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TRITICALE USAGE IN THE BIOTECHNOLOGICAL PROCESSES – BIOETHANOL AND LACTIC ACID PRODUCTION*

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Triticale shows many agronomic advantages including tolerance of acid soils, light soils, and dry conditions. This crop can be used for the ethanol production, and 8 triticale varieties were examined for it. The aim of the research was to choose a triticale variety with the lowest Falling number and the highest ethanol yield. In further research, the main aim was to see if the triticale stillage (produced during the ethanol production) can be used for the lactic acid production. According to technological parameters, the best variety is Odisej. Termal preparation of triticale was made with distilled and tap water, but without noticeable differences in the ethanol yield. During lactic acid fermentation higher acidity was recorded in the samples without agitation, while the concentration of D- and L- lactic acid was higher in the samples with agitation. Utilization of carbohydrates was the same in both cases. The Odisej variety has the best technological characteristics, low Falling number, high α-amylase activity, no need for technical enzymes during the preparation process, as well as a high ethanol yield. Triticale stillage is a good medium for the lactic acid production, which can be used for natural preservation of stillage and its application in feed.

Key words: triticale, Falling number, ethanol yield, stillage, lactic acid fermentation

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INTRODUCTION

The increased public concern about global warming has led to the development of renewable and clean energy all over the world [1]. The production of biofuels, such as ethanol and biodiesel, has been rapidly expanding in recent years, a trend that is expected to continue given the global interest in low or no carbon fuels and alternatives to petroleum (although this will partly depend on future oil prices) [2]. Ethanol produced from renewable biomass such as starch, sugar or lignocellulosic materials is believed to be one of the solutions. Major concerns have been raised in the last five years regarding a potential conflict between food and fuel, and the impact on the food access and food prices for the poor, although it has also been noted that biofuels do not necessarily adversely affect food security since they can be produced on lower quality lands [3, 4]. One of the solutions could be the use of triticale.

Triticale is the first successful human-made cereal grain, made by crossing wheat and rye [5]. Triticale combines the best characteristics of both parents: wheat's qualities for making various products with rye's robustness for adaptability to difficult soils, drought tolerance, cold hardiness, disease resistance and low-input requirements. One of the traits that initially made triticale attractive as a crop was its good protein nutritional value, particularly its high lysine content (for a cereal), the main limiting amino acid in cereal grains [5]. Triticale is known for exhibiting a high autoamylolytical enzyme activity, and this characteristic provides processing triticale without using any or less additional saccharifying enzymes than for others grains [5]. All of these characteristics make triticale a good resource for the ethanol production, but also there is a possibility for further usage of by-products. The by-product remaining after fermentation and distillation is known as whole stillage [6]. Stillage can be used as a fed, and there is always a question of how to storage it. The proliferation of spoilage organisms and food-born pathogens can be prevented by low pH and high concentrations of preservatives such as lactic and acetic acids. Lactic acid bacteria (LAB) can be homo- and hetero-fermentative, but only the homo-fermentative LAB are available for the commercial production of lactic acid [7]. The most LAB used for commercial productions belong with the genus *Lactobacillus*.

The aim of the research was to choose a triticale variety (among 8 of them) with the lowest Falling number and the highest ethanol yield. In further research, the main aim was to see if the triticale stillage (produced during ethanol production) can be used for the lactic acid production.

EXPERIMENTAL PART

Eight triticale varieties were used in the experiments from experimental trials, from the Institute for Crops and Vegetables (Rimski Šančevi location), Novi Sad (Serbia). Falling number was determined for monitored triticale varieties [8], in order to define their amylolytical enzymes [9]. The autoamylolytical enzyme system (AAQ) is defined as the percentage yield of the ethanol obtained without the addition of

saccharifying enzymes, compared with the ethanol yield obtained with the addition of an optimum combination of technical enzymes [10].
Instant dry active baker's yeast *Saccharomyces cerevisiae* provided from Alltech Fermin, Senta, Serbia was used as a producing microorganism [11].
All enzymes (Termamyl 120 L and San Super 240 L) were kindly provided from Novozymes® (Denmark) and were handled and stored according to the manufacturer's recommendations.
Mashing was carried out by using automated mashing water bath (Glasbläserei, Institut für Gärungs Gewerbe, Berlin). Mashing of ground triticale samples was done with distilled and tap water. The complete procedure was as given in [11].
After the ethanol fermentation, mash was distillated, ethanol was realized and stillage was given as the main by-product.
For pH correction (~ 6.0), 1M NaOH was added to triticale stillage before enzymatic hydrolysis.
Enzymatic hydrolysis of triticale stillage was conducted in the automated mashing water bath (Glasbläserei, Institut für Gärungs Gewerbe, Berlin) with the following enzymes (kindly provided from Novozymes ®): Termamyl (85 °C with 30 min rest), SAN Super 360L (55 °C with 30 min rest) and Celluclast (45 °C with 30 min rest). At the end of hydrolysis, the temperature was decreased to room temperature.
Enzymatic-treated triticale stillage was centrifuged (C-28A, BOECO, Germany) at 4000 rpm for 20 min. Supernatant was taken, sterilized by tyndallization (3 days at 100 °C for 30 min), and used as a medium for lactic acid fermentation.
The *Lactobacillus* strain, *Lactobacillus fermentum* PL-1 (obtained from the chair of Biochemical Engineering and Biotechnology, Faculty of Technology and Metallurgy, Belgrade) was used and it was stored on MRS agar slants (HiMedia, Mumbai, India) at 4 °C.
The strain was double subcultured. The first subculturing was the incubation on MRS agar slants at 30 °C for 24 hours, and after that, the second subculturing was the incubation in MRS broth under the same conditions. Triticale stillage was inoculated by a homogeneous strain suspension, with 3.33 % (v/v) inoculum, and before that $CaCO_3$ were added to the triticale stillage sample (0.58 g/100 mL stillage, according to the fact that each mol of glucose gives 2 mol of lactic acid and each mol of $CaCO_3$ utilize 2 mol of lactic acid for the creation of lactate [12]).
The inoculated triticale stillage sample (volume of 25 mL) was divided into 50 mL narrow neck Erlenmeyer flasks. Half of them were put in the AQUATHERM® water bath shaker (model G-86, 50 Hz, 6 Amp, NEW BRUNSWICK SCIENTIFIC CO., INC.), and they were incubated at 150 rpm and 30 °C for 72 hours, while the other half of flasks were incubated without agitation. Every 24 hours, the samples of lactic acid fermentation were taken in doublets and the analyses were repeated twice.
pH value was measured by electronic pH meter (HI 9321, Hanna Instruments), double calibrated using 4.01 and 7.00 pH buffers.
Titratable acidity (g/100 mL) was measured, and calculated (multiplied with the factor for lactic acid) according to the methods for determination of acidity [13, 14].

The fermentation broth was centrifuged (C-28A, BOECO, Germany) at 3000 rpm for 15 min. Supernatant was taken, and prior to the analysis, proteins were removed from the supernatant [15], which was then used for determination of glucose and maltose, as well as for the concentration D- i L- lactic acid by enzymatic kits (Megazyme, Bray, Ireland).

RESULTS AND DISCUSSION

The ethanol yields obtained from triticale samples during fermentations with and without the addition of technical enzymes, as well as the Falling number (FN) and Autoamylolytical Quotient, are shown in Table 1.

Table 1. Falling number, ethanol yield, and Autoamylolytical Quotient determined in triticale varieties

Triticale variety	Falling number, s	Ethanol yield, g/100 g DM*		Autoamylolytical Quotient (%)
		Without technical enzymes	With technical enzymes	
Pegaz	220	31.15	34.53	90.21
Panter	170	35.06	37.27	94.07
NST 21/06	66	36.26	38.50	94.24
Odisej	64	37.85	38.03	99.55
Jutro	82	37.11	37.61	98.65
Orion	160	32.46	36.73	88.37
Orao	180	31.26	36.20	86.35
Oganj	66	37.49	38.76	99.30

(DM*-dry matter)

The low FN is characterized by high amylolytic activities in triticale varieties, which is a favorable property for the production of ethanol. According to the results obtained for the triticale varieties: Oganj, Jutro and Odisej produced almost equal ethanol yields in both procedures - with and without the addition of technical enzymes, implying the fact that technical enzymes would not be necessary in the preparation step of the ethanol production from these varieties.

Triticale in which the lowest Falling number was determined gave the highest ethanol yield with and without the addition of technical enzymes. These triticale varieties (Odisej, Oganj and Jutro) also had the highest autoamylolytical quotient. The highest

autoamylolytical quotient was determined for Odisej (99.55%) implying that this variety has a sufficient amount of amylolytic enzymes to degrade native starch. High autoamylolytical quotient was determined for Oganj (99.30%) as well, while Orao had the lowest quotient (86.35%). The variety Odisej was chosen for further work.

The mashing was done with distilled and tap water. The recorded ethanol yield with distilled water was 37.76 g/100 g DM, while for the mash with tap water it was 37.78 g/ 100 g DM, so there is not a noticeable difference in the ethanol yield with different water type.

The next observation point was to see if triticale stillage is a good medium for lactic acid fermentation by *Lactobacillus fermentum* PL-1. The fermentation was conducted in two different ways: with and without agitation of the fermentation broth. The reason for this kind of research was to see if there was any difference in the amount of produced lactic acid with and without agitation. Also, the experiment designed in this way should give a better prospective if the $CaCO_3$ becomes more soluble with agitation, and in that way improves lactic acid fermentation by converting lactic acid into Ca-lactate and maintaining the pH [16].

Figure 1 shows pH values and titratable acidity during lactic acid fermentation with and without agitation of fermentation broth.

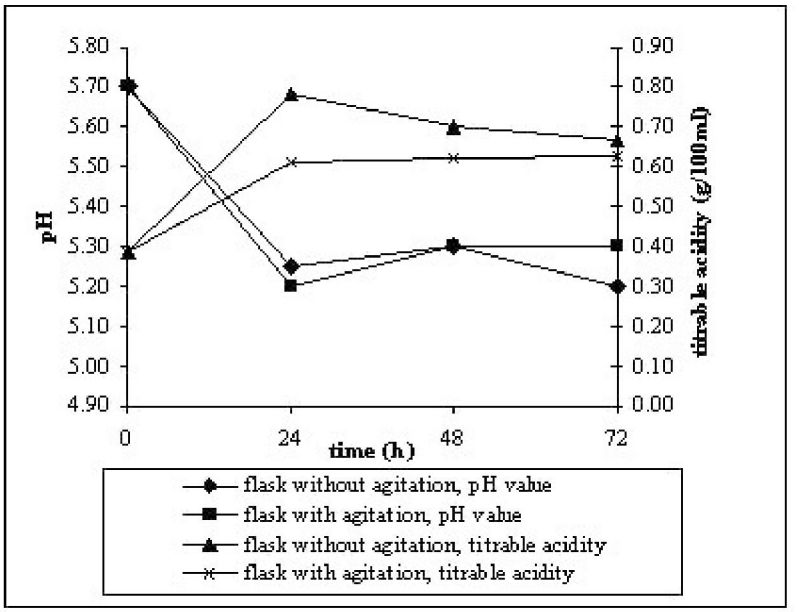

Figure . pH change and titratable acidity during lactic acid fermentation period by *Lactobacillus fermentum* PL-1

A decrease of pH value was slightly lower in the samples without agitation. Bigger titratable acidity was measured in the samples without agitation in the first 24 hours, but after that there was slight difference between the samples with and without agitation.

In further research, the results obtained by titration were verified by application of enzymatic kits for determination of L- and D- lactic acid concentration (0, 24, 48, and 72 h) and for maltose and glucose concentration in the fermentation broth (0 and 72 h). Figure 2 shows the results of L- and D- lactic acid determination. Table 2 shows the results for glucose and maltose determination.

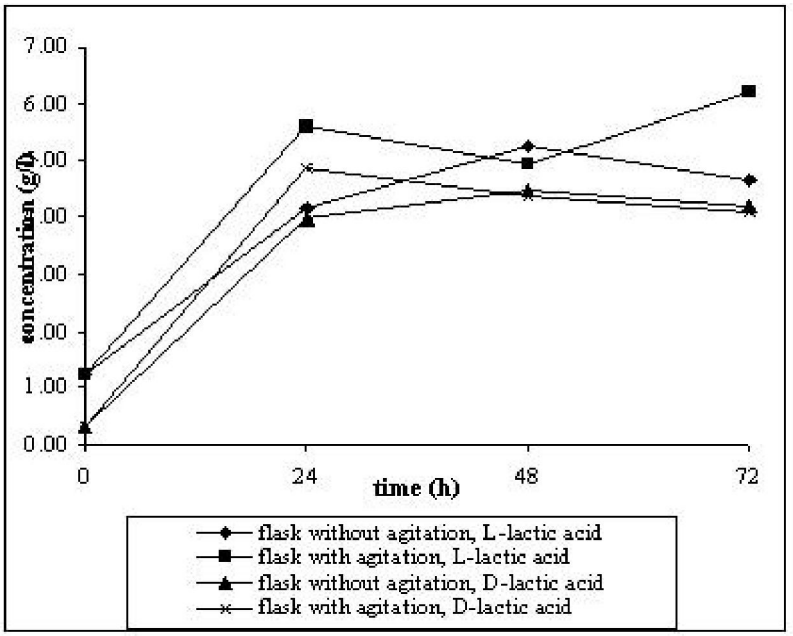

Figure 2. L- and D- lactic acid determination during the lactic acid fermentation by *Lactobacillus fermentum* PL-1

The results show that the higher concentration for L-lactic acid was different during the fermentation, but in the 72 h sample with agitation had a higher concentration. The concentration of D-lactic acid was higher in the samples with agitation in the first 24 hours, but to the end of fermentation a higher concentration was recorded for the samples without agitation. This may appear due the bigger precipitate of D-lactic acid with $CaCO_3$ (compared with precipitate of L-lactic acid). In both cases, a higher concentration was recorded for L-lactic acid.

Table 2. Glucose and maltose concentration during the lactic acid fermentation

time (h)	glucose (g/l)		maltose (g/l)	
	without agitation	with agitation	without agitation	with agitation
0	17.92		3.74	
72	BD*	0.12	BD*	BD*

(BD- beyond detection range of used kit)

The results show that, in the samples without agitation, both glucose and maltose were utilized almost completely and that the residual concentration was beyond the detection range of the used kit. For the samples with agitation, a very low concentration of glucose was detected, while the maltose concentration was beyond the detection range of the used kit.
According to the achieved results, it could be seen that agitation does not have impact on the pH value of the fermentation broth, which implies that agitation does not have impact on the buffer capacity of the broth. The bigger acidity was recorded for the samples without agitation, but enzymatic tests are much more accurate for determination of lactic acid, and the given results show better results for the samples with agitation. This occurrence could be explained by the fact that $CaCO_3$ transformed lactic acid into Ca-lactate during the fermentation [17]. Ca-lactate reduce the inhibition of the lactic acid bacteria activity, which appears at the produced lactic acid. In this way, only a part of lactic acid stayed dissolved in the triticale stillage and only that part was measured during titration [16]. In this case, $CaCO_3$ was better dissolved with agitation, more Ca-lactate was produced and the fermentation was improved. This can explain higher acidity in the samples without agitation, but again acquiring better results by enzymatic tests for lactic acid concentration in the samples with agitation. Utilization of carbohydrates was the same in both cases, which implies that the fermentation was over within 72 hours in both cases, and that during the fermentation, both glucose and maltose were taken for lactic acid bacteria metabolism [18].

CONCLUSION

Triticale can be one of the best solutions for the ethanol production. While it is not so useful for human nutrition, it is a good crop for the ethanol production and its by-product, stillage, could be used further as the fed. Among 8 triticale varieties, the best results (the lowest Falling number and the highest ethanol yield) were for the variety Odisej. The ethanol yield was the same whatever, distilled or tap water, were used, and this is very useful for the industrial application. Stillage, which is a by-product of the ethanol production, can be used as the medium for lactic acid fermentation, and in this way there is a possibility for the ecological way of fed preservation. Better results (higher concentration of L- and D-lactic acid) were achieved when the lactic acid fermentation was performed with agitation, due to better dissolving of $CaCO_3$, which reduced the inhibition of the lactic acid bacteria activity.

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IZVOD

PRIMENA TRITIKALEA U BIOTEHNOLOŠKIM PROCESIMA – PROIZVODNJA BIOETANOLA I MLEČNE KISELINE

(Originalan naučni rad)

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Tritikale ima mnogo agronomskih prednosti, i može rasti na kiselim i siromašnim zemljištima, kao i pri sušnim uslovima. Tritikale se može koristiti za proizvodnju bioetanola, i ispitano je 8 sorti ovog žita. Cilj rada je bio izabrati sortu tritikalea, sa najnižim brojem padanja i najvišim prinosom bioetanola. Dalji cilj istraživanja je bio da se ispita da li džibra dobijena nakon proizvodnje bioetanola može da se koristi za proizvodnju mlečne kiseline. Na osnovu tehnoloških parametara najbolja sorta je Odisej. Proces termičke pripreme tritikalea izvođen je sa destilovanom i vodom za piće, pri čemu nisu uočene razlike u prinosu bioetanola. Tokom mlečnokiselinske fermentacije uočena je nešto veća kiselost kod uzoraka bez mešanja, veće koncentracije D- i L- mlečne kiseline kod uzoraka sa mešanjem, dok je utrošak glukoze bio isti u oba slučaja. Sorta Odisej ima najbolje tehnološke karakteristike, nizak broj padanja, visoku α-amilaznu aktivnost, nisu neophodni tehnički enzimi za proces pripreme i daje visok prinos bioetanola. Džibra tritikalea je dobra podloga za proizvodnju mlečne kiseline što je moguće iskorititi za prirodno konzervisanje džibre i njenu primenu u stočnoj ishrani.

Ključne reči: tritikale, broj padanja, prinos bioetanola, džibra, mlečnokiselinska fermentacija

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