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BIOETHANOL FROM SWEET SORGHUM

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Objective

The vegetable oil and the vegetable alcohol are environmentally friendly motor fuels for replacing crude oil. These materials are usable as motor fuels alone or as fuel additives. The bioethanol can be used as petrol additive up to 20%, the optimal ratio of petrol:ethanol is 85:15. Generally the ethanol is produced from sugar or starch by fermentation with yeasts or bacteria, following by distillation. For ethanol production the raw material should contain sugar, starch or cellulose. The production of bioethanol is a very energy consuming process (e.g. concentration, distillation of alcohol), the energy balance of the conventional bioethanol process is negative. It means that the use of bioethanol as motor fuel could be cost effective only in the case of the permanent and remarkable increase of the price of crude oil, and/or in the case of using other alternative energy sources (e.g. wastes as raw material, biogas etc.) for bioethanol production.

In Europe the main raw material of bioethanol is the beetroot, wheat, maize, in North-America it is the maize and wheat, and while in South-America it is the sugar-cane. Beside these, ethanol can be produced from cellulose-containing materials, e.g. maize stalk, forest-products wastes, grasses or sorghum.

The sweet sorghum belongs to the grass family of *Poaceae*. The most important types of sorghum are *Sorghum vulgare var. technikum*, *Sorghum vulgare var. frumentaceum*, *Sorghum bicolor L. Sorghum vulgare var. Saccharatum*, *Sorghum vulgare var. Sudenense*.

The sorghum is a warm requiring plant, Hungary lies in the northern border of the sorghum culture area. The region of south Great Plain is appropriate for the sorghum culture, the yield is 80-120 t/ha, and even in weak soils (which are unsuitable for maize production) can be achieved 60-70 t/ha green mass [1]. Other authors give 47-52 t/ha annual yield for different types of sweet sorghum.

The juice content of sweet sorghum is in the range of 60-65% [2], and by others it is near 50% [3]. The sugar content of the juice is in 15-20% range. The anaerobic fermentation results in ethanol and carbon dioxide: the theoretical alcohol yield is 51% of the sugar content, i. e. 51 g ethanol can be produced from 100 g sugar. The commonly used yeast for ethanol production is *Saccharomyces cerevisiae*.

The aim of our work was to characterise two sweet sorghum varieties (Monori and Cellu) samples by sugar and dry matter content, and to determine the juice yield and the characteristics and self-life curve of the juice. The optimal conditions of fermentation, the ethanol yield were investigated also.

Methods and Materials

Two varieties of sweet sorghum (*Sorghum bicolor* var. Monori and Cellu) were investigated.

The particle size of sample was 0.5-2 cm. after chopping. The dry material content of the chopped sorghum, juice and bagasse were determined by drying at 105°C. The sugar content was determined by refractometer (BRIX%) and by spectrophotometrically, by means of 3,5 dinitro-salicylic acid method [4], after calibration. The juice extraction from chopped sorghum was carried out with a stainless steel screw-type extruder machine, the power of the engine was 750 W, the rotary speed was 50 1/min or 98 1/min. During self-life experiments 200 g chopped sweet sorghum or 200 ml freshly extracted juice was stored at 15, 20 or 25°C in a thermostat for 12 h. Before the fermentation the yeasts were pre-fermented aerobically, in a 200 ml juice-water mixture (50-50%), with 10 g dried selected yeast (*Saccharomices cerevisiae*), at 38°C during 30 min. This pre-fermented yeast culture was used for anaerobic fermentation at 6.25% concentration. In the case of experiments carried out with non-selected Baker's yeast the concentration of yeast were 0.85, 1.25 or 1.67 m%. The anaerobic fermentations were carried out in a 500 ml continuously mixed fermenter, the temperature was either 20 °C or 30 °C. The duration of anaerobic fermentation changed between 24-72 h. The pH was adjusted to 4.5 with 1 M H_2SO_4 solution. In some cases yeast nutritive was added, containing 1 mol/dm^3 diammonium-hydrogen phosphate (DAP) and 3 mol/dm^3 urea. Investigating the effect of cellulase enzyme for the juice fermentation, 15 μl of cellulase enzyme (Cellulast 1.5L, Novozymes A/S, Denmark, 700 U/g) and 15 μl of β-glucosidase enzyme (Novozym 188, Novozymes A/S, Denmark, 250 U/g) were added to 400 ml juice.

The distillation of fermented juices was carried out in two steps, the alcohol content of the second fraction was determined by a refractometer and a gas chromatograph (Agilent 6890N-5976 GC-MS).

Results and Discussion

In the first series of experiments the characteristics of sweet sorghum varieties were determined.

Table 1. Sugar content and juice yield of different parts of sweet sorhum varieties

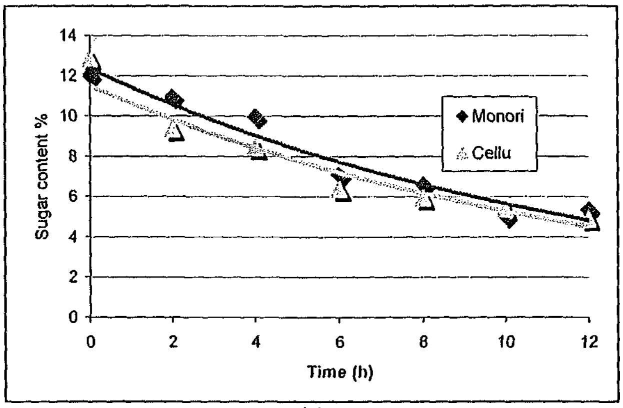
	Monori		Cellu	
	Juice yield	Sugar (m%)	Juice yield	Sugar (m%)
Chopped raw material	66.3%	12.0	61.8%	12.8
Leaves	52.3%	10.5	50.7%	10.3
Truss	31.4%	10.6	29.4%	8.5

The results show that the juice yield was higher in the case of Monori sample which can be explained by the less fibrillar structure of this sorghum variety, because the difference of the dry matter content could not cause this difference. The juice yield from leaves or truss was less, than from chopped whole plant, but the difference (concerning the mass ratio of these parts in the chopped material) was not so large, it did not give grounds for the separation of the different parts of plant.

In consideration of the extremely high microbe count of sweet sorghum, during our work the most important aspect was to minimize the loss of sugar, so the change of sugar content of harvested sorghum and juice was followed. The chopped samples were stored aerobically, at 20°C, and the sugar content of freshly pressed juice was measured in every 2 hours. The Fig. 1. shows that the sugar content both in the chopped material and in the juice decreases very rapidly, in absence of any preservation. This loss of sugar content is more than 50%. The measurements were carried out at 15°C and 25°C, in these cases the decrease of sugar content did not differ significantly from the results received at 20°C.

In the next series of experiments the alcohol yield of sorghum by use of different yeasts were investigated: wild yeast (natural flora of squeezed juice), non selected Baker's yeast (*Saccharomices cerevisiae) and* selected *Saccharomices cerevisiae* (T22 and F).

The results showed that the self-fermentation is considerable. Similar ethanol yield (30 %) was observed using either selected yeasts or Baker's yeast, but the amount of microbes was much smaller in the case of selected variant, and the yeast concentration did not affect the alcohol yield. In order to get comparable results, the next series of experiments were implemented with 0.3% selected *Saccharomices cerevisiae.*



(a)

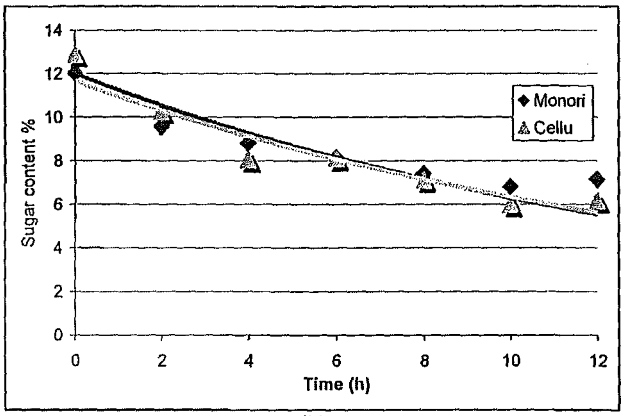


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***Figure 1.** The change of sugar content of chopped sweet sorghum (a) and the juice (b) at 20°C during aerobic storage*

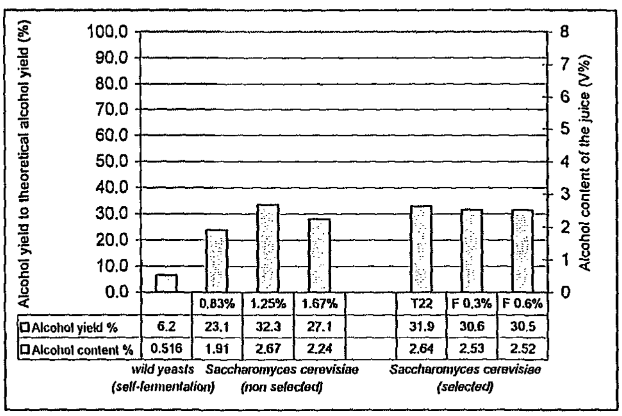
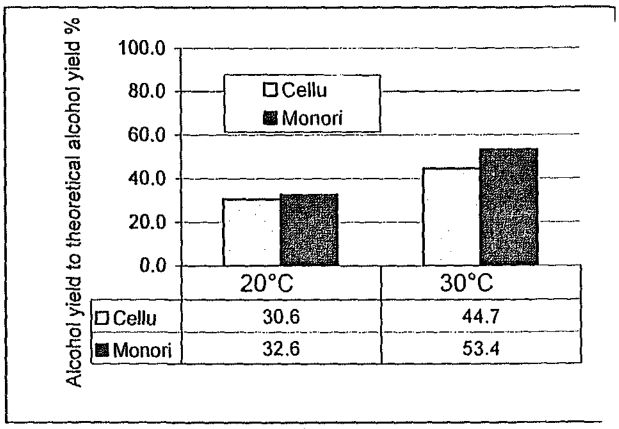


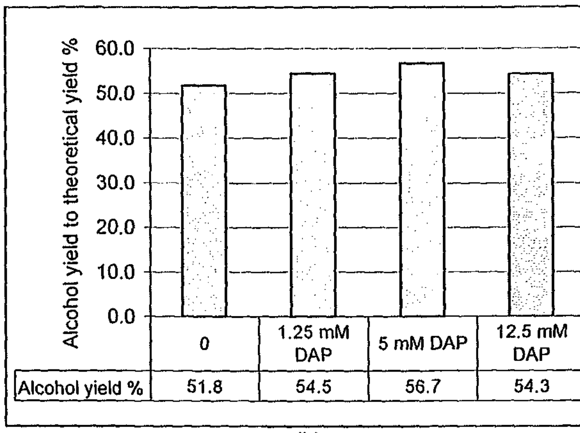
***Figure 2.** Alcohol content and alcohol yield to theoretical yield of fermented Cellu juices, the fermentation temperature was 20°C, with different yeasts applied in different concentration*

After the fermentation the product was twice distilled, and the composition of the alcohol fraction was analysed by a GC-MS. Besides the ethanol, a little amount (less, than 1% of the total alcohol content) of methanol, propanol and acetaldehyde was detected by using of Baker's yeast. In the case of selected yeasts, the ethanol did not contain any impurities.

In the next series of experiments the effect of fermentation temperature and the amount of added nutritive on the alcohol yield were examined (Fig. 3.).



(a)



(b)

***Figure 3.** The effect of fermentation temperature (a) and the amc of added nutritive (b) on the alcohol yield/theoretical alcohol yie ratio*

It was found, that the alcohol yield was higher in the cas Monori both at 20°C and 30°C as well. The fermentatio 30°C produced significantly higher alcohol yield (45-5 during 24 h fermentation time. Examining the effect of nutr on the alcohol yield, the pH was adjusted to 4.5, and DAP added to the samples in different concentrations. The re showed, that the DAP caused only a little increase in alc yield. The optimal concentration of the DAP was 5 mM.

It was examined, that the alcohol yield could be enhance adding cellulase or glucosidase enzymes, regarding tha juice contains a lot of filamentous, floating material. O basis of lignocellulose content of the juice, the theor alcohol yield increase is about 10%. (Fig. 4.)

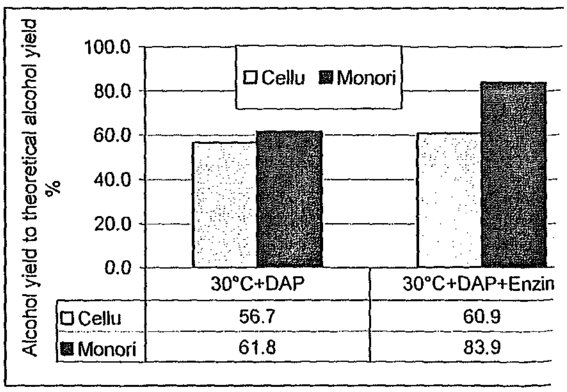


***Figure 4.** The changes of alcohol yield by addition of cellula β-glucosidase enzymes during fermentation at 30°C, pH=4.5, DAP, 24 h*

Comparing the alcohol yield from sugar only, and from sugar and lignocellulose by addition of enzymes, it was found, that the addition of enzymes considerably enhances the alcohol yield, more than it is expected theoretically on the basis of lignocellulose content, in the case of Monori instead of 60% to about 85%. It can be explained if we consider that the enzymes took part of the glucose production instead of other reaction pathways.

Conclusions

In this work the amount of fermented alcohol from sweet sorghum juice was determined, and the effect of operation parameters on alcohol yield was investigated. The juice was not pre-treated or filtered. It was found, that the maximal (theoretical) alcohol yield could not be achieved, but it can be enhanced by optimization of fermentation parameters, and adding enzymes to the juice.

Acknowledgement

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