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IMMOBILIZATION OF YEAST ON GRAPES FOR MEAD PRODUCTION

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Abstract: The aim of the study was to determine the effect of freeze-drayed grape berries used for the immobilization of yeast on the fermentation rate of honey wort. Two varieties of white grape berries were added to the wort: Seyval Blanc and Jutrzenka in the doses of 1, 2 and 5 g/dm^3. The addition of higher doses of grapes (5 g/dm^3) increased fermentation rate and concentration of ethanol (to 12,6% vol.) and decreased acetic acid synthesis by yeast and volatile acidity (to 0,9 g/l) in young mead.
Keywords: mead, immobilization, grape, honey, fermentation

INTRODUCTION

Mead is an alcoholic beverage obtained by fermenting mead wort, which contains between 9% and 18% of ethanol by volume. Mead wort is produced by diluting bee honey with the appropriate amount of water or fruit juice. Depending on the proportion to which honey is diluted, different types of mead are obtained; the finest at 1:0.5 (honey: water), or at 1:1, 1:2 and 1:3. Previous experiments have shown that honey contains numerous substances that can inhibit the metabolism of yeast **(Sroka, Tuszyński, 2007**). The rate od fermentation depends on concentration of inhibitors such as fatty acids (hexanoic, octanoic, decanoic acid), proteins (enzymes), furfural and hydroxymethylfurfural (**Viegas, Sá-Correia, 1995, Bison, 1999, Cabral et al., 2001, Malherbe et. al., 2007).** Furan derivatives are formed during heating (boiling) of the mead worts by the hydrolysis of carbohydrates **(Kahoun et al., 2008)**. The inhibitors interact synergistically with high osmotic pressure and the increasing concentration of ethanol during fermentation. **Roldán et al. (2011**) investigated influence of pollen addition at concentrations ranged from 10 to 50 g/L as a fermentation activator on the fermentation kinetics and the quality of meads. The results showed that pollen addition improved fermentation rates, alcohol yields, and the final characteristics of meads.

The aim of the study was to determine the effect of freeze-dried grape berries on the fermentation kinetic *Saccharomyces cerevisiae* yeast in mead wort. In the experiments two strains of grape Seyval Blanc and Jutrzenka were used. In the obtained young meads, the content of ethyl alcohol, sugars, reducting sugars, extract, pH, titratable acidity and volatile acidity were determined.

MATERIAL AND METHODS

Buckwheat honey (Bartnik Sadecki, Poland) was mixed with potable water in the proportion 1:2 (v/v), heated and gently boiled for 10 min, then topped up with diammonium hydrogen phosphate (V) (0.4 g/l) and citric acid (0.25 g/l), the extract was checked after mixing. The hot wort (0.2 l) was poured into 0,5 l bottles, freeze-drayed grape Jutrzenka or Seyval Blanc berry was added (1, 3 or 5 g/l), stopped with sterile fermentation trap tubes. After the wort was cooled down to approximately 30°C, a precisely defined amount of starter yeast was added (0.5 g/l calculated for the dry substance); this yeast was prepared in a three-stage culture (agar medium, 9% brewer's wort, still culture (10 ml) and shaken culture (100 ml). The fermentation progressed at room temperature (20°C to 22°C). All fermentation experiments were conducted three times. The ethanol content, extract, pH, the titratable and volatile acidity were determined using official methods **(O.I.V., 2012).** The reducing sugars were determined using method with 3,5-dinitrosalicylic acid **(Miller 1959).**

RESULTS AND DISCUSSION

In the experiments two white grapes were used. Freeze-dried particles from Jutrzenka and Seyval Blanc grape berry was added to mead wort prior fermentation. Grape particles were applied as a matrix for immobilization of *Saccharomyces cerevisiae* yeast. Experiments were made in triplicate. The control samples (mead worts) were inoculated without the addition of grapes particles.

In the conducted experiments it was found that supplementation of mead wort significantly affected the fermentation. The rate of fermentation was investigated on the basis of mass losses. Among the analysed samples, during the fermentation, the highest weight changes occurred in the case of honey wort with the addition of 5 g/l grape particles (Fig 1 and 2). Other samples were characterized by similar kinetics of fermentation in comparison to the control.

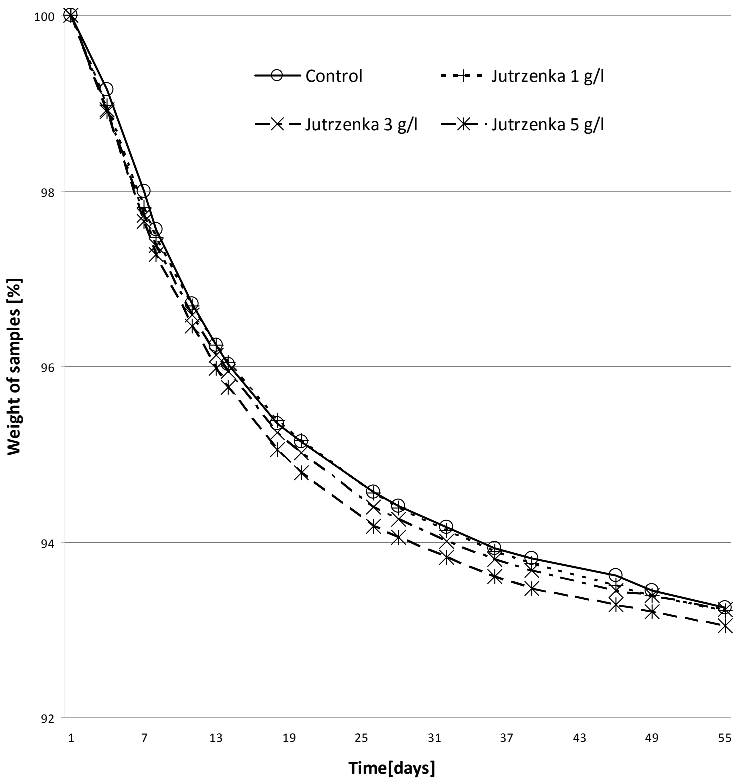

Fig 1. Fermentation kinetics of samples contained 1, 3 or 5 g/l Jutrzenka grapes

Tab. 1. Chemical composition of mead wort and juices from Jutrzenka and Seyval Blanc grapes

	pH	Total extract	Total sugar	Reducting sugar	Titratable acidity
		[g/l]			
Mead wort	3.5	35	357.2±0,3	305.9±0,3	1.3
Jutrzenka grape juice	3.3	26	245.7±0,3	196.8±0,0	9.0
Seyval Blanc grape juice	3.6	24	213.6±0,3	157.5±0,3	6.2

Yeast cells could be adsorbed on the grape surface, cause adhesion of cells, and suspend particles present in wort. The presence of grape particles may lead also to formation of complex agglomerates. Fragmented grapes could also bound different surfactant active molecules, including inhibitors of fermentation.

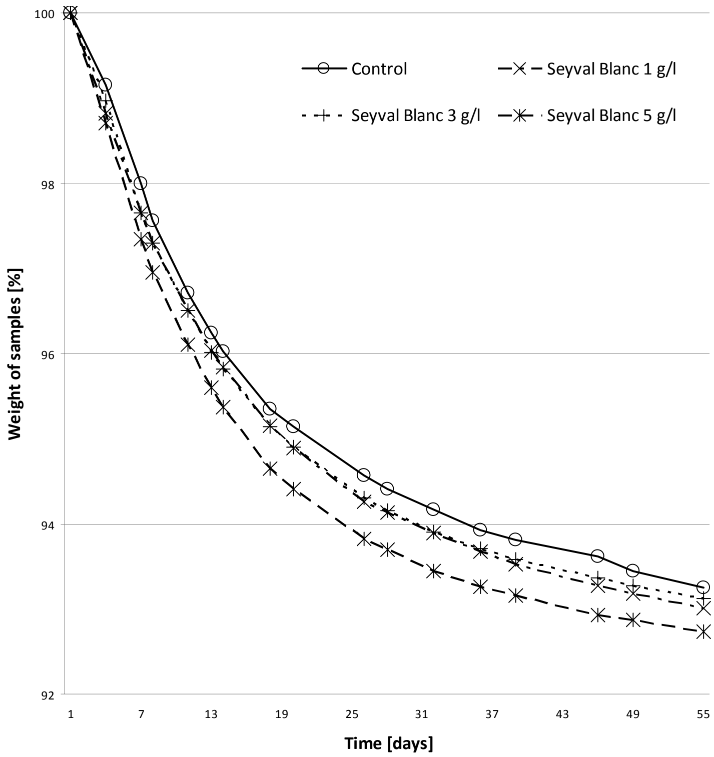

Fig 2 Fermentation kinetics of samples contained 1, 3 or 5 g/l Seyval Blanc grapes

Tab. 2. Chemical composition of mead

Addition of dried grapes [g/l]		Etanol	pH	Total extract	Total sugar	Reducting sugar	Titratable acidity	Volatile acidity
		[% vol.]		[g/l]				
Control	-	11.8±0.1^{a}	3.39±0.01^{b}	280±2	144.8±0.5^{e}	137.7±0.0^{f}	3.48±0.00^{a}	1.11±0.00^{d}
Jutrzenka	1	12.4±0.2^{b}	3.37±0.03^{ab}	225±2	162.5±0.5^{g}	139.4±0.6^{g}	3.57±0.08^{ab}	0.95±0.02^{b}
	3	12.4±0.2^{b}	3.35±0.01^{a}	230±2	147.9±0.5^{f}	129.9±0.6^{e}	3.64±0.04^{b}	0.95±0.02^{b}
	5	12.5±0.2^{bc}	3.33±0.02^{a}	154±2	90.8±0.6^{b}	82.3±0.6^{b}	3.66±0.04^{b}	0.90±0.03^{a}
Seyval Blanc	1	12.4±0.1^{b}	3.36±0.01^{ab}	166±2	96.3±0.6^{c}	117.8±0.3^{c}	3.66±0.04^{b}	1.04±0.02^{c}
	3	12.5±0.2^{bc}	3.36±0.01^{ab}	145±2	103.2±0.2^{d}	125.2±0.3^{d}	3.53±0.08^{a}	1.05±0.00^{c}
	5	12.6±0.2^{bc}	3.34±0.01^{a}	129±2	73.3±0.6^{a}	81.2±0.6^{a}	3.64±0.04^{b}	0.95±0.02^{b}

The same letters in column mean that differences within a particular group of samples are not statistically significant (at $p<0.05$)

Both the immobilization of yeast and the adsorption of inhibitory substances affect the acceleration of the mead production process, and increasing evolving of CO_2. Immobilization of microorganisms by adhesion eliminates the problem of diffusion resistance, occurring during the immobilization of microorganisms in the gels **(Sroka, Rzędowski, 1991)**. Yeast cells adsorbed on the surface are characterized by higher fermentation rate than the cells growing inside the gel **(Olejnik, Czaczyk, 1998)**. The fermentation can be inhibited by high concentrations of carbon dioxide (oversaturated liquid) and the early settling of yeast cells **(Thomas et al. 1994).** The released of CO_2 bubbles causes intensive agitation of liquid and increases mass transfer by rising the flocculated yeast cells to the surface.

The ethanol concentration in fermented samples with grapes were increased. The greatest concentration of ethyl alcohol (12.6% vol.) was found in worts with -5 g/l Seyval Blanc grape. Grape addition positively affected the efficiency of fermentation, and the produced young meads contain from 12.4 to 12.6% vol. ethyl alcohol, that was approximately from 5.0% to 6.8% more compared with control (Tab 2.).
The level of- total sugars in samples with grape were from 73.3 g/l (Seyval Blanc) to 90.8 g/l (Jutrzenka), compared to 144.8 g/l in control samples. These results confirm the better utilization of the substrates and increase the resistance of yeast cells on the toxic effect of ethanol after suplementation with grape particles. In young meads fermented with addition 5 g/l Seyval Blanc grapes were 54% less compounds of the total extract compared with control (Tab. 2).
Grape juices obtained from Jutrzenka and Seyval berrys had a relatively high acidity, 9.0 and 6.2 respectivly (tab. 1). Addition of grape particles to wort increased the level of total acidity and decreased pH of young mead (Tab. 2). This phenomenon is interesting because honeys and mead worts usually have low acidity.
Volatile acidity is another important parameter that influence quality of the finished product. The volatile acidity increases when yeast lives in stress condition. Acetic acid is a main component (about 90%) of the volatile acidity. Intensive rise of the volatile acidity is characteristic during wort fermentation with a high content of sugars. High osmotic pressure and unfavorably conditions of fermentation increase the synthesis of acetic acid by yeasts **(Fleet, 1993**). Stress factors inhibit reduction of acetaldehyde to ethyl alcohol by yeast cells. This process leads to synthesis and accumulation of higher concetration of acetic acid in the substrate **(Erasmus et al., 2003).**
The experiments showed a significant effect of the addition of grape particles on the volatile acidity. Samples fermented with addition of 0.5 g/l grape particles were distinguished by approximately 14.4-19.0% lover volatile acidity compared to the control. Reduction of volatile acidity (Tab 2) in supplemented samples was probably due to decrease of acetic acid synthesis. The decrease of acetic acid synthesis by cells of *S. cerevisieae* may be caused by a change in conditions or by a limitation of stress factors and confirms that protective effect of used grapes. This effect can be explained by several mechanisms. Particles– can lead to coagulation, precipitation or adsorption of fermentation inhibitors, flocculation and/or formation of protective microenvironment, that is characteristic for the immobilized cells.

CONCLUSION

- Supplementation with freeze-dried grapes increaes fermentation rate of mead worts.
- Immobilization of *Saccharomyces cerevisiae* yeast on the grape particles increases the concentration of ethanol in young meads
- The addition of grapes to mead wort decreases acetic acid synthesis by yeast cells and volatile acidity of mead

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