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Analysis of volatile compounds in beer of extruded rice as adjunct by headspace sampling-gas chromatography

Dongliang Zhang, Yanfei Cao, Yulu Wang, Hongjun Li*
School of Agricultural Engineering and Food Science, Shandong University of Technology, No.12 Zhangzhou Road, Zhangdian District, Zibo, Shandong Province, Chian.
Corresponding author: Hongjun Li

Abstract: *It is necessary to analyze the concentration of aroma volatiles in beer since the volatile compounds are very important factors determining the quality of final product. In this article, a simple, reliable, and sensitive static headspace-capillary gas chromatography spectrometry (HS-GC) method was developed in quantificating volatile compounds in beer of extruded rice as adjunct volatile compounds, i.e. acetaldehyde, N-propanol, ethyl acetate, isobutyl alcohol, isoamyl alcohol, isoamyl acetate, ethyl hexanoate and ethyl octanoate were separated and quantificated from beer using HS-GC. The proposed method showed excellent analytical characteristics of repeatability, recovery, accuracy, and limit of detections. HS-GC method was strongly recommended in determining the volatile compounds in beer.*

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I. Introduction

Beer is a complex mixture that constituents widely vary in nature and concentration levels, and it consists mainly of water and ethanol with about 0.5% of soluble solids [1]. Extrusion plays an important role in the modern cereal-based industry especially for the production of snack from corn, wheat, rice and oats[2]. Rice has become an attractive ingredient in the extrusion industry for its bland taste and ease of digestion[3].

It was known that beer flavors were produced by the metabolism of yeasts[4 - 5]. And the flavor is the results of a complex interaction of chemical compounds, such as, alcohols, aldehydes, acids, esters, ketones, sulphur compounds, etc.. Beer flavor could be influenced by different substances both individually and in a synergistic or antagonistic sense [6]. Beer flavor and fresh note were mainly influenced by volatile compounds. However, some volatile compounds contributed greatly to the beer flavor, while others were merely in building the background flavor of the product [7]. Flavor is considered as the main index in evaluating the quality of beer. And it is a very important factor for both brewers and consumers in commercial market. Therefore, it is necessary to develop a fast and reliable method in evaluating beer flavor. From the 1960s, several studies have focused on beer volatile compounds [8]. Advances in analytical equipments and methods have helped to analyze the volatile compounds in beer. Studies of volatile compounds in beer or other food have been reviewed previously[9-10]. Gas chromatography (GC) is the conventional technique for the detection and identification of aroma components [11]. Flame ionization detector (FID) is a sensitive detecting system for gas chromatography [12].

Recently, many different volatile compounds in beer have been identified. Some of them reduced in concentration during maturation, which followed fermentation [11]. Qualification technique of volatile compounds in regular beer has been studied before. However, there were few published articles about beer of extruded rice as adjunct. The objective of this work aimed to investigate the flavor compounds in beer of extruded rice as adjunct using HS sampling and GC. We focused on eight kinds of volatile compounds, which were selected as representative compounds responsible for the beer flavor.

II. Materials And Methods

2.1 Reagents and materials

The ethanol standard (chromatographic purity) was supplied by SaiFu Technology Co. (Tianjin, China). The standards (chromatographic purity), acetaldehyde, isobutyl alcohol, N-propanol, isoamyl alcohol, isoamyl acetate, ethyl hexanoate and ethyl octanoate were obtained from SiYou Fine Chemicals Co. (Tianjin, China). And the ethanol standard (analytical purity) was purchased from Kermel Chemical Reagent Co. (Tianjin, China).

Twenty mL headspace vials and the aluminum crimp caps were produced by Derian Instrument Co. (Shanghai China). The 1.000 mL micro injectors were obtained from Dragon Co. (Shanghai, China). Single screw extruder was provided by the Laboratory for Intensive processing of agricultural products of Shandong University of Technology.

2.2 Sample Preparation

Extrusion of rice was performed using a single screw extruder, which had a provision in adjusting the screw speed from 0 to 2000 r min^{-1}. The optimum parameters of extrusion system were as follows: diameter of die nozzle was Φ = 12mm×3, extrusion temperature was T = 60 oC, moisture of material was W = 20%, speed of screw was n = 200 r min^{-1}. After the extrusion pretreatment was finished, 60 kg malt and 40 kg extruded rice were saccharified. The saccharification procedure of extruded rice was shown in Figure 1. The ratio of material to water was 1: 4.86, and the getting ratio of wort was 76.26%.

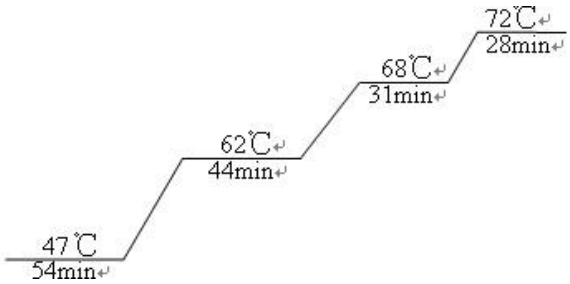

Figure 1 The Saccharification technology of extruded rice

Beer of extruded rice as adjunct sample was obtained directly from the fermentation tank. The beer was produced in our laboratory by simulating the traditional brewing method: wort was fermented in a 100 L fermentation tank at 12 oC for about 10 days, then cooled to 0 oC and stored in the tank for another 10 days. About 350 mL beer was added to 500 mL flask iodine and immediately covered with glass stopper, placed in the refrigerator (0-5 oC) until the foam disappeared (about 10 min). A 200 mL sample was added to a 250 mL volumetric flask, then 25 μL N-butyl alcohol was added as internal standard, finally the mixture was constant volume to 250 mL by beer mentioned above. Ten mL mixture was transferred to 20 mL sample vial, then the vial was tightly sealed immediately with a crimp cap and 20-mm white aluminium septa (Supelco). Every sample was repeated three times.

2.3 Preparation of mixed standard solution

In order to minimize the loss of volatile compounds, 200 mL ultrapure water and 10 mL ethanol was put into a volumetric flask (250 mL) before the standards were added. And then 25 μL acetaldehyde, 25 μL N-propanol, 12.5 μL ethyl acetate, 12.5 μL isobutyl alcohol, 25 μL isoamyl alcohol,12.5 μL isoamyl acetate, 12.5 μL ethyl hexanoate, 2.5 μL ethyl octanoate, 25 μL N-butyl alcohol were injected into the same volumetric flask with a syringe. Finally, the mixture was constant volume to 250 mL with ultrapure water.

Ten mL of standard solution was pipetted into a 20 mL headspace vial, subsequently the vial was tightly sealed immediately with crimp caps and 20-mm white aluminium septa. The experiment was carried out with a standard solution composed of : 78.80 mg L^{-1} acetaldehyde, 80.36 mg L^{-1} N-propanol, 45.00 mg L^{-1} ethyl acetate, 40.09 mg L^{-1} isobutyl alcohol, 81.25 mg L^{-1} isoamyl alcohol, 43.80 mg L^{-1} isoamyl acetate, 8.71 mg L^{-1} ethyl hexanoate, 7.88 mg L^{-1} ethyl octanoate, and 80.95 mg L^{-1} N-butyl alcohol. The quantity of the flavor compounds was qualified using internal standard method, and N-butyl alcohol was added as internal standard material.

2.4 HS-GC Equipment and Conditions

The separation, detection and quantification of the volatile compounds released from the beer samples was performed using an Agilent 6890N capillary gas chromatograph with a flame ionization detector(FID), (Agilent Co., USA), connected to a static headspace auto-sampler (Agilent 7694E headspace auto-sampler, Agilent Co., USA). This sampler applies the principle of time-controlled injection. Compounds were separated on an Agilent HP-5 capillary column (30.0 m × 0.32 mm i.d., 0.25 μm film thickness). The chromatograph was fitted with 5% phenyl methyl siloxane. Column intinial temperature was 45 oC for 5 min and increased to 180 oC with 10 oC min^{-1}. The flow rate of the carrier gas (Nitrogen) was maintained 3.5 mL min^{-1}.

The operating conditions of the HS were as follows : vial quilibration temperature, vial quilibration time, oven temperature, vial pressurization time, and loop fill time, were set 85 oC, 30 min 95 oC, 0.13 min, and 0.13 min, separately. The interface of both modules (HS and GC) was an inert transfer line heated at 100 oC.

III. Results And Discussion

3.1 The GC chromatograms of beer and mixed standard solution

There are a great number of volatile compounds, and it remains hard to determine every kind of trace volatile fraction. In this study, static headspace-capillary gas chromatography spectrometry (HS-GC) method was used to analyze the concentration of volatile compounds in beer of extruded rice as adjunct. The GC chromatograms of beer and mixed standard solution were shown in Figure 2 and Figure 3.

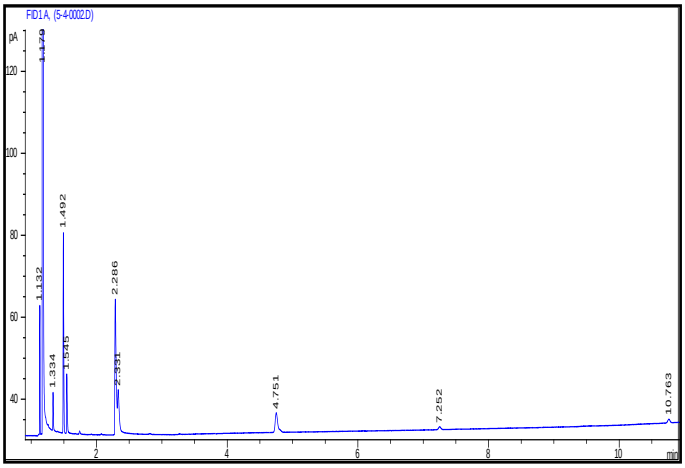

Figure 2 The GC chromatogram of beer product

The corresponding compounds and their retention time of the peaks in Fig.2 were Acetaldehyde (1.132min), ethanol (1.179min), N-propanol (1.334min), ethyl acetate(1.492min), isobutyl alcohol(1.545min), isoamyl alcohol (2.286min), isoamyl acetate (4.751min), ethyl hexanoate (7.252min) and ethyl octanoate (10.763min). Figure 2 and Figure 3 demonstrated that the curve had favorable peak shape and stable baseline, and no doublets and ghost peaks was appeared in the chromatogram. In order to avoid possible matrix effects on the calibration efficiency, the internal standard addition method was used in the calibration of the selected compounds.

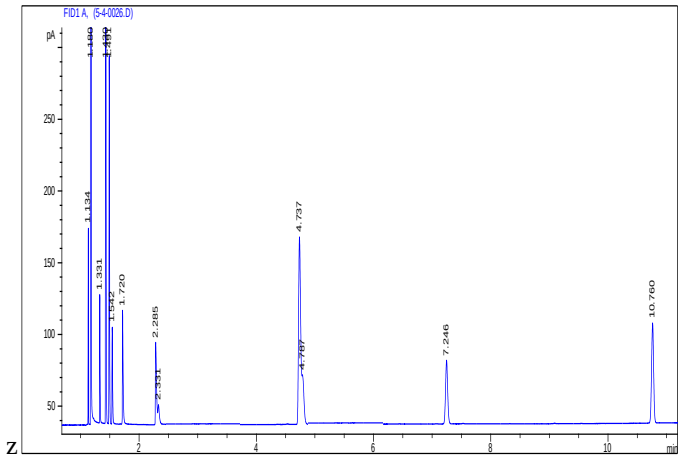

Figure 3 The GC chromatogram of mixed stand sample

3.2 Qualitative analysis

The chromatographic peaks of volatile flavor substances were identified by the retention time of the individual reference standards. Headspace chromatographic profiles were compared with known reference flavor standard compounds for identification purpose.

Eight kinds of volatile compounds were separated and identified: acetaldehyde, ethanol, ethyl acetate, isobutyl alcohol, N-propanol, isoamyl alcohol, isoamyl acetate, ethyl hexanoate and ethyl octanoate.

3.3 Quantitative analysis of flavor compounds

Volatile compounds was determined and quantified with an FID. Signals of FID were stored and integrated using computer software. The concentration of each volatile component (Ci) was calculated according to Eq. (1).

$$Ci = \frac{Ai \times Cs \times fi}{As} \quad (1)$$

The index Cs refers to the concentration of the internal standard; As is the peak area of the internal standard; Ai is the peak area of a particular volatile compound; fi identifies the calibration factor. Among the indexes in the Eq. (1), the calibration factor fi could be obtained by the formula (2) described below:

$$fi = \frac{As \times Wi}{Ai \times Ws} \qquad (2)$$

In formula (2): As refers to the peak area of the internal standard; Ws is the concentration of the internal standard; Ai is the peak area of a particular standard; and Wi identifies the concentration of the particular standard. The concentrations of each volatile compound were listed in Table1. Table 1 showed that all studied volatile compounds were tower above the thresholds of quantification limits. Results indicated that the eight kinds of volatile compounds varied dramatically in concentrations.

A validation study was carried out by assessing precision; recovery and detection limit. The repeatability was accomplished by detecting 5 same beer samples, and detection precisions were < 5% (RSD). Recovery ratios data of volatile compounds in beer of extruded rice as adjunct were ranging from 95.3% to 99.7%.

Table 1 The concentration of volatiles composition of beer

N-butyl alcohol			beer of extruded rice adjunct	
average peak area	concentration (mg L^{-1})	volatile compound	average peak area	concentration (mg L^{-1})
60.5	80.95	acetaldehyde	14.3	22.6
		N-propanol	6.2	12.2
		ethyl acetate	38.3	11.6
		isobutyl alcohol	13.9	13.1
		isoamyl Alcohol	58.2	70.0
		isoamyl acetate	15.3	2.4
		ethyl hexanoate	2.2	0.2
		ethyl octanoate	3.0	0.3

Table 2 The recovery ratio of volatiles in beer

volatile compound	addition quantity (mg L^{-1})	content of samples (mg L^{-1})	estimated value (mg L^{-1})	recovery ratio (%)
acetaldehyde	78.80	22.6	100.6	99.2
N-propanol	80.36	12.2	92.3	99.7
ethyl acetate	180.00	11.6	187.3	97.8
isobutyl alcohol	40.09	13.1	50.7	95.3
isoamyl alcohol	81.25	70.0	149.9	99.1
isoamyl acetate	43.80	2.4	44.5	96.3
ethyl hexanoate	8.71	0.2	8.76	98.3
ethyl octanoate	7.88	0.3	7.81	95.5

IV. Conclusion

In this article, HS-GC was used in determining the concentration of volatile compounds in beer of extruded rice as adjunct. Since all quantification limits were well below the respective flavor thresholds of the studied volatile compounds, this method could be applied in beer flavor research.

Eight kinds of volatile compounds were separated and identified: acetaldehyde, ethanol, ethyl acetate, isobutyl alcohol, N-propanol, isoamyl alcohol, isoamyl acetate, ethyl hexanoate and ethyl octanoate. And the concentrations of volatile compounds were 22.6 mg L^{-1}, 12.2 mg L^{-1}, 11.6 mg L^{-1}, 13.1 mg L^{-1}, 70.0 mg L^{-1}, 2.4 mg L^{-1}, 0.2 mg L^{-1}, and 0.3 mg L^{-1}, respectively. The good sensitivity and recoveries confirmed that headspace-capillary gas chromatography spectrometry method had the potential interest in quantification the beer of extruded rice as adjunct.

Acknowledgements

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