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### MICROMALTING OF TRITICALE VARIETIES NS PAUN AND ODISEJ

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The objective of this study was to evaluate the possibility of application of two triticale varieties, NS Paun and Odisej, as partial substitutes for barley malt in wort production, based on the triticale analyses, micromalting, and analyses of the produced triticale malts, with a highlight on the activity of amylases during the malting process. The analyses of two triticale varieties showed higher extract content and diastatic power in comparison to barley malt. In the varieties NS Paun and Odisej, the extract was by 11.4% and 6.3% higher than the standard values for barley malt, respectively. Diastatic power in varieties NS Paun and Odisej was by 100% and 111.9% higher than in barley malt, respectively. In both varieties,  $\alpha$ - and  $\beta$ -amylases activities were also high. The  $\alpha$ amylase activity increased during the micromalting 34 and 20 times, respectively, in comparison to native triticale grain. The β-amylase activity also increased during micromalting, but to a lesser extent. The produced triticale malts had good technological parameters and could be used as a partial substitute for barley malt in wort production.

**KEY WORDS:** triticale, malt, micromalting

#### INTRODUCTION

Triticale is a man-made cereal between wheat and rye, hence it combines characteristics of both parents (1). Triticale was developed to merge a high yield potential and good grain quality from wheat and disease resistance and environmental tolerance which is a characteristic of rye (2). Being an environmentally more flexible than the other cereals, triticale can grow on wide climate range, marginal, high acid soils, with lack of water, which can reduce requirements for chemical protection and fertilization (3). The world production of triticale reached nearly 15 million tones in 2016. The same year in Serbia, total area planted with triticale was around 23,000 ha, with the highest average yield of 38,000 t/ha (4). Triticale grain contains many vitamins, phenolic acids with antioxidant activity (940 µg/g), and high protein (11.8-15.2%), which makes it an excellent grain for animal feed, while much less quantity is used for human consumption (5,6).

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Triticale also shows promising brewing properties. In Europe, 10-30% of malt is replaced by unmalted cereals, which significantly reduces cost of beer production (7). The chemical composition of triticale grain is closer to that of wheat, than rye, related to morphology, granule size, and amylose content (8). Even in the unmalted form, triticale shows high activity of amylolytic enzymes (9). Previous studies showed large variation of  $\alpha$ - and  $\beta$ -amylase activity, which can be a consequence of different genotypes, environment influence during cultivation, and different methods used for determination (50-1500 U/g) (10). The major part of  $\alpha$ -amylase is located in the grain pericarp, while the minor part is located in the endosperm. Proteinase of triticale grain can be found in the bran fraction (11). The content of mixed linkage β-glucan in triticale is low, while the cell walls contain predominantly pentosans, where arabinoxylan is the major factor ranging from 5.9-7.5% (12). Hence, pentosans are recognized for contribution to wort viscosity, slow wort separation, and slow beer filtration (13). Also, in addition to slow filtration, the problem can be the absence of husk (14). The amino acid composition of triticale's wort is an important parameter, owing to the fact that triticale is richer than both parental species in essential amino acids. Some studies showed that triticale grain contributes significant amount of amino acids to the wort, where lysin, limiting amino acid in cereals, had a much higher level compared to wheat or rye grain (15). Furthermore, triticale has demonstrated very high extract content, with low temperature of gelatinization (59-65 °C) and high diastatic power of malt. Also, high proteolytic activity leads wort rich in soluble nitrogen, which will give dark beer colour (16). Therefore, this kind of grain properties make triticale an interesting cereal for partial substitute for barley malt in beer production, which could be used at high adjunct ratios, without addition of commercial enzymes (9). Malting is a complex process, where modification of grain includes the synthesis and activation of enzymes, degradation of protein and cell wall, which leads to the hydrolysis of starch granules, mainly by  $\alpha$ -amylase (17).

The objective of this research was to evaluate the possibility of triticale application as a partial substitute for barley malt in wort production, based on the triticale analyses, micromalting, and analyses of the produced triticale malts, with a highlight on activity of amylases during the malting process.

### **EXPERIMENTAL**

The analysis of two triticale varieties, NS Paun and Odisej, from Rimski Šančevi experimental fields (Serbia), was performed along with micromalting and analyses of the produced triticale malts. Both triticale varieties are accepted varieties in Serbia. Standard micromalting was done at the Faculty of Technology in Novi Sad. Micromalting plant (Seeger, Germany) consists of steeping, germination, and kilning unit. In the malting process tap water was used. Samples were taken every day in order to determine activities of  $\alpha$ - and  $\beta$ -amylases. Triticale, malt, and wort analyses were performed using the standard European Brewery Convention, Analytica-EBC (18) and/or MEBAK methods (19), while  $\alpha$ - and  $\beta$ -amylases activity was determined using the assay kit Malt-amylase (Megazyme, Ireland). The study included: native triticale grain analyses; micromalting of triticale;

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following the changes during micromalting; and the analyses of produced malts. The experiments were done in triplicates. All values are expressed as means  $\pm$  standard deviation. Micromalting scheme is given in Table 1.

Table 1. Micromalting scheme

1 <sup>st</sup> day	Imersion steeping for 6 hours, t=15°C; Dry steeping for 18 hours, t=15°C
2 <sup>nd</sup> day	Imersion steeping for 4 hours, t=15°C; Dry steeping for 20 hours, t=15°C
3 <sup>rd</sup> day	Imersion steeping for 2 hours, t=15°C; Dry steeping for 22 hours, t = 15°C
4 <sup>th</sup> day	Germination at 15°C, turning over
5 <sup>th</sup> day	Germination at 15°C, turning over
6 <sup>th</sup> day	Germination at 15°C, turning over
7 <sup>th</sup> day	Germination at 15°C, turning over; Drying according to the given programme
8 <sup>th</sup> day	Drying finished, malt degermination

#### RESULTS AND DISCUSSION

The results of the analyses of triticale varieties NS Paun and Odisej are given in Table 2.

**Table 2.** Analytical parameters of triticale varieties NS Paun and Odisej

Parameter	NS Paun	Odisej	
	1 <sup>st</sup> class grain (%)	93.16±0.37	88.11±0.99
Sieving test	2 <sup>nd</sup> class grain (%)	5.24±0.58	$8.46\pm0.66$
	3 <sup>rd</sup> class grain (%)	1.6±0.64	3.43±0.63
Thousand kernel weig	36.80±0.85	37.10±1.71	
Hectoliter weight (kg/	72.3±0.48	75.0±0.33	
Vitreosity (%)	7.29±1.38	26.00±2.89	
Germinative energy, 3	98±1.73	98±1.00	
Water sensibility 3 day	0	1±0.58	
Moisture content (%)	10.07±1.28	9.51±1.34	
Proteins (% dry matter	11.69±1.84	12.88±1.91	

Values represent means  $\pm$  standard deviation calculated from three determinations

Both triticale varieties had content of the first class grain (above 85%), which indicates good quality grain (20). Analyses of thousand kernel weight and hectoliter weight showed uniform results, both in a range of good quality brewer's barley (20). The variety Odisej had the vitreosity higher than 20%, which could be a consequence of dry conditions during cultivation (21). Germinative energy indicates maturity and should be above

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96% (18). Both triticale varieties showed satisfactory germinative energy and very low water sensibility (less than 10%) (20). The protein content was slightly increased in both triticale varieties, as in a good quality brewer's barley it should be between 9-11.5% dry matter (20).

The results of triticale micromalting parameters are given in Table 3.

**Table 3.** Micromalting parameters of triticale varieties NS Paun and Odisej

Parameter	NS Paun	Odisej
Moisture after 24 h of steeping (%)	33.86±2.43	35.33±2.24
Moisture after 48 h of steeping (%)	40.72±2.11	43.45±2.53
Moisture after 72 h of steeping (%)	46.16±1.44	49.16±1.98
Moisture content of green malt (%)	45.84±1.86	49.84±2.10
Moisture content of grain (%)	5.37±0.43	5.14±0.78
Malting losses (% dry matter)	10.76±1.32	14.88±1.30
Respiration losses (% dry matter)	5.57±0.77	7.97±2.30
Rootlets (% dry matter)	5.19±0.85	6.91±1.46

Values represent means  $\pm$  standard deviation calculated from three determinations

During the micromalting, both triticale varieties received moisture well and fast. Steeping was performed evenly, which lead to a desired degree of steeping (45.5%) in both triticale varieties (19). Respiration losses should be in the range of 4-8% dry matter (20). Both varieties had optimal respiration losses and an increased rootlets loss (>5%) (20). Malt moisture content should be at most 5.5%, therefore it was optimal for both varieties (22).

The results of the analyses of malts produced from triticale varieties NS Paun and Odisej are given in Table 4.

The standard value for barley malt thousand kernel weight is 25-35 g dry matter (20). Both triticale malts had optimal thousand kernel weight. Vitreosity, which is correlated to malt degradation, in good quality malt is up to 7.5% (20). The malts produced from both triticale varieties had a very low vitreosity. The hectoliter weight lower than 55 kg shows a good malt degradation and optimal grain kilning (20). Both malts had an adequate hectoliter weight. Malt extract is the main economical criterion of malt quality. It indicates the portion of malt that dissolves in water the during mashing process. In the case of barley malt it should be higher than 79% dry matter (22). The wort extract produced from both malts was significantly higher than 79%, which indicates good malt degradation. In the varieties NS Paun and Odisej, the extract was by 11.4% and 6.3% higher than the standard values for barley malt. In 2006, the variety Odisej had a slightly lower extract content of 82.66% (23) than 2017 crop (84%). A lower amount of the aleurone layer in triticale is accompanied by a larger proportion of starchy endosperm, which can explain the higher extract yield in triticale grain (14).

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**Table 4.** Results of the analyses of malts produced from triticale varieties NS Paun and Odisej

Parameter	NS Paun	Odisej
Thousand kernel weight (g dry matter)	33.40±0.69	31.36±1.21
Hectoliter weight (kg/hL)	55.0±1.52	51.3±1.31
Vitreosity (%)	$0.54\pm0.64$	1.34±0.44
Extract content, fine grist (% dry matter)	88.03±0.73	84.00±1.07
Saccharification (minute)	<10	<10
Filtration (minute)	34	>60
Wort clarity	Clear	Opal
Wort colour (EBC units)	8.4±1.95	9.9±2.02
Wort pH	5.79±0.17	5.76±0.14
Wort soluble nitrogen (% dry matter)	1.12±0.30	1.21±0.34
Viscosity (mPas, 8.6%e)	1.616±0.30	1.995±0.21
Extract content, coarse grist (% dry matter)	87.08±0.91	83.63±1.31
Extract difference (% dry matter)	0.95±0.40	0.37±0.25
Kolbach index (%)	61.54±3.00	63.35±2.67
Hartong index 45°C (%)	59.59±2.19	59.00±2.72
Diastatic power (°WK)	520±2.65	551±2.65

Values represent means  $\pm$  standard deviation calculated from three determinations

Saccharification time indicates a high activity of amyloytic enzymes in malts with the standard value up to 15 minutes (22). Therefore, both malts had an excellent saccharification time (less than 10 minutes). The variety Odisej had unsatisfactory filtration time (>60 minutes), which could be a consequence of more intense malt kilning and unsatisfactory cytolytic degradation (20). Wort colour was higher than 4.0 EBC units for both malts, which is a standard value for pale lager malt (22). The wort pH value was adequate, as a standard wort pH value ranges from 5.5 to 6.0 pH units (22). The wort viscosity indicates the activity of cytolytic enzymes, and is a range of 1.53-1.63 mPa·s (20,21). The variety Odisej had an increased viscosity, which could be a consequence of the triticale arabinoxylans solubilization (8). A similar wort viscosity was obtained for the variety Odisej in a previous study (23), where the viscosity was 1.874 mPas. The soluble nitrogen includes nitrogen compounds which dissolve in wort during mashing, and its standard value is 0.55-0.75% (24). However, both triticale malts had a much higher soluble nitrogen content. The produced triticale malts exhibited strong proteolytic degradation according to Kolbach index (>41%) (20). Extract difference is a measure of malt degradation, especially cytolytic degradation, and it gives an insight into the malt enzymatic potential. Both triticale malts had very good degradation according to this parameter (<1.3% dry matter) (20). The Hartong index at 45°C is affected by the activity of all enzymes with the exception of  $\alpha$ -amylase, whose standard value for barley malt is above 36% (20,22). Both triticale malts had a very high Hartong index at 45°C (above



59%), which indicates a very good enzyme activity. The malt diastatic power shows its ability to degrade starch into fermentable sugars, and in pale malt it should be between 240-260°WK (18). The triticale malt diastatic power was very high (520 and 550°WK) due to the high activity of  $\alpha$ - and  $\beta$ -amylase in the produced malts. In the varieties NS Paun and Odisej, this parameter was by 100% and 111.9% higher than for barley malt. Owing to the extraordinary high enzymatic activity, triticale malts could be used as a partial substitute of barley malt when unmalted raw materials are used in wort production.

The activity of  $\alpha$ - and  $\beta$ -amylase in the investigated triticale varieties during the micromalting process are given in Figures 1 and 2. The both activities in the investigated triticale varieties increased significantly during micromalting. The variety NS Paun had a lower initial  $\alpha$ -amylase activity, but a greater increase during the malting process. The  $\alpha$ -amylase activity increased by 34 and 20 times in the variety NS Paun and Odisej, respectively. The highest increase was observed during the germination stage. At the end of the malting process, the variety Odisej had a higher  $\alpha$ -amylase activity than the variety NS Paun. The  $\beta$ -amylase activity also increased during micromalting, but to a lesser extent. The variety NS Paun, had a higher initial  $\beta$ -amylase activity and showed total increase by 1.16 times, while its activity in the variety Odisej increased by 1.44 times.

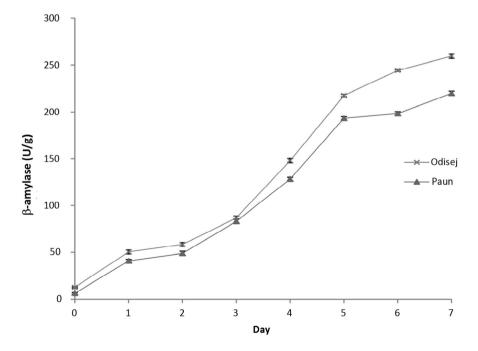
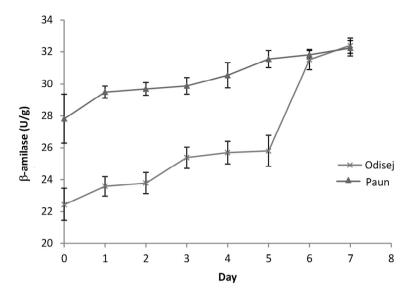


Figure 1. The α-amylase activity in triticale varieties NS Paun and Odisej during micromalting process

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**Figure 2**. The β-amylase activity in triticale varieties NS Paun and Odisej during micromalting process

#### CONCLUSION

According to the obtained results it can be concluded that the malts produced from both triticale varieties had a very high extract content and outstandingly good diastatic power, which indicates a high activity of the amylolytic enzymes. The triticale malt diastatic power was two times higher than in standard barley malt, which can guarantee improved starch degradation. Also, a great triticale characteristic is a low temperature of gelatinization, which can reduce energy costs during the mashing process. Both triticale malts had an increased wort colour, hence they could be suitable for production of dark beer. The variety Odisej showed a slower filtration and increased wort viscosity compared to the variety NS Paun, which could be a consequence of the arabinoxylans solubilization. This study suggests that triticale is more economically acceptable than barley, which is a great advantage in the wort production. The produced triticale malts had good technological parameters, and hence they could be used as a partial substitute for barley malt in the wort production.

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# МИКРОСЛАДОВАЊЕ СОРТИ ТРИТИКАЛЕА НС ПАУН И ОДИСЕЈ

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Циљ овог рада је процена могућности примене тритикалеа као делимичне замене јечменог слада у производњи сладовине, што подразумева анализу тритикалеа, микросладовање и анализе произведеног слада тритикалеа, уз праћење активности амилаза током процеса сладовања. У поређењу са јечменим сладом, обе сорте тритикалеа су имале врло висок екстракт и дијастатску снагу. У сорти НС Паун, екстракт је био повећан за 11,4% у односу на стандардне вредности за јечмени слад. Сорта Одисеј је остварила повећан екстракт за 6,3%. У сортама НС Паун и Одисеј, дијастатска снага је била повећана за 100% и 111,9% у односу на јечмени слад. Такође, активности  $\alpha$ - и  $\beta$ -амилазе су биле повишене. Активност  $\alpha$ -амилазе је код сорте НС Паун повећана 34 пута, док је код сорте Одисеј повећана 20 пута у поређењу са нативним зрном тритикалеа. Активност β-амилазе је такође повећана током микросладовања, али у мањој мери. Произведени слад тритикалеа је показао добре технолошке параметре и могућност употребе као делимичне замене јечменог слада у производњи сладовине.

Кључне речи: тритикале, слад, микросладовање

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