Concentrated and stored raw sugar beet juice as a raw material for the yearlong bioethanol production

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Abstract
The concept of yearlong bioethanol production from raw sugar beet juices which are obtained in sugar factories through extraction of sugar beet cossettes with water was analyzed. Ethanol was produced from (I) fresh raw, (II) fresh raw concentrated and (III) raw, concentrated and stored for 8 months sugar beet juices. Solid substance concentration in the concentrated raw juice was approximately 70 g/ 100 g. All these juices were found to be excellent raw materials for ethanol synthesis. The raw juice fermentation yield was close to 87% theoretical ethanol productivity. Neither juice concentration nor keeping for 8 months affected ethanol production from the raw sugar beet juice. The fermentation yields for the concentrated raw juice and the concentrated juice kept for 8 months were close to 85 and 87% theoretical productivity, respectively. Fermentation of 100 dm^3 of the concentrated juice yielded around 40 dm^3 spirit 100% v/v. The concentrated raw sugar beet juice can be kept for at least 8 months and used in bioethanol synthesis all round the year.

Keywords: bioethanol, biofuels, biomass, sugar beets, raw sugar beet juice, alcoholic fermentation.

1. Introduction

Reforms to the sucrose market in EU countries resulted in considerable changes, modernization and innovations in sugar industry aiming at increased economic vitality and effectiveness of sucrose manufacturing, wider product assortment, increased competitiveness at the market and adaptation of the technology to regulations on environment protection [1,2,3]. Potential solution to problems encountered by sugar factories is production of bioethanol apart from sucrose [4,5,6]. Current concerns about the growing exploitation of fossil fuels more than a half of which is combusted in vehicle engines [7,8]. resulting in massive emissions of pollutants to the environment [9] have motivated the development of alternative fuels including biofuels derived from renewable resources [10,11,12,13]. In Europe production of biofuels is based on rapeseed and cereals while the usage of other crops has been limited [14]. Sugar beets rate among underestimated raw materials that can be converted to bioethanol. It is to note that the volume of ethanol produced from sugar beets harvested from 1 ha is roughly 2-3-fold greater compared to that derived from maize, potatoes, wheat and rye (6.62 m^3, 3.52 m^3, 3.55 m^3, 2.76 m^3, and 2.03 m^3, respectively) harvested from 1 ha [15]. The reforms to sugar market mentioned above resulted in trials on industrial bioethanol production from sugar beets in many EU countries. These trials proved that sugar factories could produce both sucrose and bioethanol [4,5,6,16,17,18]. The latter has been obtained from the sugar beet thick juice, the run-off syrup [4,6,17] and the thin juice[18]. However, some researchers proposed that also the raw juice which is rich in nutrients could be fermented to bioethanol [5,16,19]. The raw juice obtained in extractors can be divided into 2 streams. One of them can be used to produce sucrose while the second can be mixed with by-products remained after sucrose separation and fermented to ethanol. Because the quantity of raw juice could surpass the capacity of fermentation tanks a part of this juice could be concentrated and kept until used. This concept is interesting because the accumulated, concentrated raw juice could be used by sugar factories in bioethanol production not only during the campaign but all round the year. Therefore effects of concentration and long-term keeping on potential of raw sugar beet juice as a substrate for alcoholic fermentation should be estimated.

2. Objectives

The principal objective of this study was to estimate effects of concentration and storage for 8 months of the raw sugar beet juice on bioethanol synthesis. Also optimum conditions of alcoholic fermentation of worts based on 3 variants of raw sugar beet juices were determined.

3. Materials and methods

A batch of raw sugar beet juice produced in a sugar factory through extraction of cossettes was used throughout the study. The juice contained 17.2 g/100g solid substance (s.s.). It was concentrated in an evaporator to around 73 g/100g s.s. A part of the concentrated raw sugar beet juice was kept for 8 months at around 15°C. Fermentation trials were conducted by using:

I. The fresh raw juice;
II. The fresh concentrated raw juice;
III. The concentrated raw juice after keeping for 8 months.

3.1. Wort preparing

Worts subjected to alcoholic fermentation were based on:

- fresh raw sugar beet juice containing 17.2 g/100g s.s.,
- fresh concentrated raw sugar beet juice diluted to 17.2, 20.0 and 23.0 g/100g s.s.,
- concentrated raw sugar beet juice kept for 8 months and diluted to 17.2, 20.0 and 23.0 g/100g s.s.

After sterilization through autoclaving (0.4 MPa, 121°C, 20 min) the worts were acidified with sulfuric acid to pH=4.8 and either supplemented with mineral salts or not.

3.2. Preparing yeast inoculum

Processes of alcoholic fermentation were conducted by dried distillery yeast *Saccharomyces cerevisiae* race D_2. Their dose was 2 g s.s./dm^3 wort. Before fermentation the yeast were decontaminated at pH=2 for around 20 min by using 25% sulfuric acid. This process caused the death of the weakest cells (10-20% all cells) and almost completely eliminated the undesired microbial contamination.

3.3. Fermentation

Fermentation processes were conducted in 6 dm^3 flat-bottom flasks containing 4 dm^3 of a wort. A part of worts was supplemented with diammonium hydrophosphate $(NH_4)_2HPO_3$ and magnesium sulfate $MgSO_4 \cdot 7H_2O$ (0.2 and 0.06 g/dm^3, respectively).

To initiate fermentation the worts were inoculated with the yeast race D_2 (2 g s.s./dm^3). After inoculation the flasks were closed with stoppers equipped with fermentation pipes filled with glycerol and kept in a thermostated room at 29±1°C. Fermentation was controlled by measurements of a decrease in mass of the worts caused by CO_2 evolving.

3.4. Ethanol distillation

When fermentation was complete ethanol was separated by distillation by using the system consisting of 500 cm^3 distillation flask, Liebig cooler, air bath, thermometer and 100 cm^3 volumetric flask used to collect the distillate.

3.5. Analysis of raw materials and products

The raw sugar beet juices were analyzed by methods used in sugar industry [20] for: pH, solid substance (s.s.), concentrations of sucrose and invert sugar. Worts (before and after fermentation) were analyzed by methods used in distillery industry for: total extract (before fermentation), apparent extract (after fermentation), true extract (after fermentation and ethanol distillation), pH, concentrations of sucrose, reducing compounds and ethanol [21].

4. Results and discussion

4.1. Raw sugar beet juice characteristics

Results of physicochemical analyses of the fresh raw sugar beet juice and concentrated juices derived from the first one (either fresh or kept for 8 months) are shown in Table 1. Because of the high sucrose concentration the raw sugar beet juice is an appropriate substrate for alcoholic fermentation. Sucrose was assayed by polarimetric method based on measurements of an angle of polarized light plane rotation (this angle grows proportionally to sucrose concentration). A decrease in sucrose concentration during the storage of the concentrated raw juice coincided with a rise in the content of reducing sugars (table 2) which were released from pectin and sucrose. Their appearance could be also caused by the growth of unwanted microflora at the surface of juice samples. Monosaccharides such as glucose and fructose rotate the plane of the polarized light in the opposite direction compared to sucrose and thereby they decrease an output of polarimetric sucrose measurements. Despite the drop in sucrose content the juice was easily fermented by yeasts which assimilated monosaccharides. Our results indicate that batches of concentrated and stored raw juice should be subjected to detailed physicochemical and microbiological analyses. Another advantage of the raw sugar beet juice is the relatively high content of compounds other than sucrose (circa 3 – 10 g/100g s.s.) which are mostly inorganic substances assimilated by yeasts [22]. Roots of sugar beets contain circa 1.4 – 1.7 g/100g nitrogen and mineral compounds which are extracted along with sucrose and can serve as nutrients for yeasts. Their presence can decrease costs of alcoholic fermentation because supplementation of worts is not necessary.

Table 1
Physicochemical characteristics of raw sugar beet juices

	Fresh raw juice	Fresh concentrated raw juice	Concentrated raw juice after keeping for 8 months
Solid substance (g/100g)	17.2	73.2	71.0
Sucrose (g/100g)	15.4	62.5	48.5
Compounds different than sucrose (g/100g)	1.8	10.7	22.5
pH	5.7	5.7	5.6
Reducing substances (g invert/100cm^3)	0.471	3.560	7.450

Table 2
Characteristics of worts based on raw sugar beet juices before and after fermentation

	Fresh raw juice	Fresh concentrated raw juice			Concentrated raw juice after keeping for 8 months			
Solid substance in wort (g/100g)	17.2	17.2	20.0	23.0	17.2	20.0	23.0	20.0*
pH before fermentation	4.8	4.8	4.8	4.8	4.8	4.8	4.8	5.55
pH after fermentation	4.3 ±0.1	4.3 ±0.1	4.4 ±0.1	4.4 ±0.1	4.0 ±0.1	4.1 ±0.1	4.2 ±0.1	4.0 ±0.1
Apparent extract (%)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
True extract (%)	3.5 ±0.15	3.5 ±0.15	3.8 ±0.2	4.2 ±0.2	2.4 ±0.1	2.8 ±0.1	3.8 ±0.2	4.2 ±0.2
Sugars before fermentation (g sucrose/100cm^3)	15.59	15.45	17.82	20.66	14.59	16.93	19.33	16.93
Sugars after fermentation (g sucrose/100cm^3)	0.034 ±0.002	0.119 ±0.006	0.050 ±0.002	$2 \cdot 10^{-4}$ ±0.000	0.046 ±0.002	0.041 ±0.002	0.033 ±0.002	0.136 ±0.007
Reducing compounds before fermentation (g/100cm^3)	0.471	0.838	0.958	1.131	1.738	2.131	2.460	2.131
Reducing compounds after fermentation (g/100cm^3)	0.095 ±0.005	0.057 ±0.003	0.121 ±0.006	0.164 ±0.008	0.181 ±0.009	0.425 ±0.022	0.967 ±0.043	0.492 ±0.027
Reducing compounds after inversion, before fermentation (g/100cm^3)	16.88	17.10	19.72	22.88	17.10	19.95	22.81	19.95
Reducing compounds after inversion, after fermentation (g/100cm^3)	0.131 ±0.006	0.182 ±0.009	0.173 ±0.008	0.165 ±0.008	0.230 ±0.012	0.468 ±0.026	1.002 ±0.054	0.635 ±0.033
The degree of sugars assimilation (%)	99.23 ±0.19	98.94 ±0.17	99.12 ±0.18	99.28 ±0.20	98.65 ±0.16	97.65 ±0.14	95.61 ±0.11	96.82 ±0.12
Ethanol concentration (% v/v)	9.25 ±0.1	8.80 ±0.1	10.20 ±0.15	12.00 ±0.15	9.44 ±0.1	11.00 ±0.15	12.35 ±0.15	11.30 ±0.1
Ethanol yield (% theoretical yield)	87.02 ±0.92	83.54 ±0.95	83.92 ±1.23	85.17 ±1.06	85.22 ±0.9	85.11 ±1.16	83.58 ±1.02	87.44 ±0.77
Duration of fermentation (h)	45 ±1	47 ±1	50 ±1	68 ±1	95 ±2	100 ±2	145 ±2	94 ±2
20* - the wort without supplements and pH regulation								

4.2. Analysis of raw sugar beet juice-based worts

Worts used in fermentation trials were based on the following raw sugar beet juices:

- fresh raw juice taken directly from an extractor,
- fresh raw juice concentrated to circa 73 g/100g solid substance,
- the concentrated raw juice kept for 8 months.

The worts were analyzed before and after alcoholic fermentation (Table 2).

4.3. Effects of concentration and storage on fermentation dynamics

The dynamics of alcoholic fermentation of the 3 sorts of worts is shown in figures 1 and 2. Fig. 1 presents results for the fresh raw juice and the fresh raw juice concentrated to circa 73 g/100g s.s. while Fig. 2 shows results for the concentrated juice which was kept for 8 months. Duration of successive fermentation phases is displayed in Table 3.

Analysis of these data (Fig. 1 and 2, Table 3) proves that fermentation was most dynamic for the fresh raw sugar beet juice. It was complete within 45 h. The initial and main fermentation phases lasted for 3 and 21 h, respectively. The fermentation was the longest (145 h) when worts contained the concentrated raw sugar beet juice which was kept for 8 months and diluted to 23 g/100g s.s. The data shown in Fig. 1 and 2 and in Table 3 demonstrate that the fresh raw sugar beet juice is the most appropriate substrate in ethanol production in terms of fermentation time. Concentration of this juice protecting it from physicochemical changes and microbial contamination results in relatively longer alcoholic fermentation processes. Fermentation of worts prepared from the fresh concentrated raw juice was only slightly longer (by circa 5%) compared to the fresh juice-based worts. When worts were obtained from the concentrated juice which was kept for 8 months fermentation processes were 2 or even 3-fold longer (94, 145 and 45 h, respectively). Fermentation of worts containing the latter juice diluted to 17 g/100g s.s. and 23 g/100g s.s. lasted for 4 and 6 days, respectively. Both the solid substance content in the wort and the storage of concentrated juice affected the duration of initial and main fermentation phases (and thereby the time needed to complete the whole process). However, wort concentration itself does not significantly affect the rate of fermentation because the difference in duration of fermentation of worts with the same solid substance content prepared from the fresh and concentrated sugar beet juices was only 5%.

Table 3
Dynamics of raw sugar beet juices fermentation

	Raw sugar beet juices							
	Fresh raw juice	Fresh concentrated juice			Concentrated juice after keeping for 8 months			
	Solid substance contents in worts (g/100g)							
	17.2	17.2	20.0	23.0	17.2	20.0	20.0*	23.0
Duration of the initial fermentation phase (h)	3±0.5	8±0.5	3±0.5	8±0.5	10±0.5	21±1	21±1	23±1
Duration of the main fermentation phase (h)	21±1	27±1	24±1	30±1	51±1.5	52±1.5	46±1.5	75±1.5
Duration of the whole fermentation process (h)	45±1	47±1	50±1	68±1	94±2	100±2	100±2	145±2
20.0* - the wort without supplements and pH regulation								

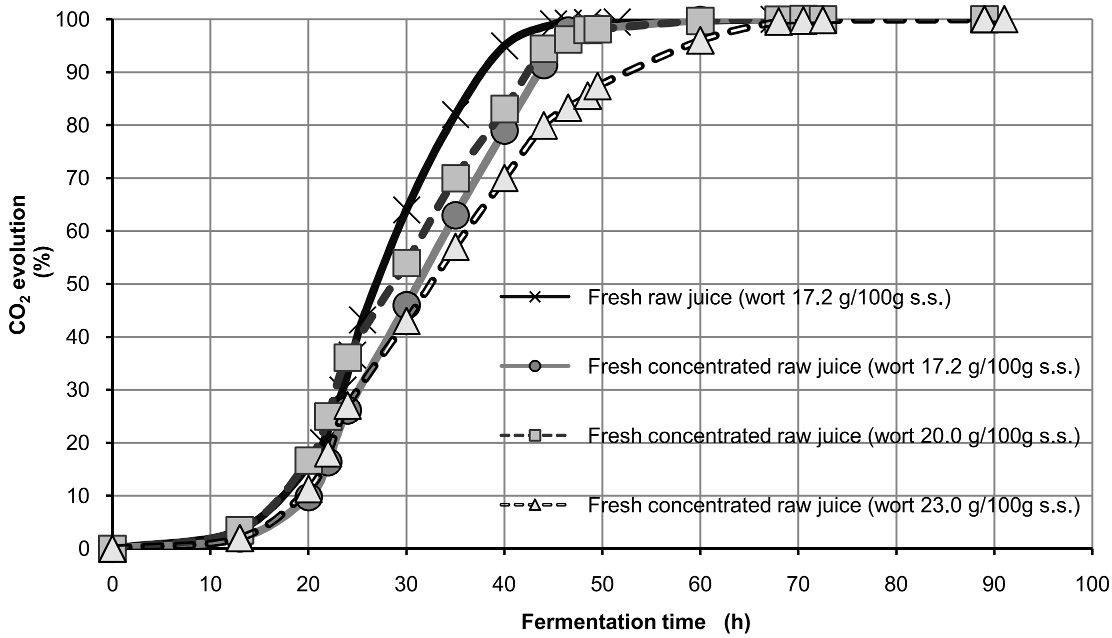

Fig. 1. Dynamics of fermentation of raw sugar beet juice-based worts

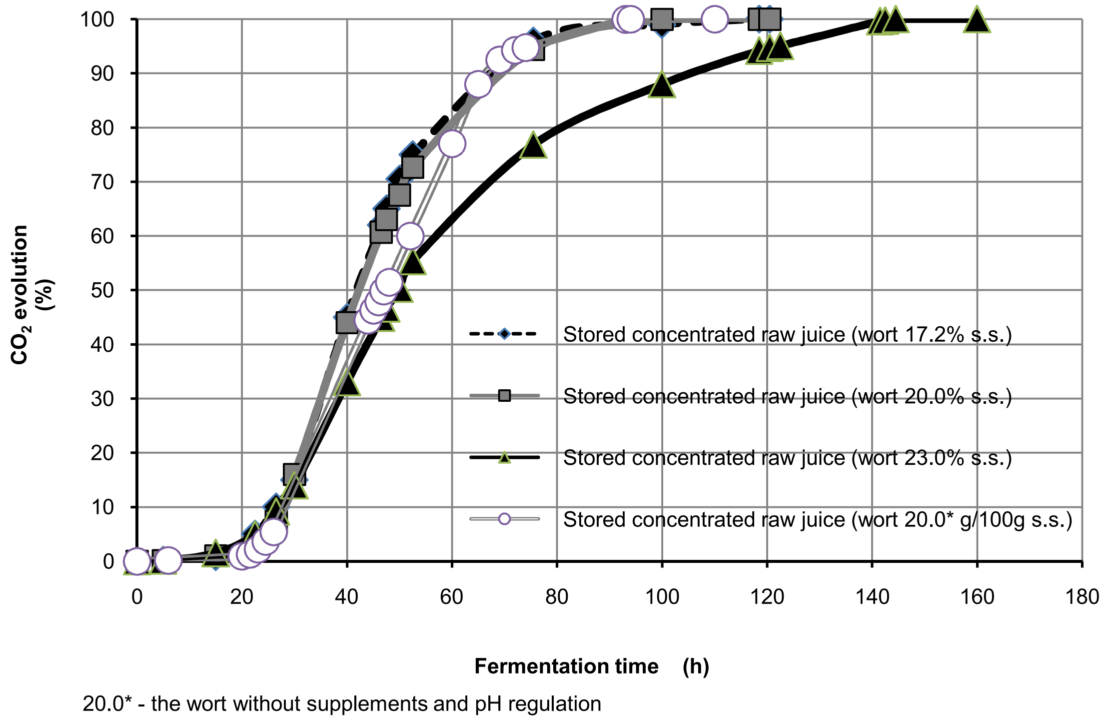

Fig. 2. Dynamics of fermentation of worts prepared from concentrated raw sugar beet juice kept for 8 months

4.4. Effects of concentration and storage on raw sugar beet juice fermentation coefficients

Worts prepared from the raw sugar beet juices were analyzed before and after fermentation processes for: apparent and true extracts, ethanol concentration, reducing compounds concentration, invert content and pH. Results which are shown in Table 2 prove that concentration and storage of the raw juice affected almost all parameters of alcoholic fermentation. The ultimate apparent extract content in worts was 0.0 g/100g s.s. (it is a true extract but its level is decreased because of ethanol presence) thereby providing evidence that all the trials were successful. The true extract concentration in these fermented worts was low and ranged between 2.4 and 4.2%. Worts prepared from the fresh raw juice and fresh concentrated raw juice were characterized by the higher content of the true extract when fermentation was finished compared to fermented worts based on the concentrated juice kept for 8 months (Table 2). A part of remaining extract which was not converted to ethanol can be used as a feed for animals [23] thereby improving the economic vitality of biofuel production. The ultimate ethanol concentration in all the worts was relatively high and varied between 8.80 and 12.35% v/v. It was the highest in worts with the greatest initial sugar concentration (Table 2). Besides, the ultimate ethanol concentration in fermented worts obtained from the concentrated and kept for 8 months, raw sugar beet juice was higher (9.44 – 12.35% v/v) compared to that in worts obtained from the fresh concentrated juice (8.80 – 12.0% v/v). This difference is thought to result from a rise in total soluble sugars being a consequence of partial hydrolysis of protopectin contained in the sugar beet raw juice and/or hydrolytic activity displayed by bacteria of the genus *Cellulomonas* contaminating the stored juice [24]. Supplementation of the worts with mineral salts only slightly affected the yield of ethanol synthesis. It is to note that the fermentation yield was higher by circa 2.5% when worts containing the concentrated and stored for 8 months, raw juice which was diluted to 20.0 g/100g s.s. prior to fermentation were supplement-free. The high alcohol yield and the lack of necessity of supplementing the worts with mineral salts render process costs relatively low and make the raw sugar beet juice an attractive raw material for bioethanol production. The lowest fermentation yield (circa 84%) and the lowest degree of sugar assimilation (96%) was observed for worts based on the concentrated raw juice, kept for 8 months and diluted to 23.0% s.s. before the process (Table 2, Fig. 3). The degree of sugar assimilation was higher in fermented worts obtained from the fresh concentrated sugar beet juice (98.94% – 99.28%) compared to that of worts derived from the juice kept for 8 months (95.61% – 98.65%). The most acceptable source of fermented sugars were the fresh concentrated and the fresh raw sugar bet juices (degrees of sugar assimilation of 99.28% and 99.23%, respectively) (Fig. 3). The highest yields of ethanol (versus the theoretical productivity) were achieved when worts were prepared from either the stored concentrated raw sugar beet juice which was not supplemented with minerals and whose pH was not adjusted before the fermentation or from the fresh raw juice (87.44% and 87.02%, respectively) (Fig. 4).

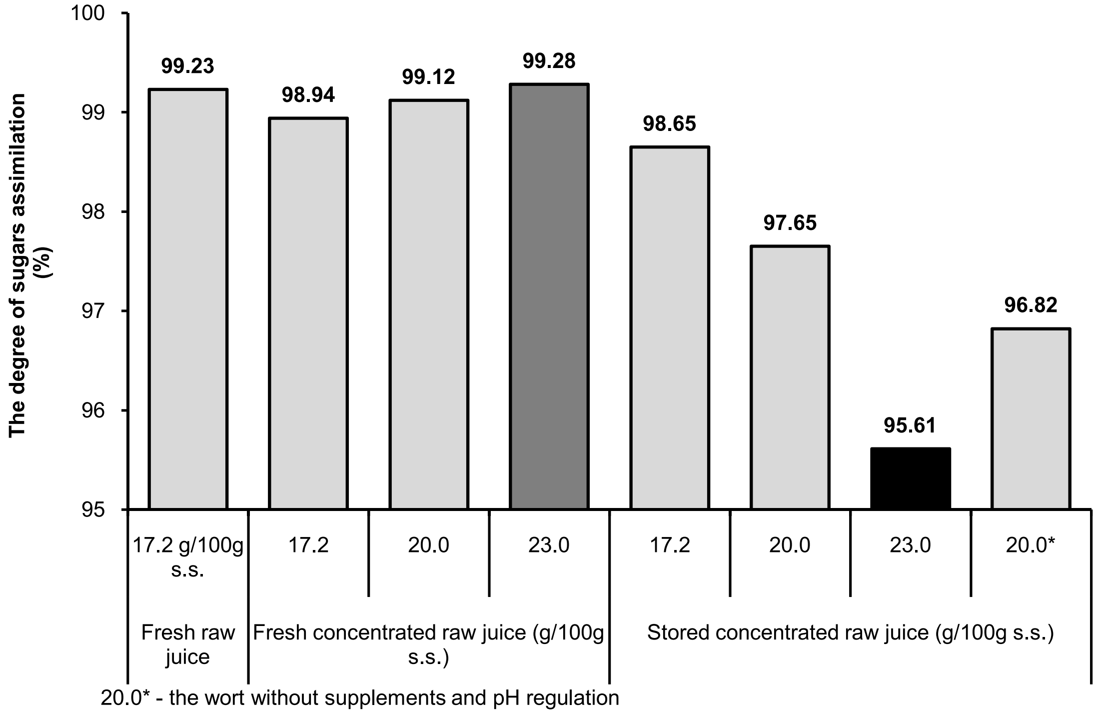

Fig. 3 The degree of sugar assimilation by yeasts

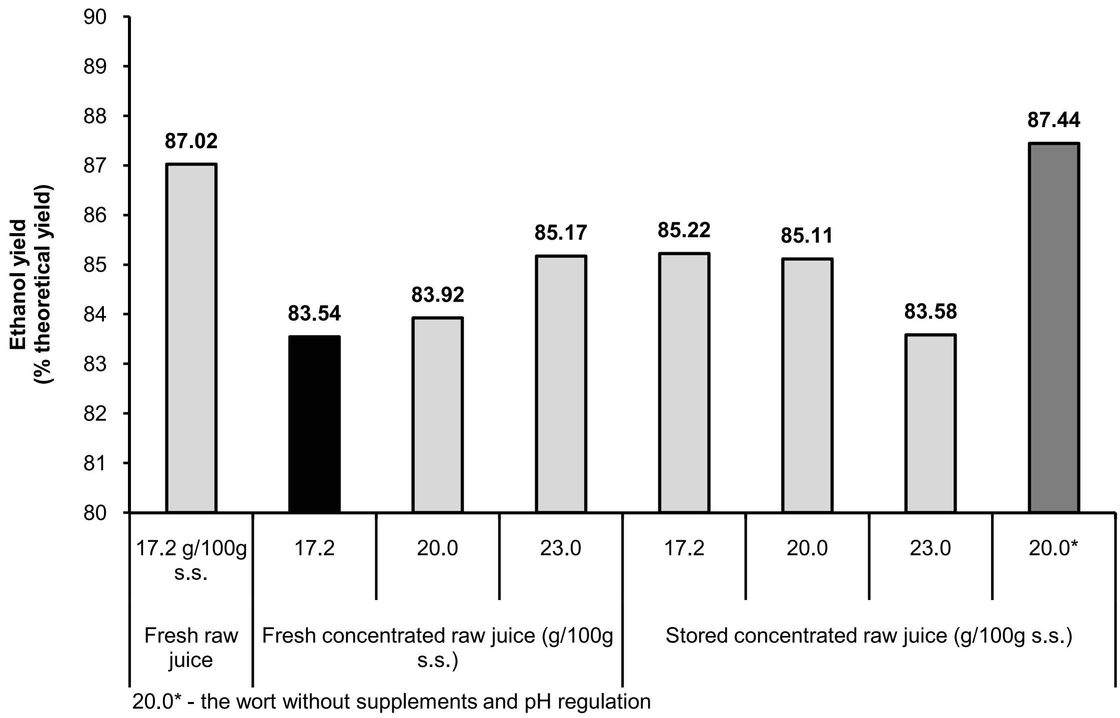

Fig. 4 Ethanol yields from worts based on raw sugar beet juices

5. Summary

The raw sugar beet juice (fresh, fresh concentrated, concentrated and stored for 8 months) was found to be an excellent raw material for ethanol production because of high concentrations of sugars and mineral compounds. This study showed that the only necessary technological operation yielding fermentation worts is dilution of the concentrated raw sugar beet juice. Results shown in table 2 and Fig. 1 – 4 provide evidence that worts based on the raw sugar beet juice need neither supplementing with mineral salts nor pH regulation. The yield of fermentation conducted by race D_2 of distillery yeast was satisfactory for all the worts and depended on the solid substance content and pretreatment of the raw juice. It was slightly higher when worts were not enriched with mineral salts and their pH was not adjusted to 4.8 before fermentation. The volume of spirit 100% derived from 100 dm^3 juice was the greatest when the concentrated raw sugar beet juice was diluted to 20.0 g/100g s.s. (Fig. 5). At this wort density 39 dm^3 spirit 100% was obtained from 100 dm^3 of the stored concentrated raw sugar beet juice supplemented with mineral salts and as much as circa 40 dm^3 (by 2.7% more) without the supplementation. The wort density of 20% s.s. was found to be optimum also for other by-products from sugar beet processing that were used in ethanol production [25]. Our experiments revealed that when the density of worts was by 3.0% higher (23.0 g/100g s.s.) the overall time of fermentation processes was increased by 26.0%. The most suitable worts contained 20.0 g/100g s.s and were based on the stored concentrated raw sugar beet juice with unregulated pH and free from supplements. Their fermentation was most dynamic (lasted for only 94 h – Fig. 2) and the ethanol yield was the greatest (87.44% - Fig. 4). Conducting of industrial bioethanol production from the raw sugar beet juice under these conditions would reduce the number of technological operations related to wort preparing and costs of supplements and acids.

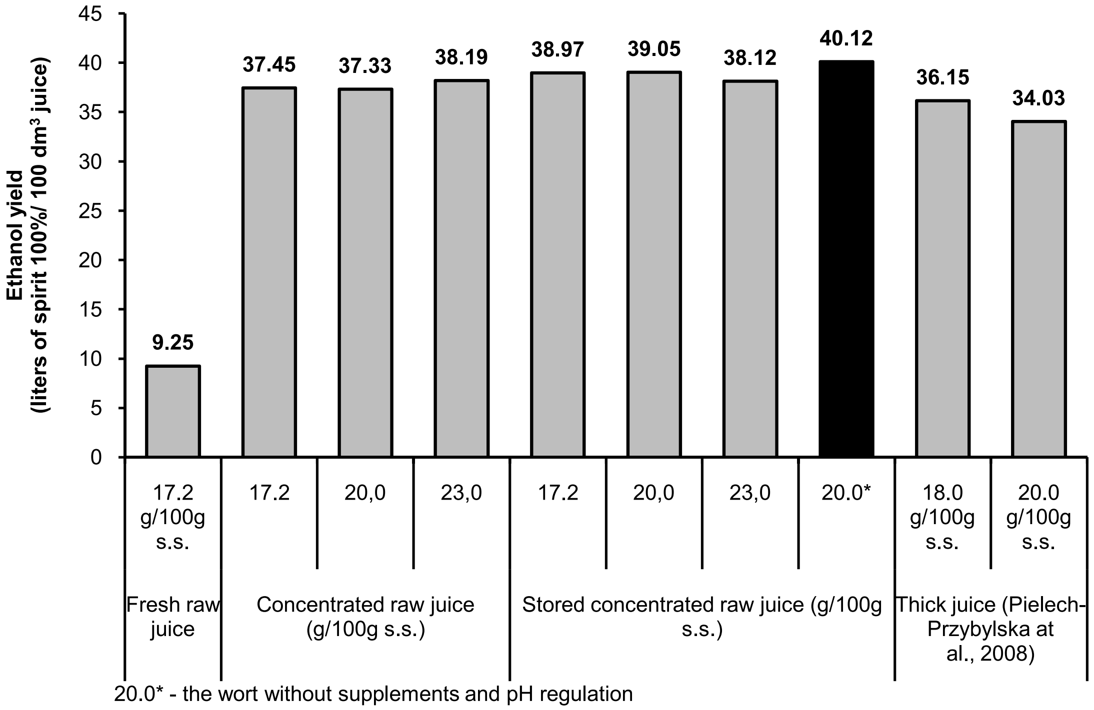

Fig. 5 Volumes of spirit 100% v/v obtained from 100 dm^3 of raw sugar beet juices

6. Conclusions

1. Fermentation trials revealed that changes resulting from concentration of the raw sugar beet juice had no significant impact on process duration.
2. Keeping of the concentrated raw sugar beet juice for 8 months resulted in slower alcoholic fermentation processes.
3. Ethanol yields from concentrated raw sugar beet juices either fresh or stored for 8 months were at the same level as for the fresh raw juice.
4. The degree of sugar assimilation by yeasts from worts containing the fresh raw sugar beet juice was higher compared to worts based on the concentrated juice kept for 8 months.
5. The ultimate ethanol concentration in worts prepared from the stored concentrated raw sugar beet juice was higher compared to worts based on the fresh concentrated juice.
6. The ultimate true extract content in all fermented worts based on the fresh concentrated raw sugar beet juice was higher compared to worts obtained from the concentrated juice kept for 8 months.

7. References

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