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## THE APPLICATION OF NATURAL ORGANIC COMPOUNDS IN BAKERY INDUSTRY

*Investigations include the analysis of the impact of commercial products: complex additive (0.1, 0.3, and 0.5%), L-ascorbic acid (0.002, 0.004 and 0.012%), diacetyl ester of tartaric acid with monoglycerides (DATEM E472e, 0.1, 0.3, and 0.5%), α-amylase (0.002, 0.006 and 0.012%), xylanase (0.004, 0.012 and 0.024%), alcohol extract of rosemary, thyme or sage (0.5, 1.0 and 2.0%), as well as the combination of complex additive and rosemary, thyme and sage extract on rheological characteristics of dough. The study includes amylograph, farinograph and extensograph analysis of dough with and without additives (control sample). The volume of lost CO<sub>2</sub> gas (mL) is the lowest in dough samples with an added combination of complex additive and thyme extract (0.05 and 0.5%) and rosemary extract (2.0%). In the samples with thyme extract (1.0% added, the volume of lost gas is at a level of samples with added complex additive, DATEM, and L-ascorbic acid.*

When wheat flour, salt, water and additives are combined in order to prepare dough, complex cascades of physical and chemical events have been reported to take place during mixing [1,2]. The formation of hydrogen bonds between glutamine residues also explains the effects on dough rheology that have been observed on esterification of glutamine residues and the addition of D<sub>2</sub>O, rather than H<sub>2</sub>O, with the former decreasing and the latter increasing the resistance to extension [3]. Based on their mobilities on acidic-buffer polyacrylamide gel electrophoresis, the monomeric gliadins ( $M_r = 3 \times 10^4 - 7.5 \times 10^4$ ) have been divided into three groups:  $\omega$ -gliadins, which have the lowest mobility and lack cysteine, followed by  $\gamma$ -gliadins and  $\alpha/\beta$ -gliadins [4].

Improvers that are used in the bakery industry include oxydo-reductive substances, enzyme products, emulsifiers, etc. Complex improvers are utilized the most, which mostly fill deficiencies in terms of technological quality of flour. Oxidative substances play a role in the formation of spatial gluten network, through oxidation of sulphydryl -(SH) groups into disulfide bridges -(S-S)-. The most widely used oxidative component in the bakery industry is the dehydro form of L-ascorbic acid (DHAA), which greatly impacts the physical properties of gluten [5]. L-Ascorbic acid decreases the time and energy needed for mixing [6]. As a strong oxidative medium it induces webbing of the gluten matrix, and improves rheological characteristics of dough. Oxi-

dative glutathione (GSSG) reacts with protein tiols during mixing of dough and blocks tiol groups, which supposedly do not have a negative effect on rheological characteristics of dough, and assist in the creation of inter-protein -S-S- bridges [7].

From the moment when flour, water, and yeast are mixed, the well-organized enzymatic systems begin to function. Amylase activity affects dough consistency through its effect on the starch granules. The damaged starch granules have high water absorption and holding capacity and when this starch is broken down by the action of amylose, the released water helps to soften the dough. This action also helps to improve dough extensibility and gas retention [8].

Mono-and diacylglycerols esterified to mono- and diacetyl tartaric acid (DATEM) are anionic oil-in-water emulsifiers that are used to improve the quality of bread. These kinds of emulsifiers, also called dough strengtheners, when added to dough improve mixing tolerance, gas retention and resistance of the dough to collapse. In terms of the final product, this substance improves loaf volume and endows it with resilient texture, fine grain as well as slicing properties [9]. Emulsifiers such as DATEM may promote the aggregation of gluten proteins in dough by binding to the protein hydrophobic surface. This produces a strong protein network, which in turn will yield bread with better texture and enlarged volume. Hydrophilic emulsifiers may also form lamellar liquid-crystalline phases in water, which associate with gliadins. The formation of such structures allows the expansion of gas cells and contributes to dough elasticity, resulting in increased bread volume [10].

In recent days, there has been a general increase in the number of consumers who reject all synthetic addi-

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tives in food, including antioxidants. Thus, over the last few years, this had led to an increase in the use of natural antioxidants, especially those of vegetable origin, and obtained in the form of extracts. Rosemary (*Rosmarinus officinalis* L.) extracts have been applied to different oils, retarding the oxidation more successfully than other natural and synthetic antioxidants such as butylated hydroxynasole and butylated hydroxytoluene.

Owing to its pronounced oxido-reductional properties, the alcohol extract of the thyme herb (*Thymus vulgaris* L.), which belongs to a group of antioxidants of natural origin and holds a great significance and usage in numerous branches of industry, is of interest in this study. Active components of thyme herb have complex chemical content. The main ingredients of thyme include essential oils, tannins, phenols (tymol guayacol), triterpenes, etc. Due to its chemical content, thyme is used in medicine and pharmaceutical industry for manufacturing of commercial products. It is used in teas, by itself or in combination with other herbs. It is also used in food industry, as fragrance for liquors and other alcoholic beverages.

Because of its pronounced oxido-reductive properties, the alcohol extract of sage herb (*Salvia officinalis* L.) is also of interest in this study. The main ingredients of sage include essential oils, tannins (rosemary acid), bitter compounds of diterpene type (carnosol, picrosalvin, etc), flavonoids, diterpenes, etc. Sage is widely used in pharmaceutical, cosmetic and food industries. It is an ingredient utilized in numerous commercial products and can be used on its own or in combination with other herbs (chamomile, cantarion, rosemary, etc.). In addition to being used in preparation of commercial products, it is also used in teas (by itself or in combination with other herbs), which have a wide application in treating many illnesses. Sage contains a high concentration of one of the most active natural antioxidants – rosemelanol-9-ethyl-aether. Sage is utilized to great extent in cooking, food and beverage industries. As of 1965, sage can be utilized for human consumption, and as of 1970, essential sage oil is acceptable for food consumption, within limits, due to the presence of thujone. Because it contains pronounced aromatic properties, it is often used as a spice as well as a fragrance for alcoholic liquors and beverages. It is of particular importance to mention that due to its chemical content, it is utilized as a natural antioxidant in the food industry. Sage is used to extend the shelf life of meat and meat products, during preparation of various beverages, in beer industry, baking and confectionery [11].

The purpose of this study was to evaluate the effects of the additions of Rosemary, Thyme and Sage extracts on rheological characteristics of dough and fermentation of dough.

## MATERIAL AND METHODS

### Materials

Throughout the experiment, dough was prepared with wheat flour and 55.5% water, 2% salt, 3.5% yeast (30% DM, Alltech-Fermin, Senta), calculated on flour weight basis. Commercial wheat flour (wet gluten – 23.5%, moisture – 14.2%, ash 0.5% – DM, proteins – 10.2% DM), tap water and commercial table salt were used for mixing. The following flour additives were used: commercial complex, L-ascorbic acid (BASF, Germany) (0.001, 0.002 and 0.006% w/w), diacetyl ester of tartaric acid with monoglycerides – Datem (Beldem, Belgium) (0.1, 0.3 and 0.5% w/w), fungal  $\alpha$ -amylase (Beldem, Belgium) (0.001, 0.003 and 0.006% w/w), xylanase (Beldem, Belgium) (0.002, 0.006 and 0.012% w/w) and alcohol extract of rosemary, thyme and sage herb. Commercial complex improver S-500 Acti Plus (Puratos, Belgrade), which contains emulsifier E472e, antioxidant E300 and enzyme complex (0.1%, 0.3% and 0.5% w/w).

Characteristics of alcohol extracts of rosemary herb (*Rosmarinus officinalis* L.) were: dry matter (DM) content, 1.2%; antioxidative activity,  $EC_{50}$ ,  $0.022 \pm 0.0003$  mg/mL; total flavonoids,  $76.2 \pm 1.03$  mg rutin/g DM; pH 6.3; refraction index,  $n_D^{20}$ , 1.37; relative density, 0.89 g/cm<sup>3</sup> (0.5, 1.0, and 2.0% w/w flour). Characteristics of alcohol extracts of thyme herb (*Thymus vulgaris* L.) were: 1.3% DM; antioxidative activity,  $EC_{50}$ ,  $0.023 \pm 0.0017$  mg/mL; total flavonoids,  $107.7 \pm 0.72$  mg rutin/g DM; pH 6.4; refraction index  $n_D^{20}$ , 1.37; relative density, 0.89 g/cm<sup>3</sup>; (0.5, 1.0, and 2.0% w/w flour). Characteristics of alcohol extracts of sage herb (*Salvia officinalis* L.) were: 2.1% DM; antioxidative activity,  $EC_{50}$ ,  $0.021 \pm 0.0002$  mg/mL; total flavonoids,  $91.8 \pm 0.57$  mg rutin/g DM; pH 6.4; refraction index,  $n_D^{20}$ , 1.37; relative density, 0.89 g/cm<sup>3</sup> (0.5, 1.0, and 2.0% w/w flour).

### Methods

#### Flour characterization

Flour samples were analyzed for moisture percentages, wet gluten and the crude ash contents of flour samples were determined [12]. For protein determination, flour samples were analyzed for nitrogen percentage [12], and this was used to compute protein content (N% × 5.7).

#### Extracts characterization

Alcohol extracts of rosemary, thyme and sage herbs were produced through extraction with 70% ethanol (occasionally mixing during 24 h) [13].

Anti-oxidative activity of extracts was determined according to a DPPH (2,2-diphenyl-2-picrylhydrazyl) test. The content of total flavonoids in extracts was determined using a spectrophotometric method with alu-

minium chloride, which is based on a formation of favo-noid-aluminium complex [14]. Determining of dry matter content, pH values, index refraction, relative density were performed according to a method Ph. Eur. [13].

#### Dough characterization

Characterization of dough was determined by farinograph, extensograph and amylograph (Brabender, OHG, Germany) [12,15].

#### Gas production and dough development

A Rheofermentometer F3 (Chopin, Villeneuve-La-Garenne Cedex, France) was used to measure the maximum height of dough, Hm (mm) and the amount of CO<sub>2</sub> produced (CO<sub>2</sub>, mL/3h) following the procedure by Huang, *et al.* [16] with a minor modification, the amount of dough was adjusted to 250 g. The test was conducted at 28.5 °C for 3 h.

#### Statistical analysis

All samples were prepared and analyzed in triplicates. Height and diameter of each replication were taken three times and the average value of three measurements was reported. The results were statistically tested by analysis of variance and the means were compared by the Scheffe test at a significance level of 0.05, using software Statistica 8.0, series 0608c.

## RESULTS AND DISCUSSION

### Farinograph

Results of farinograph analyses of dough prepared from flour without additives (control sample) and flour with additives (complex additive, Datem, L-ascorbic acid, α-amylase, xylanase and extracts of rosemary, thyme and sage), are shown in Table 1.

Water absorption capacity varies from 54.5% (α-amylase 0.006%) to 56.2% (xylanase 0.006%). The samples used do not have a significant influence ( $p = 0.068$ ) on the rate of water absorption with respect to control sample (55.5%). In relation to the control sample (1.5 min), the development of dough changes in samples with added L-ascorbic acid (2.0), rosemary extract, or a combination of complex additive and thyme (1.7), as well as sage extracts (1.0%). Other additives do not have a significant influence ( $p = 0.072$ ) on the development of dough. In relation to the control sample (0.5 min), the stability of dough significantly increases ( $p = 0.038$ ) with the addition of: Datem-a (0.3%), L-ascorbic acid (0.002%), rosemary extract (1.0%), thyme extracts (0.5 and 1.0%) and sage extract (1.0%), and significantly decreases ( $p = 0.036$ ) with the addition of rosemary extracts (0.5 and 2.0%), combination of complex additive and thyme extract (0.05 and 0.5%). The degree of softening of dough significantly decreases ( $p = 0.041$ ) with an elevation of concentration of complex additive

and Datem and sage extract (0.5%). Addition and increase in concentration of α-amylase has a negative effect on the degree of softening of dough. Combining of complex additive and rosemary extract has a positive effect on the degree of softening of dough in relation to the control sample, which is at a level of results attained with the addition of complex additive (0.5%) and Datem (0.3 and 0.5%). A sample with added thyme extract (0.5%) has a particularly favorable impact on the degree of dough softening. A degree of softening is elevated with an increase of extract concentration, but all samples with an added extract have a more favorable level of softening in relation to samples with other additives and the control sample. Test sample with added sage extract (0.5%) has the most favorable impact on the quality number. The rest of the additives do not have a significant influence ( $p = 0.062$ ) on the quality number in relation to control sample, therefore all samples have been sorted into quality class b1, except for those with added α-amylase and added sage extract (1.0%), whereby an increase in concentration results in diminished quality number, based on significantly increase ( $p = 0.039$ ) of degree of softening of the sample. Samples with added α-amylase in concentrations of 0.001, 0.003 and 0.006% are sorted into b2, b2 and c1 quality class respectively, similarly to literature results [15].

### Extensograph

The results of extensograph analyses of doughs prepared from flour without additives (control sample) and flour with additives (complex additive, Datem, L-ascorbic acid, α-amylase, xylanase and extracts of rosemary, thyme and sage), are shown in Table 2.

The energy of dough samples with additives is higher in relation to the control sample. The energy of dough is significantly reduced ( $p = 0.041$ ) with the increase of the concentration of complex additive and rosemary extract. The highest values of energies of dough are found for samples with added complex additive (0.1%), L-ascorbic acid (0.006%), rosemary extract (0.5%), thyme extract (0.5%), sage extract (0.5%) and combination of complex additive and sage extract (0.05 and 0.5%). In samples of dough with added sage extract, an increase of energy in relation to the control sample is at a level of samples where the complex additive is added. Other additives have a lower influence on the energy increase of dough. Resistance of dough is diminished in relation to the dough sample with the addition of Datem, α-amylase, xylanase and thyme extract, and significantly increases ( $p = 0.038$ ) with the inclusion of complex additive, L-ascorbic acid, rosemary extract and sage extract. The greatest dough resistance occurs in samples with the addition of complex additive (0.3%), L-ascorbic acid (0.006%), rosemary extract (2.0%) and sage extract (2.0%), which indicates that these additives have a significant influence ( $p = 0.032$ ) on strengthening

Table 1. Farinograph dough indicators with and without additives

Additive, %	Water absorption capacity, %	Dough development min	Dough stability min	Softening degree FU	Quality number	Quality group
Control						
0	55.5±0.3	1.5±0.1	0.5±0.05	70±0.3	59.4	b1
Complex additive						
0.1	55.0±0.2	1.5±0.2	0.5±0.05	80±0.3	60.8	b1
0.3	55.5±0.3	1.5±0.2	0.5±0.05	70±0.3	56.2	b1
0.5	55.0±0.1	1.5±0.1	0.5±0.05	60±0.3	62.6	b1
Datem						
0.1	55.2±0.4	1.5±0.2	0.5±0.05	70±0.3	58.3	b1
0.3	55.5±0.5	1.5±0.1	1.0±0.1	60±0.5	65.1	b1
0.5	55.0±0.2	1.5±0.1	0.5±0.05	60±0.4	59.4	b1
L-Ascorbic acid						
0.001	55.2±0.2	2.0±0.1	0.5±0.05	70±0.3	59.2	b1
0.002	55.3±0.4	1.5±0.2	1.0±0.1	80±0.4	55.8	b1
0.006	55.3±0.5	2.0±0.2	0.5±0.05	70±0.3	55.1	b1
Rosemary extract						
0.5	55.5±0.4	1.7±0.2	0.2±0.05	70±0.5	56.2	b1
1.0	55.5±0.5	1.7±0.1	0.7±0.04	70±0.3	56.2	b1
2.0	55.3±0.3	1.7±0.2	0.2±0.05	80±0.3	58.7	b1
Thyme extract						
0.5	55.2±0.3	1.5±0.1	1.0±0.1	40±0.3	68.8	b1
1.0	55.3±0.2	1.5±0.2	1.0±0.1	50±0.3	66.2	b1
2.0	55.0±0.5	1.2±0.2	0.7±0.05	60±0.4	62.6	b1
Sage extract						
0.5	55.3±0.3	1.5±0.1	1.0±0.1	60±0.3	63.1	b1
1.0	55.0±0.5	1.7±0.1	0.2±0.02	80±0.5	54.3	b2
2.0	55.3±0.2	2.0±0.2	0.5±0.05	70±0.3	56.2	b1
$\alpha$ -Amylase						
0.001	55.0±0.4	1.5±0.1	0.5±0.02	90±0.4	52.8	b2
0.003	55.0±0.5	1.5±0.2	0.5±0.01	100±0.5	47.7	b2
0.006	54.5±0.3	1.5±0.3	0.5±0.02	110±0.3	43.5	c1
Xylanase						
0.002	55.5±0.4	1.5±0.2	0.5±0.05	75±0.3	56.6	b1
0.006	55.5±0.6	1.5±0.2	1.0±0.1	60±0.4	62.4	b1
0.01	56.2±0.5	1.5±0.1	0.7±0.02	80±0.5	55.1	b1
Complex additive + rosemary extract						
0.05 + 0.5	55.6±0.2	1.5±0.2	0.5±0.01	60±0.4	60.4	b1
Complex additive + thyme extract						
0.05 + 0.5	55.6±0.2	1.7±0.2	0.2±0.02	80±0.3	54.3	b2
Complex additive + sage extract						
0.05 + 0.5	55.6±0.1	1.5±0.2	0.5±0.04	80±0.3	53.3	b2

of gluten, similar to results of Šebešić *et al.* [1]. Extensibility of dough gently significantly increases ( $p = 0.042$ ) in relation to the control sample when additives are used in minimum concentrations. Extensibility of dough diminishes as the increase concentration of additives. Dough samples with added xylanase have the

greatest extensibility, while a sample with L-ascorbic acid (0.006%) added to it has the lowest degree of extensibility. Related number of dough samples with additives is disturbed and contains extremely high values with an increase in the concentration of additive, which occurs as a result of increased resistance and reduction

Table 2. Extensograph dough indicators with and without additives

Additive, %	Energy, cm <sup>2</sup>	Resistance, EU	Extensibility, mm	Ration of resistance/extensibility, R/E
Control				
0	77.5±1.3	310±4.3	136±1.3	2.28
Complex additive				
0.1	99.5±2.1	390±5.1	141±2.1	2.76
0.3	83.0±1.2	540±3.2	105±1.2	5.14
0.5	70.5±1.1	380±5.3	119±1.0	3.19
Datem				
0.1	85.5±1.4	200±4.2	156±2.1	1.85
0.3	86.0±1.6	290±5.2	158±1.7	1.83
0.5	77.0±1.2	260±2.2	158±1.4	1.64
L-Ascorbic acid				
0.001	102.0±1.3	385±4.4	147±1.5	2.62
0.002	96.6±1.1	445±3.3	130±2.1	3.42
0.006	103.4±1.2	570±3.4	117±1.7	4.87
Rosemary extract				
0.5	95.0±0.1.5	380±4.4	134±1.6	2.83
1.0	94.0±1.3	390±3.4	139±1.4	2.80
2.0	89.0±1.3	440±5.3	122±1.4	3.60
Thyme extract				
0.5	72.5±1.2	260±3.5	154±1.3	1.68
1.0	82.5±1.4	260±4.2	158±1.4	1.64
2.0	67.0±1.6	290±7.2	132±1.2	2.19
Sage extract				
0.5	93.5±1.5	380±3.4	139±2.1	2.73
1.0	93.0±1.7	370±5.5	136±2.2	2.72
2.0	84.5±0.3	430±4.5	122±2.1	3.52
$\alpha$ -Amylase				
0.001	82.5±1.2	280±4.4	163±1.4	1.72
0.003	77.6±1.4	280±3.3	150±1.5	1.87
0.006	83.5±1.2	290±4.4	155±1.3	1.87
Xylanase				
0.002	82.9±1.2	270±5.4	170±1.3	1.59
0.006	69.0±1.5	230±6.2	158±2.1	1.46
0.01	67.5±1.7	220±3.3	172±3.3	1.28
Complex additive + rosemary extract				
0.05 + 0.5	84.0±1.2	390±4.3	126±1.2	3.09
Complex additive + thyme extract				
0.05 + 0.5	86.5±1.1	350±5.3	140±2.1	2.50
Complex additive + sage extract				
0.05 + 0.5	96.0±1.1	440±4.3	133±1.3	3.31

in extensibility of dough. Typically, an increase in concentration of complex additive, L-ascorbic acid and rosemary extract results in sudden elevation of resistance and a significantly decrease ( $p = 0.036$ ) in extensibility. An especially negative relationship between resistance and extensibility in relation to the control sample occurs

in samples with added complex additive (0.3%) and L-ascorbic acid (0.006%), similar to results of Davidović *et al.* [17].

Indicators shown in the extensograph (Table 2) reveal that all additives strengthen the gluten of wheat flour, increase energy and resistance, and decrease ex-

tensibility of dough. A significant increase ( $p = 0.038$ ) in resistance and decrease of extensibility results in change of the R/Ex (resistance/extensibility) values.

### Amylograph

Results of amylograph analyses of dough prepared from flour without additives (control sample) and flour with additives (complex additive, Datem, L-ascorbic acid,

$\alpha$ -amylase, xylanase and extracts of rosemary, thyme and sage) are shown in Table 3.

The characteristic of flour control sample and samples with additives (complex additive, Datem, L-ascorbic acid,  $\alpha$ -amylase, xylanase and extracts of rosemary, thyme and sage), is low amyloytic activity (350–470 AU). Maximum viscosity (amyloytic activity) of flour samples with additives significantly increases ( $p = 0.028$ ) in

Table 3. Amylograph dough indicators with and without additives

Additive, %	Maximal temperature, $t_{\max}$ / min	Maximal viscosity, $\mu_{\max}$ , amylograph unit
Control		
0	74.5±1.3	350
Complex additive		
0.1	75.25±1.1	360
0.3	76.5±1.2	360
0.5	78.25±0.3	360
Datem		
0.1	76.5±1.4	390
0.3	77.5±1.2	435
0.5	79.5±1.1	470
L-Ascorbic acid		
0.001	74.0±1.2	390
0.002	74.5±1.5	380
0.006	74.5±1.3	370
Rosemary extract		
0.5	74.5±1.3	390
1.0	74.0±1.2	395
2.0	73.75±1.1	400
Thyme extract		
0.5	74.5±1.5	360
1.0	74.5±1.3	380
2.0	74.5±1.2	400
Sage extract		
0.5	73.0±1.5	375
1.0	74.0±1.3	385
2.0	74.5±1.2	400
$\alpha$ -Amylase		
0.001	75.0±1.1	355
0.003	75.25±1.2	360
0.006	73.75±1.4	380
Xylanase		
0.002	73.0±1.5	390
0.006	73.0±1.2	370
0.01	74.0±1.1	350
Complex additive + rosemary extract		
0.05 + 0.5	75.0±1.6	410
Complex additive + thyme extract		
0.05 + 0.5	75.0±1.2	400
Complex additive + sage extract		
0.05 + 0.5	74.0±1.2	400

relation to the control sample. Addition of complex additive as well as an increase in concentration does not have a significant influence ( $p = 0.062$ ) on an increase in amylolytic activity in relation to the control sample. Positive effect on amylolytic activity in relation to control sample is achieved by inclusion and increase in concentration of Datem and extracts of rosemary, thyme and sage. Inclusion and increase in the concentration of  $\alpha$ -amylase to a lesser extent, also results in significantly increase ( $p = 0.041$ ) of amylolytic activity in relation to the control sample. The addition of xylanase has a significantly increase ( $p = 0.039$ ) in amylolytic activity of flour in relation to the control sample, only in concentration of 0.002%, further increase in concentration of xylanase results in the lowering of amylolytic activity of flour. Addition of combination of complex additive and rosemary, thyme or sage extracts, has a significantly increase ( $p = 0.038$ ) on amylolytic activity of flour relative to the control sample and samples with other additives. Indicators provided on the amylograph (Table 3) show that the optimal effect on flour with low amylolytic activity is achieved by adding Datem and extract of rosemary, thyme or sage.

### Dough development

The results of rheofermentometer analyses related to the development of dough prepared from flour without additives (control sample) and flour with additives (complex additive, Datem, L-ascorbic acid,  $\alpha$ -amylase, xylanase and extracts of rosemary, thyme and sage), are shown in Table 4.

Maximum development of dough  $H_m$  (mm) significantly increases ( $p = 0.029$ ) in all samples with additives in relation to the control sample. The samples that reach the greatest dough development contain the following additives: thyme extract (0.5%), complex additive (0.3%), Datem (0.5%), rosemary extract (1.0%), sage extract (1.0 and 2.0%),  $\alpha$ -amylase (0.006%) and xylanase (0.01%). A favorable influence on  $H_m$  occurs in samples with added combination of complex additive and rosemary extract, 0.05 and 0.5% respectively. The time of maximum raising of  $T_1$  (h:min), as an indicator of dough tolerance during fermentation, does not change significantly ( $p = 0.064$ ) in respect to the control sample with the addition and increase in the concentration of complex additive and Datem. Dough samples with added extracts of rosemary, thyme and sage and L-ascorbic acid have an increased tolerance during fermentation, expressed through time  $T_1$ , while samples with added  $\alpha$ -amylase and xylanase have the shortest tolerance, similar to the results of Huang *et al.* [16]. Complex additive and extracts of rosemary, thyme and sage, as opposed to other additives, do not decrease tolerance of

dough expressed as time difference  $T_2-T'_2$  (h:min) in respect to the control sample. The height of dough development  $h$  (mm) at the end of the analyses is greatest in samples with complex additive (0.3%), and rosemary extract (1.0%) and sage extract (2.0%).

### Gas production

The results of rheofermentometer analyses, related to the production and retention of gas in the dough, which is prepared from flour without additives (control sample) and flour with additives (complex additive, Datem, L-ascorbic acid,  $\alpha$ -amylase, xylanase and extracts of rosemary, thyme and sage) are shown in Table 5.

The time of release of gases  $T_x$  (h:min) is extended, in respect to the control sample, in samples where complex additive, Datem, extract of rosemary and extract of sage are used as additives, and is shortened in samples where L-ascorbic acid,  $\alpha$ -amylase, xylanase and thyme extract are used as additives. The total volume of gas retained in dough (mL) is larger in respect to the control sample in the samples where complex additive (0.3%), rosemary extract (1.0%), sage extract (1.0 and 2.0%) and ascorbic acid (0.001%) are added. Addition of thyme extract, Datem,  $\alpha$ -amylase and xylanase has a significant influence on the increase ( $p = 0.029$ ) of gas retention. A dough sample with an added combination of complex additive and extract of rosemary, thyme or, of sage has a favorable influence on the retention of gas. The volume of lost  $\text{CO}_2$  gas (mL) is the lowest in dough samples with an added combination of complex additive and thyme extract (0.05 and 0.5%) and rosemary extract (2.0%). In samples with thyme extract (1.0%) added, the volume of lost gas is at a level of samples with added complex additive, Datem, and L-ascorbic acid. Samples with added  $\alpha$ -amylase and xylanase exhibit a very large volume of released gas in respect to the control sample and samples with other additives. The volume of retained gas (mL) is larger in dough samples with an addition of complex additive (0.3%), L-ascorbic acid (0.001%) and rosemary extract (1.0%), sage extract (1.0 and 2.0%) and dough sample with an added combination of complex additive and sage extract, while it is lower in samples with added Datem,  $\alpha$ -amylase and xylanase, thyme extract (1.0%), in agreement to the results of Huang *et al.* [16]. Samples that have a favorable retention coefficient of gases (%) are those that contain the following additives: complex additive, Datem, L-ascorbic acid, rosemary extract, thyme extract (1.0%), sage extract, combination of complex additive and thyme extract (0.05 and 0.5%) and control sample, while samples that have an unfavorable retention coefficient contain  $\alpha$ -amylase and xylanase and thyme extract (1.0%), combination of complex additive and thyme extract (0.05 and 0.5%).

Table 4. Reofermentometric indicators of dough development with and without additives

Additive, %	$Hm^a$ / mm	$h^a$ / mm	$100(Hm-h)/Hm$	$T_1$ (h:min) <sup>b</sup>	$T_2$ (h:min) <sup>b</sup>	$T^*_{2}$ (h:min) <sup>b</sup>	$T_2-T^*_{2}$ (h:min) <sup>b</sup>
Control							
0	46.7±1.3	38.4±2.3	17.8	2:07	2:57	1:19	1:37
Complex additive							
0.1	60.8±2.3	52.8±2.1	13.2	2:06	2:54	1:22	1:31
0.3	77.2±1.4	62.1±2.3	19.6	2:06	3:09	1:31	1:37
0.5	62.7±1.2	42±2.1	33	2:12	2:39	1:30	1:09
Datem							
0.1	49.3±1.2	43.2±2.3	12.4	2:04	3:27	0:00	0:00
0.3	53.5±1.1	43.4±2.1	18.9	1:51	2:31	1:19	1:12
0.5	67.6±1.4	39.8±1.3	41.1	2:03	2:30	1:30	1:00
L-Ascorbic acid							
0.001	51.1±2.1	44.8±2.3	12.3	2:48	3:27	0:00	0:00
0.002	47.4±2.2	40.8±2.3	13.9	2:27	3:43	0:00	0:00
0.006	51±1.3	44.7±2.3	12.4	2:01	3:30	0:00	0:00
Rosemary extract							
0.5	47.5±2.3	40.1±2.3	15.6	2:18	3:04	1:36	1:28
1.0	53.5±1.3	45.2±3.3	15.5	2:37	0:36	1:51	1:45
2.0	26.2±1.5	8.3±0.3	35.7	2:18	1:49	1:37	0:12
Thyme extract							
0.5	87.4±1.6	0	100	3:21	3:21	0:00	0:00
1.0	48.2±2.1	41.4±0.3	14.1	2:18	3:18	1:15	2:03
2.0	57.4±1.8	0	100	2:19	2:22	0:07	2:15
Sage extract							
0.5	32.1±2.2	2±0.1	93.8	2:19	3:13	1:36	1:37
1.0	57±1.3	49.7±2.1	12.8	2:42	3:31	1:48	1:45
2.0	59.6±2.3	52.7±1.3	11.6	3:03	4:15	1:54	2:21
$\alpha$ -Amylase							
0.001	47.3±2.3	0	100	0:55	0:57	0:46	0:10
0.003	56±1.3	0.9±0.03	98.4	1:34	1:55	1:01	0:54
0.006	58.1±1.3	50±2.3	13.9	1:30	2:19	0:55	1:24
Xylanase							
0.002	53±2.2	45.8±3.3	13.6	1:21	1:58	0:51	1:07
0.006	53.8±1.2	2.7±0.3	95	1:24	1:39	0:57	0:42
0.01	57.2±3.2	39.2±2.3	16.9	1:07	1:33	0:49	0:43
Complex additive + rosemary extract							
0.05 + 0.5	55±1.3	0	100	1:46	2:42	1:12	1:30
Complex additive + thyme extract							
0.05 + 0.5	39±1.2	0	100	0:55	0:57	0:45	0:02
Complex additive + sage extract							
0.05 + 0.5	35.7±2.2	28.6±1.3	19.9	1:55	3:07	1:15	1:52

<sup>a</sup>Maximum height and height and of dough development, respectively; <sup>b</sup>time of dough development

Table 5. Reofermentometric indicators of production of gases in dough with and without additives

Additive, %	Hm <sup>*</sup> / mm	T <sub>1</sub> (h:min)	T <sub>x</sub> (h:min)	Total volume, mL	Lost CO <sub>2</sub> , mL	Retention volume, mL	Retention coefficient, %
Control							
0	19.12±1.3	0:31±0.3	0:42±0.3	1855±3.1	82±1.1	1782±5.3	97.1
Complex additive							
0.1	85.2±2.3	1:36±0.2	1:09±0.2	2001±3.3	87±1.3	1914±7.7	95.6
0.3	90.2±3.3	1:33±0.1	1:06±0.1	2233±4.3	97±2.1	2136±8.5	95.7
0.5	87.9±2.3	1:37±0.1	1:07±0.1	2195±5.2	75±1.1	2120±9.9	96.6
Datem							
0.1	61.2±2.1	1:58±0.1	1:34±0.2	1627±4.3	103±1.1	1524±6.3	93.7
0.3	82.9±2.2	1:43±0.1	1:12±0.1	1775±5.3	83±2.2	1632±7.3	95.2
0.5	87.5±3.2	1:37±0.1	1:07±0.1	1728±5.6	77±1.2	165±8.4	95.5
L-Ascorbic acid							
0.001	89.1±2.2	1:37±0.1	0:54±0.1	1931±6.6	74±1.1	1857±6.9	96.2
0.002	86.3±3.2	1:51±0.1	0:48±0.1	1861±7.1	95±3.2	1766±8.9	94.9
0.006	85.6±2.1	1:42±0.2	0:55±0.1	1900±4.2	86±2.1	1814±7.7	95.5
Rosemary extract							
0.5	72.6±1.2	2:25±0.2	1:24±0.1	1803±3.2	60±2.1	1743±6.6	96.7
1.0	66.0±2.2	2:37±0.1	1:07±0.1	1900±4.1	101±2.3	1800±5.5	94.7
2.0	81.8±3.1	0:30±0.1	0:04±0.1	1816±3.3	57±1.3	1760±6.9	96.9
Thyme extract							
0.5	72.2±2.3	0:19±0.1	0:19±0.2	1541±3.2	413±2.3	1128±8.9	73.2
1.0	84.1±1.2	0:27±0.1	0:36±0.1	1823±4.3	94±3.3	1729±7.9	94.8
2.0	80.7±2.3	0:27±0.1	0:36±0.1	1407±3.2	303±5.3	1104±8.8	78.5
Sage extract							
0.5	70.6±2.3	2:30±0.4	1:24±0.1	1834±5.3	53±2.3	1782±5.5	97.1
1.0	72.9±2.3	2:04±0.5	1:13±0.1	1916±2.1	78±4.3	1838±7.7	96.0
2.0	63.3±1.3	0:42±0.1	1:40±0.1	1907±3.2	86±2.3	1821±8.8	95.5
$\alpha$ -Amylase							
0.001	10.61±1.3	0:33±0.1	0:21±0.1	1537±4.1	394±4.3	1143±7.8	74.4
0.003	107.6±2.3	0:39±0.1	0:19±0.1	1758±3.2	532±3.3	1226±8.8	69.7
0.006	107.0±3.3	0:42±0.1	0:19±0.1	1777±6.2	522±5.3	1254±9.9	70.6
Xylanase							
0.002	115.0±2.3	0:33±0.2	0:19±0.1	1504±3.2	388±6.3	1116±7.7	74.2
0.006	109.7±3.1	0:36±0.1	0:21±0.1	1451±2.2	296±2.3	1155±8.8	79.6
0.01	110.3±2.2	0:37±0.1	0:19±0.1	1317±4.1	225±4.3	1092±9.6	82.9
Complex additive + rosemary extract							
0.05+0.5	87.8±2.1	0:30±0.1	0:42±0.1	1910±2.1	86±3.3	1824±6.7	95.5
Complex additive + thyme extract							
0.05+0.5	86.0±2.3	1:54±0.1	0:49±0.1	1375±2.2	40±5.3	1334±8.7	97.1
Complex additive + sage extract							
0.05+0.5	89.3±3.3	0:27±0.2	1:00±0.1	1995±1.1	78±6.3	1917±5.8	96.1

## CONCLUSIONS

The volume of the lost CO<sub>2</sub> gas (mL) is the lowest in dough samples with added rosemary (2.0%), thyme (1.0%) or sage (0.5%) extracts. The results of the analysis justify the application of alcohol extracts of rosemary, thyme and sage herbs as flour additives, in order

to improve the rheological properties of dough, and encourage further research in the application of natural raw materials as additives in the bakery industry. Test sample with added sage extract (0.5%) has the most favorable impact on the quality number. The greatest dough resistance occurs in samples with the addition of complex additive (0.3%), L-ascorbic acid (0.006%), rose-

mary extract (2.0%) and sage extract (2.0%), which indicates that these additives have a significant influence on strengthening of gluten.

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**IZVOD****PRIMENA PRIRODNIH ORGANSKIH JEDINJENJA U PEKARSKOJ INDUSTRIJI**

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(Naučni rad)

Ispitivan je uticaj različitih aditiva na tehnološke karakteristike hlebnog testa. Primjenjeni su: kompleks aditiva (0,1, 0,3 i 0,5%), L-askorbinske kiseline (0,002, 0,004 i 0,012%), diacetil estar vinske kiseline sa monoglicerideima (Datem E472e, 0,1, 0,3 i 0,5%),  $\alpha$ -amilaza (0,002, 0,006 i 0,012%), ksilanaza (0,004, 0,012 i 0,024%), alkoholni ekstrakti ruzmarina (0,5, 1,0 i 2,0%), ekstrakt timijana (0,05 i 0,5%), ekstrakt kadulje (0,5, 1,0 i 2,0%) kao i kombinacija kompleksnih aditiva i ruzmarina, timijana i kadulje na reološke osobine hlebnog testa. Studija obuhvata amilografsku, farinografsku i ektenzografsku ispitivanja hlebnog testa sa i bez dodatka aditiva. Rezultati analiza u potpunosti opravdavaju korišćenje alkoholnih ekstrakata ruzmarina, timijana i kadulja kao aditiva u hlebnog testu, s obzirom na poboljšanje reoloških karakteristika testa. Kao značajan doprinos primene ovih ekstrakata može se istaći i činjenica da se dobija specijalna vrsta hleba koja se može okarakterisati kao funkcionalna hrana. Kompleksni aditivi i ekstrakt ruzmarina imaju pozitivan efekat na nivo omeđavanja testa u odnosu na kontrolni uzorak. Energija testa je značajno smanjena ( $p = 0,041$ ) sa povećanjem koncentracije kompleksa aditiva i ekstrakta ruzmarina. Najveće vrednosti energije testa nalaze se za uzorce sa dodatkom kompleksa aditiva (0,1%), L-askorbinske kiseline (0,006%), ekstrakta ruzmarina (0,5%), ekstrakta timijana (0,5%), ekstrakta žalfije (0,5%) i kombinacija kompleksnih aditiva i ekstrakta kadulje (0,05 i 0,5%). Visina testa (mm) na kraju analize je najveći u uzorcima u kojima je kompleks aditiva – 0,3%, ekstrakta ruzmarina – 1,0% i ekstrakta žalfije – 2,0%. Izdvojeni  $\text{CO}_2$  (ml gase) najniži je u uzorcima testa sa dodatkom ekstrakta ruzmarina (2,0%), timijana (1,0%) ili kadulje (0,5%). Rezultati analize opravdavaju primenu alkoholnog ekstrakta ruzmarina, timijana i kadulje radi poboljšanja reoloških osobina testa. Testo sa dodatkom ekstrakta žalfije (0,5%) ima najpovoljniji uticaj na kvalitetni broj. Najveći otpor testa se javlja u uzorcima sa dodatkom aditiva kompleksa (0,3%), L-askorbinska kiselina (0,006%), ekstrakta ruzmarina (2,0%) i ekstrakta žalfije (2,0%), što ukazuje da su ovi aditivi imaju značajan uticaj na jačanje glutena.

Ključne reči: Testo • Reološke osobine • Ekstrakt ruzmarina • Ekstrakt tamjana • Ekstrakt kadulje • Amilograf • Farinograf • Ekstenziograf • Reofermentometar

Key words: Dough • Rheological properties • Rosemary extract • Thyme extract • Sage extract • Amylograph • Farinograph • Extensograph • Rheofermentometer