Sugar Beet as a Raw Material for Bioethanol Production

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Abstract

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Overproduction of sugar causes a reduction in the acreage under sugar beet. That is why new non-food technologies for exploitation of agricultural products are sought. Utilization of beet for liquid fuel production could be one of them. The aim of experiments with sugar beet raw juice fermentation was to verify the possibility to return a part of distiller's slops back to the fermentation process and thereby to obtain stillage with higher content of dry solids. This would bring about energy savings during slops thickening and drying. Tests with recycling of different portions of stillage (20, 25 and 30%) back to the fermentation stage were carried out. No significant increase in dry solids content in mash was found and therefore no energy savings during thickening can be expected. The only savings can be made in water consumption that is replaced by slops.

Keywords: sugar beet; fermentation; bioethanol; ETBE; MTBE; *Saccharomyces cerevisiae*; stillage recycling

The Czech Republic provides favourable conditions for sugar beet cultivation approximately on 120 thousand hectares of arable land. Sugar beet is cultivated in the most fertile regions where it is impossible to leave the land unused. Considering an average sugar yield of 5 t per ha, the production of 600 thousand tons of sugar would be possible. This amount is by 150–180 thousand tons higher than the domestic consumption of sugar (LOUČKA & KADLÍK 1994; ADAMEC *et al*. 1999). Such quantities of sugar cannot be exported to other countries due to the customs barriers and legislation and that is why the area of arable land under sugar beet is reduced every year (ADAMEC *et al*. 1999).

For these reasons new ways of using agricultural products outside the food industry are sought. A solution to this problem could be a possibility to use agricultural residues as renewable energy sources (RES). This question is obviously connected with the situation on the market with fossil fuels. The biomass yield would be necessary to increase by 50–100% to be competitive with fossil fuels (HUGHES & WILTSEE 1995). For the future it is possible to consider that rising prices of crude oil together with strong efforts to improve technologies for biofuel production could equalize the prices of fossil fuels and biofuels. Very important is also the legislative and economic support of biofuel production followed by research maintenance (WALKER 1995).

European Commission published the White Paper on RES in November 1997. In this document the Commission sets the goal of doubling the proportion of RES from 6 to 12% by 2010 (COM 599 of 26.11. 1997). The document also involves a 5% mandatory proportion of RES in engine fuels by 2005. Just for a comparison, the current proportion of RES in the Czech Republic is 0.5% (MELZOCH *et al*. 1999).

All these aspects, including the endeavour of the Czech Republic to approach the European Community countries and their legislation, imply that the Czech Republic will have to be more concerned in the increasing use of RES.

The idea to use ethanol as a source of energy is not new. The oldest evidence about alcohol used as an engine fuel comes from 1890. Between the world wars about 4 million cars used gasoline blended with 25% vol. of ethanol (CHEREMINISOFF 1979).

Ethanol can be used as a versatile fuel, energy source, either in pure state or after blending with gasoline or diesel oil. Ethanol has very acceptable properties as low toxicity for humans, low volatility, low evaporation and

photochemical activities and high heat of vaporization (threefold higher than gasoline) (U.S. Department of Energy 1997; WYMAN 1996). A high octane number of ethanol, namely 140, is another advantage. On the other hand, fuel efficiency of alcohol is lower than that of gasoline and 1 l of gasoline has to be compensated by 1.2–1.5 l of alcohol (TREU 1996). Another disadvantage of fuel blending with ethanol is the increased corrosion of inner parts of fuel tank and engine, which needs to use more resistant materials (Novo Nordisk 1985). Gasoline blending with ethanol up to 5% vol. does not require any constructional adaptations of the engine (TREU 1996; Community Position EC No. 39/97, 1997), up to 10% vol. improves the octane number by 3–4 units on average (Novo Nordisk 1985). The blend above 20% vol. of ethanol requires a carburettor adaptation. Another possibility how to use nonaqueous ethanol is its chemical modification on ETBE (ethyl tertiary butyl ether) (DIANA *et al.* 1998). 10% of gasoline blending with ETBE equals 4.5% of neat ethanol. ETBE can be used as a substitution of MTBE (methyl tertiary butyl ether). The original use of MTBE was to replace lead as an octane-enhancing additive. In comparison with ETBE, MTBE has higher toxicity and more negative effects on the environment due to its slow biodegradability (STEFFAN *et al.* 1997; JAVANMARDIAN & GLASER 1997; MO *et al.* 1996; KELLER *et al.* 1998). MTBE has a higher residence time in the environment. In contrast, ethanol is a naturally occurring intermediate produced during the fermentation of organic matter and is expected to rapidly biodegrade in essentially all environments with conditions (i.e. temperature, pH, and pressure) that support microbial activity (SHIBLOM *et al.* 1994; ULRICH 1999). Other positives of ETBE are high value of octane number, anti-knock properties, lower corrosive impacts on the engine and lower toxicity than in MTBE (JOHANSON *et al.* 1995; WALKER 1995; GERHARTZ 1987; WYMAN 1996). Replacement of 15% of gasoline would reduce carbon dioxide emissions by one half and unburned carbohydrates by one third. The major environmental concern about the increased use of ethanol fuels is an increase in emissions of reactive aldehyde (DIANA *et al.* 1998). Data on 10% ethanol/gasoline blend indicate acetaldehyde levels more than twice as high as those from gasoline engines and formaldehyde levels higher about 30%. The emissions of carbon monoxide and volatile organic compounds show a decrease (WYMAN 1996).

Besides the above-mentioned power reasons there are a lot of factors which support bioethanol production:

– Bioethanol program can solve problems with overproduction of agricultural products.
– Optimal utilization of agricultural potential, better exploitation of agricultural production and arable land in marginal regions with less favourable climatic conditions.
– Maintenance of a certain level of employment in agriculture due to an increase in acreage.

– Lower dependence on crude oil import.
– Utilization of waste material from bioethanol production that has high values of BOD and COD as a fertilizer or feeding mixture and therefore restriction of its treatment in sewage water. It would improve the economics of the whole technology.

The negatives issue from a disproportion in the prices of renewable and fossil fuels. It results from the fact that the charges for elimination of negative effects on the environment connected with their exploitation and mining are not incorporated in the price of fossil fuels. This should be solved by a complex system of ecological taxes which would not affect the incomes of the state budget (ŠAFAŘÍK 1999).

Some debates about the biofuel problems have been focused on the energy balance question. In literature it is possible to find a number of studies comparing the energy content (heat or combustion) of ethanol products and by-products with that of biomass and fuels consumed in the production process.

Roughly speaking, it is possible to express the energy balance, i.e. total efficiency of ethanol production, as a ratio of energy input in products and energy costs for its production (DOBRZYCKI 1992).

$$E = \frac{\text{energy input in products (including by-products)}}{\text{energy consumption (treatment and production)}}$$

It is necessary to mention that different authors base their calculations on different premises and that is why they publish completely different data. Naturally, the ratio *E* differs for different raw materials. For instance TREU (1996) indicates that energy needed for bioethanol production is higher than energy that can be obtained by ethanol combustion and the only advantageous raw materials are sorghum, cassava or molasses.

Many papers have been focused on the total energy effectiveness of bioethanol production from sugar beet. It is considered that energy needed for sugar beet processing to obtain ethanol is equal to 25–50% of the energy cost of produced ethanol. The costs depend on the technique of treatment and that is why it is possible to find variant numbers qualifying the total efficiency in a wide range from 67% (loss) to 300% (very optimistic estimation) (DOBRZYCKI 1992).

From an economic point of view and in comparison with cereals, sugar beet and intermediates from beet processing are very good raw materials for alcohol production due to their content of fermentable sugars which can be directly used for fermentation without any modification.

Molasses is a traditional raw material for distilleries in the Czech Republic and about 90% of ethanol production comes from this raw material nowadays. Molasses production has decreased every year since 1997 and the estimated last year's production of 118 thousand tons is not sufficient for domestic alcohol production. This insuffi-

ciency was partly covered by an increased import of 34 508 t in 1998/99 (ADAMEC *et al.* 1999).

Sugar beet – Disadvantage of direct beet and beet pulp fermentation is a slow release of sugars from pulp into the fermented solution. The second aspect is a sort of problematic storability of beet that brings about sugar loss due to enzyme action (BERGHALL *et al.* 1997; KUNTEOVÁ 1997).

Raw juice contains about 15–20% of dry solids. Raw juice purity ranges between 85 and 90% that means there are about 85–90% of sugars and 10–15% of nonsugars in dry matter. Considering these facts, raw juice can be used straightaway after pH adjustment for fermentation. All these properties together with a relatively low price in comparison with other intermediates from beet processing make the raw juice a very profitable material for alcohol production. Its only disadvantage is low storability (HINKOVÁ *et al.* 2000) and easy decomposition by the action of microorganisms.

Contrary to molasses, raw juice contains all nonsugars that are usually removed by purification process in further steps of sugar beet processing. Hence, these nonsugars remain in a broth and after the fermentative process pass to stillage making its composition very suitable for addition into fodder. Additional advantage is a low content of inorganic salts (especially potassium) (HINKOVÁ *et al.* 2000). Another suggestion how to decrease the volume of stillage from juice fermentation is based on a recycling of the part of distillation residue for sugar extraction from the raw material (GUTSCHIREITER 1987).

Thin juice is very suitable for ethanol production but the biggest disadvantage is a very small or hardly any possibility of its storage because the concentration of sugars is almost ideal for microorganism growth. For these reasons it is necessary to supply the distillery very fluently with this material, which requires a contact between the distillery and the sugar plant.

Thick juice is a relatively pure and highly concentrated sugar solution (RDS content 60–65%, polarization 55–65%, purity 90–95%) that is obtained by the concentration and thickening of thin juice on evaporators. This eliminates problems with storability that is comparable with molasses. On the other hand, the production of thick juice is very complicated and expensive, and it consequently influences the ethanol price too (KUNTEOVÁ 1996).

MATERIAL AND METHODS

Analytical Methods

– Refractometric dry solids (RDS) content (= saccharization) – measured directly on digital refractometer ABBEMAT (Dr. Kernchen, Germany). Results are expressed in % (w/w).

– Polarization – determined on polarimeter SUCROMAT VIS/NIR (Dr. Kernchen, Germany) at the wave length 589 nm after precipitation of diluted sample (13 g of sample filled with distilled water in 100 ml volumetric flask) with Herles I and II agents and filtration.

– Purity – calculated as a ratio of polarization to RDS multiplied by 100.

– Content of sucrose and invert sugars (= sum of glucose and fructose) – analysed on HPLC with autosampler AS 54 (Ecom s.r.o., Czech Republic), column filling: ionex OSTION LG KS 0800 Ca^{2+}, mobile phase: demineralized water with flow rate 0.5 ml/min at the temperature 80°C, compact glass cartridge columns for impurities interception were implemented: HEMA BIO 1000 Q (3 × 30 mm), HEMA BIO SB (3 × 30 mm) (Tessek Separon, Czech republic), sample dosing was provided with autosampler AS 54 (Ecom s.r.o., Czech Republic), detection was by refractometer RIDK 101 (Laboratorní přístroje Praha, Czech Republic), data collection, integration and evaluation were done by computer program CSW 17 (Data Apex, Czech Republic).

– pH – measurement of diluted sample (13 g of sample filled with distilled water in 100ml volumetric flask) on apparatus THERM 2290-3 (ALMEMO, Germany) with the glass electrode.

– Ethanol – in fermentation broth and thereby the ethanol yield was determined together with sugars on HPLC under the above-mentioned conditions. Ethanol concentration in the distillate was measured by a specific-gravity bottle.

– Ethanol yield – expressed as a percentage of theoretical yield that would be achieved if all present sugars were absolutely transferred to ethanol and carbon dioxide.

– Ash – conductivity measurement of diluted sample (5 g of sample into 100 ml of distilled water) at the temperature 20°C. The conductometer was OK-104 (Radelkis, Hungary). The amount of soluble ash (A) is calculated from the formula (FRIML & TICHÁ 1986):

$$A = 0.0018\,a\ (b - c) \times\ (0.9 + S/1000)$$

where: t – temperature of measured solution (°C)
b – conductivity of solution (mS/cm)
c – conductivity of distilled water used for sample dilution (mS/cm)
S – refractometric dry solids (saccharization) of the sample (% w/w)
a – a correction factor for temperature obtained from the formula:

$$a = 1/1 + 0.023\ (t - 20)$$

– Anion and cation content – measured on isotachophoretic analyzer IONOSEP 900.1 (Recman, Czech Republic).

Raw Material

Samples of sugar beet raw juices were taken from sugar factories Bašnice and Hrochův Týnec during the sugar

Table 1. Composition of raw juice concentrates

Analytical quantity	Raw juice concentrate		
	Bašnice	Hrochův Týnec	Dobrovice
Saccharization (% w/w)	66.49	69.97	69.38
Polarization (% w/w)	61.28	47.54	56.04
Sucrose content* (% w/w)	58.98	56.51	–
Purity* (% w/w)	88.71	80.76	80.77
pH	6.7	4.22	6.26
Reducing sugars (% w/w in RDS)	2.84	11.87	–
Cation composition (% w/w in RDS)			
NH_4^+	0.02	0.04	–
K^+	0.68	0.75	–
Na^+	0.05	0.06	–
Ca^{2+}	0.11	0.14	–
Mg^{2+}	0.08	0.12	–

*calculated and determined by HPLC

campaign 1996/1997 and sugar plant Dobrovice in the campaign 1997/1998. Juices were concentrated on a climbing film evaporator (Armfield, England) and stored for a period of 4 months. Table 1 shows the composition of concentrates before dilution and fermentation.

Raw juice from the sugar plant Hrochův Týnec had a very low value of pH that influenced invert sugar formation by sucrose hydrolysis. This higher invert sugar content also influenced the value of polarization that is apparently lower than the real sucrose content determined by HPLC. That means during the thickening of raw juice of lower pH, the inversion of sucrose is higher and consequently the purity is reduced. Such a concentrate could be used readily for fermentation where all sugars are fermented. However, this way is not acceptable for crystallization to obtain sugar where sugar losses would be higher and that is why pH should be adjusted (increased) before treatment.

Microorganisms

– Dried yeasts *Saccharomyces cerevisiae*, strain DF 639, were used for fermentation to ethanol. The yeasts (produced by SIHA, Germany) are enriched with nutrients and their biggest advantage is they do not need be propagated before application.
– French commercially produced bakery yeasts *Saccharomyces cerevisiae*.
– Distillery yeasts *Saccharomyces cerevisiae* from the Culture Collection of the Department of Fermentation Chemistry and Bioengineering were maintained on malt agar slants at 4°C. The inoculum was grown on complete medium at 30°C for 24 hrs in Erlenmeyer flasks placed on the rotary shaker.

Fermentation with Three Different Types of Yeasts
Saccharomyces cerevisiae

To compare the overall fermentation efficiency and osmotic tolerance of different yeasts, different fermentation media from concentrates from Bašnice and Hrochův Týnec (Table 1) were prepared. Raw juice concentrates were diluted with water to the resultant concentration of fermentable sugars 16% (Series B-16) and 20 % (Series B-20, HT-20). Fermentation with a higher concentration of fermentable sugars in the medium (25%) was carried out as well (Series HT-25). That means four different fermentation media were prepared (Table 2) and each inoculated with 3 different types of yeasts. Thus 12 experiments were carried out (Table 3). Before fermentation, pH of mash was adjusted with 10% sulphuric acid (v/v) and 10% sodium hydroxide (w/w) to the value of 5. Each Erlenmeyer flask contained about 500 g of mash. The addition of yeasts was about 1.2 g of yeast dry solids per 1000 g of mash. Mashes were analysed before and after fermentation for sugar and ethanol content (Table 3).

Table 2. Composition of mash for fermentation with different kinds of yeast *Saccharomyces cerevisiae*

Analytical quantity	Mash designation			
	B-16	B-20	HT-20	HT-25
RDS (% w/w)	17.30	21.64	23.56	28.94
Amount of fermentable sugars (% w/w)	15.86	19.78	21.06	26.24

B – Bašnice; HT – Hrochův Týnec

Table 3. Comparing of mash composition before and after fermentation with different kinds of yeast *Saccharomyces cerevisiae*

Analytical quantity	Original	After fermentation			Original	After fermentation		
		dried yeast	bakery yeast	distillery yeast		dried yeast	bakery yeast	distillery yeast
Bašnice		B-16				B-20		
Weight of mash (g)	500.00	459.50	462.10	462.20	500.00	451.40	456.90	451.40
RDS (% w/w)	17.30	5.69	5.77	5.87	21.64	7.52	6.77	7.51
Polarization (% w/w)	15.94	0.30	0.44	0.42	19.92	0.26	-0.66	0.40
Sucrose content (% w/w)	15.36	0.12	0.13	0.04	19.16	0.21	0.17	0.04
Reducing sugars (% w/w)	0.50	0.04	0.04	0.13	0.62	0.22	0.33	0.15
Amount of fermentable sugars (% w/w)	15.86	0.16	0.17	0.17	19.78	0.43	0.50	0.19
pH	5.00	4.22	4.00	3.95	5.00	4.29	4.08	3.98
Hrochův Týnec		HT-16				HT-25		
Weight of mash (g)	500.00	457.40	461.50	458.50	500.00	449.80	451.60	445.60
RDS (% w/w)	23.56	8.81	8.65	8.44	28.94	11.69	11.02	11.00
Polarization (% w/w)	14.78	0.68	0.78	0.76	18.40	0.22	0.00	0.54
Sucrose content (% w/w)	18.50	0.18	0.24	0.11	23.04	0.02	0.26	0.22
Reducing sugars (% w/w)	2.56	0.10	0.08	0.19	3.20	0.54	0.63	0.10
Amount of fermentable sugars (% w/w)	21.06	0.28	0.32	0.30	26.24	0.56	0.89	0.32
pH	5.00	4.30	4.27	4.18	5.00	4.44	4.34	4.22

Table 4. Composition of mash series F_{20} with 20% recycle of distiller's stilage

	Mash designation					
	F_{20}-0	F_{20}-1	F_{20}-2	F_{20}-3	F_{20}-4	F_{20}-5
Amount of mash component (g)						
Raw juice	440.6	143.3	139.6	110.9	111.0	114.5
Water	1259.6	297.3	300.4	329.4	329.9	325.7
Stillage	0.0	110.6	110.1	110.1	110.6	110.0
Total weight	1700.2	551.2	550.1	550.4	551.5	550.2
Amount used for fermentation	500.6	500.6	500.3	500.3	500.6	500.3
Composition of mash						
RDS (%)	18.89	16.98	16.03	14.91	15.36	15.46
Fermentable sugars (%)	16.62	16.34	15.58	14.44	15.03	15.08
Ash (%)	0.40	0.35	0.50	0.47	0.48	0.46
pH	4.73	4.86	4.93	4.85	4.65	4.52

Fermentation ran under anaerobic conditions for 60 hrs at a temperature of 30°C in 1000ml Erlenmeyer flasks. After fermentation, the yeasts were separated by filtration with addition of approximately 1% of kieselguhr on a pressure filter ZTF1.2 (Mikropur, Czech Republic). The liquid part was distilled in Claisen condenser. The fractions up to a temperature of 97°C were collected.

Recycling of Distiller's Slops Back to the Fermentation Stage

The medium was prepared by dilution of raw juice concentrate Dobrovice (Table 1) with water and addition of distillation stillage from the previous fermentation. The amount of added distiller's slops was 20, 25 and 30% of the total mash weight. The ratio of water to juice concen-

Table 5. Composition of mash series F_{25} with 25% recycle of distiller's stilage

	Mash designation					
	F_{25}-0	F_{25}-1	F_{25}-2	F_{25}-3	F_{25}-4	F_2-5
Amount of mash component (g)						
Raw juice	440.6	141.8	135.1	110.4	110.3	110.6
Water	1259.6	271.1	278.3	303.4	302.9	302.7
Stillage	0.0	138.0	137.6	138.0	137.7	137.6
Total weight	1700.2	550.9	551.0	551.8	550.9	550.9
Amount used for fermentation	500.6	500.5	500.0	500.2	500.6	500.2
Composition of mash						
RDS (%)	18.89	16.94	16.19	14.97	14.91	14.78
Fermentable sugars (%)	16.62	17.03	16.44	15.20	15.35	15.24
Ash (%)	0.40	0.50	0.42	0.41	0.45	0.43
pH	4.73	4.71	4.89	4.90	4.53	4.70

Table 6. Composition of mash series F_{30} with 30% recycle of distiller's stilage

	Mash designation					
	F_{30}-0	F_{30}-1	F_{30}-2	F_{30}-3	F_{30}-4	F_{30}-5
Amount of mash component (g)						
Raw juice	440.6	126.6	128.3	110.3	109.8	110.8
Water	1259.6	234.1	258.2	274.9	275.1	275.6
Stillage	0.0	152.0	164.9	165.2	165.6	165.3
Total weight	1700.2	512.7	551.4	550.4	550.5	551.7
Amount used for fermentation	500.6	487.5	500.1	500.1	500.3	500.2
Composition of mash						
RDS (%)	18.89	16.17	16.78	16.01	15.78	15.17
Fermentable sugars (%)	16.62	17.13	17.02	15.06	15.11	15.48
Ash (%)	0.40	0.44	0.53	0.47	0.48	0.49
pH	4.73	4.85	4.81	4.76	4.79	4.77

trate was chosen according to the resultant concentration of fermentable sugars which ranged between 14.5 and 17.0% (w/w). Tests ran in three parallel series designated F_{20}, F_{25} and F_{30} with different amounts of stillage in fermentation broth (Series F_{20}-20%, F_{25}-25% and F_{30}-30% of stillage). Totally five consecutive fermentation steps (designated F1–F5) were carried out in each series. The composition of mashes and the amount of all components are shown in Tables 4–6.

About 500 g of medium was transferred into Erlenmeyer flasks (1000 ml in volume) and inoculated with dried yeasts *Saccharomyces cerevisiae* at an amount of 0.6 g per kg. Fermentation ran in thermostat under anaerobic conditions at a temperature of $29 \pm 1°C$ for 72 hrs. Alco-

hol was distilled off and the slops were used for concentrate dilution in the following step.

RESULTS AND DISCUSSION

Fermentation with Three Different Types of Yeasts
Saccharomyces cerevisiae

After fermentation with different types of yeasts, the mashes were analysed for RDS, polarization, sucrose, reducing sugar and fermentable sugar content (Table 3). It is evident from a comparison of analytical values of mashes before and after fermentation that with the higher initial level of fermentable sugars in the medium, the value of residual sugars is also higher. That means the yeasts
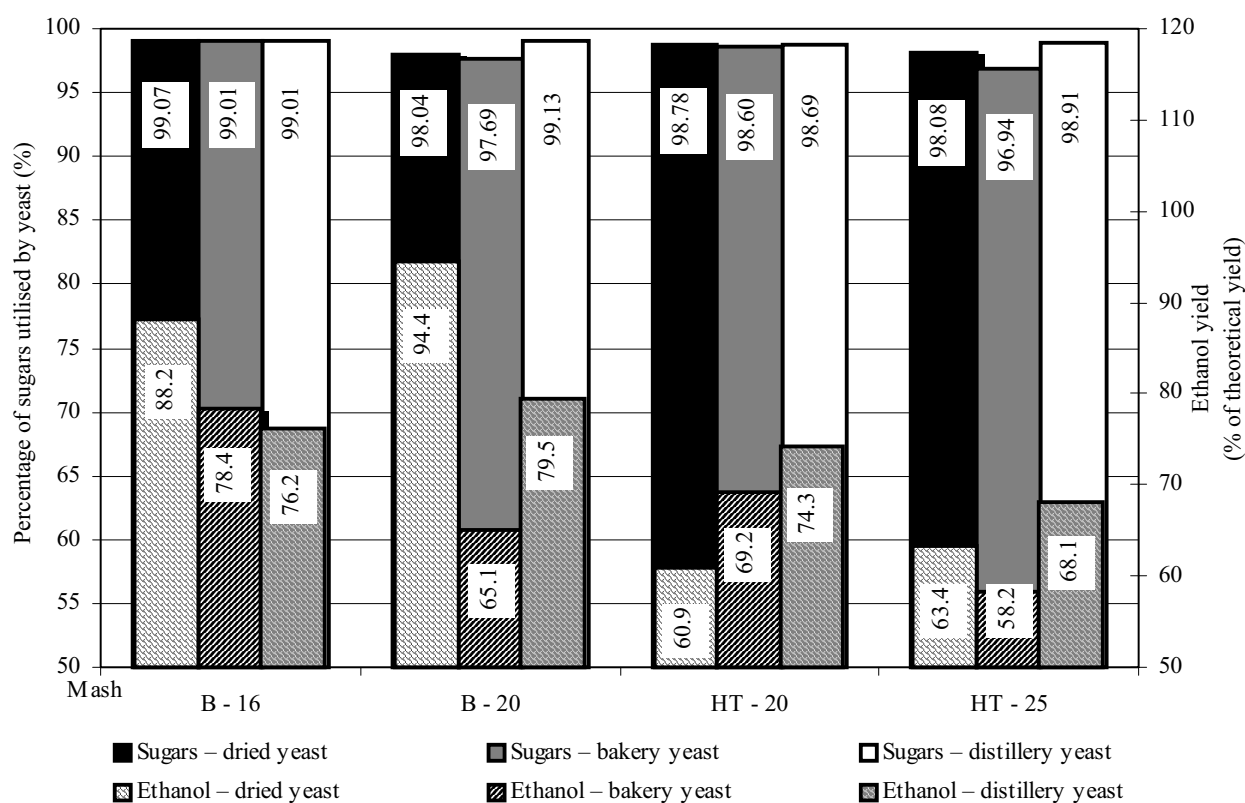
Fig. 1. Efficiency of fermentation using different yeast *Saccharomyces cerevisiae*

were not capable to exploit all sugars due to increasing osmotic pressure.

This phenomenon is also confirmed by comparing the percentage of utilized sugars that was calculated as a ratio of residual to entering sugars multiplied by 100 (Fig. 1). Mashes with the initial value of fermentable sugar up to 20% (Series B-16, B-20, HT-20) had the residual amount of sugars lower than 0.5% (w/w). In all mashes sugars were exploited at least from 97–99%. Naturally, these facts also influenced the ethanol yields. Generally, bakery yeasts gave lower yields than the other two types of yeasts. In broth with the initial lower level of sugars, dried yeasts gave more ethanol than distillery yeasts. However, distillery yeasts showed higher tolerance to osmotic pressure and provided higher yields in mashes with the initial higher amount of sugars. Beyond this fact, it is also obvious that the entering raw juice influenced the exploitation of sugars and thereby the ethanol yield (Fig. 1). Comparing the ethanol yields of mashes with the initial comparable content of fermentable sugars (Series B-20, HT-20) it is seen that fermentation of raw juice "Hrochův Týnec" gave lower yields.

Regarding all these facts it was evident that another fermentation test should be carried out with dried yeast that produces reasonable ethanol yields and enables high percentage of sugar exploitation. Another advantage that cannot be omitted is an incomparably easier way of dried yeast application.

Recycling of Distiller's Slops Back to the Fermentation Stage

The aim of these experiments was to verify the possibility to return a part of distiller's slops back to fermentation and thereby to obtain stillage with higher content of dry solids. This would bring about energy savings during slops thickening and drying. Another reason of stillage recycling was to save technological water used for juice concentrate dilution and make the use of all unused nutrients possible in the following steps. The general goal was to propose and verify a new non-waste technology (FOŠENBAUER 1999). A disadvantage of stillage recycling is the concentration of some undesirable products (e.g. metabolites of fermentation, inhibitors and salts) which can negatively influence yeast activity. That is why the amount of slops which are recycled is very important and it was a subject of our investigation.

The initial mash composition in all series differs in the ratio of added stillage. An absolute amount of stillage in individual steps was almost the same. It is possible to see from the data (Table 7) that the amount of added juice

Table 7. Mass balance in all single steps of mash processing with recycling stillage (20–30 %)

Mass balance	Mash designation					
	F_{20}-0	F_{20}-1	F_{20}-2	F_{20}-3	F_{20}-4	F_{20}-5
Weight before fermentation	500.61	500.62	500.25	500.30	500.56	500.31
Weight after fermentation	462.80	464.55	479.64	467.58	470.95	468.81
Weight decrese during fermentation	37.81	36.07	20.61	32.72	29.61	31.50
Weight of distiller's slops	387.58	389.23	422.44	394.68	405.27	398.25
	F_{25}-0	F_{25}-1	F_{25}-2	F_{25}-3	F_{25}-4	F_{25}-5
Weight before fermentation	500.61	500.53	500.04	500.16	500.59	500.17
Weight after fermentation	461.92	466.44	480.35	467.53	470.63	469.56
Weight decrese during fermentation	38.69	34.09	19.69	32.63	29.96	30.61
Weight of distiller's slops	383.49	392.15	423.92	389.91	402.60	399.51
	F_{30}-0	F_{30}-1	F_{30}-2	F_{30}-3	F_{30}-4	F_{30}-5
Weight before fermentation	500.64	487.48	500.05	500.09	500.28	500.19
Weight after fermentation	463.65	456.99	482.03	468.33	470.59	469.52
Weight decrese during fermentation	36.99	30.49	18.02	31.76	29.69	30.67
Weight of distiller's slops	387.79	391.79	429.86	394.60	403.07	402.11

Table 8. Composition of mash after fermentation with stillage recycling (20–30%)

Composition after fermentation	Mash designation					
	F_{20}-0	F_{20}-1	F_{20}-2	F_{20}-3	F_{20}-4	F_{20}-5
RDS (%)	6.76	6.06	9.43	5.62	5.53	6.10
Ash content (%)	0.52	0.46	0.54	–	0.48	0.41
Fermentable sugars (%)	0.16	0.30	6.16	0.31	0.51	0.77
Ethanol (%)	8.71	8.28	5.67	7.32	6.91	7.10
Ethanol yield (%)	90.05	83.37	59.37	84.44	79.97	79.88
	F_{25}-0	F_{25}-1	F_{25}-2	F_{25}-3	F_{25}-4	F_{25}-5
RDS (%)	6.66	6.37	9.28	6.67	5.76	6.23
Ash content (%)	0.55	0.50	0.55	–	0.52	0.54
Fermentable sugars (%)	0.18	0.35	5.36	0.48	0.84	0.94
Ethanol (%)	8.71	8.30	5.61	7.30	6.87	6.91
Ethanol yield (%)	89.88	84.42	60.93	83.44	78.21	79.12
	F_{30}-0	F_{30}-1	F_{30}-2	F_{30}-3	F_{30}-4	F_{30}-5
RDS (%)	6.11	6.05	9.16	6.46	6.24	6.65
Ash content (%)	0.49	0.52	0.54	–	0.53	0.50
Fermentable sugars (%)	0.17	0.39	5.41	0.54	0.67	0.74
Ethanol (%)	8.66	7.84	5.26	7.04	6.73	6.69
Ethanol yield (%)	89.70	83.60	60.49	84.86	78.29	77.40

Table 9. Composition of distiller´s slops from fermentation with stillage recycling (20–30%)

Composition of slops	Slops designation					
	F_{20}-0	F_{20}-1	F_{20}-2	F_{20}-3	F_{20}-4	F_{20}-5
RDS (%)	4.62	4.58	8.82	4.88	4.34	4.78
Ash content (%)	0.54	0.48	0.55	0.53	0.50	0.51
Fermentable sugars (%)	0.15	0.17	6.44	0.29	0.57	0.72
Ethanol (%)	0.23	0.21	0.15	0.27	0.11	0.20
	F_{25}-0	F_{25}-1	F_{25}-2	F_{25}-3	F_{25}-4	F_{25}-5
RDS (%)	4.38	4.24	8.98	4.70	4.62	4.55
Ash content (%)	0.54	0.51	0.56	0.55	0.56	0.53
Fermentable sugars (%)	0.17	0.23	5.78	0.30	0.38	0.58
Ethanol (%)	0.21	0.21	0.12	0.15	0.10	0.21
	F_{30}-0	F_{30}-1	F_{30}-2	F_{30}-3	F_{30}-4	F_{30}-5
RDS (%)	4.93	4.33	8.04	4.66	4.72	5.24
Ash content (%)	0.52	0.51	0.56	0.58	0.56	–
Fermentable sugars (%)	0.22	0.42	8.40	0.57	0.65	0.88
Ethanol (%)	0.29	0.18	0.27	0.17	0.10	0.11

concentrate decreases in the following steps and on the contrary, the amount of added water slightly grows. Logically, it causes a slightly declining trend of fermentable sugar and dry solids content. On the other hand, the amount of ash is balanced in all steps. Considering that the amount of stillage is the same and total weight of mash too, the effect of concentration of inorganic compounds in stillage is thereby negligible.

The course of fermentation is evident from Table 7 which shows mass balance in all single steps of mash processing. The total decrease in weight slows down during fermentation (from 38 to 30 g) and indicates deceleration of fermentation process. The differences between mashes with different amounts of stillage are insignificant.

The low value of weight decrease in the third step (Mash $F_{20/25/30}$-2) was caused by a failure of thermostat when the temperature during fermentation increased up to 40°C and this temperature shock influenced the yeast activity and subsequently all results obtained in this step.

The course of fermentation was also investigated in terms of the composition of mashes in different stages of processing (Table 8). It is evident that the values of dry solids content do not show an increasing trend. That is why the presumption of mash concentration due to 20–30% stillage recycling and therefore energy savings during mash distillation were not confirmed. Similarly, the ash content did not rise either. Thus it is possible to suppose that no significant concentration of salts could be expected. On the other hand, the values of unused fermentable sugars slowly increased (omitting again the value in the third step where fermentation was not effective due to a ther-

mostat failure), which affirmed a reduction in yeast efficiency.

Comparing ethanol yields in individual steps (Table 8), it is obvious that the yield was slowly decreasing and the total decrease in the fifth step was by 10–12% lower in comparison with fermentation without stillage addition (steps $F_{20,25,30}$-0). The difference between mashes with different portions of added stillage was not significant. Comparing the values of slops (Series F_{20} = –1.76, F_{25} = –2.40 and F_{30} = –2.41) we can see that with the higher amount of added stillage the decrease in ethanol yield is faster.

In parallel, the quality of obtained distiller's slops was investigated in terms of RDS, sugar, ash and ethanol content (Table 9). The results are similar to those of the mash composition after fermentation: neither dry solids increase nor growth of ash content were not significant. Similarly, the ratio of unused fermentable sugars in slops (Series F_{25}, F_{30}) increased twice or four times (Series F_{20}) in comparison with the first step of fermentation.

CONCLUSION

The first series of experiments was focused on selection of a suitable type of yeasts and did not demonstrate any large differences between the used yeasts. The ratio of exploited fermentable sugars ranged in the interval of 97–99% for all experiments. However, a slight variation of ethanol yield values is evident. Generally, bakery yeasts gave lower yields than the other two types of yeasts. In broth with the initial lower level of sugars, dried yeasts SIHA gave more ethanol than distillery yeasts. This is

due to the presence of additional nutrients which are supplemented to commercially produced dried yeasts. A high price of dried yeasts is the main disadvantage of their use. On the other hand, it is compensated by higher ethanol yields and easy applicability to fermentation because there is no need of propagation and the yeasts can be readily added at a precise amount. For these reasons, dried yeasts SIHA were used in all further experiments.

During recycling of stillage back to the fermentation stage at an amount of 20–30% of the mash weight, one cannot expect a significant increase in dry solids content of distiller's slops and therefore any energy savings during stillage thickening. The only savings can be expected in water consumption that is replaced by distiller's slops. In this ratio of stillage return, the ethanol yields will decrease with hardly any effect on the amount of added stillage. That is why it would be worthwhile to test a higher amount of stillage recycling and its influence on the course of fermentation and ethanol yields.

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Řepa by mohla být využita pro výrobu kapalných paliv, konkrétně bioethanolu. Cílem fermentačních pokusů s řepnou surovou šťávou bylo ověřit možnosti vracení části lihovarských výpalků zpět do fermentace, a tím získat výpalky s vyšším obsahem sušiny. To by přineslo úsporu energií při zahušťování výpalků a jejich sušení. Byly provedeny testy, při nichž se recyklovalo 20, 25 a 30 % výpalků. Po pěti cyklech recyklace nebylo pozorováno žádné výrazné zvýšení obsahu sušiny a tak nelze předpokládat, že by došlo k nějakým výraznějším úsporám energie během sušení. Jediné úspory je možné očekávat ve spotřebě technologické vody, která je zčásti výpalky nahrazena.

Klíčová slova: cukrová řepa; fermentace; bioethanol; ETBE; MTBE; *Saccharomyces cerevisiae*; recyklace výpalků

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