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ETHANOL DETERMINATION IN SUGAR BEET FERMENTATION LIQUID BY FULL EVAPORATION HEADSPACE GAS CHROMATOGRAPHIC METHOD

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ABSTRACT. This paper reports a full evaporation (FE) headspace gas chromatographic (HS-GC) method for rapid determination of ethanol in sugar beet fermentation liquid. In this method, a very small volume,10 µL of sugar beet fermentation liquid sample is introduced into a headspace sample vial and heated up to a temperature of 105 ºC, when a full evaporation can be achieved within 3 min. The ethanol in the headspace of the vial is then measured by GC-FID. The full evaporation technique showed a good reproducibility, with a relative standard deviation less than 2.54% for six measurements. The method was verified by using spiked fermentation samples with different volumes of standard ethanol solution when ethanol concentrations were determined together with their corresponding confidence intervals, the relative standard deviations and the recovery degrees. The relative standard deviations ranged from 1.18% to 2.59% and the recovery degrees ranged from 88.7% to 92.6%. The present method is simple, rapid and requires no sample pretreatment.

Keywords: *ethanol, sugar beet fermentation liquid, full evaporation headspace*

INTRODUCTION

Bioethanol can be a suitable alternative to replace fossil fuels and represents one of the key for reducing pressure on the levels of atmospheric carbon. Bioethanol can be produced from fermentation of corn grain (wheat, barley and rye), sugar beet, sugar cane and vegetable residues [1, 2]. Today, bioethanol produced from cereals and biodiesel from rape seed are the two biofuels obtained from agriculture in Europe.

The bioconversion of sugar beet to bioethanol contains three steps: extraction of sugars from sugar beet chips (hydrolysis), fermentation of sugars and bioethanol distillation/analysis [3].

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Ethanol can be produced either from molasses, which is a by-product after the sugar is extracted, or directly obtained from sherbet.

In present work, sugar beet was converted to bioethanol production by direct fermentation (without previous hydrolysis) of its sugars (sucrose) by solid state fermentation (SSF) [4].

The quantification of ethanol during the fermentation process plays an important role in food industry researches. Many methods are used for ethanol determination from the fermentation liquids like colorimetry [5,6] enzymatic methods [7,8], dichromate oxidation [9], high performance liquid chromatography [10,11] and gas chromatography [12,13].

The fermentation liquids have a very complex composition, thus, together with the suspended yeast they contain many non-volatile compounds such as sugars, colored substances etc. Therefore laborious and time-consuming pretreatment procedures are necessary to remove the compounds that interfere in the ethanol quantification. For this reason it is necessary to develop simple and accurate methods for ethanol quantification during the fermentation processes.

Headspace gas chromatography (HS-GC) proved an effective technique for measuring volatile species in samples with complex matrices [14,15]. The advantage of the headspace sampling is that direct liquid or solid probing is avoided and complex sample matrix in a liquid or solid sample can be simplified or even eliminated in its vapor phase. The full evaporation (FE) technique was one of the early headspace - gas chromatographic techniques. It utilizes the headspace sampler as an evaporator rather than an enclosed static vapor-liquid equilibrium space to achieve quantitative analysis of volatile species in samples. The technique was initially developed by Markelov and Guzowski [16] in 1993. A very small sample size was used in a sample vial to achieve a near-complete evaporation or transfer of analytes from a condensed matrix into a vapor phase in the headspace of the sample vial in a very short period of time; therefore sample pretreatments are not required. The method is based on the near-complete transfer of the analyte into the vapor phase and then the measurement of the volatile species in the headspace by GC [17]. This method has been successfully used in several studies reported in the literature [18-20].

The objective of the present study was to develop a simple full evaporation headspace GC method for the rapid determination of ethanol from sugar beet fermentation liquid samples. The method has been developed using a (5%-phenyl)-methylpolysiloxane column, a non-polar capillary column with greater thermal stability and with a wide range of applications, including the analysis of alcohols [21]. After the fermentation process was finished the ethanol formed was separated from the fermentation liquid using a fractionated distillation process. The ethanol thus obtained was further analyzed using the same full evaporation headspace GC technique for the identification of methanol and C3-C5 higher saturated alcohols. In order to have a good

separation of C3-C5 higher saturated alcohols the non-polar column was replaced with a polar column, with a polyethylene glycol stationary phase. Although for this column damage occurs at lower temperatures and lower oxygen levels, its separation characteristics proved useful in the case of C3-C5 higher saturated alcohols.

RESULTS AND DISCUSSION

Analysis of sugar beet fermentation sample using FE-HS-GC-FID method

The GC chromatogram of a sugar-beet fermentation sample analyzed using FE headspace analysis is shown in Fig.1. It can be seen that the ethanol, the major component present in the sample gave the highest peak in the chromatogram at the retention time of 4.430 min, and is clearly separated from methanol, another volatile compound identified in the fermentation sample.

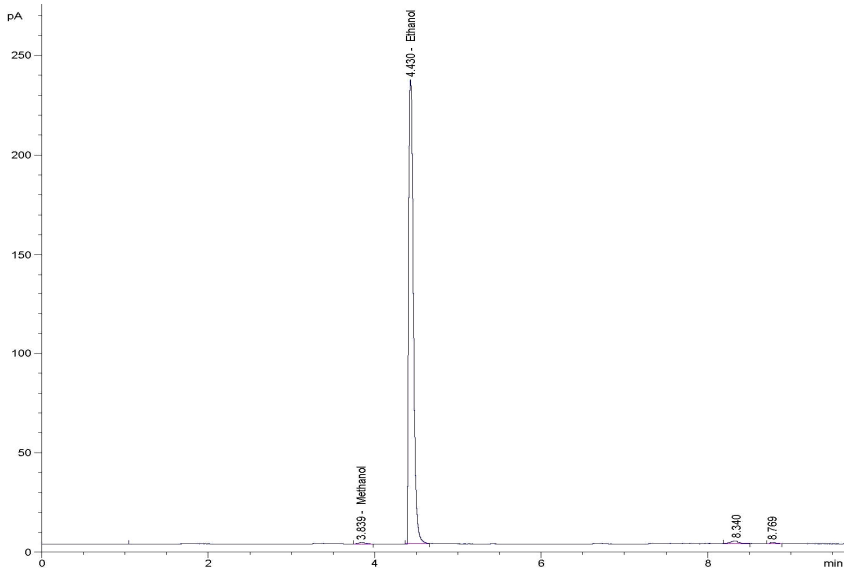

Figure 1. Chromatogram of a sugar-beet fermentation sample analyzed using FE-HS-GC method

Method validation

In the present study an external standard calibration was used for the ethanol quantification by full evaporation headspace method using a standard ethanol solution of 10 g/L. The calibration was realized by adding different volumes (5, 10, 15, 20, 25, 50 µl) of the standard ethanol solution into a set of headspace vials followed by immediate capping of headspace

vials, sample incubation in the headspace oven and gas chromatographic analysis. After the GC analysis of the standard solutions used for calibration, the following calibration curve was obtained:

$$y = 3.32(\pm 0.06) \times m + 309(\pm 16.4)$$

where *y* represents the gas chromatographic response (ethanol area) and *m* represents the absolute amount (in µg) of ethanol from the sample analyzed. The method was linear between 50 and 500 µg, with a regression coefficient of 0.9994 (n=6). The 95% confidence intervals ($\alpha = 0.05$, 4 degrees of freedom) for the slope and y-intercept of the calibration curve were 3.32±0.17 and 309±45.5 respectively. The full evaporation technique showed a good reproducibility, with a relative standard deviation less than 2.54% for six measurements.

The present method was verified by using spiked fermentation samples with ethanol. The sugar beet fermentation liquid used as reference was analyzed by FE-HS-GC in order to determine the initial content of ethanol (before adding ethanol). The initial concentration of ethanol in the sugar beet fermentation sample was 3260 mg/L. A volume of 500 µL fermentation sample was spiked with the following volumes of 10 g/L standard ethanol solution: 5, 10, 15, 20, 25 µL. Thus, the recovery grade of full evaporation method could be determined by comparing the theoretical values of ethanol from the spiked samples with the measured values using this technique. The variation of the methodology was expressed as confidence intervals (95%) of the ethanol concentrations measured. Table 1 shows ethanol concentrations, the relative standard deviations and the recovery degrees for the determination of ethanol concentration from the spiked sugar beet fermentation samples.

Table 1. Verification of the FE-HS-GC method using spiked fermentation samples with different volumes of standard ethanol solution. Theoretical and measured concentrations (mean ± confidence interval), relative standard deviations (RSDs) and recovery degrees in the analyses of spiked fermentation samples (n=3)

Spiked sample no.	Initial ethanol concentration of fermentation sample (mg/L)	Added volume of standard ethanol solution (µL)	Ethanol concentration of spiked samples (mg/L)		RSD[b] (%)	Recovery degree[d] (%)
			Theoretical values	Measured values[a]		
1	3260	5	8260	7360±474	2.59	89.1
2	3260	10	13260	11762±418	1.43	88.7
3	3260	15	18260	16872±494	1.18	92.4
4	3260	20	23260	21050±675	1.29	90.5
5	3260	25	28260	26169±765	1.18	92.6

[a] Mean ± confidence interval (n=3, confidence level: 95%)
[b] Relative standard deviation
[d] Mean value (n=3)

The results obtained are similar with those reported by Li et al. [18].

Identification of methanol and higher alcohols in distilled ethanol sample using FE-HS-GC-FID method

Ethanol formed during sugar beet fermentation process was separated from the fermentation liquid by fractionate distillation. The FE-HS-GC method was also used to identify methanol and higher alcohols that are present in distilled ethanol resulted from the sugar beet fermentation process. The headspace operating conditions used were the same as for ethanol quantification but for the separation process, a polar capillary column has been used, in order to separate the alcohols. The chromatogram obtained for the distilled ethanol sample is shown in Fig.2.

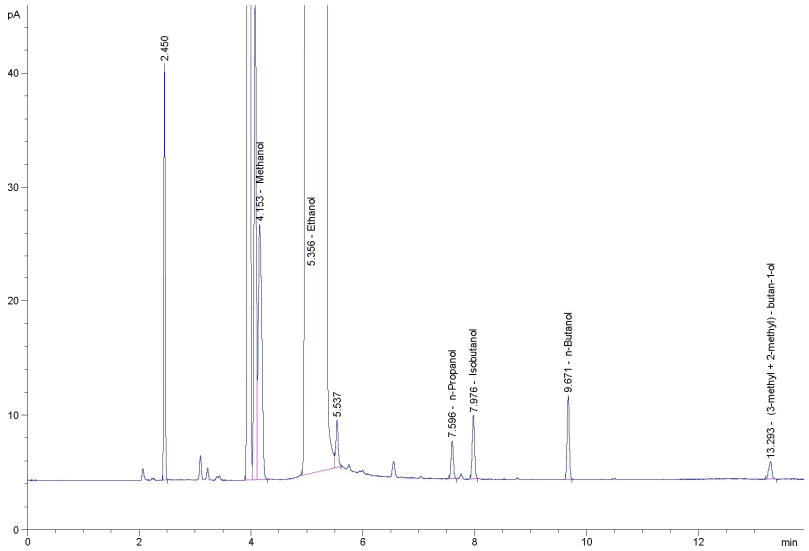

Figure 2. Chromatogram of a distilled ethanol sample analyzed using FE-HS-GC method

It can be seen that these alcohols are clearly separated with the exception of 3-methyl and 2-methyl-butan-1-ol, that being isomers are very difficult to be separated. The alcohols identified and their corresponding retention times are shown in Table 2.

Table 2. Identified alcohols in distilled ethanol using FE-HS-GC method

	Retention time RT (min)	Alcohol
1	4.153	Methanol
2	7.596	n-Propanol
3	7.976	Isobutanol
4	9.671	n-Butanol
5	13.293	(3-methyl+2-methyl)-butan-1-ol

Thus, the FE-HS-GC method could be successfully used for the identification of methanol and higher alcohols. Further studies are necessary in order to determine if this method is suitable for the quantification of methanol and higher alcohols from distilled ethanol.

CONCLUSIONS

A full evaporation headspace gas chromatographic method has been used to determine the ethanol in sugar beet fermentation liquid. The present method is simple, rapid, and accurate and can be successfully used to monitor ethanol formation during fermentation processes. It also can be used to identify higher alcohols that are present in distilled ethanol.

EXPERIMENTAL PART

Materials and methods

All chemicals used in this study were of analytical reagent-grade. Absolute ethanol, methanol, n-propanol, isobutanol, 3-methyl-isobutanol and 2-methyl-isobuthanol were purchased from Merck (Darmstadt, Germany). A standard ethanol solution of 10 g/L in water was used for external standard calibration. The analyzed sample was obtained from a sugar-beet fermentation process in a lab fermentation system. Headspace vials for the autosampler, magnetic caps with PTFE/silicone septa were purchased from Agilent Technologies (Palo Alto, CA, USA).

Gas chromatographic analysis

All measurements were carried out using an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a CTC Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and a flame ionization detector (FID). The GC operating conditions used for ethanol quantification were the following: HP-5 capillary column (J&W Scientific 19091J-413, length 30 m, inner diameter 320 μm and film thickness 250 μm); temperature program: 35 °C held for 10 min; helium carrier gas 1.2 ml/min, inlet temperature 250°C; detector temperature 250°C; the hydrogen and air flow rates 40 and 400 ml/min, respectively; helium makeup flow 30 ml/min. The GC operating conditions used for the identification of higher saturated alcohols were the following: DB-WAX capillary column (J&W Scientific 123-7032, length 30 m, inner diameter 320 μm and film thickness 250 μm); temperature program: 35 °C (5 min hold) to 150 °C at 5 °C/min then to 250 °C (2 min hold) at 20 °C/min; inlet temperature 220 °C; detector temperature 280 °C; helium carrier gas 1.5 ml/min; hydrogen and air flow rates 40 and 400 ml/min, respectively; helium makeup flow 30 ml/min.

The optimal parameters of the sample preparation and headspace operating conditions used in this study were selected according to other studies [17] in order to have complete ethanol evaporation. A volume of 10 μl

of sample solution was introduced into a 20 ml headspace vial followed by immediate capping of the headspace vial. The vial was placed in the headspace oven of the CTC Combi Pal autosampler and a 2.5 ml gastight headspace syringe was used to extract 1 ml of the gas phase in the headspace vials and inject into the GC.

Headspace operating conditions used for ethanol quantification and also for the identification of higher saturated alcohols were as follows: incubation time 5 min, incubation temperature 105 °C, agitation speed 750 rpm, syringe temperature 110°C, syringe flushing time 1 min.

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