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DETERMINATION OF DIACETYL AND 2,3-PENTANEDIONE IN BEER BY GC/MS USING SOLID-PHASE EXTRACTION COLUMNS

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A new GC/MS method for the determination of diacetyl and 2,3-pentanedione was investigated. Diacetyl and 2,3-pentanedione were derivatized with 1,2-diaminobenzene to form 2,3-dimetylquinoxaline and 2-ethyl-3-methylquinoxaline, respectively. The amounts of formed 2,3-dimetylquinoxaline and 2-ethyl-3-methylquinoxaline were proportional to the concentrations of diacetyl and 2,3-pentanedione present in the sample. 2,3-Dimetylquinoxaline and 2-ethyl-3-methylquinoxaline were extracted by solid-phase extraction (SPE) columns and determined by gas chromatography using a mass selective detector. This method was applied for the determination of diacetyl and 2,3-pentanedione concentrations in beer. Extraction by SPE columns proved to be very simple and reliable. The method can be used for simultaneous determination of diacetyl and 2,3-pentanedione concentrations in beer in a great number of beer samples.

KEY WORDS: vicinal diketones, gas chromatography, mass selective detector, beer

INTRODUCTION

Beer is malt beverage obtained from an alcoholic fermentation of the aqueous extract of malted barley with hops. The production of beer is a multistage process involving biological conversion of raw materials to final product.

Fermentation is performed by yeast. Brewing yeast fermentation performance; that is, the ability of yeasts to consistently metabolize wort constituents into ethanol and other fermentation products in order to produce beer with satisfactory quality and stability, is influenced and controlled by a number of factors (genetic characteristics,

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cell physiology, nutritional availability and physical enviroment) (1). Some by-products are obtained during the process affecting significantly the taste, smell and other beer characteristics, and their formation, as well as degradation, are connected with yeast cells metabolism (2). The most important by-products of beer fermentation are vicinal diketones, higher alcohols, esters, aldehydes and S-compounds (3).

Diacetyl (2,3-butanedione) is the main aroma constituent of green beer. The beer has a sweet, nonspecific taste, when diacetyl concentrations in beer are higher than the limit values, and the increased concentrations result in buttery aroma. The taste threshold for diacetyl is 0.10 mg/dm^3. 2,3-Pentanedione is similar to diacetyl, however, the taste threshold is higher (near 1.0 mg/dm^3) resulting in honey-like aroma (4). Reduction of diacetyl and 2,3-pentanedione is simultaneous with other processes during ripening of beer, so it can be considered as an important criterion for maturity level estimation of a certain beer. The formation and reduction of vicinal diketones proceeds in three phases: formation of precursors, transformation of precursors and diketone reduction (5).

The formation and reduction of diacetyl and 2,3-pentanedione is affected by yeast (6, 7), fermentation temperature (8, 9), wort composition (10-12), aeration and amount of dissolved oxygen (13, 14), pH (15-17), bacterial infection (18, 19) and pressure (20).

The aim of these experiments was to determine precisely concentrations of diacetyl and 2,3-pentanedione in beer.

EXPERIMENTAL

Chemicals, supplies and samples

Diacetyl (2,3-butanedione), 2,3-pentanedione, 2,3-hexanedione, 1,2-diaminobenzene (for the synthesis) and ethanol (for chromatography) were purchased from Merck, dichlormethan (p.a.) from "Zorka" Šabac and SPE Octadecyl (C_{18}) columns N° 7020-02, volume 3 cm^3 and packed with 200 mg of octadecylsilane were purchased from "J.T. Baker". Diacetyl and 2,3-pentanedione mass concentrations were determined in commercial bottled beer.

Gas chromatography (GC) with flame ionisation detection (FID) and experimental conditions

A Hewlett Packard 5890 Series II gas chromatograph was used of diacetyl solution analyses. The gas chromatograph was equipped with flame ionisation detector (FID). The gas chromatography (GC) separation was carried out on a Hewlett Packard 20 M (Carbowax 20 M) (50 m x 0.32 mm and 0.3 μm film thickness) capillary column. Carrier gas was helium at constant flow rate 1.1 cm^3/min; column head pressure was 70 kPa. Constant flow rate of gases through the detector was: hydrogen 30 cm^3/min, air 400 cm^3/min and nitrogen 30 cm^3/min. The following temperature program was used: injector and detector temperatures 200°C and 250°C, respectively, initial temperature 40°C, without holding, temperature increase 8°C/min to 200°C and held at this temperature for 1 minute. The retention time for diacetyl varied between 14.612-14.662 min. The injected sample volume was 1 – 10 μL (split ratio 1 : 20).

Gas chromatography with mass selective detection (GC/MS) and experimental conditions

The GC/MS analyses were carried out using a Hewlett Packard model G 1800 A GC SYSTEM combined with Hewlett Packard mass selective detector (using SCAN technique in the range 45 – 425 Da) with autosampling and autoinjection (Hewlett Packard 6890). The separation was accomplished on a Hewlett Packard 5 MS (methyl syloxane) column (30 m × 0.25 mm and 0.25 μm film thickness). The carrier gas was helium at constant flow rate 0.8 cm^3/min. The following temperature program was used: injector and detector temperatures 250°C and 280°C, respectively, initial temperature 50°C, without holding, temperature increase 40°C/min to 200°C, without holding at 200°C, and 20°C/min to 280°C, and held at this temperature for 7.25 min. The whole cycle lasted 15 min. The retention times of derivates of diacetyl, 2,3-pentanedione and 2,3-hexanedione (internal standard) i. e. 2,3-dimethylquinoxaline, 2-ethyl-3-methyl-quinoxalin and 2-methyl-3-n-propyl-quionoxalin were: 4.60, 4.90 and 5.20 min, respectively. The injected sample volume was 1 μL (in splitless mode).

Sample preparation for GC with FID

Diacetyl in 5% ethanol solution was used in the experiments, according to MEBAK method (21). Before use, the sample was intensively shaken for 5 min to obtain homogenous diacetyl solution. Diacetyl was weighed on analytical balance, 66.3 mg and transferred to a 100 cm^3 flask (corresponding to 6.63 mg/dm^3 mass concentration).

Sample preparation for GC/MS

Content of diacetyl and 2,3-pentanedione was determined by calibration method. Samples containing 0.05 – 1.00 mg/dm^3 of diacetyl and 0.005 – 1.000 mg/dm^3 of 2,3-pentanediene were prepared. Internal standard (2,3-hexanedione) was added to the samples in the same amount as well as 1cm^3 of 1,2-diaminobenzene solution. 1,2-Diaminobenzene was added for derivatization of diacetyl, 2,3-pentanedione and internal standard. Solution of 1,2-diaminobenzene was obtained by dissolving 20 mM in hydrochloric acid (1 M HCl solution). The samples were heated at 30°C for 15 min in water bath.

The derivates of diacetyl, 2,3-pentanedione and internal standard i. e. 2,3-dimethyl-quinoxalin, 2-ethyl-3-methyl-quinoxalin and 2-methyl-3-n-propylquinoxalin were concentrated and extracted on SPE column. The reaction between diacetyl and 1,2-diaminobenzene is presented in Fig. 1. The reaction between 2,3-pentanedione and 1,2-diaminobenzene is presented in Fig. 2.

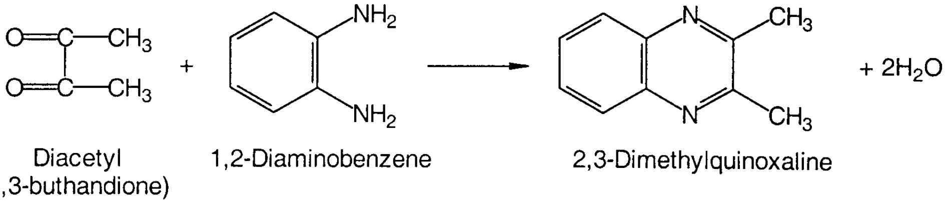


Fig. 1. Reaction between diacetyl and 1,2-diaminobenzene

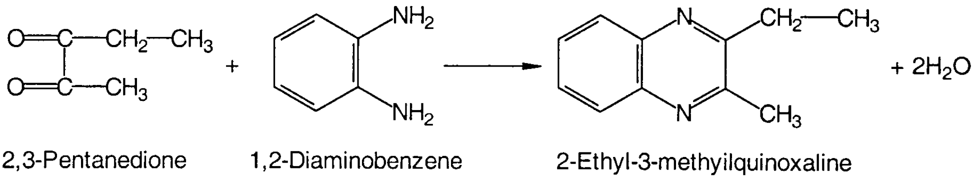


Fig. 2. Reaction between 2,3-pentanedione and 1,2-diaminobenzene

The derivatized diacetyl, 2,3-pentanedione and internal standard were transferred to SPE columns. SPE octadecyl columns are nonpolar and they bind the nonpolar compounds e.g. derivates of vicinal diketones. The derivates of diacetyl, 2,3-pentanedione and internal standard were extracted with dichlormethan. After that, dichlormethan was evaporated. The dry residue disolved in 200 μL of ethylacetate was transferred to a vial, closed with rubber stopper and Al-cap. 1 μL of each sample was injected into the GC/MS.

The aim of further investigations was to apply the GC/MS method for determination of diacetyl and 2,3-pentanedione concentration in beer. The same concentration of internal standard was added to original beer samples as to samples for calibration. Beer samples with different concentration of diacetyl and 2,3-pentanedione, and same concentrations of internal standard were also prepared. The determination of diacetyl and 2,3-pentanedione in original beer samples and beer samples with the addition of diacetyl and 2,3-pentanedione was performed as described above (sample preparation for calibration).

RESULTS AND DISCUSSION

In order to determine the detection threshold (lowest concentration of investigated substance that can be determined), the diacetyl mass concentration i. e. volumes of diacetyl solutions injected were lowered (in the range from 663 ng/μL to 332 ng/μL).

The lowest detected mass concentration was 332.0 ng/μL. Varying the chromatographic parameters (threshold (THRSH), attenuation (ATT 2^) and peak width (PK WD)), lower concentrations of diacetyl were determined. In order to optimize the chromatogram, a number of analyses were performed varying the mentioned chromatographic parameters. Mass concentration of diacetyl injected into the GC was 102.0 ng/μL. First, the threshold was increased, to value –3 (THRSH = -3). Then the attenuation was increased, to value –4 (ATT 2^ = -4). The attenuation value –4 was not satisfactory so attenuation values –1 (ATT 2^ = -1) and –2 (ATT 2^ = -2) were used for further analyses. The best results were obtained applying attenuation value –1. This value was used for further analyses. The peak width was also varied in the experiments, from 0.04 to 1.00 min. The lowest standard deviation was obtained with the peak width 0.12 min (PK WD = 0.12). Five measurements were performed using peak width 0.12 min and the results are presented in Table 1.

Chromatographic parameters used in these analyses are listed above the chromatogram presented in Fig. 3. Limit of detection – LOD and limit of quantitation – LOQ were calculated according to ACS Committee on Enviromental Improvement (22).

Table 1. Determination of diacetyl mass concentration 102.0 ng/μL using peak width 0.12 min.

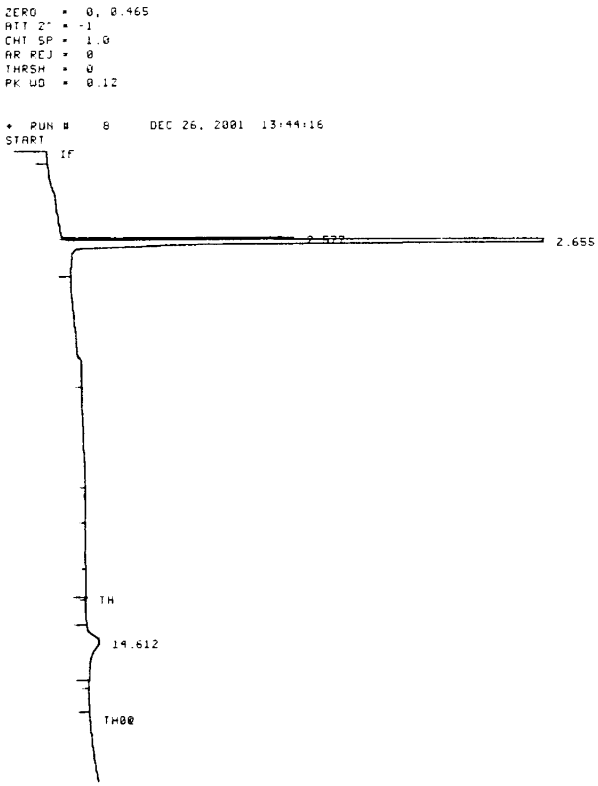
N° of analysis (N)	Retention time (minutes)	Area (X_i)	Diacetyl mass concentration in prepared sample (ng/μL)
1.	14.612	2831	100.70
2.	14.662	2733	97.21
3.	14.630	2977	105.89
4.	14.622	2646	94.12
5.	14.615	3151	112.08

Table 2. Statistical data obtained by diacetyl determination using GC with FID

	Diacetyl mass concentration in prepared sample (ng/μL)
$\overline{X}$	102.0
SD	6.40
LOD	19.2
LOQ	64.0
CV	6.27%

The lowest diacetyl mass concentration determined using GC with FID was 102.0 ng/μL. The mass concentrations of diacetyl in beer are 0.1 mg/dm^3, and that is 1000 times lower value than the lowest concentration determined in these experiments. In order to determine the content of diacetyl and 2,3-pentanedione in the beer, extraction methods should be used. Due to the complex composition of beer and the fact that retention time depends on sample composition and conditions of GC work, GC with mass selective detector (MS) was used in further work. This device gives the retention time but also the mass spectra of investigated compounds. In this way, the identification is more accurate than with the use of other types of detectors. The average match quality for determined compounds was 92%.

The chromatogram of the test solution prepared for calibration (extraction performed on SPE column) is presented in Fig. 4.



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ZERO    =  0, 0.465
ATT 2^  = -1
CHT SP  =  1.0
AR REJ  =  0
THRSH   =  0
PK WD   =  0.12

+  RUN #    8     DEC 26, 2001  13:44:16
START
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Fig. 3. Chromatogram of the test solution analysed using GC with FID

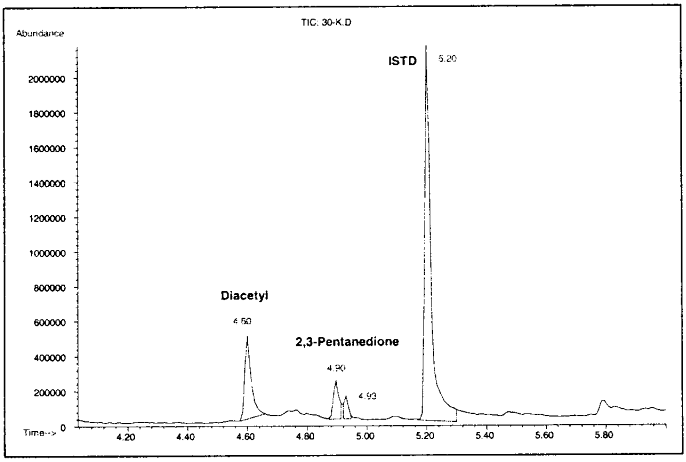

Fig. 4. Total ion chromatogram of derivates of diacetyl (2,3-dimethylquinoxalin), 2,3-pentanedione (2-ethyl-3-methylquinoxalin) and internal standard 2,3-hexanedione (2-methyl-3-n-propylquinoxalin)

Table 3. presents the results of the diacetyl and 2,3-pentanedione determination in beer using GC/MS. Table 4. presents statistical data (mean value ($\bar{x}$), standard deviation (*SD*), limit of detection -*LOD*, limit of quantitation -*LOQ* and coefficient of variation (*CV*)) obtained for the results presented in Table 3.

Statistical data show that the GC/MS method for the determination of diacetyl and 2,3-pentanedione is more sensitive and accurate than the method using GC with FID.

Chromatograms of an original beer sample and the beer sample with the addition of diacetyl and 2,3-pentanedione are presented in Figure 5. The samples were prepared by extraction on SPE column. The solution obtained after extraction on SPE column was clear and the determination simple.

Table 3. Determination of diacetyl and 2,3-pentanedione in the sample of beer with five replications using GC/MS

N° of analysis (N)	Diacetyl		2,3-Pentanedione	
	Mass concentration in prepared sample (ng/μL)	Mass concentration in beer (mg/dm^3)	Mass concentration in prepared sample (ng/μL)	Mass concentration in beer (mg/dm^3)
1.	0.45	0.090	0.20	0.039
2.	0.44	0.088	0.22	0.043
3.	0.45	0.090	0.23	0.045
4.	0.46	0.092	0.20	0.039
5.	0.44	0.088	0.19	0.037

Table 4. Statistical data obtained by diacetyl and 2,3-pentanedione determination using GC/MS

	Diacetyl		2,3-Pentanedione	
	Mass concentration in prepared sample (ng/μL)	Mass concentration in beer (mg/dm^3)	Mass concentration in prepared sample (ng/μL)	Mass concentration in beer (mg/dm^3)
$\bar{x}$	0.45	0.090	0.21	0.041
SD	0.0077	0.0015	0.0148	0.0030
LOD	3.0077	0.0045	0.0444	0.0090
LOQ	0.077	0.015	0.148	0.030
CV	1.71%		7.06%	

To determine diacetyl and 2,3-pentanedione by gas-chromatography with flame ionization detector, it is necessary to preconcentrate the sample. Extraction, liquid-liquid, solid-phase extraction - SPE and solid-phase microextraction - SPME can be used for the preconcentration.

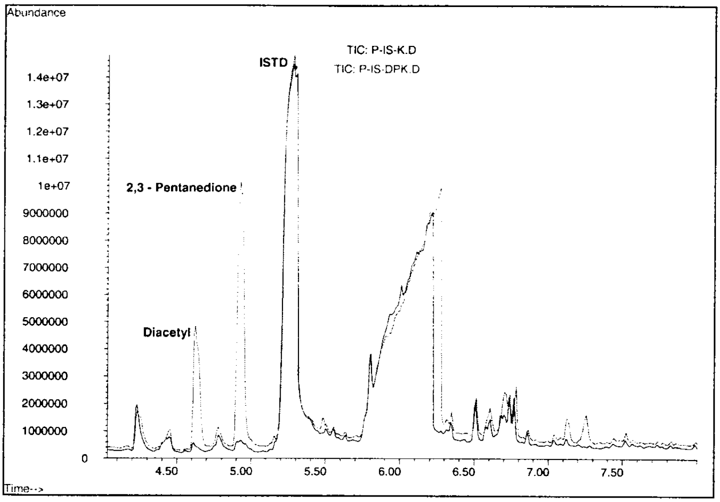

Fig. 5. Total ion chromatograms of beer samples with and without addition of diacetyl and 2,3-pentanedione extracted on SPE column

The use of gas chromatography with mass selective detection is recommended for the determination of diacetyl and 2,3-pentanedione content because of the complex composition of beer and because of the fact that the retention time depends on the sample composition. Besides, this procedure gives the retention time as well as the mass spectrum of the compound investigated, resulting in more specific identification of investigated compounds. If compared LOD and LOQ values calculated for data obtained by using gas chromatograph with flame ionisation detector and gas chromatograph with mass selective detector, it can be concluded that sensitivity is 800 times higher by using gas chromatograph with mass selective detector.

Extraction on SPE columns proved to be simple and reliable. This method is simple and can be used for simultaneous determination of diacetyl and 2,3-pentanedione content in a large number of beer samples.

REFERENCES

1. Walker, G. M.: Yeast Technology in Yeast Physiology and Biotechnology, John Wiley and Sons, Chichester, England, (1998) pp. 283-284.
2. Nedović, V.: Fermentacija piva u Imobilisani ćelijski sistemi u fermentaciji piva, Monografija, Zadužbina Andrejević, Beograd, (1999) p. 14.
3. Kunze, W.: Proizvodnja piva u Tehnologija sladarstva i pivarstva (Technologie Brauer und Malzer, prevod), Jugoslovensko udruženje pivara, Beograd, (1998) p. 308.
4. Fix, G. J.: Diacetyl: Formation, Reduction, and Control, *Brewing Techniques*, **1** (1993) 20.
5. Boulton, C. and D. Quain: Biochemistry of fermentation in Brewing yeast and Fermentation, Blackwell Science, United Kingdom (2001) pp. 128-129.

6. Haukeli, A. D. and S. Lie: Production of Diacetyl, 2-Acetolactate and Acetoin by Yeasts during Fermentation, *J. I. Brewing*, **78** (1972) 229-232.
7. Engan, S.: Beer Composition: Volatile Substances in Brewing Science, Volume 2, Edited by Pollock, J. R. A., Academic Press Inc. London Ltd., John Wiley and Sons Ltd., Bristol, Great Britain (1981) p. 130.
8. Engan, S.: Beer Composition: Volatile Substances in Brewing Science, Volume 2, Edited by Pollock, J. R. A., Academic Press Inc. London Ltd., John Wiley and Sons Ltd., Bristol, Great Britain (1981) p. 131.
9. Kunze, W.: Proizvodnja piva u Tehnologiji sladarstva i pivarstva (Technologie Brauer und Malzer, prevod), Jugoslovensko udruženje pivara, Beograd, (1998) p. 310.
10. Lodahl, M.: Malt extracts: Cause and concern, *Brewing Techniques*, **1** (1993) 26-28.
11. Fix, G. J.: Diacetyl: Formation, Reduction, and Control, *Brewing Techniques*, **1** (1993) 24-25.
12. Boulton, C. and D. Quain: Biochemistry of fermentation in Brewing yeast and Fermentation, Blackwell Science, United Kingdom (2001) pp. 130-131.
13. Mändl, B., Geiger, E. und A. Piendl: Über die Bildung von Acetohydroxysäuren und das Verhalten von vicinalen Diketonen während der Gärung, *Brauwissenschaft*, **27** (1974) 57-66.
14. Engan, S.: Beer Composition: Volatile Substances in Brewing Science, Volume 2, Edited by Pollock, J. R. A., Academic Press Inc. London Ltd., John Wiley and Sons Ltd., Bristol, Great Britain (1981) p. 132.
15. Haukeli, A. D. and S. Lie: The Influence of 2-Acetohydroxy Acids on the Determination of Vicinal Diketones in Beer and During Fermentation, *J.I.Brewing*, **77** (1971) 538-543.
16. White, F. H. and T. Wainwright: Analysis of Diacetyl and Related Compounds in Fermentations, *J. I.Brewing*, **81** (1975) 46.
17. Pajunen, E. and V. Mäkinen: The effect of pH and agitation on beer fermentation, European Brewery Convention. Proceedings of the 15th Congress, Nice. Elsevier Scientific Publishing Company, Amsterdam, The Netherlands, (1975) 525-538.
18. Engan, S.: Beer Composition: Volatile Substances in Brewing Science, Volume 2, Edited by Pollock, J. R. A., Academic Press Inc. London Ltd., John Wiley and Sons Ltd., Bristol, Great Britain (1981) p. 132-133.
19. Fix, G. J.: Diacetyl: Formation, Reduction, and Control, *Brewing Techniques*, **1** (1993), pp. 23-24.
20. Liebs, P., Krüger, M. und H. C. Wolter: Untersuchungen zum schnellen Diacetylabbau in Bier durch *Saccharomyces carlbergensis*, *Nahrung*, **14** (1970) 33-38.
21. MEBAK–Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission, Band III, 1. Gaschromatographie, 1.2. Vicinale Diketone, 1.2.1. Vicinale Diketone–Headspace-Methode, 2. Auflage, Vollständig neubearbeitet, Selbstverlag der MEBAK, D-85350 Freising-Weihenstephan (1996) s. 18-21.
22. ACS Committee on Enviromental Improvement: Guidelines for data acquisition and data quality evaluation in enviromental chemistry, *Anal. Chem.*, **52** (1980) 2242-2249.

ОДРЕЂИВАЊЕ ДИАЦЕТИЛА И 2,3-ПЕНТАНДИОНА У ПИВУ ПОМОЋУ GC/MS УЗ ПРИМЕНУ SOLID-PHASE EXTRACTION КОЛОНА

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Истраживана је нова GC/MS метода за одређивање диацетила и 2,3-пентандиона. Диацетил и 2,3-пентандион су дериватизовани са 1,2-фенилендиамином при чему настају 2,3-диметилквиноксалин и 2-етил-3-метилквиноксалин. Настале коли-чине 2,3-диметилквиноксалина и 2-етил-3-метилквиноксалина су биле пропорцио-налне концентрацијама диацетила и 2,3-пентандиона у узорку. 2,3-диметилквино-ксалин и 2-етил-3-метилквиноксалин су екстраховани помоћу SPE колона и одре-ђивани гасном хроматографијом са масено-селективним детектором. Ова метода је примењена за одређивање концентрација диацетила и 2,3-пентандиона у пиву. Екстракција помоћу SPE колона се показала једноставном и поузданом. Метода се може користити за истовремено одређивање садржаја диацетила и 2,3-пентандиона у већем броју узорака пива.

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