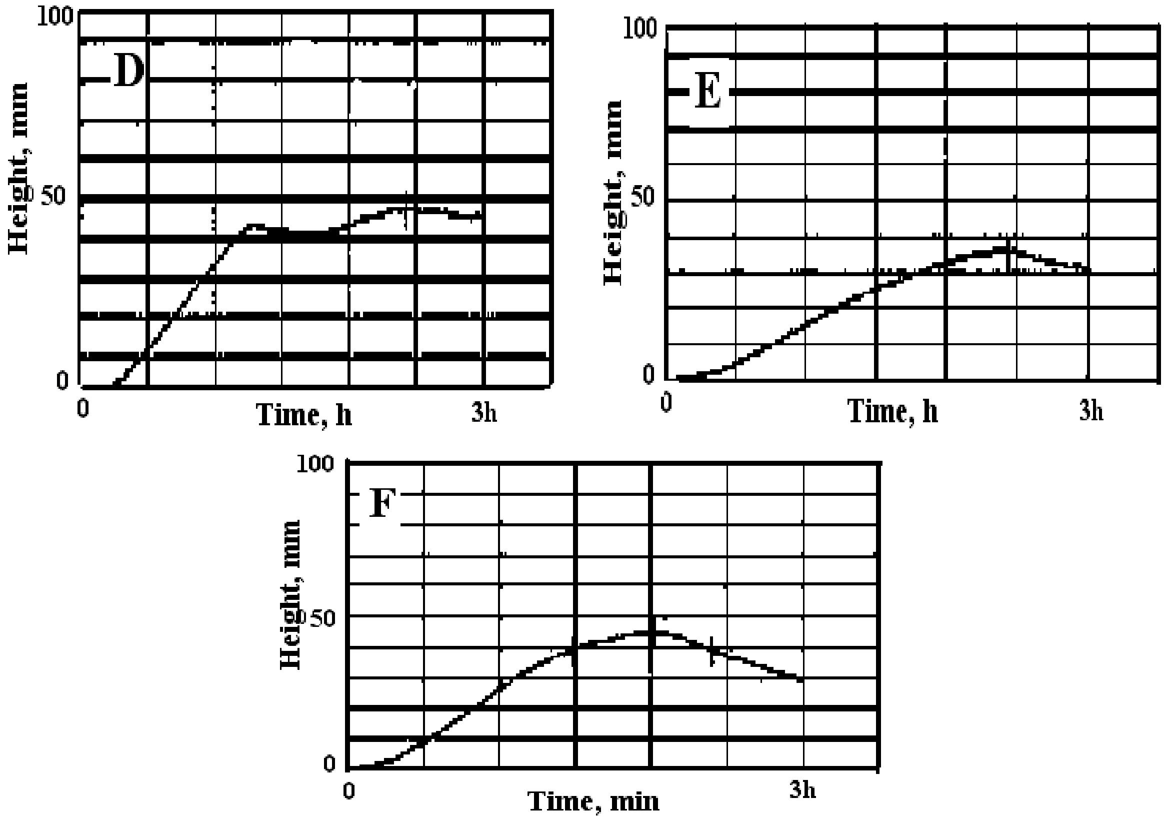

Figure 2. Dough development determined with the Chopin rheofermentometer for: D-compressed yeast, E-instant dry yeast, F-active dry yeast

From the point of view of the quantities of carbon dioxide registered during the fermentation, there is a correspondence between the gas volume registered at the Chopin rheofermentometer and the quantity of the formed carbohydrates. Table 4 shows that for the active dry yeast and for the active instant dry yeast, the minimum values of the gas volume was below the fermentation interval of 60-90 minutes which corresponds to the transition from glucose fermentation to fructose fermentation. For the dough prepared by using the compressed yeast, the minimum carbon dioxide emission is registered in the fermentation interval of 150-180 minutes, a moment which corresponds to the transition towards an incomplete activation of maltase in the dough system.

Table 4. The variation of the gas volume (mean ± standard deviation) during the fermentation, measured with the Chopin rheofermentometer

The analyzed sample	**Fermentation time (minutes)**					
	30	**60**	**90**	**120**	**150**	**180**
Dough produced with compressed yeast	60±0.3	70±0.5	73±0.5	77±0.5	81±0.4	45±0.2
Dough produced with instant dry yeast	27±0.2	38±0.3	36±0.2	42±0.7	50±0.6	52±0.7
Dough produced with active dry yeast	41±0.6	58±0.2	55±0.5	68±0.6	70±0.5	72±0.5

Conclusions

• It was observed that in the first hour of fermentation the dough sugar content increases, then in the interval 1-2 hours, glucose and fructose are used in fermentation, and after that, in the interval 2-3 hours of fermentation, especially fructose and maltose are used by the yeasts;

• From the concentration of fermentable carbohydrates during the three hours of fermentation it is noticed that the highest fermentation rate is present in the active dry yeast, followed by the compressed yeast and by the active instant dry yeast;

• From the point of view of the quantities of released carbon dioxide, the highest values are registered at the compressed yeast, followed by the instant dry yeast and by the active instant dry yeast, which indicates that the compressed yeast presents the most intensive fermentative activity of the three commercial forms of analyzed yeasts;

• Contrary to expectations, of the three forms of analyzed yeasts, the active dry yeast adapts most easily to the fermentation of sugars whereas the compressed yeast presents the highest fermentation activity.

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