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ASSESSMENT OF FERMENTATION RESULTS OF *HANSENIASPORA (KLOECKERA)* STRAINS ISOLATED IN FINGER LAKES' WINERIES

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Hanseniaspora (*Kloeckera*) yeast strains were isolated from several Finger Lakes' wineries (New York State, USA), which belonged to species *H. uvarum* and *H. osmophila*, and were examined by the ability of fermentation in Chardonnay grape juice and synthetic medium, respectively. One-way analysis of variance (ANOVA), post hoc range tests, and pairwise multiple comparison analysis were used to estimate the glucose, fructose consumption and the ethanol, acetic acid production after fermentation in both media. The strains of *H. uvarum* formed quite a large amount of acetic acid (average 0.753 g l^{-1}) in the grape juice. The isolated *H. osmophila* strain (FL392) produced an average amount of 68.06 g l^{-1} of ethanol in the same medium. The compounds analyzed in synthetic medium for *H. uvarum* and *H. osmophila* isolates were observed in lesser amounts than in grape juice.

Keywords: *Hanseniaspora* (*Kloeckera*), statistical analysis, wine aromatic compounds, wine yeasts

It is acknowledged and well established that a range of different yeasts genera (*Hanseniaspora*/*Kloeckera*, *Torulopsis*, *Hansenula*, *Debaryomyces*, *Candida*, *Saccharomyces*) has considerable influence on the elaboration of final composition of aromatic substances during the spontaneous alcoholic fermentation of must into wine (LAFON-LAFOURCADE, 1983; FLEET et al., 1984; HEARD & FLEET, 1985; 1988; FLEET & HEARD, 1993; SCHÜTZ & GAFNER, 1993; EGLI et al., 1998). Moreover, this characteristic is affected by other factors, such as temperature, pH, nutrients and the growth level of each yeast genus (KUNKEE & AMERINE, 1970; BENDA, 1982; LAFON-LAFOURCADE, 1983). In accordance with that, wine is a complex alcoholic beverage, which is abundant in diverse flavours. Although, several researchers have already followed and scrutinized the production and composition of different aromatic

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substances along grape must fermentation (HOLLOWAY & SUBDEN, 1991; GIL et al., 1996; EGLI et al., 1998; CIANI & FATICHENTI, 1999), it is still not entirely understood that how exactly the chemical and biological pathways derive from each other relative to the formation of characteristic aromatic compounds in wine (LAFON-LAFOURCADE, 1983; FLEET, 1990).

There has been much discussion concerning the benefit and disadvantage of *Hanseniaspora* (*Kloeckera*) yeast strains during grape fermentation. It was supposed earlier that the strains of these genera are suppressed after a short time in the fermentative process as the alcohol content increases. However, several researchers experienced that they can reach maximum populations of 10^6–10^7 cells ml^{-1}; therefore they probably significantly contribute to the overall fermentation (HEARD & FLEET, 1985; FLEET, 1990; MORENO et al., 1991; ROMANO et al., 1997). There are scientists who ponder on these species as positive agents during the fermentation, where they can produce useful aromatic compounds, e.g., acetoin, 2,3-butanediol, glycerol and other higher alcohols (MATEO et al., 1991; ROMANO et al., 1992; ROMANO et al., 1993; HEROLD et al., 1995; GIL et al., 1996; ROMANO & SUZZI, 1996; ROMANO et al., 1997; CIANI & MACCARELLI, 1998; ROMANO et al., 1998). Others consider them spoilage microorganisms and would like to prevent their function and restrain their activity during fermentation because of the high formation of acetic acid, acetaldehyde, ethyl acetate and other off-flavors (VELÁZQUEZ et al., 1991; GAFNER & SCHÜTZ, 1994; SPONHOLZ, 1994; CIANI & PICCIOTTI, 1995; CIANI & MACCARELLI, 1998). For these reasons, experimental fermentations were conducted using *Hanseniaspora* (*Kloeckera*) and *Saccharomyces* yeast strains in grape juice as well as in synthetic medium to scrutinize harmful or beneficial effects of the apiculate yeasts (SHARF & MARGALITH, 1983; HERRAIZ et al., 1989; 1990; SCHÜTZ & GAFNER, 1993; ZIRONI et al., 1993; BATAILLON et al., 1996). Others inoculated the medium with *Hanseniaspora* (*Kloeckera*) strains only and conducted fermentations to study their biology (SHARF & MARGALITH, 1983; GAO & FLEET, 1988; HEARD & FLEET, 1988; ROMANO et al., 1993; CHAROENCHAI et al., 1998; CIANI & FATICHENTI, 1999).

In our experiment, we subjected Chardonnay grape juice and synthetic medium to fermentation by using different *H. uvarum* and *H. osmophila* strains originating from Finger Lakes' wineries (NY, USA). These data were compared and estimated by statistical analysis.

1. Materials and methods

1.1. Yeast strains

The 17 yeast strains used in this study were isolated from 11 different wineries in the Finger Lakes region, NY, USA, and are listed in Table 1. Yeasts were grown on Phytone™ yeast extract agar plates at 25 °C (72 g l^{-1}; Becton Dickinson, Cockeyville, MD, USA) and stored at 5 °C. Type strains of *Hanseniaspora uvarum* and *H. osmophila* were purchased from Centraalbureau voor Schimmelcultures (CBS Yeast

Division, Baarn & Delft, The Netherlands) and used as reference strains (Table 1). The strains isolated in Finger Lakes wineries were previously characterized by physiological and different molecular methods (BUJDOSÓ et al., 2001).

Table 1. Reference and isolated strains of genera *Hanseniaspora (Kloeckera)* used in the study

Strain	Species	Origin
CBS 279[a]	*Hanseniaspora uvarum*	Inst. Brewing, Tokyo, Japan
FL73	*Hanseniaspora uvarum*	Delaware, Wagner Vineyards[b]
FL194	*Hanseniaspora uvarum*	Chardonnay, Wagner Vineyards[b]
FL200	*Hanseniaspora uvarum*	Riesling, Wagner Vineyards[b]
FL519	*Hanseniaspora uvarum*	Gewurztraminer, Wagner Vineyards[b]
FL529	*Hanseniaspora uvarum*	Seyval, Wagner Vineyards[b]
FL592	*Hanseniaspora uvarum*	Ravat, Wagner Vineyards[b]
FL176	*Hanseniaspora uvarum*	Ives, Lakewood Vineyards[b]
FL355	*Hanseniaspora uvarum*	Riesling, Hermann J. Wiemer Vineyard[b]
FL436	*Hanseniaspora uvarum*	Chardonnay, Dr. Frank's Wine Cellars[b]
FL562	*Hanseniaspora uvarum*	Gewurztraminer, Lameroux Landing Wine Cellars[b]
FL599	*Hanseniaspora uvarum*	Chardonnay, Prejean Winery[b]
FL602	*Hanseniaspora uvarum*	Ravat, Wagner Vineyards[b]
FL722	*Hanseniaspora uvarum*	Riesling, Glenora Wine Cellars[b]
FL775	*Hanseniaspora uvarum*	Vignoles, Hunt Country Vineyards[b]
FL843	*Hanseniaspora uvarum*	Riesling, Standing Stone Vineyards[b]
FL871	*Hanseniaspora uvarum*	Cabernet Franc, Swedish Hill Vineyard[b]
CBS 106[a]	*Hanseniaspora osmophila*	Bark of tree, København, Denmark
FL392	*Hanseniaspora osmophila*	Baco Noir, Fulkerson Vinery[b]

[a] Reference strains purchased from Centraalbureau voor Schimmelcultures (CBS), Baarn & Delft, The Netherlands

[b] Isolated in Finger Lakes region, NY, USA; identified at Cornell University, New York State Agricultural Experiment Station, Wine Research Program, NY, USA (BUJDOSÓ et al., 2001)

1.2. Preparation of grape juice and synthetic medium

The frozen Chardonnay juice was thawed at room temperature and sedimented in Sorvall RC-5C refrigerated superspeed centrifuge (Du Pont Company, Instrument Products, Biochemical Division, Newtown, CT, USA) at 4 °C. The grape juice was supplemented with 1 g l^{-1} of diammonium phosphate (Difco). After that, it was filter sterilized using Stainless Steel Dispensing Vessel (Millipore Corporation, Bedford, MA, USA) and pressed through filters with nitrogen gas (15 kPa). Double filter membranes were used for filtration in the sizes of pre-filter glass fiber membrane and 0.2 μm filter membrane (Gelman Sciences, Ann Arbor, MI, USA). The sugar content of the grape juice was determined by refractometer (Atago Co., Ltd., Japan). The synthetic medium was made up from the following components: glucose (110 g l^{-1}), fructose (110 g l^{-1}), DL-malate (6 g l^{-1}), D-tartrate (3 g l^{-1}), Yeast Nitrogen Base without amino acids or ammonium sulfate (1.7 g l^{-1}), citric acid (200 mg l^{-1}), 30 ml of amino acid stock solution [L-arginine (26.67 g l^{-1}), L-glutamine (26.67 g l^{-1}), L-glutamate (6.67 g l^{-1}), L-phenylalanine (5 g l^{-1}), L-alanine (4 g l^{-1}), L-threonine (4 g l^{-1}), L-aspartate

(3.33 g l^{-1}), L-isoleucine (3.33 g l^{-1}), L-leucine (3.33 g l^{-1}), L-methionine (3.33 g l^{-1}), L-proline (3.33 g l^{-1}), L-serine (3.33 g l^{-1}), γ-amino-n-butyric acid (3.33 g l^{-1}), L-aspargine (1.67 g l^{-1}) and L-histidine (1.67 g l^{-1})] and 10 ml of vitamin stock solution [sodium bicarbonate (50 g l^{-1}), myo-inositol (9.8 g l^{-1}), nicotinic acid (200 mg l^{-1}), pyridoxine·HCl (160 mg l^{-1}), calcium pantothenate (60 mg l^{-1}), thiamine·HCl (60 mg l^{-1}), folic acid (19.8 mg l^{-1}) and biotin (12.3 mg l^{-1})]. The synthetic medium was also completed with diammonium phosphate (1 g l^{-1}, Difco), and the pH was adjusted to 3.5 with sodium hidroxide (Sigma) and filtered using 0.2 μm Nalgene Top Filter (Nalge Nunc International, Rochester, NY, USA). The filter sterilized Chardonnay grape juice and synthetic medium were refrigerated until their utilization.

1.3. Pilot fermentation and analysis of grape juice and synthetic medium

Ten ml aliquot of filter sterilized Chardonnay grape juice (from 1998, 22° Brix, pH = 3.5, 30 ppm SO_2 added in the winery) originated from Lamoreaux Landing Wine Cellars (Finger Lakes region, USA) and synthetic medium were inoculated with strains of *Hanseniaspora* (*Kloeckera*) isolated at Finger Lakes wineries respectively (Table 1). Initial cell number was 10^6 cells ml^{-1} in both fermenting media. The fermentation was accomplished in 300 ml Erlenmeyer flasks containing 100 ml grape juice as well as 100 ml synthetic medium separately, and they were shaken 140 r.p.m. at 30 °C. Plastic bubblers were placed on top of the flasks containing 50% alcohol for preventing contamination. Weight decrease was measured frequently and fermentation was considered finished when the weight change was found constant. All the fermentations were done in triplicate. Fermentations were vigorous in either medium accompanied by considerable foam development. After about 3 days, experiments were stopped and samples were refrigerated until they were used for the following investigations. The amount of D-glucose and D-fructose (kit no. 139106), acetic acid (kit no. 148261) and ethanol (kit no. 176290) was measured by specific enzymatic methods (Boehringer Mannheim, Indianapolis, IN, USA).

1.4. Statistical methods

One-way analysis of variance (ANOVA) was applied to investigate whether there would be any difference between the means of the utilization of glucose, fructose and the production of ethanol, acetic acid among the strains belonging to genera *Hanseniaspora* (*Kloeckera*) in both media. Means were calculated from three independent measurements after fermentation for all the components. To determine the differences that were between the means, a Post Hoc Range Test was completed. Pairwise multiple comparisons were determined to test the dissimilarity between each pair of means applying the method of Tukey's honestly significant difference test. This method was also employed to create homogeneous subsets according to the discrimination for the components utilized and produced by those strains in both media. Linear regression analysis was considered as well to determine the relationship between pathways within grape juice and synthetic medium (SPSS Inc., 1999).

2. Results

2.1. Fermentation in grape juice and synthetic medium

Strains belonging to the genera *Hanseniaspora* (*Kloeckera*) were previously identified and differentiated by physiological tests and diverse molecular methods (BUJDOSÓ et al., 2001). The same strains were subjected to fermentation experiments. The fermentation was mainly focused on the consumption of glucose and fructose. There were interests also in the productions of ethanol and acetic acid, because these outcomes are contributed fundamentally to the fermenting juice during the activity of *Hanseniaspora* (*Kloeckera*) strains. Chardonnay grape juice and synthetic medium were used in these experimental fermentations.

The strains of *H. uvarum* originating from Finger Lakes region showed similar results (Table 2). Considering all the measurements, an average utilization of 55.38 g l^{-1} of glucose (ranging between 46.10 and 63.37 g l^{-1}) and 61.80 g l^{-1} of fructose (52.63 and 70.01 g l^{-1}) were typical for the strains of *H. uvarum*. An average production of 38.26 g l^{-1} of ethanol (32.47 and 42.49 g l^{-1}) and 0.753 g l^{-1} of acetic acid (0.589 and 0.929 g l^{-1}) were characteristic of the same strains in the fermentation of Chardonnay grape juice. The reference strain of *H. uvarum*, coded as CBS 279 behaved slightly differently. The average utilization of glucose was weaker, 43.94 g l^{-1}; for fructose it was almost the same, 60.16 g l^{-1}; the ethanol production, 31.47 g l^{-1} and the amount of acetic acid, 0.684 g l^{-1} were less compared to the rest of the strains.

The strain, FL392 (*H. osmophila*) that was isolated from Baco Noir in Fulkerson Winery (Finger Lakes region, NY, USA) also performed remarkably. The utilization of glucose from three independent experiments increased to an average of 102.80 g l^{-1}, with a lower usage of fructose, 76.71 g l^{-1}. The ethanol production of this strain extended to 68.06 g l^{-1} that was 1.5 times more than for the mean of the strains of *H. uvarum*. The acetic acid production of that strain was not that significant in relation to the others, an average of 0.710 g l^{-1}.

A fermentation trial was also done in synthetic medium. Although using conditions similar to the natural grape juice regarding ingredients and fermentation specifications, the experiments ended in different results. Taking into consideration all the data, an average usage of glucose for the strains of *H. uvarum* was barely different from that in grape juice, 59.32 g l^{-1} (ranging between 48.69 and 72.87 g l^{-1}) and for fructose was 58.76 g l^{-1} (47.41 and 68.28 g l^{-1}). The ethanol production was an average of 38.25 g l^{-1} (19.69 and 38.23 g l^{-1}) and that for the acetic acid was 0.439 g l^{-1} (0.193 and 0.721 g l^{-1}). For reference strain CBS 279, the assimilation of glucose and fructose was close to the majority of the strains of *H. uvarum* (55.02 g l^{-1} and 55.67 g l^{-1}); although the percentage of ethanol was less (24.64 g l^{-1}), and the acetic acid production was somewhat higher (0.489 g l^{-1}).

Table 2. Results of fermentations conducted in Chardonnay grape juice and synthetic media inoculated by *H. uvarum* (line 1–17) and *H. osmophila* (line 18) strains isolated at Finger Lakes' wineries

Strain	Chardonnay grape juice				Synthetic media			
	Glucose utilization ($g\ l^{-1}$)	Fructose utilization ($g\ l^{-1}$)	Ethanol ($g\ l^{-1}$)	Acetic acid ($g\ l^{-1}$)	Glucose utilization ($g\ l^{-1}$)	Fructose utilization ($g\ l^{-1}$)	Ethanol ($g\ l^{-1}$)	Acetic acid ($g\ l^{-1}$)
1. CBS 279[a]	43.94±4.55[b]	60.16±3.92	31.47±1.48	0.684±0.037	55.02±7.24	55.67±6.15	24.64±2.03	0.489±0.183
2. FL73	56.60±2.87[c]	62.19±0.00	38.96±1.10	0.775±0.027	59.34±2.17	56.25±5.22	28.28±4.90	0.387±0.102
3. FL194	56.75±3.03	63.50±0.75	39.23±0.32	0.752±0.038	56.46±2.59	54.95±5.58	25.60±4.78	0.301±0.094
4. FL200	53.44±4.55	68.71±0.75	41.49±1.34	0.822±0.094	63.52±6.29	65.38±1.32	34.27±3.53	0.522±0.097
5. FL519	53.30±3.49	68.71±1.56	40.61±1.09	0.735±0.016	57.76±0.75	61.32±6.09	29.55±5.15	0.558±0.067
6. FL529	53.29±6.12	53.94±1.56	33.24±0.56	0.642±0.007	57.04±7.24	53.07±5.00	25.94±2.80	0.441±0.114
7. FL592	56.61±2.93	63.35±3.29	39.61±0.23	0.820±0.060	63.52±4.65	60.74±4.37	31.28±3.94	0.554±0.144
8. FL176	59.06±3.95	57.26±1.32	39.07±0.40	0.769±0.028	63.23±9.50	57.27±4.37	30.59±3.11	0.371±0.131
9. FL355	53.01±3.76	61.32±0.87	37.35±0.75	0.740±0.046	58.05±4.97	61.76±5.85	29.24±5.17	0.341±0.032
10. FL436	50.13±4.91	61.32±0.00	35.27±0.89	0.724±0.019	54.88±5.36	64.08±1.39	29.20±3.57	0.355±0.018
11. FL562	58.77±3.48	58.42±0.50	36.92±1.36	0.801±0.046	57.76±2.24	56.25±8.50	27.83±5.05	0.406±0.046
12. FL599	58.62±3.68	64.08±0.66	40.72±0.88	0.724±0.056	56.46±2.99	59.58±1.50	27.59±3.06	0.436±0.092
13. FL602	52.87±2.45	59.87±0.50	36.85±0.60	0.718±0.063	58.33±5.39	61.18±0.66	29.47±4.84	0.461±0.133
14. FL722	53.44±5.93	54.52±0.66	33.27±0.71	0.641±0.046	54.02±1.94	49.44±3.51	22.68±3.31	0.409±0.108
15. FL775	57.33±3.96	64.95±1.75	40.07±1.51	0.851±0.049	65.53±6.09	63.50±0.75	34.12±2.82	0.541±0.042
16. FL843	56.32±4.19	64.08±1.39	39.80±1.26	0.757±0.028	62.22±3.89	58.57±5.25	29.94±7.02	0.430±0.010
17. FL871	56.75±3.89	62.63±1.89	39.61±0.59	0.774±0.020	61.07±3.49	56.83±4.52	28.32±5.83	0.503±0.063
18. FL392	102.80±3.25	76.70±8.69	68.06±4.21	0.710±0.036	82.36±3.45	68.07±0.65	44.85±3.62	0.528±0.030

[a] Reference strain purchased from Centraalbureau voor Schimmelcultures, Baarn & Delft, The Netherlands
[b] Data presented for each strain is the mean of three independent fermentation experiments
[c] i.e. ±2.87: Standard deviation

The strain of *H. osmophila* (FL392) performed differently from the rest of the strains. The glucose utilization was stronger (82.37 g l^{-1}), yet it was somewhat lower for fructose (68.07 g l^{-1}). Its ethanol aggregation was not extreme in the synthetic medium (44.85 g l^{-1}), however it was above the average of the strains of *H. uvarum*. The acetic acid amount produced was more than the average for the strains of *H. uvarum* (0.528 g l^{-1}) as well. Interestingly, the pH did not change notably in either medium remaining around the initial value of 3.5.

2.2. One-way ANOVA and post hoc range test

One-way analysis of variance (ANOVA) was applied to analyze whether means for the utilization of glucose, fructose and the production of ethanol and acetic acid are statistically equal between strains in both mediums. Since the significance level of ANOVA was P = 0.000 almost in all the cases (except for fructose P = 0.002 and acetic acid P = 0.042 in synthetic medium), Tukey's honestly significant difference test was chosen to determine more exactly the differences existing among the means. Those homogeneous subsets are shown in Tables 3 and 4, which contain the strains considered not significantly different for the components utilized and produced in both mediums. Strains that contained more "X" indicated that those could not be placed unambiguously into one subset.

In Chardonnay grape juice the glucose consumption of strains was separated in three homogeneous subsets (Table 3). The reference strain CBS 279 was not that extreme in the utilization of glucose, since it was considered homogeneous to several other strains. Yeast strain FL392 seemed very different, hence it was divided into another subset. According to the consumption of fructose strains belonged to five homogeneous subsets by their means, and strain FL392 was differentiated again. Comparing the means according to the production of ethanol, cultures were classified in six homogeneous subsets. FL392 was again considered different from the rest of the strains. Regarding the production of acetic acid they appeared more equal. Three quite large homogeneous subsets were produced indicating the similarity of the strains according to their means.

In synthetic medium, relative to the utilization of glucose, they were considered very homogeneous with one exception; strain FL392 (Table 4). In compliance with the consumption of fructose they seemed more alike having three quite large homogeneous subsets. In the production of ethanol these strains were considered homogeneous almost entirely excluding strains FL392, FL200, FL775, in contrast to the outcomes experienced for the utilization of glucose in the same medium. There was only one homogeneous subset relative to the production of acetic acid regarding the means for strains.

Table 3. Multiple range analysis for the consumption of glucose, fructose and production of ethanol, acetic acid in Chardonnay grape juice after fermentations conducted by strains of *H. uvarum* and *H. osmophila* isolated at Finger Lakes' wineries, NY, USA

Strain[a]	Homogeneous subsets[b] by the utilization of glucose	Strain	Homogeneous subsets by the utilization of fructose	Strain	Homogeneous subsets by the production of ethanol	Strain	Homogeneous subsets by the production of acetic acid
CBS 279[c]	X	FL 529	X	CBS 279	X	FL 722	X
FL436	XX	FL 722	XX	FL 529	XX	FL 529	X
FL602	XX	FL 176	XXX	FL 722	XX	CBS 279	XX
FL355	XX	FL 562	XXX	FL 436	XXX	FL 392	XX
FL529	XX	FL 602	XXX	FL 602	XXX	FL 602	XXX
FL519	XX	CBS 279	XXX	FL 562	XXX	FL 436	XXX
FL722	XX	FL 355	XXXX	FL 355	XXXX	FL 599	XXX
FL200	XX	FL 436	XXXX	FL 73	XXX	FL 519	XXX
FL843	XX	FL 73	XXX	FL 176	XXX	FL 355	XXX
FL73	X	FL 871	XX	FL 194	XXX	FL 194	XXX
FL592	X	FL 592	XX	FL 592	XX	FL 843	XXX
FL194	X	FL 194	XX	FL 871	XX	FL 176	XXX
FL871	X	FL 599	XX	FL 843	XX	FL 871	XXX
FL775	X	FL 843	XX	FL 775	XX	FL 73	XXX
FL599	X	FL 775	XX	FL 519	XX	FL 562	XX
FL562	X	FL 200	X	FL 599	XX	FL 592	XX
FL176	X	FL 519	X	FL 200	X	FL 200	XX
FL392	X	FL 392	X	FL 392	X	FL 775	X

[a] Pairwise multiple comparisons were applied to test the difference between each pair of means according to the method of Tukey's honestly significant difference test.
[b] Tukey's honestly significant difference test was used also to show the homogeneous subsets for the strains by components using SPSS 9.0 statistical software package (significance level, P=0.05). Strains that were not considered significantly different by the means were assembled in identical column (homogeneous subset). "X" symbolized the mean of three independent fermentation results according to the component examined by each strain. Strains whereat belonged more than one "X" implied that it could not be ranged into one subset unambiguously.
[c] Reference strain purchased from Centraalbureau voor Schimmelcultures, Baarn & Delft, The Netherlands

Table 4. Multiple range analysis for the consumption of glucose, fructose and production of ethanol, acetic acid in synthetic media after fermentations conducted by strains of *H. uvarum* and *H. osmophila* isolated at Finger Lakes' wineries, NY, USA

Strain[a]	Homogeneous subsets[b] by the utilization of glucose	Strain	Homogeneous subsets by the utilization of fructose	Strain	Homogeneous subsets by the production of ethanol	Strain	Homogeneous subsets by the production of acetic acid
FL722	X	FL 722	X	FL 722	X	FL 194	X
FL436	X	FL 529	XX	CBS 279	X	FL 355	X
CBS 279[c]	X	FL 194	XXX	FL 194	X	FL 436	X
FL194	X	CBS 279	XXX	FL 529	X	FL 176	X
FL599	X	FL 73	XXX	FL 599	X	FL 73	X
FL529	X	FL 562	XXX	FL 562	X	FL 562	X
FL519	X	FL 871	XXX	FL 73	X	FL 722	X
FL562	X	FL 176	XXX	FL 871	X	FL 843	X
FL355	X	FL 843	XXX	FL 436	X	FL 599	X
FL602	X	FL 599	XXX	FL 355	X	FL 529	X
FL73	X	FL 592	XXX	FL 602	X	FL 602	X
FL871	X	FL 602	XXX	FL 519	X	CBS 279	X
FL843	X	FL 519	XXX	FL 843	X	FL 871	X
FL176	X	FL 355	XXX	FL 176	X	FL 200	X
FL200	X	FL 775	XX	FL 592	X	FL 392	X
FL592	X	FL 436	XX	FL 775	XX	FL 775	X
FL775	X	FL 200	XX	FL 200	XX	FL 592	X
FL392	X	FL 392	X	FL 392	X	FL 519	X

[a] Pairwise multiple comparisons were applied to test the difference between each pair of means according to the method of Tukey's honestly significant difference test.
[b] Tukey's honestly significant difference test was used also to show the homogeneous subsets for the strains by components using SPSS 9.0 statistical software package (significance level, P=0.05). Strains that were not considered significantly different by the means were assembled in identical columns (homogeneous subset). "X" symbolized the mean of three independent fermentation results according to the component examined by each strain. Strains whereat belonged more than one "X" implied that it could not be ranged into one subset unambiguously.
[c] Reference strain purchased from Centraalbureau voor Schimmelcultures, Baarn & Delft, The Netherlands

2.3. Linear regression analysis of compounds utilized and produced in fermentation

Linear regression analysis was used to evaluate the relationship between components utilized and produced for the strains of *H. uvarum* isolated at Finger Lakes' wineries ($n = 48$). The coefficients of correlation were also examined for the glucose, fructose utilized and the ethanol, acetic acid produced after the fermentation in both mediums. There was a weak significant connection between the utilization of glucose and the production of ethanol ($R = 0.322$; $P = 0.026$) in Chardonnay grape juice during the fermentation of these strains. That relationship was similar also regarding the consumption of glucose and the yield of acetic acid ($R = 0.347$; $P = 0.016$). There was a stronger significant correlation, however, between the utilization of fructose and the production of ethanol ($R = 0.789$; $P = 0.000$, Fig. 1). The consumption of fructose and the production of acetic acid were also related significantly to each other ($R = 0.489$; $P = 0.000$). A statistical relationship was found to exist between the utilization of glucose and the production of ethanol in synthetic medium ($R = 0.651$; $P = 0.000$).

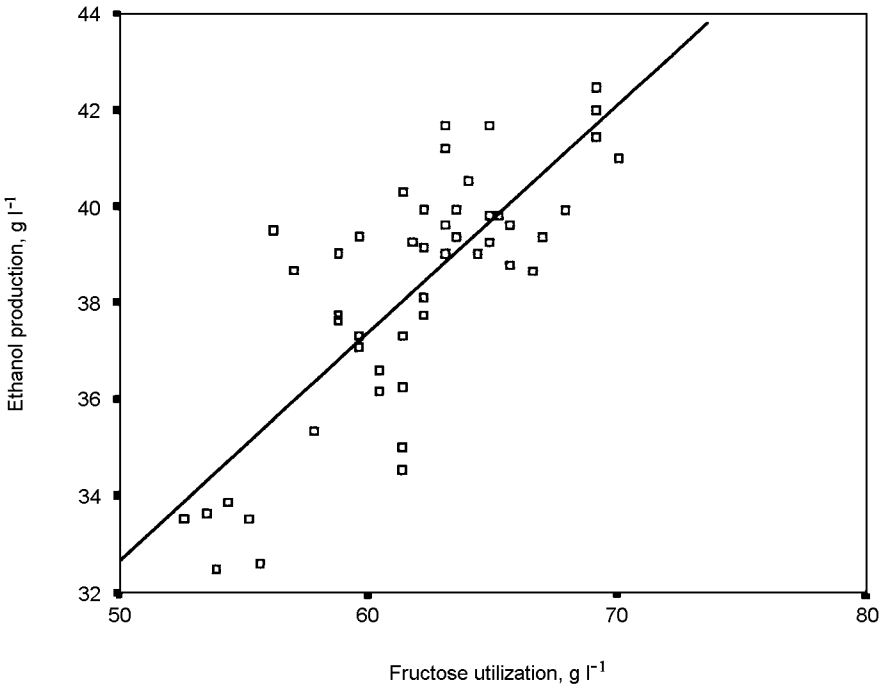

Fig. 1. Significant connection between the fructose utilization and the production of ethanol for the strains of *H. uvarum* isolated at Finger Lakes' wineries and fermented in synthetic medium ($R = 0.789$; $P = 0.000$). Y = 8.865+0.476x

The consumption of glucose and the production of acetic acid seemed to evolve from each other significantly (R = 0.472; P = 0.001). By the utilization of fructose and the production of ethanol that pathway was also found significantly correlated (R = 0.593; P = 0.000). However, there was no significant connection between the consumption of fructose and the production of acetic acid in synthetic medium (R = 0.100; P = 0.498). Ethanol and acetic acid productions were also examined statistically in both mediums. It turned out that the connection between these two compounds was stronger in Chardonnay grape juice (R = 0.661; P = 0.000) than in synthetic media (R = 0.261; P = 0.074).

3. Discussion

Different investigations were conducted on the strains isolated at Finger Lakes' wineries. First they were inoculated in Chardonnay grape juice and synthetic medium. The consumption of glucose and fructose by the strains of *H. uvarum* and the strain of *H. osmophila* (FL 392) was in good correlation with the results experienced earlier (CIANI & FATICHENTI, 1999). Interestingly, during the fermentation of grape juice it seemed that the average fructose utilization (approx. 56%) was greater than the average glucose consumption (approx. 50%) for the strains of *H. uvarum*. Some researchers observed similar results in synthetic medium (CIANI & FATICHENTI, 1999). The preferred consumption of fructose, however, can be advantageous by the strains of *H. uvarum* (CIANI & FATICHENTI, 1999). According to other scientists it was revealed that difficulties emerge for the strains of *S. bayanus* at the end of fermentation (SCHÜTZ & GAFNER, 1995). Cells in their late stationary phase, which still predominate during the fermentation, are inhibited more and more in the utilization of glucose due to factors such as increased amounts of ethanol, extreme anaerobic conditions, and decreased nutrition (SCHÜTZ & GAFNER, 1995). In accordance with that, it might help to avoid sluggish fermentation using particular strains of *H. uvarum*, which could change the glucose-fructose ratio towards the percentage of glucose due to their growth (CIANI & FATICHENTI, 1999). The strain of *H. osmophila* (FL392) utilized most of the glucose and much less from fructose, which was noted for this species by other scientists as well (CIANI & FATICHENTI, 1999). Nevertheless, the ethanol production of that strain was outstanding. It was almost two times more than the average for the strains of *H. uvarum*. The data concerning the acetic acid production by *H. uvarum* strains was in good correlation with the earlier result (CIANI & MACCARELLI, 1998). However, there were other researchers who experienced higher amount than that produced by the strains of this species (SCHÜTZ & GAFNER, 1993; CIANI & MACCARELLI, 1998). In contrast to the work of others (FLEET & HEARD, 1993) we found smaller amount of acetic acid produced by the strains of *H. uvarum* in grape juice (an average of 0.753 g l^{-1}). Several scientists also experienced less productivity of acetic acid by this species (ROMANO et al., 1992; CIANI & PICCIOTTI, 1995). In synthetic medium the average consumption of

glucose and fructose was almost equal (approx. 53%) for the strains of *H. uvarum*. This was in contrast to some researchers who experienced greater fructose utilization in synthetic medium by that species (CIANI & FATICHENTI, 1999).

Reference strain *H. uvarum* (CBS 279) fermented much slower in both media than the rest of the strains. This function can be attributed to the fact that this strain was isolated originally from a brewery, which might be responsible for the different productivity in dissimilar environments. The reference strain *H. osmophila* (CBS 106) could not proliferate either in grape juice or in synthetic medium. This yeast strain was isolated from the bark of a tree, which could also induce the incapacity to grow and multiply under different conditions. Isolated strain FL392 showed repeatedly higher glucose utilization than fructose, which resulted in greater ethanol production in contrast to *H. uvarum* strains in that medium, although the amount produced was lower, compared to grape juice. Generally the production of ethanol and acetic acid for each strain was at higher concentrations in grape juice compared to synthetic medium. Also, the overall average sugar consumption was almost the same (approx. 118 g l^{-1}) for the strains of *H. uvarum* in both mediums, although the average production of ethanol and acetic acid varied in these mediums.

One-way ANOVA and multiple range analysis were used to determine diversity among strains that were isolated in the Finger Lakes' wineries in the consumption of glucose, fructose and the production of ethanol, acetic acid in both media. Considering the strains of *H. uvarum* there were more homogeneous subsets in grape juice than in the synthetic medium. Several times strain FL392 was divided into different subsets in both mediums, which indicated its dissimilarity to the rest of the strains in its physiological characteristics. Regression analysis was applied to determine the relationship between the variables. The strength of the connection between the variables was described by correlation coefficients. In contrast to others (CIANI & MACCARELLI, 1998) significant positive correlation was found between the production of acetic acid and the formation of ethanol in both mediums by our investigation.

Several researchers find the species of genera *Hanseniaspora* (*Kloeckera*) important and as a feasible new trend because of their possible contribution to the fermentation of grape juice and must with positive flavours in wine (HEARD & FLEET, 1988; ROMANO et al., 1992; ZIRONI et al., 1993; CIANI & MACCARELLI, 1998). According to some other scientists these yeast strains produce undesirable flavours during fermentation (VELÁZQUEZ et al., 1991; CIANI & PICCIOTTI, 1995).

In relation to our results, it is obvious that the strains of *H. uvarum* isolated at Finger Lakes' wineries are significant acetic acid producers. However, that experience stands against the fact what Maudy Th. Smith disclosed in the chapter "Diagnosis of the genus *Hanseniaspora*" of "The yeast, a taxonomic study" published by Kurtzman and Fell (SMITH, 1998), which is definitely mistaken, that acetic acid is not produced by this genus. There are also publications proving that the strains of *Hanseniaspora* (*Kloeckera*) produce notable amount of acetic acid during grape fermentation (BENDA, 1982; LAFON-LAFOURCADE, 1983; VELÁZQUEZ et al., 1991; FLEET & HEARD, 1993; CIANI & PICCIOTTI, 1995; CIANI & MACCARELLI, 1998).

After the investigation of the isolated *H. osmophila* strain that produced quite a large amount of ethanol it might be that further strains belonging to this species are able to form similar percentage of alcohol. Considering this experience there is a possibility for that the strains of *Hanseniaspora* will be used as a new trend in the wine fermentation technology. Therefore it is worth giving attention to the species of genera *Hanseniaspora* (*Kloeckera*) to continue additional investigations with them in the future.

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