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THE INFLUENCE OF SELECTED MICROORGANISMS ON ETHANOL YIELD FROM JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.) TUBERS*

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The yield of ethanol production by bacteria *Zymomonas mobilis,* distillery yeast *Saccharomyces cerevisiae* and *Kluyveromyces* yeast have been studied using Jerusalem artichoke tubers. Two cultivars of Jerusalem artichoke: Albik and Rubik were used. Both acid and enzymatic hydrolysis were used to hydrolyse inulin into fermentable sugars. There was no use to hydrolyse inulin prior to fermentation with *Kluyveromyces* yeast. Fermentation with bacteria and distillery yeast was carried out at pH 5.5, 30°C while the time was 72–96 h. The conditions were: pH 4.5, 35°C and 72–96 h when using *Kluyveromyces* yeast for fermentation. Fermentation of Jerusalem artichoke tubers by bacteria *Zymomonas mobilis* gave 86.11% and 90.02% theoretical yield of ethanol after acid and enzymatic hydrolysis of inulin, respectively. The distillery yeast *Saccharomyces cerevisiae* or *Kluyveromyces* yeast used for the mash fermentation allowed obtaining lower ethanol yield (80–84.15% of theoretical yield).

INTRODUCTION

A great attention has been given to search for high yielding raw materials for ethanol production [Barta, 1993]. Jerusalem artichoke (*Helianthus tuberosus* L.) is a rich source of carbohydrates. Jerusalem artichoke contains 11–20% of carbohydrates where 70–90% is inulin and inulids. Inulin consists of linear chains of about 35 D-fructose residues (inulids consist of less than 30 residues) linked in the β (2→1) position. This chain is terminated by a D-glucose residue linked to fructose by an α (1→2) bond [Rosa *et al.*, 1986]. Jerusalem artichoke can grow very well in poor land, shows high tolerance to frost and various plant diseases, may have high tubers (even 90 t/ha) and carbohydrates (5–14 t/ha) yield [Swanton *et al.*, 1992]. These advantages make this plant competitive to conventional food crops.

Due to its chemical composition, Jerusalem artichoke can be used comprehensively, for example the tubers can be eaten fresh or cooked, used for fructose syrups production, while the overgrown part constitutes good material for silage and forage [Barta, 1993]. Using Jerusalem artichoke tubers for ethanol production it is important to apply proper microorganisms which can efficiently and quickly ferment inulin or sugars after hydrolysis of inulin. Distillery yeast and bacteria *Zymomonas mobilis* are not adapted to correctly ferment high molecular weight β-fructosides, such as inulin [Guiraud *et al.*, 1982; Margaritis *et al.*, 1982; Sachs *et al.*, 1981]. The use of yeast with inulinase activity and high fermenting capacity would allow fermentation of Jerusalem artichoke tubers without prior hydrolysis of inulin and inulids [Margaritis *et al.*, 1981; Pekić *et al.*, 1985; Toran-Diaz *et al.*, 1985; Williams *et al.*, 1982].

The purpose of the study was to compare ethanol yield in the fermentation process of two cultivars of Jerusalem artichoke tubers: Albik and Rubik depending on hydrolysis of inulin (acid or enzymatic) and used microorganisms (bacteria *Z. mobilis*, distillery yeast, yeast with inulinase activity).

MATERIALS AND METHODS

Biological material. Jerusalem artichoke tubers of two cultivars: Albik and Rubik were used as a medium for ethanol production. They were obtained from Plant Breeding and Acclimatization Institute, National Centre for Plant Researches in Radzików, Poland. The tubers were harvested in autumn 2000 and kept frozen about 6 months at minus 15°C. The strains used in this study were bacteria *Zymomonas mobilis* 3881 and 3883, distillery yeast *Saccharomyces cerevisiae* Bc16a and D_2 and yeast with inulinase activity: *Kluyveromyces fragilis* and *Kluyveromyces marxianus*.

Media for inocula. The medium for inocula of bacteria and distillery yeast contained (g/L): glucose 80, yeast extract 10, KH_2PO_4 1, $(NH_4)_2SO_4$ 1, and $MgSO_4 \times 7H_2O$ 0.5 [Nowak, 1999]. The inocula were prepared by growing the bacteria and distillery yeast for 20 h at 30°C. The medium for inocula of *Kluyveromyces* yeast contained (g/L): malt extract 3, yeast extract 3, peptone 5, glucose 10. The yeast grew on this medium for 20 h at 32°C in a rotary shaker [Duvnjak *et al.*, 1982]. Yeast extract, malt extract and peptone was from BTL Łódź, Poland.

Media for fermentation. There were acid and enzymatic hydrolyses used to hydrolyse inulin into fermentable sugars before fermentation by bacteria and distillery yeast. Acid hydrolysis was conducted using concentrated sulfuric acid (H_2SO_4) (pH 2.0, 100°C, 60 min) [Williams *et al.*, 1982].

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Enzymatic hydrolysis was conducted with inulinase (Sigma-Aldrich, about 17 U/g) from *Aspergillus niger* (0.02 g of enzyme/1 kg of tubers, pH 5.0, 55°C, 60 min). After the hydrolysis, the pH was adjusted to 5.0–5.5 for bacteria and distillery yeast. Inulinase was not inactivated after the hydrolysis. Using yeast with inulinase activity the Jerusalem artichoke tubers were only sterilized before fermentation and the pH was adjusted to 4.5 [Margaritis *et al.*, 1981].

Fermentation. Fermentation was carried out in Erlenmeyer flasks filled with 200 g of mash from Jerusalem artichoke tubers and 10% by weight of inocula (density of cells of bacteria was 1.24×10^{10} mL^{-1}, of distillery yeast *S. cerevisiae* 4.74×10^{9} mL^{-1}, of yeast with inulinase activity *K. fragilis* and *K. marxianus* 5.0×109 mL^{-1}). Fermentation was carried out at 30°C after inoculation with bacteria or distillery yeast and at 35°C after inoculation with *Kluyveromyces* yeast [Margaritis *et al.*, 1982; Toran-Diaz *et al.*, 1985].

Analytical methods. Dry matter was determined by dry weight method and reducing sugars by the dinitrosalicylic acid method [Miller, 1959]. The ethanol concentration in the culture medium was measured after distillation by density method. The fermentation yield was expressed as percentage of theoretical yield referred to the carbo-hydrates fermented during fermentation (total amount of reducing sugars, obtained after acid hydrolysis, decreased in amount of reducing sugars determined after fermentation) and as grams of alcohol produced per 1 gram of carbo-hydrate fermented.

The statistical evaluation of the obtained results was carried out with analysis of variance (n=3, α=0.05).

RESULTS AND DISCUSSION

Jerusalem artichoke (*H. tuberosus* L.) tubers, as a rich source of carbohydrates, were used for alcohol production using different microorganisms: bacteria, distillery yeast, and yeast with inulinase activity.

Jerusalem artichoke (*Helianthus tuberosus* L.) tubers of two cultivars: Albik and Rubik used in this research, had almost identical dry matter (about 23%). Table 1 shows the content of fermentable sugars in Jerusalem artichoke tubers after the hydrolysis with sulfuric acid and inulinase. Higher contents of sugars were obtained after acid (78.15% dry matter) than enzymatic hydrolysis (18.18% dry matter). The enzymatic hydrolysis did not inactivate the enzyme, that is why the significant part of inulin could be hydrolysed just during the fermentation of mash. Sterilization process, for yeast with inulinase activity used for fermentation, marginally caused the decomposition of inulin into fermentable sugars (Table 1).

The mash from Jerusalem artichoke tubers, after acid and enzymatic hydrolysis and sterilization, was the medium for alcoholic fermentation. Figure 1 shows the influence of bacteria strain, cultivar and kind of hydrolysis of inulin before fermentation on the ethanol yield. Using bacteria *Z. mobilis* 3881 for fermentation of tubers after enzymatic hydrolysis of inulin, 86.11% and 90.02% of theoretical yield of ethanol was obtained for cultivar Albik and Rubik, respectively (Figure 1). After acid hydrolysis, bacteria fermented the medium with lower ethanol yield: 82.19% and 86.11%, respectively. The results obtained for bacteria *Z. mobilis* are similar, or in some cases lower than those achieved by Toran-Diaz *et al.* [1985] and Margaritis *et al.* [1981]. The fermentation of mashes with *saccharomyces cerevisiae* after enzymatic hydrolysis of inulin allowed obtaining from 82.19 to 84.15% of theoretical yield of ethanol (Figure 2). Lower yield was obtained for acid hydrolysed medium: 76.32% and 80.23% theoretical yield of ethanol (Figure 2) which were of about 6–33% higher than in the

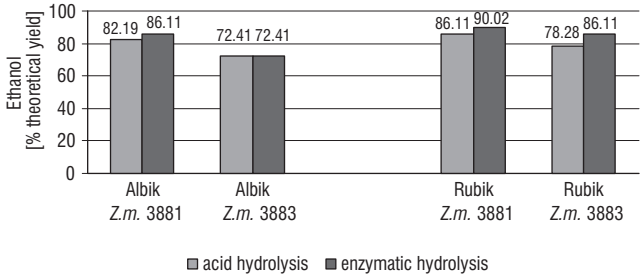

FIGURE 1. Ethanol yield from Jerusalem artichoke tubers using bacteria *Zymomonas mobils*.

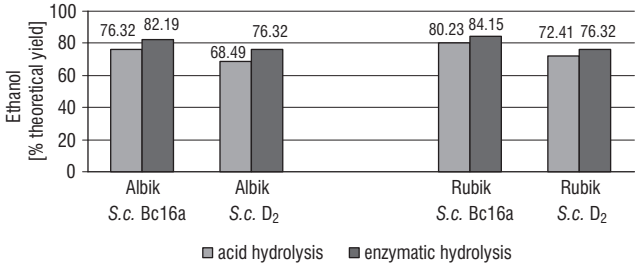

FIGURE 2. Ethanol yield from Jerusalem artichoke tubers using distillery yeast *Saccharomyces cerevisiae*.

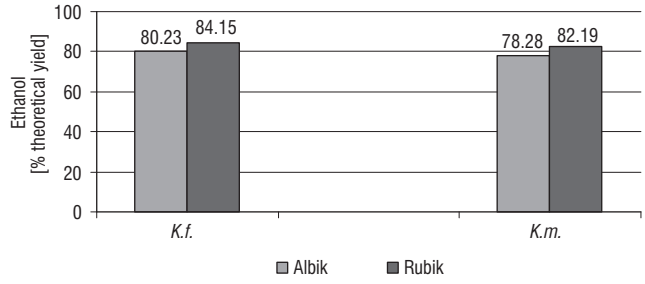

FIGURE 3. Ethanol yield from Jerusalem artichoke tubers using yeast *Kluyveromyces fragilis* and *Kluyveromyces marxianus*.

TABLE 1. Content of fermentable sugars in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers.

Cultivar	Raw tuber		Mash after					
			hydrolysis with H_2SO_4		hydrolysis with inulinase		sterilization	
	[g/L]	[% d.m.]	[g/L]	[% d.m.]	[g/L]	[% d.m.]	[g/L]	[% d.m.]
Albik	11.29	4.94	178.81	78.15	41.60	18.18	13.85	6.05
Rubik	10.51	4.62	167.60	73.64	38.78	17.04	12.48	5.48

research by Sachs *et al.* [1981] and of about 9–28% than in that of Duvnjak *et al.* [1982]. It was confirmed that the influence of hydrolysis on the ethanol yield from Jerusalem artichoke tubers is statistically significant (Table 2). The enzymatic hydrolysis of inulin, compared with acid hydrolysis, made it possible to obtain better results of alcoholic fermentation, but the mashes fermented with bacteria were usually of higher ethanol yield regardless of the hydrolysis method of inulin. From the tested bacteria, strain 3881 was more effective than strain 3883. Distillery yeast Bc16a fermented the tested samples with higher ethanol yield than yeast D_2 (Table 2).

Using yeast with inulinase activity for fermentation, similar ethanol yield was obtained as in the samples fermented with distillery yeast: 80.23–84.15% of theoretical yield (Figure 3). The presented yields are comparable with those obtained by other authors [Duvnjak *et al.*, 1982; Margaritis *et al.*, 1981, 1982] or higher than these reported by Rosa *et al.* [1986]. The advantage of using yeast with inulinase activity, was the possibility of omitting the preliminary hydrolysis of inulin into fermentable sugars.

Microorganisms used for fermentation of Jerusalem artichoke tubers utilized in 81 to about 99% fermentable sugars available in the medium (Table 2). The highest contents of utilized sugars were observed after enzymatic hydrolysis of inulin before fermentation. This could mean that the enzymatic hydrolysis is more preferable than the acid one when the medium is used for fermentation.

It should be noted that most studies published so far on alcohol production from Jerusalem artichoke were conducted on juice from tubers. The fermentation of juice is easier to handle than fermentation of mash from tubers but obtaining the juice increases the costs of the production process. The ethanol yields obtained in the research are relatively high and make the possibility of better utilization of the tubers with omission of the process of extracting the juice and developing the pulp.

The obtained results confirm high fermentation ability of bacteria *Z. mobilis* as microorganisms alternative to commonly used distillery yeast or even to yeast with inulinase activity. In spite of the fact that using Jerusalem artichoke tubers for alcohol production is known for years, recently this plant is becoming more and more popular. Jerusalem artichoke meets basic requirements for materials used in distilling of alcohol and can be more and more efficiently processed to ethanol using modern fermentation techniques.

CONCLUSIONS

1. Acid hydrolysis of inulin in mash of Jerusalem artichoke tubers allowed obtaining higher contents of fermentable sugars comparing to enzymatic hydrolysis. But the mashes after enzymatic hydrolysis were characterised by more effective fermentation and higher degree of consumed sugars.

2. During fermentation, bacteria *Zymomonas mobilis* produced ethanol with higher yield than the yeast strains used. Strain *Z. mobilis* 3881 stood out better productivity than strain 3883.

3. The enzymatic hydrolysis of inulin in mashes from cultivar Rubik and fermentation using bacteria *Z. mobilis* 3881 allowed obtaining high ethanol yield (about 90% of theoretical yield).

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TABLE 2. Fermentation of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers by selected microorganisms.

Cultivar	Process before fermentation	Microorganism	Ethanol [%,v/v]	Ethanol [g/g]	Fermented sugars [%]
Albik	acid hydrolysis	*Z.m.* 3881	9.1	0.42^{a}	95.98
		Z.m. 3883	8.2	0.37^{b}	96.84
		S.c. Bc16a	8.2	0.39^{c}	96.88
		S.c. D_2	7.4	0.35^{d}	96.94
	enzymatic hydrolysis	*Z.m.* 3881	9.9	0.44^{e}	98.71
		Z.m. 3883	7.4	0.40^{f}	81.51
		S.c. Bc16a	9.4	0.42^{a}	99.10
		S.c. D_2	8.8	0.39^{c}	99.78
	sterilization	*K.f.*	9.1	0.41^{g}	96.86
		K.m.	9.1	0.40^{g}	97.40
Rubik	acid hydrolysis	*Z.m.* 3881	8.7	0.44^{E}	93.45
		Z.m. 3883	7.4	0.37^{B}	95.21
		S.c. Bc16a	8.2	0.41^{G}	93.44
		S.c. D_2	7.4	0.37^{B}	94.34
	enzymatic hydrolysis	*Z.m.* 3881	9.7	0.46^{D}	99.61
		Z.m. 3883	9.1	0.44^{E}	96.85
		S.c. Bc16a	9.1	0.43^{A}	99.10
		S.c. D_2	8.2	0.39^{C}	99.40
	sterilization	*K.f.*	8.7	0.43^{A}	90.78
		K.m.	8.7	0.42^{A}	91.28

Z.m. – Zymomonas mobilis; *K.f. – Kluyveromyces fragilis*; *S.c. – Saccharomyces cerevisiae*; *K.m. – Kluyveromyces marxianus*. Means within columns with different letters differ significantly (α=0.05). Small letters are for cultivar Albik and capitals for Rubik.

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WPŁYW WYBRANYCH DROBNOUSTROJÓW NA WYDAJNOŚĆ ALKOHOLU ETYLOWEGO Z BULW TOPINAMBURU (*HELIANTHUS TUBEROSUS* L.)

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Przedmiotem pracy było określenie wydajności produkcji alkoholu etylowego z bulw topinamburu (*Helianthus tuberosus* L.) przy użyciu bakterii *Zymomonas mobilis,* drożdży gorzelniczych *Saccharomyces cerevisiae* oraz drożdży posiadających aktywną inulinazę *Kluyveromyces fragilis* i *Kluyveromyces marxianus*. Badania prowadzono na rozdrobnionych bulwach topinamburu dwóch genotypów: Albik i Rubik. W celu rozłożenia inuliny i inulidów bulw topinamburu do cukrów fermentujących, zastosowano hydrolizę kwasową i enzymatyczną (tab. 1). W przypadku stosowania drożdży zawierających aktywna inulinazę, proces ten nie był konieczny. Fermentacje (tab. 2) rozdrobnionych bulw z użyciem bakterii i drożdży gorzelniczych prowadzono przy pH 5,5, w temperaturze 30°C i czasie 72–96 godzin. Używając drożdży z rodzaju *Kluyveromyces* do fermentacji stosowano pH 4,5, temperaturę 35°C i czas 72–96 godzin.

W wyniku przeprowadzonych badań stwierdzono, że stosując bakterie *Zymomonas mobilis* do fermentacji rozdrobnionych bulw topinamburu uzyskiwano po hydrolizie kwasowej i enzymatycznej inuliny, odpowiednio 86,11% i 90,02% wydajności teoretycznej (rys. 1). Wykorzystując do fermentacji zacierów drożdże gorzelnicze *Saccharomyces cerevisiae* lub z rodzaju *Kluyveromyces*, otrzymywano niższą wydajność etanolu (80–84,15% wydajności teoretycznej) (rys. 2, 3).