THE INFLUENCE OF TABLE SALT, WHITE SUGAR AND COMPLEX BAKERY ADDITIVE ON THE FERMENTATIVE ACTIVITY OF *SACCHAROMYCES CEREVISIAE*

UTICAJ KUHINJSKE SOLI, KONZUMNOG ŠEĆERA I KOMPLEKSNOG PEKARSKOG ADITIVA NA FERMENTATIVNU AKTIVNOST *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

This study determined the influence of the addition of commercial bakery improver (0.1-0.9 %), table salt (1-7 %), and white sugar (1-9 %) into dough on fermentative activity (FA) of yeast, and consequently CO₂ production, to improve dough handling and quality of wheat bread by using an optimized additive combination. The FA of yeast cells was evaluated using fermentograph SJA device. The addition of white sugar in the amount of 1-4 % and complex additive in the amount 0.1-0.5 % enhances FA of yeast. Further increase of the amount of these two compounds decreases the FA. The addition of table salt in the amount of 1-7 % negatively affected the FA. The optimal amounts of complex additive, table salt and white sugar, obtained by response surface methodology (RSM) using Design Expert software, which provided maximum FA of 1171.31 ml CO₂/gdm/2h were the following: 0.41, 1.0 and 3.91 % based on flour weight, respectively.

Key words: fermentative activity, bakery yeast, *Saccharomyces cerevisiae*.

REZIME

Savremena proizvodnja pekarskih proizvoda podrazumeva primenu različitih komercijalnih preparata za poboljšanje fizičkih i hemijskih karakteristika testa, pa samim tim i poboljšanje senzornih i organoleptičkih osobina pekarskih proizvoda sa ciljem da se zadovolje ukus i zahtevi protošača i dobiju visoko kvalitetni proizvodi uz minimalne troškove. Fermentativna aktivnost (FA) ćelija pekarskog kvasca je jedan od osnovnih faktora koji utiče na fizičku strukturu pekarskih proizvoda. Visoka FA pekarskog kvasca podrazumeva intenzivno oslobađanje ugljendioksida u testu, što za posledicu ima dobijanje pekarskih proizvoda poroznije strukture i boljih organoleptičkih osobina. U ovom radu je utvrđen uticaj dodatka komercijalnog kompleksnog pekarskog poboljšivača (0.1-0.9%), kuhinjske soli (1-7%) i konzumnog šećera (0.9-1%) u brašno na fermentativnu aktivnost (FA) pekarskog kvasca u testu, odnosno na njegovu sposobnost proizvodnje CO₂ u cilju olakšanja obrade i pripreme testa, kao i povećanja kvaliteta pšeničnog hleba dodatkom optimalne kombinacije aditiva. FA pekarskog kvasca određena je SJA fermentografom. Ustanovljeno je da se dodatkom šećera u količini 1-4% i aditiva u količini 0,1-0,5% u brašno povećava FA kvasca u testu. Dalje povećanje količine ove dve komponente je negativno uticalo na metabolizam kvasca, odnosno FA. Dodatak kuhinjske soli u količini 1-7% je negativno uticao na FA kvasca. Optimalne količine aditiva, kuhinjske soli i konzumnog šećera, dobijene metodom odzivne površine (RSM) pomoću softvera Design Expert su: 0,41%, 1,0 % i 3,91% na masu brašna, pri čemu se ostvaruje maksimalna FA kvasca od 1171,31 ml CO₂/gdm/2h.

Ključne reči: fermentativna aktivnost, pekarski kvasac, *Saccharomyces cerevisiae*.

INTRODUCTION

Fermentative activity (FA) of bakery yeast cells is one of the main factors that affect the physical structure of the baked goods. High FA of yeast cells involves intense release of carbon dioxide in the dough. Namely, CO₂ produced by yeast causes the gluten to stretch, and some escape, but most of the gas is retained and is trapped within the matrix (Hutkins, 2006). Consequently, it creates foam-like structure of dough that is the prerequisite for a rapid heat flow through the dough, which enables porous structure and better organoleptic properties of baked goods. Further, fermentation rates and yeast cells metabolism are mainly affected by temperature and osmotic pressure. Osmotic pressure in dough can be very high, even 35 to 100 atmospheres, and mainly depends on the proportions of sugar and salt used (Poitrenaud, 2006). The leavening of doughs is highly dependent on osmotolerance of yeast (Oda and Ouchi, 1990). In the dough, carbon sources are constituted of small quantities of glucose, fructose and sucrose. Glucose and fructose

can be directly metabolised as they are diffused through the cellular membrane. There is a greater preference for glucose, which is the first to be used in the dough. By decreasing the glucose concentration, the yeast uses the fructose in the dough. The sucrose is hydrolysed to glucose and fructose by yeast invertase in the first minutes of mixing. Therefore, at the end of this process, the dough only has glucose, fructose and maltose. The introduction of sucrose in the dough is a very common practice in bakeries. By introducing this type of disaccharide in the dough, we deal with an increase of fermentable glucid quantity. As a consequence, yeast activity is accelerated. The process occurs until a maximum degree of sucrose is added; afterwards, yeast activity is inhibited (Voica and Codină, 2009). Yeasts used in baking are often exposed to high concentrations of sugars. Industrial bakers' yeasts express high levels of invertase activity, which cleaves sucrose rapidly to effectively double osmotic pressure of sweet doughs (Evans 1990). Many large scale bakeries add sucrose (about 1 % to 3 %) as an additional source of readily fermentable sugars (Voica and Codină, 2009). Sweet

bread doughs can contain up to 30 % of added sucrose per weight of flour, which exerts a severe osmotic stress (Reed and Nagodawithana, 1991). Table salt is generally considered to be an important chemical compound (additive) in bakery production, since it affects palatability, taste, and the structural and physical properties of bread. The salt is used in bakery production for stabilizing yeast fermentation rate, strengthening the dough, enhancing the flavor of the final product, and increasing the dough mixing time (Miller and Hoseney, 2008). It acts as a seasoning and flavours the dough, as a color enhancer by reducing the action of sugar within the dough, and controls the texture by strengthening the gluten. By slowing down the yeast action, it allows the fermentation to be controlled and retards bacteria growth. High concentration of salt decreases yeast activity in the dough, thus retarding gas production (Lynch et al., 2009). The salt gives strength to the gluten, enabling the dough to efficiently hold carbon dioxide, which is released into the dough as a by-product of the yeast fermentation. On the other hand omitting the salt from the formula results in dough that is slack and sticky in texture, work-up is difficult, bread volume is poor, and bread as final product is quite tasteless (Luchian and Canja, 2010).

Besides white sugar and salt, modern bakery production involves the use of various commercial products to improve the physical and chemical characteristics of the dough, and thus improve the sensory and organoleptic properties of baked goods in order to meet the consumer's tastes and requirements, and produce high quality products at minimum costs. Besides, bread improvers (additives) are used in bakery industry in order to simplify the work of bakers, smooth out the dough more quickly, enhance machinability, and increase yields. Complex improvers include oxidative and reductive substances, enzyme products, emulsifiers, etc. (Davidović et al., 2010). The most widely used oxidative component in the bakery industry is the oxidized form of L-ascorbic acid (DHAA), which greatly impacts the physical properties of gluten by decreasing the time and energy needed for mixing (Lillard and Seib, 1982; Dahle and Murthy 1970). As a strong oxidative medium it induces webbing of the gluten matrix, and improves rheological characteristics of dough (Every et al., 1999). The addition of different kinds of emulsifiers such as mono- and diacylglycerols esterified to mono- and diacetyltartaric acid (DATEM) also called dough strengtheners improves bread characteristics. They substances progress mixing tolerance, gas retention and resistance of the dough to collapse, promote the aggregation of gluten proteins in dough by binding to the protein hydrophobic surface, form lamellar liquid-crystalline phases in water, which associate with gliadins. In effect we can obtain bread with better texture, elasticity, and increased volume (Ribotta et al., 2004). Enzymes, usually amylases are often used in commercial bread improvers because their activity affects dough consistency through the effect on the starch granules which enables softening the dough and improving dough extensibility and gas retention (Dubois et al., 1990).

The addition of table salt, white sugar and commercial bakery improver into dough, affects the metabolic functions of bakery yeast *Saccharomyces cerevisiae*, and thus its FA. Although there are some studies dealing with the effect of salt or sugar on FA of yeast, the influence of the concurrent interactions of sugar, salt and commonly used commercial additive containing L-ascorbic acid, emulsifiers and enzyme complex on the FA determined by using SJA device has not been reported before. Thus, findings that are concerning the influence of these three commonly used compounds, as well as their combination

on the FA of yeast could contribute to gain necessary knowledge for process development and optimization.

MATERIAL AND METHOD

Throughout the experiment, dough was prepared with domestic wheat flour T-500 (water adsorption capacity 53-57 %) with an average quality like start material. Flour quality tests indicated following values: 12.1 % moisture, ash 0.42 %, protein 0.45 %. Flour was taken from a package of 25 kg. Fresh compressed commercial baker's yeast, *Saccharomyces cerevisiae* (31.7 % of moisture), was provided by a local producer Alltech-Fermin, Senta. Table salt and white sugar (99.7 % pure sucrose) were obtained from local factories. The domestic commercial complex additive was composed of an emulsifier (mono- and diacetyl ester of tartaric acid of mono and diglycerides of fatty acids), anti-caking agent (calcium carbonate), ascorbic acid ($C_6H_8O_6$), and enzymatic complex flour as filler. According to the manufacturer recommendations it should be dosed in an amount of from 0.15 to 0.45 % on the weight of flour in the bread making process, and from 0.3-0.5 % to the flour for the production of baked goods. The FA was analyzed according to Serbian standards methods (Pravilnik o kvalitetu i drugim zahtevima za pekarski kvasac "SI.list SRJ", br.9/2002 i "SI.list SCG", br. 56/2003) following the procedure: 280 g of flour, sugar (1-9 % w/w based on flour weight) and commercial complex additive (0.1-0.9% w/w based on flour weight) previously heated to 35 °C were transferred in suitable enamel jug also preheated at 35 °C. Pressed bakery yeast (5 g) was suspended in 160 ml of table salt solution (1-7 % w/w based on flour weight) in tap water (35 °C). The mixing was performed on laboratory farinographic mixer during 5 min. Formed dough sample was placed into a preheated mold and transferred to SJA fermentograph chamber at 35 °C. Fermentograph plotter registered the changes in the volume of CO₂ during 2 h of the fermentation. The experimental design and statistical analysis were performed using Stat-Ease software (Design- Expert 7.0.0 Trial, Minneapolis, MN, USA). Box-Behnken design (Andre and John, 1987) was applied to evaluate optimal process conditions and the individual and combined effects of the three independent variables (contact time, initial dye concentration and solution pH) on the response (adsorption capacity). The total of 17 experiments were conducted in the study toward the construction of a quadratic model. A quadratic second-degree polynomial equation (1) approximated in this study can be described as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 \quad (1)$$

where Y is the response (predicted FA); X_1 , X_2 and X_3 are independent variables (complex additive amount, table salt amount and white sugar amount added in % on flour weight bases); β_0 is the constant coefficient, β_1 , β_2 , β_3 are linear coefficients, β_{11} , β_{22} , β_{33} are the quadratic coefficients, β_{12} , β_{13} and β_{23} are interaction coefficients. The optimization process includes estimation of the coefficients by fitting them in a mathematical model that fits best the experimental conditions, prediction of the fitted model response and checking the adequacy of the model. The levels of chosen independent variables which predominantly affect the extent of yeast FA and the design matrix are given in Tables 1 and 2, respectively. The goodness of fitting and polynomial equations terms significances were determined through appropriate statistical methods (coefficient of determination R^2 , F -value at a probability P of 0.05). The adequacy and significance of the quadratic models was assessed by the analysis of variance (ANOVA).

Table 1. Values of factors in Box–Behnken design

Factor	Name	Low value	High value
X ₁	Additive (% w/w)	2.0	6.0
X ₂	Salt (% w/w)	30	50
X ₃	Sugar (% w/w)	0	60

Table 2. Box-Behnken matrix and responses (X₁: complex additive (% w/w); X₂: Salt (% w/w); X₃: Sugar (% w/w); Y: Fermentative activity of yeast cells (ml CO₂/gdm/2h)

X ₁	X ₂	X ₃	Y
0.1	1	5	1174.21
0.1	4	1	665.17
0.1	4	9	411.72
0.1	7	5	128.33
0.5	1	1	999.89
0.5	1	9	914.34
0.5	4	5	755.00
0.5	4	5	784.94
0.5	4	5	718.64
0.5	4	5	748.25
0.5	4	5	752.45
0.5	7	1	130.47
0.5	7	9	67.37
0.9	1	5	1099.35
0.9	4	1	643.78
0.9	4	9	410.65
0.9	7	5	101.59

RESULTS AND DISCUSSION

Second order polynomial equation has been obtained as a result of following response function:

$$Y = 1076,01585 + 283,92991X_1 - 105,88664X_2 + 75,71413X_3 + 10,02567X_1X_2 + 3,17489X_1X_3 + 0,46786X_2X_3 - 378,67245X_1^2 - 7,26666X_2^2 - 9,90238X_3^2$$

fitting by multiple regression. The adequacy and significance of the quadratic models were assessed by the analysis of variance (ANOVA) (Table 3). The developed regression model was proved to be significant and adequate ($P < 0.05$), with only 1.15 % of the total variations not explained by the model ($R^2 = 0.985$).

Table 3. Analysis of variance (ANOVA) for the experimental results (* significant at $P < 0.05$; ** not significant)

Model	F-value	P-value
X ₁	50.01	< 0.0001*
X ₂	0.44	0.5288**
X ₃	403.42	< 0.0001*
X ₁ X ₂	11.51	0.0115*
X ₁ X ₃	0.13	0.7269**
X ₂ X ₃	0.024	0.8823**
X ₁ ²	0.029	0.8701**
X ₂ ²	3.53	0.1024**
X ₃ ²	4.11	0.0822*
X ₃ ²	24.11	< 0.0017*

Fig. 1-3 presents the response surface plots (3D) of the individual and interactive effects of process parameters on FA. The results presented in Fig.1 clearly implied that the addition of table salt in the amount 1-7 % negatively affected the FA,

probably as a consequence of osmotic stress. This outcome was expected concerning findings reported previously, implying that the addition of salt in the dough has a retarding effect on the activity of the yeast, and consequently gas formation (Lynch et al., 2009; Lucian and Canja, 2010). This appears to be the result of increased osmotic pressure and the action of sodium and chloride ions on the membrane of yeast cells. However, if there is no salt, the yeast will ferment too quickly resulting in gassy, sour dough and baked products with open grain and poor texture. In this sense, the salt aids in controlling the rate of fermentation (Miller and Hosene, 2008).

Fig. 1. Response surface plots of the interaction of table salt and complex additive sugar addition on FA.

From Fig. 2 it is notable that the addition of white sugar in the amount of 1-4 % and complex additive in the amount 0.1-0.5 % enhances FA of yeast. Further increase of the amount of these two compounds decreases the FA.

Fig. 2. Response surface plots of the interaction of white sugar and complex additive addition on FA

From Fig. 3, it is obvious that the addition of salt shows more negative influence on FA than the addition of the same amount of sugar.

Fig. 3. Response surface plots of the interaction of white sugar and table salt addition on FA

CONCLUSION

The prediction of optimal reaction conditions was made by use of desirability function concept and Design Expert software. Maximal FA (1171.31 ml CO₂/gdm/2 h) should be ensured with the following amounts of additives: 0.41 % (complex additive), 1.0 % (table salt) and 3.91 % (white sugar) based on flour weight. These findings could contribute to gain necessary knowledge for process development and optimization of bread production from high quality wheat flour commonly used in bakery production. Still, further investigation is needed to explore the addition of these compounds on FA during the bread production from different types of dough such as for example: dough prepared from wheat flour of poor technological quality by substitution of barley and millet flour (Živančev et al., 2016), or flour of wholemeal spelt enriched with plant proteins (Šimurina et al., 2015).

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