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SCIENTIFIC PAPER

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INFLUENCE OF YEAST EXTRACT ENRICHMENT ON FERMENTATIVE ACTIVITY OF *Saccharomyces cerevisiae* AND TECHNOLOGICAL PROPERTIES OF SPELT BREAD

Article Highlights

- Yeast extract, salt and white sugar slightly decreased yeast fermentative activity
- Yeast extract increases volume and decrease firmness of spelt wheat bread
- In sweet bread with sugar content over 5% the effect of yeast extract is the opposite
- Maximal bread elasticity was obtained at yeast extract content of 1.57%

Abstract

Considerable interest in the consumption of bread from ancient spelt wheat with superior nutritional properties, easy digestibility and pleasant taste and aroma, has arisen recently. Yeast extract (YE) is nowadays frequently used as a natural flavor enhancer for improving organoleptic properties such as meaty, cheesy and savory attributes of different food products. YE is a natural product from the baker's yeast industry with high nutritional richness containing amino acids, peptides, nucleotides, vitamins and minerals. In this work, the effect of YE, table salt and white sugar content on fermentative activity of yeast and technological properties of spelt bread was investigated by response surface methodology (RSM) aiming to define the optimal conditions for obtaining a new functional product. The addition of YE, salt and white sugar slightly decreased yeast fermentative activity, while CO_2 retention in dough was found to depend more on its chemical composition. YE addition (up to 5%) was found to increase volume and decrease firmness of bread prepared from dough with sugar content up to 5%, while the opposite effect was established in bread with higher sugar content. Maximal bread elasticity was obtained for YE content of 1.57%, table salt content of 2.01% and sugar content of 7.15%.

Keywords: yeast extract, spelt wheat, fermentative activity, bread quality.

Bread has been a traditional staple of human diet obtained by dough baking since the Neolithic era, for over 12,000 years [1]. Dough is a complex mixture formulated with water, grain flour, salt, sugar, and shortening agents usually fermented by baker's yeast (*Saccharomyces cerevisiae*) as starter. Common wheat (*Triticum aestivum* L. spp *vulgare*) flour is most frequently used in bread production because of its gluten protein fraction that permits retention of the CO_2 in the viscoelastic dough matrix [2]. An attractive and healthier alternative with higher nutritional value for bread making to common wheat is its ancient subspecies spelt (*Triticum aestivum* L. spp *spelt*). Spelt is a cereal suitable for cultivation by organic methods without or with less requirements of fertilization and herbicides or pesticides, and heavy chemical protection [3,4]. It is harvested as hulled grain and consequently it contains higher insoluble dietary fiber and protein content [5]. The nutritive value of spelt wholemeal bread is highly appreciated because of its lower starch content, higher fiber and protein content, more rapidly digested starch and less rapidly digested proteins than common wheat, which make it more suit-

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able for consumers [6,7]. Spelt seems to have a higher content of B complex vitamins (B1, B2, B3, B9 and B12) and lipids with a more favorable fatty acid profile, as well as higher percentage of minerals such as magnesium, phosphorus, iron, copper and zinc than common wheat [3]. Besides, spelt bakery products are very popular because of their pleasant aroma and taste [4].

Owing to health-related necessity, it is of great interest to improve bread nutritional quality by using spelt and enriching its formulation, and lowering its salt content. One of the strategies proposed to reduce the sodium chloride content of food is the enrichment with other taste enhancers. Yeast extract (YE) commercially marketed as liquid, paste or powder is widely used as a natural flavor enhancer for improving the organoleptic properties (meaty, cheesy and savory attributes) and increasing the nutritive value (amino acids, peptides, nucleotides, vitamins, minerals) of various types of food products such as soups, gravies, meat products, sauces, and as a flavoring in snacks and spice mixtures [8,9]. YE is a valuable natural source of glutamate and nucleotide in many processed foods and represents a cost-effective replacement for nucleotides and monosodium glutamate (MSG), which has no nutritional benefits [8]. YE is a concentrate of the soluble fraction of yeast biomass, made by removing cell walls of the yeast culture. Different yeast species, especially primarily-grown baker's yeast *S. cerevisiae*, have been employed for YE production. A variety of methods for yeast cell wall disruption, generally based on autolysis, plasmolysis or hydrolysis, are applied. Autolysis is the most frequent manufacturing practice, particularly for food grade YE production. In difference to the other two procedures, the autolysis enables production of low-sodium yeast extract gaining wide acceptance in the food industry, particularly in convalescent and infant food formulations. Autolysis is the self-digestion of viable yeast cells by the yeast's own hydrolytic enzymes such as protease and nuclease, which break down insoluble macromolecules like proteins and nucleic acids to soluble products of peptides, amino acids (mainly glutamate), nucleotides and amino acid derivates. Savory flavors in YE derive from the degradation of cell proteins and RNA to yield taste enhancers such as glutamic acid, peptides and ribonucleotides [8,9]. Besides, during the production process of YE the Maillard reaction occurs generating specific color and aroma compounds which contribute to the pleasant odor and taste of YE [10]. The use of YE in flavorings in food production in the European Union is subject to Regulation (EC) no. 1334/2008 and has been given GRAS status (generally recognized as safe) [11].

Yeast-mediated dough fermentation is a crucial stage in bread production because it determines the unique qualities, such as texture, flavor, and taste of bread [8]. Baker's yeast cells utilize fermentable sugars in dough, which are present in small amounts at the beginning of dough preparation and/or formed through the action of amylases in flour, or added as supplement, and generate ethanol and carbon dioxide (CO_2) which is responsible for dough leavening during the fermentation phase and the oven rise. The incorporation of sucrose in the dough is a very common practice in bakeries. Wheat flour contains only modest amounts of maltose and glucose, and although wheat flour contains amylases that can release fermentable sugars from starch, sugar availability may still be growth-limiting. In order to overcome this issue, bakeries add sucrose (about 1% to 3%) as an additional source of readily fermentable sugars. Table salt needs to be included in the dough formulation because it has a strengthening effect on the gluten network, which enables the retention of gas in the dough matrix. Also, salt acts as a color enhancer by reducing the action of sugar within the dough. Table salt is usually used in amounts of 1-2% of flour mass for improving bread taste [12]. In the present study, 0-2.5% of table salt and 0-10% of white sugar were added to flour mass in dough, in order to examine the influence of YE on yeast fermentative activity and technological quality of standard, salted and sugared bread up to the amount which is usually used in bakery production. Secondary yeast metabolites such as acetic acid, succinic acid, glycerol, and aroma compounds are also produced during dough fermentation. These metabolites have a crucial influence on dough rheology and bread texture, volume, flavor composition and taste [2,12]. Generated CO_2 causes the gluten to stretch; some escapes, but most of the gas is retained and trapped within the dough matrix producing bubbles, while the alcohol is simply evaporated [13]. YE may be employed to supplement fermentation media and provide certain yeast nutrients, particularly amino acids as an organic nitrogen source [14]. Although the role of YE addition as a source of vitamins, minerals, and amino acids in brewing and distilling fermentations is widely described in the scientific literature, little information is available concerning its role in dough fermentation.

In contrast to the advantages regarding the nutritional features, spelt flour was reported to form dough with lower stability and elasticity and higher extensibility, due to higher content of gliadins and lower content of glutenins in gluten than common wheat [15]. In general, spelt bread is characterized by lower specific volume, darker crumb and crust color, increased hardness, and has been described as poor compared to common wheat [7,15]. Technological properties of bread are dependent on the yeast fermentation process in dough. Further, fermentation in dough is directly associated with the characteristics of yeast culture, its fermentative activity and gas formation ability, and fermentation conditions such as chemical composition of dough, especially carbohydrate concentration, which is dependent on raw materials used [12]. Thus, it is necessary to investigate the multiple influences of white sugar, table salt and YE content on the fermentative activity, and, consequently, on the technological properties of spelt bread, and to optimize their amount.

In our previous research we have found that spelt bread enriched with YE showed enhanced nutritive value, had better acceptance with general consumers, and showed better quality evaluated by organoleptic analysis [16]. Bread samples enriched with 5% of YE, 1.5% of salt and 0% sugar were previously determined as being the best from the aspect of bread quality parameters. The addition of YE up to 5% improved appearance without deteriorating texture descriptors and bread crumb quality, while the taste became more complex, but without increasing the salty taste. Also, the addition of sugar up to 10% in samples with YE, improved most sensory characteristics of spelt bread [16]. However, there is a lack of knowledge about the influence of different amounts of YE on the fermentative activity of yeast in dough and quality properties such as bread volume, firmness and elasticity. To our knowledge, no comprehensive analysis of the combined influence of YE, salt and sugar addition on the fermentative activity of baker's yeast and the quality of spelt bread has been reported yet.

In this work, the response surface methodology (RSM) was used to evaluate the effect of multiple factors such as YE, salt and sugar content and their interaction on the fermentative activity of baker's yeast in dough, and, finally, on the technological characteristics of wholemeal organic spelt bread enriched with YE as new functional food with enhanced nutritional value. The novelty of this work lies in the aspects of the development of the best formulation of wholemeal spelt bread enriched with YE with the best technological properties. The results presented may be valuable for both scientific and practical reasons.

MATERIALS AND METHODS

Materials

Spelt flour from organic spelt grown in the year 2018 in Bačko Gradište, Serbia, by "Spelta Jevtić" was used for wholemeal bread production. Table salt was produced by "So Product" d.o.o, Stara Pazova, Serbia. White sugar was produced by "Crvenka" Sugar Factory A.D., Crvenka, Serbia. Dry instant baker's yeast and food grade low-sodium YE powder produced by autolysis were kindly provided directly from the baker's yeast factory.

Chemical analysis

Spelt flour, YE powder and dry baker's yeast were analyzed according to the standard AOAC methods to determine the content of moisture (method 925.10 for flour, and 966.18 for YE and dry yeast), ash (method 923.03), total nitrogen and protein (method 928.87 for flour and 962.10 for YE and dry yeast), starch (method 945.37), sugar (method 939.03), crude fiber (method 920.86), and total fat (method 922.06) [17]. Determination of the reducing sugar content was carried out by the AACC method 80-68.01 [18]. The content of copper, zinc, magnesium, calcium and iron was estimated using an atomic absorption spectrophotometer as per the AOAC standard method 985.29 [17]. The sodium chloride content of YE was determined by the standard AOAC method 935.47 [17].

Fermentative activity of yeast in dough

The fermentative activity of baker's yeast was analyzed according to the following procedure: 280 g of spelt flour and white sugar (0, 5 and 10 mass% per flour mass) previously heated to 35 °C were transferred into a suitable enamel jug also preheated at 35 °C. Instant baker's yeast (0.5% per flour mass) was added and the mixture was dry stirred on a laboratory farinographic mixer for 1 min. Appropriate weighs of table salt (1.5, 2 and 2.5 mass% per flour mass), YE powder (0, 2.5 and 5% per flour mass) and ascorbic acid (0.006% per flour mass) were suspended in the appropriate amount of tap water (35 °C). The amount of tap water was adjusted to obtain final water content of 60% per flour mass in each dough sample, taking into consideration the amount of moisture for each ingredient. Suspension was poured into the dry mixture and mixing was performed on a laboratory farinographic mixer for 5 min. The formed dough sample was placed into a preheated mold and transferred into a SJA fermentograph (Nässjö, Sweden) chamber thermostated at 35 °C. The volume of CO_2 (mL) released during the first hour of dough fermentation was registered on the fermentograph recorder. Then, the dough was hand mixed in the same manner for each sample and returned to the chamber to monitor the volume of CO_2 (mL) released during the second fermentation hour. Each experiment was conducted in triplicate using the three chambers of the SJA fermentograph. The fermentograph plotter registered the changes in the volume of CO_2 during 2 h of fermentation. Fermentative activity was presented as the average volume of CO_2 (mL) produced by one gram of yeast dry mass during 2 h.

Bread-making procedure

Bread was baked according to the AACC method 10-09.01 [18]. The content of main ingredients for dough preparation was in the same ratio as for the fermentative activity analysis.

Bread properties

Each bread sample was weighed and its volume was assessed 24 h after baking by the non-contact AACC method 10-16.01 (Volumetric and Dimensional Profile of Baked Products by Laser Topography-Vol-Scan Profiler method) [18] using a benchtop laser-based scanner Volscan Profiler (Stable Micro Systems, England). Data were presented as the mean of three measurements. Crumb texture of breads was determined instrumentally on a texture analyzer TA.XTplus (Stable Micro Systems, England, UK) according to the AACC method 74-10A modified to record crumb elasticity in addition to crumb hardness [18]. A 36 mm aluminum cylindrical probe and a 5 kg load cell were used. Pre-test speed, test speed and post-test speed were 1.0, 1.7 and 1.7 mm/s, respectively. Testing was performed in bread samples with dimensions of 3 cm×2 cm×2 cm (length×width×thickness). Breads were evaluated at 40% compression strain. Crumb hardness was recorded as the peak force during compression. Crumb elasticity was calculated as the area during the withdrawal of the first compression divided by the area of the first compression, and is an indicator of the speed of crumb recovery.

Experimental design and statistical analysis

Response surface methodology (RSM) was used as a widely applied statistical modeling technique to optimize spelt bread composition on the basis of simultaneous consideration of several factors at many different levels and their interaction using a small number of observations. For optimization and statistical analysis, the StatSoft Statistica software, for Windows, program ver. 12 (Statistica 2012) was used. Box-Behnken design was applied to evaluate the optimal bread composition and the individual and the combined effects of the three independent variables (content of YE, salt and sugar) on the responses (fermentative activity of yeast, bread volume, bread elasticity and crumb texture). A quadratic second-order polynomial (SOP) equation (1) was fitted to the response surface generated by the experiment:

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

where the dependent variables are the responses of yeast fermentative activity (Y_1), the responses of bread volume (Y_2), the responses of crumb hardness (Y_3), and the responses of crumb elasticity (Y_4); the independent variables are the quantity of YE (X_1), the quantity of salt (X_2), and the quantity of sugar (X_3); β_0 is the constant coefficient, β_1, β_2, β_3 are the linear coefficients, β_{11}, β_{22}, β_{33} are the quadratic coefficients, β_{12}, β_{13} and β_{23} are the 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 fermentative activity, spelt bread volume, crumb hardness and elasticity and the design matrix are presented in Table 1, respectively. The adequacy and significance of the mathematic models was assessed by the analysis of variance (ANOVA) by means of post-hoc Tukey's HSD test. For this purpose, all testing was performed in three parallel runs. 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).

RESULTS AND DISCUSSION

Chemical composition of spelt flour, yeast and yeast extract

Based on the results of the chemical composition of raw materials (Table 2) it can be concluded that spelt flour used in this work represents a valuable source of nutrients, primarily starch (59.30%), protein (15.32%) and minerals, especially Mg (304.6 mg/kg).

Chemical and mineral composition of the used instant baker's yeast is in agreement with the standard instant dry yeast quality. The used YE is characterized by high protein content (71.88%) as evaluated by the Kjeldahl method, indicating that is composed mostly of nitrogen-containing compounds and is a valuable source of peptides, amino-acids and nucleo-

Table 1. Box-Behnken matrix and responses (X_1: yeast extract (%); X_2: salt (%); X_3: sugar (%); Y_1: fermentative activity of yeast cells (mL CO_2 g^{-1} $2h^{-1}$); Y_2: bread volume (mL); Y_3: bread firmness (g); Y_4: elasticity ($g \cdot s^{-1}$)); $^{a-i}$ - different letters in the superscript in the same table column indicate statistically significant difference between values at a significance level of p < 0.05 (based on post-hoc Tukey's HSD test)

Sample	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
0	0	1.5	0	498.48±3.49^{g}	1575±18^{a}	2969.22±23.57^{hi}	32.39±0.04^{a}
1	0	1.5	5	448.43±3.60^{f}	1846±17^{f}	2084.60±39.90^{b}	35.01±0.43^{cd}
2	0	2	0	445.82±2.54^{f}	1589±14^{ab}	2917.80±27.08^{gh}	32.60±0.25^{a}
3	0	2	10	286.79±4.21^{bc}	2074±28^{g}	1270.95±3.09^{a}	38.87±0.16^{e}
4	0	2.5	5	277.66±2.63^{b}	1777±17^{c-e}	2340.57±42.00^{de}	35.15±0.36^{cd}
5	2.5	1.5	0	419.97±5.20^{e}	1583±18^{ab}	3027.03±19.66^{i}	32.99±0.19^{ab}
6	2.5	1.5	10	296.43±3.45^{c}	1825±3^{ef}	2151.75±24.41^{c}	35.15±0.40^{cd}
7	2.5	2	5	309.47±4.56^{d}	1770±26^{cd}	2359.80±27.02^{de}	39.57±0.10^{e}
8	2.5	2.5	0	312.86±5.05^{d}	1599±15^{ab}	2900.59±44.98^{g}	32.99±0.24^{ab}
9	2.5	2.5	10	189.02±1.57^{a}	1820±13^{d-f}	2190.20±34.8 ^{c}	35.87±0.43^{d}
10	5	1.5	5	315.73±3.28^{d}	1633±16^{b}	2800.59±24.10^{f}	33.15±0.17^{ab}
11	5	2	0	312.60±3.47^{d}	1770±26^{cd}	2318.70±35.71^{d}	34.92±0.22^{cd}
12	5	2	10	179.11±2.54^{a}	1765±14^{c}	2390.64±19.08^{e}	34.14±0.43^{bc}
13	5	2.5	5	179.11±2.03^{a}	1765±22^{c}	2350.70±13.20^{de}	34.99±0.55^{cd}

Table 2. Chemical composition and mineral content of raw materials

Property	Spelt flour	Instant baker's yeast	Yeast extract powder
Dry solids (%)	90.02±0.95	94.35±0.87	95.61±1.05
Ash (%)	2.75±0.05	5.50±0.14	12.95±0.35
Total Kjeldahl nitrogen (%)	2.45±0.02	7.33±0.35	11.50±0.45
Total Kjeldahl protein (%)	15.32±0.62	45.79±0.78	71.88±1.02
Starch (%)	59.30±1.04	25.79±0.38	0.00±0.00
Sugar (%)	2.12±0.08	13.43±1.10	6.95±1.08
Crude fiber (%)	2.13±0.08	0.00±0.00	0.00±00
Total fat (%)	0.54±0.08	0.58±0.06	0.16±0.02
Cu (mg/kg)	5.62±0.45	6.20±0.56	1.59±0.16
Zn (mg/kg)	24.76±0.89	56.32±1.09	54.38±0.78
Mg (mg/kg)	304.6±2.05	517.18±3.45	594.81±3.08
Ca (mg/kg)	60.99±2.12	42.19±1.63	55.9±1.89
Fe (mg/kg)	38.19±1.05	49.01±0.89	64.95±1.36

tides. These results are consistent with the data published in previous studies revealing that health benefits of YE are directly related to its high nutrient content [9,19]. The ash composition in YE indicates its potential as a source of mineral compounds, especially Mg (594.81 mg/kg), Fe (64.95 mg/kg), Ca (55.9 mg/kg) and Zn (54.38 mg/kg). YE is a valuable source of organic nitrogen (amino acids) and key metal ions used by *S. cerevisiae* for specific catalytic processes or structural reasons, thus enhancing its viability and fermentation rate.

Validation of the experimental design

The analysis of variance (ANOVA) was used to evaluate adequacy and significance of the models, as detailed in Table 3. The regression models were significant at 95% confidence levels. From Table 3 it can be seen that regarding the effect of YE, salt and sugar on the fermentative activity of yeast cells, bread volume, bread firmness, and bread elasticity, the developed quadratic models Y_1, Y_2, Y_3 and Y_4 were proven to be significant and adequate ($P < 0.05$), with only 1, 0.6, 0.2 and 4.2% of the total variations not explained by the model (R^2 = 0.990, 0.994, 0.998 and 0.958), respectively. The obtained values of the coefficients of determination were satisfactory ($R^2 > 0.95$). Hence, at least 95% of the variations in the response could be addressed to the obtained model equations. The effect of the examined variables on every examined response was approved by statistical significance of

*Table 3. Analysis of variance (ANOVA) for the experimental results (X_1: yeast extract (%); X_2: salt (%); X_3: sugar (%); Y_1: fermentative activity of yeast cells (mL CO_2 g^{-1} $2h^{-1}$); Y_2: bread volume (mL); Y_3: bread firmness (g); Y_4: elasticity ($g \cdot s^{-1}$)); * - statistically significant at a significance level of $p < 0.05$; df - degrees of freedom*

Term	*df*	Sum of squares			
		Y_1	Y_2	Y_3	Y_4
X_1	1	28749.2*	16005.9*	205892*	381.429
X_1^2	1	18.9	3568.1*	60809*	7.72951*
X_2	1	35191.5*	799.5	10076	0.39897
X_2^2	1	45.3	7574.0*	87583*	17.44415*
X_3	1	37680.8*	117849.9*	1308539*	12.04133*
X_3^2	1	29.1	289.3	761	10.85348*
X_1X_2	1	244.9	10751.1*	139989*	195.654
X_1X_3	1	123.6	67604.6*	813646*	10.28264*
X_2X_3	1	6.2	173.1	7082	0.77961
Residual variance	4	1394.4	1591.2	6762	259.239
Total sum of squares	13	134710.6	246772.4	2874518	6.113.584
R^2	-	0.990	0.994	0.998	0.958

all obtained coefficients. On the basis of the obtained values of residual variance and the total sum of squares, it can be concluded that mathematical models were adequate for the approximation of the experimental results.

Analysis of response surfaces

Second order polynomial equations have been obtained as a result of the response function (Y_{1-4}) fitting by multiple regression analyses. Obtained regression coefficients (β_0, β_1, β_2,…, β_{23}), presented in Table 4, were used to generate the 3D response surface plots (Figures 1-4) in order to illustrate the effects of different ingredients' quantities on responses of yeast fermentative activity in dough and different bread properties. The response surface plots were made using a software tool by varying two variables within the experimental range, while the third variable was kept constant at the middle level (2% table salt, and 5% white sugar).

*Table 4. Regression coefficients of SOP (X_1: yeast extract (%); X_2: salt (%); X_3: sugar (%); Y_1: fermentative activity of yeast cells (mL CO_2 g^{-1} $2h^{-1}$); Y_2: bread volume (mL); Y_3: bread firmness (g); Y_4: elasticity ($g \cdot s^{-1}$)); * - statistically significant at a significance level of $p < 0.05$*

Parameter	Y_1	Y_2	Y_3	Y_4
β_0	651.9075*	853.750*	5470.26*	-5.5623
β_1	-35.1968	-77.050*	300.23*	0.6825
β_{11}	-0.4391	6.040*	-24.93*	-0.2811*
β_2	-73.8158	813.500*	-2786.20*	40.5002*
β_{22}	-17.0163	-220.000*	748.12*	-10.5580*
β_3	-12.1372	57.675*	-203.53*	1.0412*
β_{33}	-0.1363	-0.43	0.7	-0.0833*
β_{12}	5.9466	39.400*	-142.17*	0.5315
β_{13}	0.4225	-9.880*	34.28*	-0.1218*
β_{23}	-0.4717	-2.500	15.99	0.1678

In general, table salt and sugar are essential dough ingredients commonly used in dough formulation. The viability and fermentative performance of yeast are influenced by sugar availability, salt level, vitamin and mineral composition of dough. The concentration of fermentable sugar above 5% increases osmotic pressure in dough, which causes an inhibitory effect on yeast and decreases its fermentative activity [12]. Sweet bread dough can contain up to 30% of added sucrose per weight of flour, which exerts a severe osmotic stress. Bakers' yeasts express high levels of invertase activity, which cleaves sucrose rapidly and the osmotic pressure of sweet dough is effectively doubled. High concentration of table salt also increases osmotic pressure and partly dehydrates yeast cells, while accumulation of Na^+ and Cl^- is toxic at high concentrations thus retarding gas production [12,20]. However, table salt is of crucial importance for bakery production since it stabilizes yeast fermentation rate, controls the texture by strengthening the gluten in dough, and increases the dough mixing time which affects palatability, and enhances flavor and the structural and physical properties of bakery products [21]. In the absence of salt, the yeast will ferment too quickly resulting in a gassy dough that is slack and sticky in texture and difficult to work-up, and in baked products with poor volume and texture. Besides, by slowing down the yeast action, it allows the fermentation to be controlled and retards bacterial growth [12,20]. The presence of both sugar and salt in sweet dough strongly reduces the water

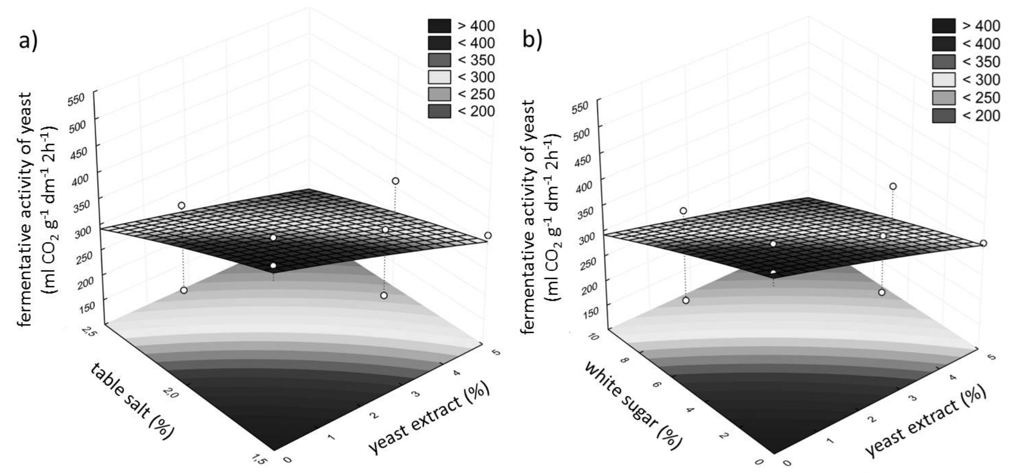

Figure 1. Response surface plots of the interaction of (a) table salt×yeast extract (white sugar content = 5%), (b) white sugar×yeast extract (table salt content = 2%), and their influence on the fermentative activity of yeast.

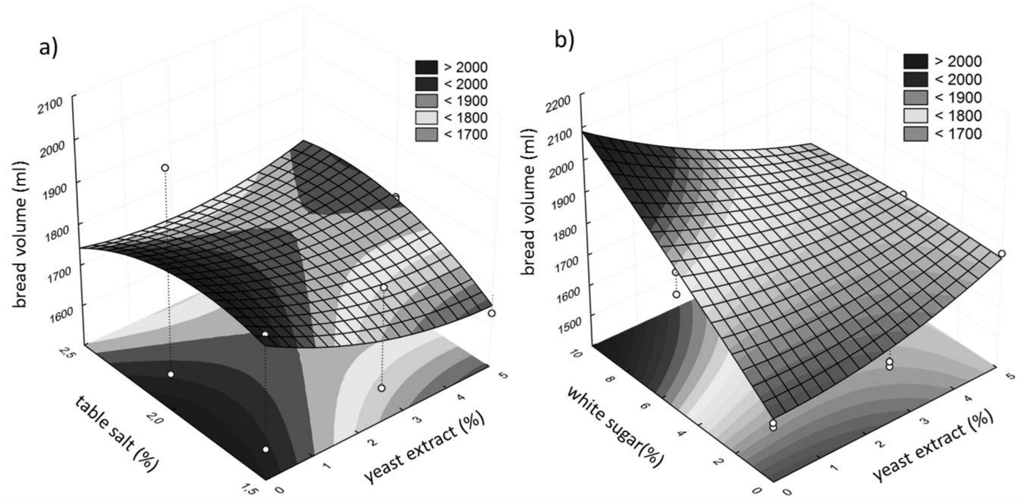

Figure 2. Response surface plots of the interaction of (a) table salt×yeast extract (white sugar content = 5%), (b) white sugar×yeast extract (table salt content = 2%), and their influence on bread volume.

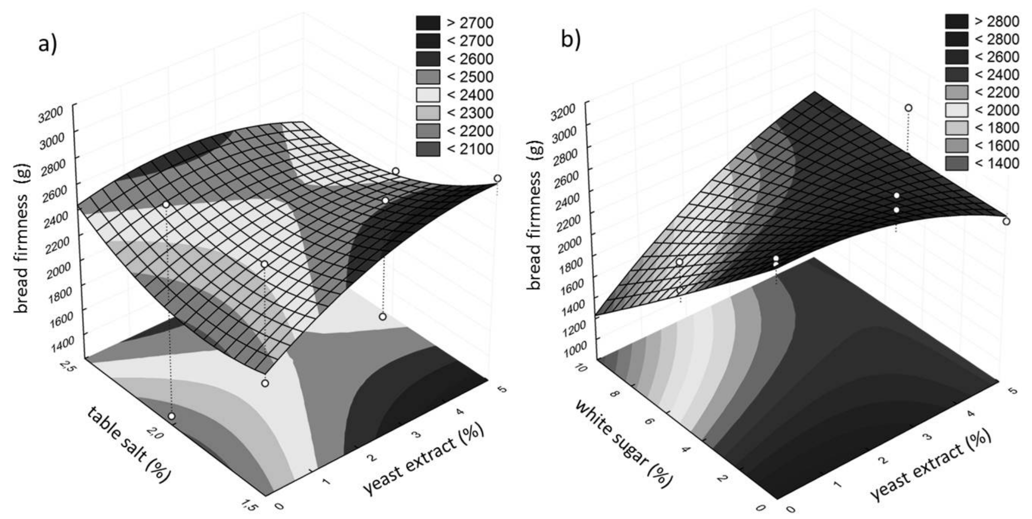

Figure 3. Response surface plots of the interaction of (a) table salt×yeast extract (white sugar content = 5%), (b) white sugar×yeast extract (table salt content =2 %), and their influence on bread firmness.

activity of the dough. Hence, the effective Na^+ concentrations in these products increase, leading to harmful effects on baking performance that are not observed in lean dough [12]. Therefore, we have examined the influence of the content of these ingredients on the fermentative activity of yeast in dough.

Regarding the fermentative activity of yeast, it can be seen that the model's terms X_1, X_2, X_3, were significant at 0.05 level (P < 0.05) while terms X_1^2, X_2^2, X_3^2 and X_1X_2, X_1X_3 and X_2X_3 had no significant effect on the yeast's fermentative activity (Table 3). Figure 1a and b depict the response surface plots (3D) of the individual and interactive effects of the process parameters on the fermentative activity of yeast. From these plots, it is noticeable that the increase of YE, salt and sugar content in dough negatively affects the fermentative activity of yeast and consequently decreases the rate of CO_2 production. Although low-sodium YE was used, according to the results of analysis the content of sodium-chloride in used YE was 0.4%, which contributes to the total salt content. A slight decrease of the fermentative activity with the addition of YE, salt and sugar was quite expected, considering its influence on the increase of the osmotic pressure in dough and the action of sodium and chloride ions on the membrane of yeast cells. Despite the fact that YE represents a nutritive source for yeast cells, its sodium chloride content has a pronounced inhibitory effect on the yeast's fermentative activity, due to its contribution to the increase of osmotic pressure. According to the second-order polynomial equation fit, the highest yeast fermentative activity was obtained when the dough was prepared with the lowest salt content (1.5%) and without sugar and YE.

Specific volume of bread is closely related to the fermentative activity of yeast, the rate of CO_2 produced by yeast, as well as to the retention of CO_2 produced which is dependent on the chemical composition of dough, especially its gluten fraction [12]. From Table 3 it is noticeable that significant models' terms (P < 0.05) for specific bread volume, region model (Y_2) are X_1, X_3, X_1^2, X_2^2, X_1X_2 and X_1X_3. To evaluate the interactive effects of YE, salt and sugar on the volume of bread, response surface plots (3D) are presented in Figure 2a and b. From Figure 2a, it is obvious that when sugar content is 5%, bread volume increased with increasing salt content up to 2% and decreasing YE content. These findings are quite expected, since salt gives strength to the gluten, enabling the dough to efficiently hold CO_2 [12]. As shown in Figure 2b, white sugar addition promoted the volume of bread within the range investigated. The addition of YE increased bread volume of samples with salt content of 2% and sugar content up to 5% (Figure 2b), while it decreased bread volume of samples with higher sugar content. According to the mathematical model, the highest bread volume of 1727.84 mL can be obtained when the dough is prepared with 1.9% of salt, 3% sugar and 3.9% of YE. It is well known that CO_2 creates a foam-like structure in dough which is the prerequisite for increasing the dough and bread volume. However, a comparison of Figure 1 and Figure 2 showed that the volume of bread failed to decrease continuously with the amount of produced CO_2. The volume of bread samples apparently depended more on the CO_2 retention capacity in the dough structure. As stated before, although salt addition leads to reduced gas production by yeast cells, the amount of CO_2 remaining in the dough is higher when salt levels are enhanced [12].

Considering the bread firmness regression model (Y_3), significant model's terms at probability of 95% (P < 0.05) are X_1, X_3, X_1^2, X_2^2, X_1X_2 and X_1X_3, while X_2, X_3^2, and X_2X_3 are insignificant (Table 3). Interactive effects of YE, salt and sugar on bread's firmness, as the response, are shown in Figure 3a and b. As observed from Figure 3a, for sugar content of 5% and salt content 1.5-2%, the addition of YE increases bread firmness. However, when salt content is in the range of 2-2.5%, YE addition has a positive effect only in the amount up to 2%, while in higher amounts it decreases bread firmness. From Figure 3b, it is obvious that at the salt content of 2%, YE and sugar demonstrated a pronounced negative effect on bread firmness.

Bread volume was increased, while bread firmness was decreased, along with an increase of the sugar content in the dough in the range of 1-10%. YE addition in amounts up to 5% had a positive effect on the volume and negative effect on the firmness of bread prepared from doughs with sugar content up to 5%, while the opposite impact on these properties was established in bread with higher sugar content. White sugar is commonly added in the amount of 5% in bread production. For spelt bread with 5% of added white sugar of flour mass, the highest bread volume and the lowest bread firmness was obtained in bread with salt content of 2.27-2.28%.

Regarding bread elasticity, the regression analysis of the data (Table 3) showed that it was significantly affected by model terms X_3, X_1^2, X_2^2, X_3^2 and X_1X_3, while the influence of X_1, X_2 and interaction X_1X_2 and X_2X_3 were found to be insignificant (P > 0.05). As shown in Figure 4a, bread elasticity was increased along with the increase of salt content up to

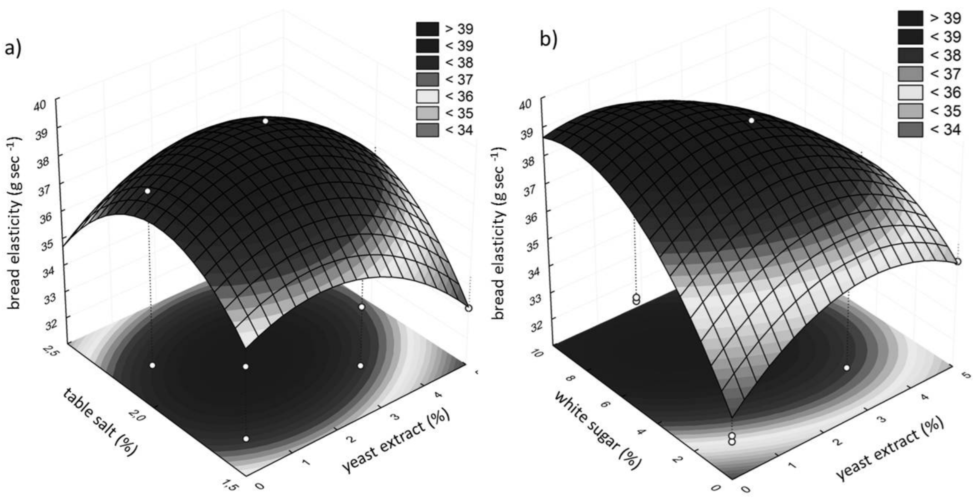

Figure 4. Response surface plots of the interaction of (a) table salt×yeast extract (white sugar content = 5%), (b) white sugar×yeast extract (table salt content = 2%), and their influence on bread elasticity.

2.01% and the increase of YE up to 1.57%. With further increase of salt and YE content, the elasticity of bread declined. From Figure 4b it is noticeable that the elasticity was pronounced with an increase of white sugar content up to 7.13% and YE content of 1.57%. Further, the increase of sugar and YE content decreased bread elasticity. According to the mathematical model, maximal elasticity of 39.47 g s^{-1} was obtained for YE, table salt and white sugar contents of 1.57, 2.01 and 7.13%, respectively.

CONCLUSION

Enrichment of wholemeal spelt bread with YE enhances its nutritive value, while its consumption contributes to reaching the daily threshold for amino acids and minerals such as Zn, Mg and Fe. The present investigation showed that the addition of YE, table salt and white sugar in dough slightly decreased the fermentative activity of yeast and the rate of CO_2 produced. The volume of bread and the retention of CO_2 in dough was more dependent on its chemical composition. Bread volume was increased, while bread firmness was decreased, along with an increase of sugar content in dough in the range of 1-10%. YE addition in amounts up to 5% increased volume and decreased firmness of bread prepared from doughs with sugar content up to 5%, while the opposite effect on these properties was established in bread with higher sugar content. For bread with common white sugar content of 5%, maximal bread volume and minimal bread firmness was obtained with salt content of 2.27-2.28%. The highest bread elasticity was obtained for YE content of 1.57%, table salt content of 2.01% and sugar content of 7.15%.

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UTICAJ DODATKA EKSTRAKTA KVASCA NA FERMENTATIVNU AKTIVNOST *Saccharomyces cerevisiae* I TEHNOLOŠKE KARAKTERISTIKE HLEBA OD SPELTE

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NAUČNI RAD

U poslednje vreme se javilo veliko interesovanje za konzumacu hleba od drevne spelta pšenice koja ima superiornu nutritivnu vrednost, lako je svarljiva i veoma je prijatnog ukusa i arome. Ekstrakt kvasca (EK) se danas često koristi kao prirodni pojačivač ukusa za poboljšanje organoleptičkih svojstava kao što su mesni, sirasti i slani ukus različitih prehrambenih proizvoda. EK je prirodni proizvod iz industrije pekarskog kvasca visoke nutritivne vrednoste koji sadrži aminokiseline, peptide, nukleotide, vitamine i minerale. U ovom radu ispitivan je uticaj sadržaja EK, kuhinjske soli i belog šećera na fermentativnu aktivnost kvasca i tehnološka svojstva hleba od spelte metodom odzivne površine (RSM) sa ciljem da se definišu optimalni uslovi za dobijanje novog funkcionalnog proizvoda. Dodatak EK, soli i belog šećera blago je smanjio fermentativnu aktivnost kvasca, a zadržavanje CO_2 *u strukturi testa u većoj meri zavisi od hemijskog sastava samog testa. Utvrđeno je da dodatak EK (do 5%) povećava zapreminu i smanjuje čvrstinu hleba pripremljenog od testa sa sadržajem šećera do 5%, dok je suprotan efekat ustanovljen kod hleba sa većim sadržajem šećera. Maksimalna elastičnost hleba dobijena je za sadržaj EK od 1,57%, kuhinjske soli od 2,01% i šećera od 7,15%.*

Ključne reči: ekstrakt kvasca, spelta, pšenica, fermentativna aktivnost, hleb.