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Quality Requirements of Bioethanol Samples Obtained from the Sugar Beet Cultivated in the Experimental Fields of Viisoara-Turda, within the Agricultural Year 2007-2008

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Abstract: This paper presents some of the results obtained during the experiences carried out within the sugar beet experimental field of Viisoara, Cluj County. In the agricultural year 2007 – 2008, several researches were conducted on sugar beet in order to obtain bioethanol, using sugar beet as main biological raw material. Taking into account the quality requirements of bioethanol, one carefully monitored the sugar beet crop evolution regarding the influence of different technology factors, such as irrigation regime and fertilization, on sugar beet yield. As a result, after applying a number of test methods, one determined the characteristics of bioethanol for each sample obtained from sugar beet in the experimental fields of Viisoara, according to the EN 15376 standard requirements, as a component of the automotive fuel for petrol vehicles, according to EN 228:2008 standard.

Keywords: sugar beet, bioethanol, quality requirements

INTRODUCTION

In accordance with the EN 228:2008 standard, the requirements and test methods for the ethanol commercialized and delivered in order to be used as a component of automotive fuel for petrol vehicles are summarized in EN 15376.

This standard (4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14) provides all the characteristics, requirements and test methods applicable to (bio) ethanol, currently recognized as necessary in order to characterize the product which will be mixed with petrol and used for automotive fuel, in a concentration of maximum 5% (v/v) (Tab. 1).

The methods used in order to characterize the bioethanol obtained from the sugar beet cultivated in the experimental field of Viisoara are as it follows (1, 2, 3, 15, 16, 17):

- Actual alcoholic strength determination by pycnometry (EC/2870/2000);
- Methanol and higher alcohols determination by gas chromatography (EC/2870/2000);
- Water determination test (Karl Fischer Method) (EN 15489:2008);
- Inorganic chloride determination by ion chromatography (EN 15492:2009);
- Copper determination by graphite furnace atomic absorption spectrometry (EN 15488:2008);
- Total acidity determination by light indicator (EN 15489:2008);
- Phosphate determination by ammonium molybdate spectrophotometric method (EN 15487:2008);
- Non-volatile matter determination by gravimetric method (EN 15691:2009);
- Sulfur determination by fluorescence in ultraviolet method (EN 15486:2008).

Tab. 1

General application requirements and corresponding test methods for ethanol

Characteristics	Unit	Limit values		Test method
		Minimum	Maximum	
Ethanol content+ higher saturated alcohols	% (m/m)	98,7		EC/2870/2000- Method I, Appendix II, Method B (EC/2870/2000)
Higher saturated alcohols (C3-C5) content	% (m/m)		2,0	EC/2870/2000- Method III (EC/2870/2000)
Methanol content	% (m/m)		1,0	EC/2870/2000- Method III (EC/2870/2000)
Water content	% (m/m)		0,300	EN 15489 (EN 15489:2008)
Inorganic chloride content	mg/l		20,0	EN 15484 (EN 15484:2008) or EN 15492 (EN 5492:2009)
Copper content	mg/kg		0,100	EN 15488 (EN 15488:2008)
Total acidity (expressed as acetic acid)	% (m/m)		0,007	EN 15491 (EN 15489:2008)
Aspect		Colorless clear		Visual inspection
Phosphorus content	mg/l		0,50	EN 15487
Total dry residue	mg/100ml	<	10	EC/2870/2000- Method II
Sulfur content	mg/kg	6,60	10,0	EN 15485 or EN 15486

Source: EN 15376

MATERIALS AND METHODS

One prevailed 16 kg sugar beet samples for each of the 54 experimental plots. The sampels were weighed and placed in labeled bags, which were transported and stored at the Research Institute for Analytical Instrumentation Cluj-Napoca. Each sample was prepared and submitted to the fermentation process, which took place in the BIOCOMB Laboratory, at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Thus, to remove the existing impurities and to ensure a higher efficiency of sugar extraction, the sugar beet roots were washed under running water and then crushed using a hammer mill.

From the resulted composition, one took samples of 15 kg net weight, which were placed in barrels labeled according to the irrigation system applied, the fertilization and, respectively, the sugar beet variety.

The dilution was made with fresh hot water at a temperature of 70 -80^{o}C, in a 2:1 ratio (2 parts water to 1 part of sugar beet pulp) in order to facilitate the extraction.

After cooling the mixture of sugar beet pulp and water at a temperature of 30^{o}C, one introduced yeast and everything was thoroughly mixed for homogenization.

One used for the fermentation process Ethanol Red yeast (*Saccharomyces cerevisiae*) and the emulsifier E 491. Ethanol Red yeast is predominantly used in industry due to its high tolerance to alcohol, maintaining its cell viability during the fermentation process.This type of yeast is able to determine the increment of the alcohol concentration in the case of high temperatures fermentation; maximum range is 48g alcohol at 100g and 18% v/v at a working temperature of 30 – 35^{o}C, with the development limits of yeast between 30-40^{o}C. One used 200g Ethanol Red yeast for each 2:1 mixture.

The leaven acidity was continuously monitored in order not to decrease the ability of yeast fermentation. One recorded the pH= 4 - 5, therefore it was no need for an additional neutralization and acidification of the leaven.

As the preparation of sugar beet roots for grinding and fermentation was carefully carried out and the environmental working place was a laboratory, the development of fungus and mold at the surface of the leaven was negligible, which is why no antiseptic was added. The disinfection of the inner surface of the barrel and fermenter was carried out with formalin (formaldehyde concentration 3%), while the lime –wash (1% concentration) was used to clean the outer surface of the barrel, respectively the fermenter.

For each sample, the fermentation of the composition was carried out in a double jacket fermenter endowed with a stirrer and a thermostat, equipment situated in the BIOCOMB Laboratory from USAMV Cluj-Napoca.

The first sample subjected to fermentation was NF1S1: non-irrigated, fertilized NPK 250+55kg N active substance/ha, sugar beet variety –Libero. The fermentation temperature was maintained at 25^{o}C, aeration was realized every 2 hours in order to encourage the yeast multiplication.

After about 24 hours, one noticed the beginning of the curled fermentation, materialized by turbulent foam at the surface of the composition. The presence of CO_2 was highlighted by the phenomenon of extinguishing matchsticks near the surface of the composition.

On the third day from the beginning of the fermentation process, when trying to light the matchstick, one noticed that this one not quenched, which was a sign that CO_2 was no longer produced. Moreover, one noticed the absence of the specific noise of fermentation, like a bubbling.

One collected a sample from the fermenter in order to determine the residual sugar from the composition, fact that was achieved by using a refractmeter designed for the determination of sugar concentration, which finally indicated a rate of 3.1% unfermented sugar in the composition; consequently, the increment of the temperature in the fermenter at 30^{o}C was demanded. Furthermore, one added an extra dose of 50g yeast, assuming that, because of the relatively low temperature, some of them died.

High temperatures, (e.g.30^{o}C), as well as yeast addition of, favored the resumption of the fermentation process, which was monitored by daily sampling so as to determine the residual sugar, as it can be seen in Table 2.

Tab. 2

Residual sugar concentration (%)

Day	4	5	6	7	8	9	10
Residual sugar concentration (%)	3,1	2,8	2,2	1,4	0,6	0,3	0,0

Consequently, for the NF1S1 sample, the fermentation process lasted 10 days which had an impact on the bioethanol acidity, the long fermentation favoring the appearence of an acetic fermetnation, to the detriment of the acoholic one.

For all other samples one took the following corrective measures: the temperature of the dilution water 80-83^{o}C; the temperature of the fermentor: 30^{o}C.

Finally, it was found that, with no exceptions, for all other samples, the fermentation process lasted, without any interrumption, for 68 – 72 hours.

One monitored the followings: the concentration of the unfermented sugar from the leaven, by taking periodic samples from the mixture subject to fermentation and their refractometric analysis, which provided clear indications regarding the conduct and

completion of the fermentation process; the temperature in the fermenter, which was maintained at 30°C throughout the fermentation process; the acidity of the leaven, by using a pH-meter, in order to maintain a pH= 4 – 5, favorable to yeast activity; the control of the leaven aspect, in order to visualize any occurrence of some bacteria and mold contamination that could compromise the results of the experiment.

The results obtained for the 6 analyzed samples are summarized in Table 3; ethanol concentration is presented both in units of mg/l as well as in mass % (m/m); overlapping chromatograms in a three-dimensional plan is presented in Figure 1.

Tab. 3

Concentration of bioethanol from the fermentation liquid samples resulted in the sugar beet fermentation process

Sample code	NF1S1	NF1S2	NF3S1	IF1S1	IF1S2	IF1S3
C_{Etanol} ,[mg/L]	42000	36700	15600	17800	25700	21200
C_{Etanol} ,[% (v/v)]	6,92	7,13	8,35	8,71	7,30	7,65

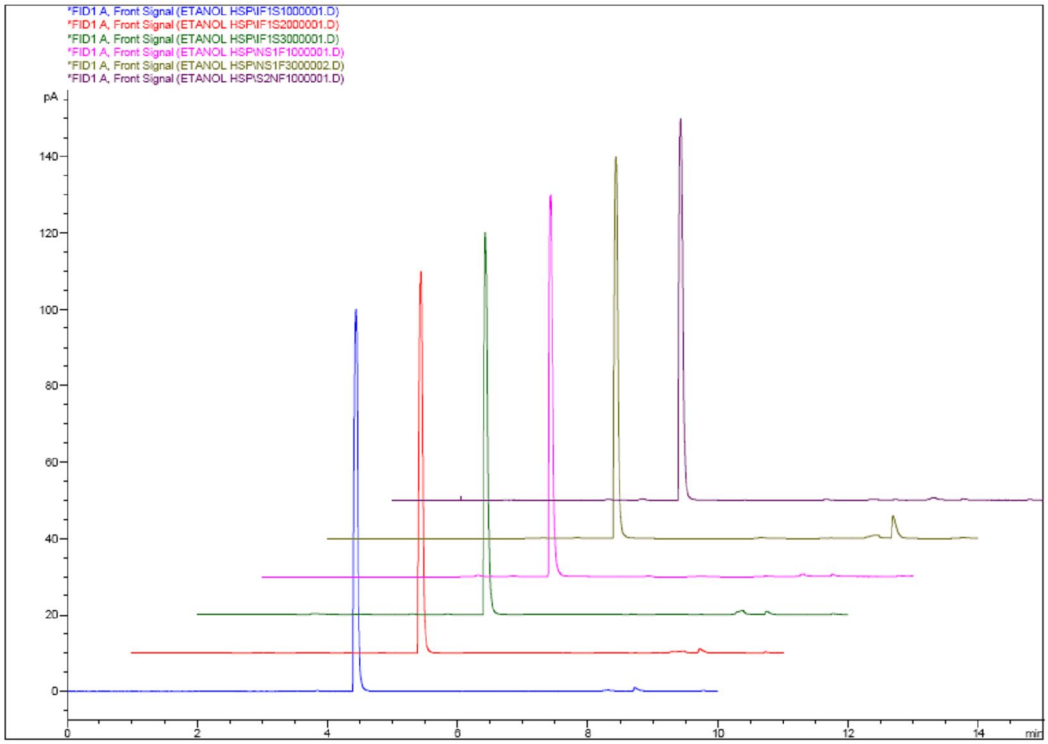

Fig. 1. Overlapping chromatograms in a three dimensional plan for the analyzed bioethanol samples NF1S1, NF1S2, NF3S1, IF1S1, IF1S2, IF1S3

After fermentation, the resulted bioethanol was separated from the fermentation liquid through a simple distillation process, respectively fractional distillation. The distillation process was repeated several times until the produced bioethanol reached an alcoholic concentration of minimum 98.7%.

The determination of methanol and higher alcohols was made by gas chromatography using an Agilent 7890N gas chromatograph with flame ionization detector, GC-FID, where the separation was achieved using the DB-WAX polar capillarity column.

The alcohols identified and analyzed in the bioethanol samples are presented in Table 4.

Tab. 4

Methanol and higher alcohols identified and analyzed in the bioethanol samples

No. of entry	Retention time (min)	Nomenclature
1.	4,160	Methanol
2.	7,993	n-Propanol
3.	9,700	Izobutanol
4.	13,942	2-methyl-izobutanol + 3-methyil-izobutanol

RESULTS AND DISCUSSIONS

The present results strictly refer to the bioethanol obtained form the sugar beet cultivated on the experimental fields of Viisoara in the agricultural year 2007-2008.

After the distillation process, one obtained different amounts of bioethanol, as it can be noticed in Table 5.

Tab. 5

Quantities of bioethanol obtained through distillation (l) from 15kg sugar beet root samples

Experimental plot	Repetition 1	Repetition 2	Repetition 3	Average	Transformation coefficient (l/t)
NF1S1	1,440	1,441	1,445	1,442	0,096
NF1S2	1,385	1,380	1,378	1,381	0,092
NF1S3	1,409	1,413	1,411	1,411	0,094
NF2S1	1,456	1,458	1,451	1,455	0,097
NF2S2	1,396	1,389	1,400	1,395	0,093
NF2S3	1,422	1,429	1,424	1,425	0,095
NF3S1	1,454	1,461	1,450	1,455	0,097
NF3S2	1,421	1,429	1,425	1,425	0,095
NF3S3	1,442	1,437	1,441	1,440	0,096
IF1S1	1,452	1,457	1,459	1,456	0,097
IF1S2	1,395	1,389	1,398	1,394	0,093
IF1S3	1,411	1,414	1,411	1,412	0,094
IF2S1	1,458	1,451	1,459	1,456	0,097
IF2S2	1,412	1,409	1,418	1,413	0,094
IF2S3	1,439	1,440	1,444	1,441	0,096
IF3S1	1,471	1,468	1,477	1,472	0,098
IF3S2	1,454	1,459	1,452	1,455	0,097
IF3S3	1,477	1,473	1,469	1,473	0,098

*The bioethanol was obtained each time from an initial quantity of sugar beet of 15kg (used as sample for the fermentation process).

Knowing the fact that one can obtain about 6,62m^3 bioethanol from an average sugar beet production of 61,7t/ha (*Bioethanol in Deutschland, Landwirschaftsverfag Munster*), it results a transformation coefficient of 0,107. It is also to be noted the fact that, if in the case of bioethanol obtained experimentally, the coefficient is smaller due to several factors, including: non-use of nutrients within the fermentation process, such as those containing nitrogen, which usually increase the efficiency of bioethanol production; distillation carried out within laboratory conditions, without making any amendments to the obtained liquid.

The bioethanol was obtained each time from an initial quantity of sugar beet of 15kg (used as sample for the fermentation process).

The values of the ethanol and higher alcohols content - % (m/m) - determined from the samples collected from Viisoara experimental plots were just above the minimum values

allowed by EN 15376 – 98,7% (m/m) –this concentration being obtained through repeated fractional distillation.

The values corresponding to the higher saturated alcohols (C3-C5) content - % (m/m) – determined from the samples prevailed from the experimental field were much below as compared with the maximum permissible limit EN 15376 – 2,0% (m/m).

As in the case of higher saturated alcohols, the methanol determination was performed by gas chromatography using Agilent 7890N gas chromatograph with flame ionization detector, GC-FID, endowment of the ICIA Environment Analysis Laboratory.

The values for the methanol content are also lower than the maximum permissible limit of EN 15376 – 1.0 % (m/m). A similar situation was encountered for water content, total acidity, phosphorus content, total dry residues and sulfur content; only the inorganic chloride and copper contents were high, being close to the maximum allowable limit value of EN 15376.

CONCLUSIONS

The ethanol samples obtained from each experimental lot located in Viisoara were analyzed within the Environmental Analysis Laboratory of the Research Institute for Analytical Instrumentation ICIA Cluj-Napoca.

One determined the characteristics of each biethanol sample, according to the requirements of the EN 15376 standard, through the use of bioethanol obtained within the experimental fields as a component of the automotive fuel for petrol vehicles, in accordance with EN 228:2008.

The values obtained through laboratory determinations allowed to formulate the following conclusions:

1. In the best interest of an efficient alcoholic fermentation, several conditions must be met, namely: appropriate trimming of roots before grinding; strictly compliance with the temperature range 80-85^{o}C of the dilution water; maintaining a temperature of about 30 ^{o}C in the fermenter, thus ensuring, on the one hand the acoholic fermentation of the leaven and, on the other hand, the completion of the fermentation process within a time period of 68- 72 hours.

2. Use, in future experiences, of a growth factor to stimulate the development of yeasts;

3. Use, in future experiences, of nitrogen compounds during the fermentation process to obtain a satisfactory yield of bioethanol;

4. Experimental factors (such as irrigation regime, fertilization and sugar beet variety) and their graduations do not influence the characteristics of bioethanol;

5. The ethanol and higher alcohols content is satisfactory and it is slightly above the minimum required by EN 15376. These values were obtained by fractional distillation a process that was repeated for several times in order to achieve the necessary concentration;

6. The low percentage content of higher saturated alcohols gives direct information on the content of bioethanol from the obtained distillation liquid. Taking into account the fact that the values of the bioethanol concentrations are obtained by the difference between the content of ethanol and higher alcohols, respectively higher alcohol content, one can conclude that the samples obtained by fractional distillation in laboratory conditions have satisfactory concentrations of ethanol, despite of the relatively small amounts, but which meet all the requirements.

A correction could increase the ethanol content and, accordingly, could greatly reduce the relatively high water content of ethanol. But the amendment requires special conditions, which can be obtained only on industrial scale.

7. The obtained bioethanol samples are characterized by small values of methanol content, phosphorus and total dry residue, which are favourable for the quality of bioethanol used as a component of petrol fuel used for vehicles.

8. The inorganic chloride and copper contents are high, being closed to the maximum allowable limit value of EN 15376.

9. The total acidity recorded slightly lower or equal values to the maximum limit provided in EN 15376, due to the long time period of the fermentation process.

10. The sulfur, known for its lubricating properties, has registered low values, less than the minimum allowed by EN 15376, which is an advantage for the bioethanol obtained, whereas high sulfur content can lead to clogged injectors for petrol.

11. The appearance of the bioethanol obtained respects the EN 15376 requirements, the fluid is colorless and clear in all the performed distillations.

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