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Alcoholic Fermentation with different *Saccharomyces* Strains and its Effect of Mash Composition

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Abstract:*The aim of this study was to determine the effect of two yeast strains (Saccharomyces cerevisiae and S. bayanus) on the fermentation with or without additional pectinase. The organic acids products (tartaric, malic, and succinic acid), carbohydrates (glucose, fructose, and sucrose) utilized, and ethanol produced were examined by HPLC. The efficiency of fermentation was affected by several parameters such as the preparation procedure of the fruits, like temperature (18°C), yeast strain applied, pH adjustment (3.2), the dosage of yeast nutrient and application of pectinase. We have found that pectinase pre-treatment and S. cerevisiae could significantly alter the amount of the examined components, which changed the quality of the end product. We have also experienced significant (p≤0.05) differences in the sugar utilisation of yeast strains as well as organic acid contents in the different stages of fermentation, where the samples fermented with spontaneous and identified yeast strains.*

Keywords: *fermentation processes, pectinase, organic acid and sugar composition, alcohol production, high performance liquid chromatography*

1. Introduction

It is well known that the fermentation process of fruits is basically determined by their compositional parameters (sugar content), the proportion of their nutritional components and health condition of fruits, including their microbiological appropriateness. The adaptation capacity and fermentation performance of the applied yeast strains are crucial factors for the elaboration of the end-product in industrial fermentation [1]. Cells function in a remarkably complex environment during the fermentation process, and they can adapt to these environmental factors, in addition to changing environmental parameters. This ability is an essential requirement against yeast strains developed for alcoholic fermentation [2]. In the past decades, however, beside specific yeast strains used in fermentation non-*Saccharomyces* strains have gained an ever-increasing role, since they are capable to imitate the processes of spontaneous fermentation, and they increase the aromatic complexity of the product, as well [3].

The natural microflora of plants has definitive importance during fermentation processes. The microbiome of blue plums is principally characterized by the presence of *Aureobasidium* sp. and *Kloeckera apiculata* (the anamorph of *Hanseniaspora uvarum*) yeasts composing more than 80% of the microflora [4]. These microorganisms also get into the mash during fruit processing, and they carry out spontaneous fermentation. With the progress of fermentation, non-*Saccharomyces* species disappear one by one; thus, *Saccharomyces cerevisiae* is responsible the main and final stage of fermentation. The population of this species is quite different concerning its oenological and other characteristics, e.g., its vulnerability against lethal toxins or its ability to produce them [5]. Moreover, the alcohol production capacity of *S. cerevisiae* strains was examined and proven in several studies not only in alcoholic fermentation but in the field of bioethanol production from sewage-slum as well [6].

Pectin is an important carbon source for bacteria and fungi decomposing the plant material. According to several publications, various microorganisms, such as different yeast species of the genera *Saccharomyces*, *Kluyveromyces, Cryptococcus, Rhodotorola, Aureobasidium pullulansand*and *Candida*

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[7- 9] are capable of producing pectinases, like polygalacturonase [10]. These enzymes have been applied for increasing the yield and purity of fruit juices for long time [11], as well as for making sugars more easily achievable for the microbiome carrying out the fermentation process.

Among environmental factors, *p*H is often considered as the most important regulatory parameter for glucose fermentation [12]. Some studies also proved that *p*H affects the production of gases and microbial metabolites in the presence of glucose [13] or alanine [14].

Organic acid and sugar content varies in different cultivars of stone fruits, what also influences the quality parameters of the fruits, because the proportion of these substances - among other things - determine the maturity stage of the fruits, as well as the quality of the product acquired from them. Former studies documented the sugar and organic acid composition of different raw fruits, e.g. that of apricot, Japanese apricot, plum [15] and peach [16]. In the fruits of the genus Prunus [plum, cherry, peach, apricot, almond] a manifold of organic acids have been identified, such as malic acid, citric acid, the quinic acid in peach [17], oxalic acid in plum [18] tartaric acid, ascorbic acid, shikimic acid, succinic acid, malic acid and fumaric acid in peach [19]. The dominant water-soluble sugars in drupes are fructose, glucose and sucrose; furthermore, stachyose [20], sorbitol [21], raffinose [22], rhamnose [23], arabinose, galactose and xylose [24].

The presence of assimilable nitrogen by the yeast during alcoholic fermentation is well known, just like the fact that the utilisation of nitrogen is regulated by several molecular processes that have already been described in *S. cerevisiae*. Latest results on this topic were published in 2005 and 2012 [25, 26], whilst on nitrogen regulation, only a single review was issued lately [27]. The authors examined several studies on the role of nitrogen metabolism, mostly among oenological circumstances. Most studies classified assimilable nitrogen as a primary and non-primary energy source, depending on the alcoholic fermentation conditions, the strains used and the classification method [28, 29].

These environmental and composition parameters influence the process of alcoholic fermentation. High-quality products can only be achieved if we use faultless raw materials, microbiologically developed starter cultures and exploiting the facilities of directed fermentation technology to the maximum. As a results of our research, by optimizing the fermentation parameters, it is possible to produce a high-quality end-product (plum wine, spirit).

2. Materials and Methods

2.1. Fruit materials

Prunus domestica „Stanley" (European plum) fruits (15 kg) were achieved from a family orchard (Levél, Hungary), in the state of complete ripeness. Plant parts inappropriate for consumption and unripen fruits were discarded right after the arrival of the material, and the processing started on the same day.

2.2. Yeast strains

Two types of *Saccharomyces* strains were used as starter cultures for fruit fermentation with a recommended dosage of 20 g /100 kg. From the two strains, the efficiency of *S. cerevisiae* has already been proven in oenology [30, 31], as well as in the fermentation process of brandy production [32] concerning optimal fermentation characteristics. For the measurements, dried strains of *S. cerevisiae* (SafŒno™ SC 22, Fermentis, France) and *S. bayanus* (Safspirit Fruit, Fermentis, France) were bought. The used *S. cerevisiae* strain has good alcohol tolerance (15%), medium kinetics and medium nitrogen requirements and well adapted to barrel fermentation with limited temperature control. The used *S. bayanus* strain recommended for fruit fermentation due to it fructophilic character. Reported as a neutral strain, but in some cases produces refined and balanced esters. Low nitrogen demand and high resistance to alcohol (17%).

Prior to use them, they were rehydrated in 10x volume of plum juice at 25°C for 30 min.

2.3. Conditions of fermentation

In the preparation process, after washing, stoning and chopping we made 5 types of samples from the 10 kg of plum material (Figure 1). Control samples were left unwashed, since we fermented it with wild yeasts (spontaneously). The initial *p*H of all samples was set to 3.2 with 20% phosphoric acid (Merck, Germany).

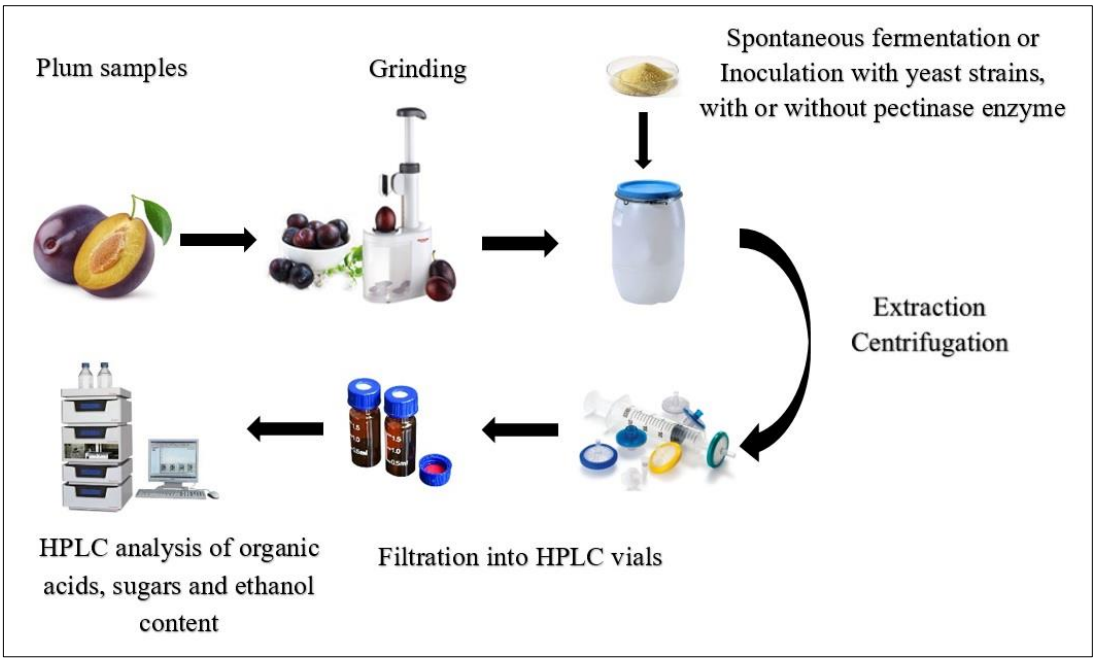

Figure 1. Flow diagram for plum mash production, analytical preparation and measurement

We carried out five types of fermentation: (1) Spontaneous fermentation - control sample (C) with no additional yeast, no *p*H adjustment or pectin digestion. (2) Sample inoculated with *S. cerevisiae* (S.c – p) with adjusted *p*H and additional yeast nutrient, but with no pectinase treatment. (3) Sample inoculated with *S. cerevisiae* with additional pectinase treatment (S.c + p), adjusted *p*H and additional yeast nutrient; (4) Sample inoculated with *S. bayanus* with additional pectinase (S.b + p), adjusted *p*H and added yeast nutrient. (5) Sample inoculated with *S. bayanus* without pectinase treatment (S.b – p), adjusted *p*H and added yeast nutrient.

For the enzyme treatment we used 2 g / 100 kg Safizym pectinase enzyme (Safizym Clean, Fermentis, France; endo-polygalacturonase (>2,450 PG/g), pectin methyl esterase (>490 PE/g) and pectin lyase (>70 PL/g)).

As for the yeast nutrient (20 g/ 100 kg) we used SpringFerm™ (Fermentis, France) which includes inactivated yeast (rich in growth factors). Detailed composition: dry matter >94%, total nitrogen 9.2-10.9%, total polysaccharides 13.3-19.3%, lipids 1.2-2.4%, mineral substance 9.2-13.6%, thiamine 130-257 ppm, calcium pantothenate 157-331 ppm, niacin 480-805 ppm and folic acid 16-5 ppm. SpringFerm™ is a fermentation activator 100% based on fully autolyzed yeasts, 9 times richer in soluble nitrogen than a basic inactivated yeast. Yeast nutrient was added of the beginning of fermentation process because the raw material (plum) has a lower nitrogen content (0.5-0.8 g/100g against to apricot, cherry, nectarine, peach) [33], however it would be necessary for yeast growth and alcoholic fermentation process. Yeast assimilable deficiency can sometimes lead to sluggish or stuck fermentation [34, 35].

Samples (2 L/sample) were put in 3 L Erlenmeyer flasks, sealed with airlock tube that released the carbon dioxide by-product. Fermentation lasted for 12 days at 18°C of temperature. We sampled six times: on day 1 (sweet mash), then on days 3, 5, 8, 10, and 12. Samples (15 mL) were stored in an ultralow freezer at -55°C (Bio-Medlab B-HL 100, China) until further analysis. Three parallel

measurements were carried out to determine the organic acid, sugar, and ethanol content of the samples.

2.4. Sample preparation

Sample preparation is detailed in Figure 1 (paragraph 2.3.) We measured 1 g of each mash sample into 250 mL Erlenmeyer flask and diluted with 20 mL of high-purity water (ZeneerPower 1, Human Corporations, Korea), then shook for 1 h on a rotary shaker (Elphan 358S, Bohemia) for extraction at room temperature (24°C).

Samples were centrifuged for 30 min at 6000 RCF (Labnet Hermle Z206A, USA) in 15 mL tube, then 1.5 mL of each supernatant was centrifuged for an additional 20 min at 14 500 RCF (Biosan Microspin 12, Latvia) in 2 mL Eppendorf tube. Subsequently, the samples were filtered through a syringe filter (polyvinylidene difluoride [PVDF nylon] 0.22 µm, Filter Bio) into 2 mL screw-topped HPLC vial (Berrytec, Germany).

2.5. HPLC analysis of organic acids

The analysis of organic acids was carried out using an ion exchange HPLC system (Jasco, LC 900, Japan), identifying the components with an UV-975 type (Jasco, Japan) detector at 210 nm wavelength. To separate the organic acids BioRad Aminex HPX-87H (USA) column was used at 35°C; the flow rate of the eluent was 0.6 mL/min. The equipment was calibrated with standard solutions of tartaric acid, malic acid, and succinic acid (Merck, Germany) of 0.05-1 mg/mL concentration diluted in the applied eluent (0.1% sulphuric acid).

2.6. HPLC analysis of sugars and ethanol

The separation of sugars and ethanol was also carried out using an ion exchange HPLC system (LC 900, Jasco, Japan); components were identified with a refractive index detector (RI 71, Merck, Germany). The column (Supelcogel H, Sigma Aldrich, USA) was kept at room temperature, and the flow rate of the eluent was 0.5 mL/min. HPLC system was calibrated for glucose, fructose, sucrose and ethanol, similarly to the method described above for the organic acid analysis. The concentration of standard solutions was between 0.5 and 10 mg/mL diluted in high purity water, which was also the eluent.

2.7. Statistical analyses

Data were expressed as the mean ($n = 3$) ± standard deviation (SD). One-way analyses of variance (ANOVA) were used to compare the significant difference for the data. The predicted values were considered significant at $p \leq 0.05$. The statistical analyses were performed using Microsoft Office Excel 2016® software.

3. Results and discussions

3.1. Changing of organic acid composition of mash samples during the process of fermentation

During fermentation organic acids released from the fruits are utilised by the yeasts for their vital functions, meanwhile other acids may also be produced. The aim of this study was to measure the change in the amount of tartaric acid, malic acid and succinic acid (Table 1).

Table 1. Changes of detected organic acid levels (tartaric acid, malic acid, succinic acid) in the samples examined during the process of fermentation

Sample	Day of fermentation	Tartaric acid (g/L)	Malic acid (g/L)	Succinic acid (g/L)
Control	1	N.D.**	6.26 ±* 0.25	3.28 ± 0.17
	3	N.D.	1.11 ± 0.05	3.08 ± 0.21
	5	N.D.	0.78 ± 0.13	2.63 ± 0.16
	8	0.07 ± 0.01	N.D.	2.26 ± 0.22

	10	0.06 ± 0.02	N.D.	2.06 ± 0.15
	12	0.10 ± 0.02	N.D.	0.95 ± 0.04
***Saccharomyces cerevisiae* with added pectinase**	1	N.D.	6.26 ± 0.25	3.28 ± 0.17
	3	N.D.	6.04 ± 0.58	3.30 ± 0.06
	5	0.07 ± 0.01	3.92 ± 0.20	3.51 ± 0.05
	8	0.23 ± 0.05	3.54 ± 0.13	3.10 ± 0.03
	10	0.33 ± 0.01	3.22 ± 0.02	3.05 ± 0.10
	12	0.50 ± 0.04	2.99 ± 0.02	3.06 ± 0.08
***Saccharomyces cerevisiae* without pectinase**	1	N.D.	6.26 ± 0.25	3.28 ± 0.17
	3	N.D.	5.49 ± 0.21	3.32 ± 0.23
	5	N.D.	4.57 ± 0.31	3.35 ± 0.48
	8	N.D.	N.D.	3.11 ± 0.12
	10	0.01 ± 0.00	N.D.	3.12 ± 0.05
	12	0.20 ± 0.01	N.D.	3.00 ± 0.06
***Saccharomyces bayanus* with added pectinase**	1	N.D.	6.26 ± 0.25	3.28 ± 0.17
	3	N.D.	5.90 ± 0.36	3.36 ± 0.28
	5	0.05 ± 0.01	5.27 ± 0.45	3.35 ± 0.13
	8	0.23 ± 0.02	3.62 ± 0.48	3.45 ± 0.13
	10	0.34 ± 0.03	3.51 ± 0.23	3.31 ± 0.17
	12	0.49 ± 0.06	3.17 ± 0.06	2.84 ± 0.11
***Saccharomyces bayanus* without pectinase**	1	N.D.	6.26 ± 0.25	3.28 ± 0.17
	3	N.D.	6.22 ± 0.15	3.30 ± 0.07
	5	N.D.	4.68 ± 0.12	3.55 ± 0.15
	8	N.D.	0.62 ± 0.02	3.58 ± 0.13
	10	0.01 ± 0.01	N.D.	3.41 ± 0.28
	12	0.13 ± 0.01	N.D.	3.10 ± 0.06

*± - Values are presented as mean ±S.D. (N=3) and evaluated by one-way ANOVA with a confidence level of 95 %
** N.D. – under limit of detection

Tartaric acid can be almost 50% of the total organic acid content in grapes, [36]; in the case of other fruits, the amount of this acid depends on manifold factors, like plant growing technology [37] or the cultivar [38]. During HPLC analyses, tartaric acid was not detected in neither case in the sweet mash on day 1. However, depending on the sample, it can be managed to detect it during the different stages of fermentation. Presumably, the measured differences are related to the application of pectinases. Research supports effect of pectinase enzyme dosage on acid content. It was observed that the pectinase enzyme treatment increased the acidity of the juice [39]. In the case of samples treated with pectinase (S.c + p, S.b + p), tartaric acid was traced (though in a miniscule amount) from the 5th day of fermentation, and its amount did not exceed the concentration of 0.5 g/L even at the end of fermentation. On the other hand, in the samples without pectinase treatment (S.c - p, S.b - p), tartaric acid only appeared on the 9th day of fermentation, and its amount was considerably lower (0.20 and 0.13 g/L) than the above mentioned results. Consequently, the amount of tartaric acid is significantly affected by the pectinase treatment as well as the *Saccharomyces* yeast strain used for inoculation. Nevertheless, this difference was no longer observed in the later stages of fermentation (from the 5th day on). The reason for this may be that the used enzyme complex (pectin methylesterase, endo-polygalacturonase, and pectin lyase) during sample preparation released the tartaric acid faster from the fruit cells than the natural enzyme system of the yeast strains used for inoculation. According to Joshi et al. (2011) addition of pectinase

significantly increased the colour, total soluble solids, titratable acidity and total sugars in the enzymatically extracted juices [39].

Concerning the whole process of mash fermentation, we can state that the addition of pectinases significantly influences the production of tartaric acid. As a result of the enzyme complex treatment, tartaric acid production started at earlier stages and its amount was also significantly higher by the end of the process.

Malic acid is a dominant organic acid in apples and stone fruits, while berries contain mostly citric acid. The results support this statement since on day 1 citric acid was not detected in any of the samples but already found malic acid at a concentration of 6.26 g/L. In the control samples, the amount of malic acid decreased by 82.2% by the 3rd day of fermentation and fell below the detectable level by the 8th day. In the case of samples without pectinase (S.c - p. and S.b - p.), these values only showed a decrease of 26.9% (S.c - p.) and 25.2% (S.b - p.) even by the 5th day. However, in the case of samples with additional pectinase (S.c + p. and S.b + p.), the amount of malic acid only declined by 47.7% (S.c +p.) and 50.6% (S.b + p.) by the 12th day of fermentation. The reason for this may be the fact that heteropolysaccharides were released by the pectinase enzyme complex during the pre-treatment and as a result yeasts used homogalacturonans (polymers of galacturonic acid linked by 1–4 bonds) and xylogalacturonans (other monosaccharides like rhamnose, fucose attached to galacturonic acid) for their vital functions instead of malic acid in course of secondary (metabolic) fermentation [40]. Based on these results, we can state that pectinase pre-treatment significantly decreased ($p \leq 0.05$) the level of malic acid.

Succinic acid is one of the most important organic acids produced by yeasts during alcoholic fermentation. This organic acid is developed by the oxidation of isocitric acid (a cyclic substrate of citric acid) in the reductive TCA cycle [41]. During measurements, we have only found a difference between the spontaneously fermented and the *Saccharomyces* inoculated samples. The initial succinic acid concentration (3.28 g/L) decreased continuously in the control sample and by the end (12th day) of fermentation, its amount was merely 0.95 g/L. On the contrary, in samples S.c + p and S.c - p it increased by the 5th day of fermentation (3.51 and 3.35 g/L), and by the end of fermentation, it slightly lowered. In samples S.b + p and S.c - p found increasing tendency till the 7th day of fermentation (3.45 and 3.58 g/L), and then a not too intense decrease was observed. The statistical analysis of succinic acid levels revealed that the amount of this organic acid is not affected significantly ($p \leq 0.05$) either by the presence of pectinases or the type of yeast strain used. Jiang (2020) has observed no significant differences between control and with enzymatic treated samples in the concentration of citric, lactic, and succinic acids after alcoholic fermentation [42].

3.2. Changing of sugar composition and ethanol content of mash samples during the process of fermentation

Table 2 shows the changes in the composition of fermentable sugars and the concentration of ethanol during the process of fermentation. Different plum cultivars have differing sugar compositions [35]. Our raw material (*Prunus domestica* „Stanley") contains the largest amount of sucrose (50.48 g/L), following by glucose (35.94 g/L) and finally fructose reaching the lowest concentration (31.80 g/L).

Table 2. The change of mean carbohydrate (fructose, glucose, sucrose) and mean ethanol content detected in the samples during fermentation

Sample	Day of fermentation	Fructose (g/L)	Glucose (g/L)	Sucrose (g/L)	Ethanol (v/v %)
Control	1	31.80 ± 0.87*	35.94 ± 0.68	50.48 ± 0.11	0.00 ± 0.00
	3	29.67 ± 1.79	35.00 ± 1.50	46.40 ± 2.38	N.D.
	5	19.06 ± 0.59	31.70 ± 1.50	32.43 ± 2.39	1.13 ± 0.03
	8	17.68 ± 0.67	15.00 ± 0.36	N.D.	3.98 ± 0.07
	10	N.D.**	N.D.	N.D.	5.25 ± 0.10

	12	N.D.	N.D.	N.D.	5.43 ± 0.05
***Saccharomyces cerevisiae* with added pectinase**	1	31.80 ± 0.87	35.94 ± 0.68	50.48 ± 0.11	0.00 ± 0.00
	3	25.45 ± 0.63	26.71 ± 0.76	0.074 ± 0.01	2.32 ± 0.06
	5	13.51 ± 0.98	6.90 ± 0.76	N.D.	4.31 ± 0.02
	8	N.D.	N.D.	N.D.	6.89 ± 0.12
	10	N.D.	N.D.	N.D.	7.86 ± 0.03
	12	N.D.	N.D.	N.D.	8.3 ± 0.11
***Saccharomyces cerevisiae* without pectinase**	1	31.80 ± 0.87	35.94 ± 0.68	50.48 ± 0.11	0.00 ± 0.00
	3	31.17 ± 1.58	32.95 ± 0.51	0.82 ± 0.19	2.16 ± 0.10
	5	21.32 ± 1.31	7.74 ± 0.10	N.D.	4.07 ± 0.04
	8	N.D.	N.D.	N.D.	6.81 ± 0.06
	10	N.D.	N.D.	N.D.	7.62 ± 0.13
	12	N.D.	N.D.	N.D.	7.71 ± 0.09
***Saccharomyces bayanus* with added pectinase**	1	31.80 ± 0.87	35.94 ± 0.68	50.48 ± 0.11	0.00 ± 0.00
	3	25.99 ± 0.42	32.42 ± 1.29	N.D.	2.24 ± 0.01
	5	14.85 ± 0.52	29.82 ± 0.75	N.D.	4.56 ± 0.06
	8	N.D.	N.D.	N.D.	6.50 ± 0.17
	10	N.D.	N.D.	N.D.	6.84 ± 0.16
	12	N.D.	N.D.	N.D.	7.15 ± 0.20
***Saccharomyces bayanus* without pectinase**	1	31.80 ± 0.87	35.94 ± 0.68	50.48 ± 0.11	0.00 ± 0.00
	3	30.2 ± 1.07	36.23 ± 1.74	N.D.	1.98 ± 0.04
	5	22.63 ± 0.49	26.43 ± 0.48	N.D.	3.50 ± 0.21
	8	N.D.	N.D.	N.D.	6.09 ± 0.08
	10	N.D.	N.D.	N.D.	6.86 ± 0.03
	12	N.D.	N.D.	N.D.	7.01 ± 0.09

*± Values are presented as mean ±S.D. (N=3) and evaluated by one-way ANOVA with a confidence level of 95 %
** N.D. – under limit of detection

While examining the fructose utilisation we saw that wild yeasts of the control sample utilised the available monosaccharides (ketohexose) much slower than the case of yeast inoculated or enzyme-treated samples. In this aspect, basic differences were found compared to the next 4 samples. In the case of samples inoculated with the 2 different yeast strains (*S. cerevisiae, S. bayanus*), we did not detect significant difference concerning fructose utilisation. If we look at the results for different preparation methods - disregarding the starter culture - we can find an unambiguous difference between samples treated with pectinases and the non-treated ones. A mean difference of 16.16% can be observed between enzyme-treated and non-treated samples on the 3rd day of fermentation. In the case of samples treated with pectinases, fructose utilization was remarkably better. By the 5th day of fermentation, this difference increased to 35.45%, naturally in favour of the enzyme-treated samples.

The background for this observation is that the components (taste and flavour components, fermentable sugars) released by the pectinases (pectin methyl esterase, polygalacturonase) became achievable for the yeast cells; thus, they were utilized more quickly. The findings were in accordance with the obtained results by Diano et al. (2008) and Hosseini et al. (2021) for the apple juice treated with the immobilized pectinase. Also, these results showed that the reducing sugar content was significantly increased after the pomegranate juice treatment by the free and immobilized pectinase which was possibly related to the liberation of the reducing sugars after the pectin hydrolysis [43, 44].

Yeast cells themselves also possess pectinases, yet if we apply artificial enzyme supplementation (produced by a selected *Aspergillus niger* strain), the efficiency can clearly be increased. Regardless of the control sample, on the 7th day of fermentation no sample contained detectable amount of fructose. According to the statistical analyses, on the 3rd day of fermentation, only samples S.c-p and S.b-p showed

no significant difference. During comparing all the other samples, found differences in the significance level of $p \leq 0.95$. By the 5th day of the fermentation process, significant differences were detected by statistical methods among all the samples concerning fructose utilisation.

As for glucose utilisation, also observed differences between the individual samples. While in the case of the fructose, pectinases were responsible for the observed utilisation differences; in the case of glucose, the inoculated yeasts resulted the differences. The reason for this is that *S. cerevisiae* produces more alcohol, and *S. bayanus* produces fragrance components more effectively. Puškaš et al. (2019) support these results with their laboratory experiments with 4 microbial strains (*Saccharomyces cerevisiae, Saccharomyces bayanus, Torulaspora delbrueckii, Metschnikowia pulcherrim*) and its combination [45].

This is supported by our results too because *S. cerevisiae* degraded 79.63% of the glucose by the 5th day of fermentation, while strain *S. bayanus* functioned on an efficiency level of 21.75% during the same period. For both starter cultures, however, we can observe that the level of glucose fell behind the minimum detectable value of the HPLC-RI system by the 8th day of fermentation; thus, the strains must have used all the glucose for their fermentation activity [46]. On the 5th day of fermentation, significant differences were detected between the following sample pairs: C and S.c-p; C and S.c + p; C and S.b + p; S.c - p and S.b + p; S.c + p and S.b + p.

Our study results show that in the case of all (but for the control) samples, yeasts used up the sucrose (disaccharide) first [47], and the amount of this sugar fell below the minimum detectable level already on the 3rd day of fermentation. Even on the 5th day of fermentation, the control sample contained a remarkable level of sucrose compared to the original amount-only 35.7% was utilised by the wild yeasts. This is a significant difference compared to the samples inoculated with the identified yeasts. From this aspect, sucrose utilization of identified yeasts turned out to be more efficient. Among the 2 applied yeasts, remains of disaccharide were detected in case of *S. cerevisiae* on the 3rd day of fermentation. Moreover, significant difference was observed between pectinase treated and untreated samples. Sample S.c + p contained 10 times higher amount of sucrose on the 2nd day of fermentation, which is a statistically significant difference. Jeong et al. (2002) in their research demonstrated the effect of pectin-degrading enzyme on increased sugar consumption and alcohol production compared to untreated samples [48].

The concentration of produced ethanol during fermentation is also shown in Table 2. It is unambiguous based on the results that a significant difference emerged between both the control sample and samples inoculated with *S. cerevisiae* and *S. bayanus* strains, as well as the samples prepared without enzyme products and pectinase-treated samples concerning the tendency of their ethanol production ($p \leq 0.05$) [48, 49].

In the control sample, ethanol production reached a detectable concentration (1.13 v/v%) only by the 5th day of fermentation, whereas in the other samples we detected ethanol concentrations between 0.98–1.32% already on the 3rd day of sampling. The measured differences also subsisted in the ongoing stages of fermentation, and the ethanol production of wild yeast was below the level we found in samples inoculated with the identified strains. According to the statistical analyses, significant differences were observed concerning ethanol production between all the samples when comparing them to each other.

The final ethanol content of the control sample was below that of samples S.c + p and S.c – p, which means a difference of 2.87 v/v% and 2.28 v/v%, respectively. This difference is also below that of *S. bayanus* strains in the case of which sample S.b + p contained 1.72 v/v% more and sample S.b - p contained 1.58 v/v% more ethanol than the respective sample of spontaneous fermentation. Other authors also reported significant differences in the alcohol yield in samples fermented spontaneously and by *S. cerevisiae* [49, 50].

4. Conclusions

Numerous *S. bayanus* strains provide lower ethanol yield in fermentation than *S. cerevisiae*, which is also supported by our studies, since *S. cerevisiae* produced 0.925 v/v% more alcohol on average by

the end of fermentation, than strain *S. bayanus*.

Due to its natural features, pectin remarkably inhibits the solubility of valuable organic compounds into the liquid phase; thus, the importance of pectin degradation lies in the fact that it releases and grants access to the components which microorganisms can utilise. In addition to the above-mentioned ones, the aim of using pectinase products for fruit-based beverages is the indirect lowering of the methanol content and the liquefaction of the mash.

It can also be determined from the results that the addition of the pectinase enzyme complex slightly affects the amount of ethanol produced. While in the case of samples treated with pectinases, an ethanol yield of 8.30% (S.c + p) and 7.15% (S.b + p) was observed, for samples S.c - p and S.b - p, this value was 7.71 and 7.01 v/v%, respectively. This means a mean difference of 0.36 v/v% concerning the ethanol concentration.

As a results of this study, we demonstrated that the outcome of the plum mash fermentation is largely dependent on the pectinase treatment and/or the additional yeast strains. Pectinase treatment significantly changed most of the majority of the examined components except succinic acid. In case of the applied yeasts, *S. cerevisiae* utilized glucose more effectively and therefore produced more alcohol than *S. bayanus.* The combined use of the *S. cerevisiae* strain and the pectinase enzyme gives the best results in terms of ethanol yield for fruit raw material. This result is also economically advantageous in the case of winemaking and distillation processes.

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