

Volatile Compounds Fingerprints for White Duck down and White Goose down Determined by Gas Chromatography-Ion Mobility Spectrometry

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Abstract

This work first describes a simple approach for the untargeted profiling of volatile compounds for distinguishing between white duck down (WDD) and white goose down (WGD) based on resolution-optimized GC-IMS combined with optimized chemometric techniques, namely PCA. The detection method for down samples was established by using GC-IMS. Meanwhile, the reason of unpleasant odors caused by WDD was explained on the basis of the characteristic volatile compounds identification. GC-IMS fingerprinting can be considered a revolutionary approach for a truly fully automatable, cost-efficient, and in particular highly sensitive method. A total of 22 compounds were successfully separated and identified through GC-IMS method, and the significant differences in volatile compounds were observed in three parts of WDD and WGD samples. The most characteristic volatile compounds of WGD belong to aldehydes, whereas carboxylic acids from WDD were detected generated by autoxidation reaction. Meanwhile, the main reason of unpleasant odor generation was possibly attributed to the high concentration of volatile carboxylic acids of WDD. Therefore, the constructed model presents a simple and efficient method of analysis and serves as a basis for down processing and quality control.

Keywords

Gas Chromatography-Ion Mobility Spectrometry (GC-IMS), Principal Components Analysis (PCA), Down, Characteristic Volatiles Fingerprints,

1. Introduction

The down and feather industry of China possesses 70% share of the global market, especially down industry with a high value. White duck down (WDD) and white goose down (WGD) are fine thermal insulator and padding, used as raw material for down products, such as jackets, bedding, pillows and sleeping bags. The residual oil and fat content of WDD and WGD during manufacturing affects the thermal property and odors characteristics of down products through volatile compounds release [1]. The requirement of oil and fat content for down products is limited in the range of 0.3% - 0.5% regulated by importers from Japan, USA and EU etc. Generally, the thermal property and odor characteristics of WGD are better than that of WDD due to different diet and living habits, led to great differences in quality and final price. Meanwhile, in the case of WDD, the existence of unpleasant odors hinders seriously the development of its industries. The research focused on volatile compounds released by WDD leading to unpleasant odor was less reported because of its complex composition. Therefore, the explanation for volatile compounds generated from WDD could be useful to define the reason of unpleasant odor, to distinguish WDD from WGD, to characterize the down level, to develop suitable deodorant using in down manufacturing process. In addition, studies on the volatile compounds from WDD and WGD could be an interesting method for down industry.

The volatile compounds in down create a challenge in their extraction, separation and quantitation due to the complexity of the matrix and their very low concentrations [2] [3]. Gas chromatography (GC) was considered as a valuable technique in flavour analysis, used to characterize odor-active compounds, as well as character impact compounds [4] [5]. Recent years, most of developed analytical methods are based on GC techniques, coupled to flame ionization detection or mass spectrometry (MS) determining different analytes as markers [6] [7]. Furthermore, determination of volatile fraction using other advanced techniques, such as electronic nose [6] and ion mobility spectrometry (IMS) with UV ionization source [8] [9], has been also described in food quality and food safety. IMS is a rapid analytical technique for volatile and semi-volatile compounds based on the gas-phase separation of ionized molecules under a weak electric field at ambient pressure through detecting the drift velocities of the ionized sample molecules [10] [11] [12] [13]. The ion mobility (K) is equal to the proportion between the drift velocity (v_d) and the strength of electric field (E), given by Equation (1) [10]. The ion mobility is related to the mass of the sample species and its collision cross section (CCS) with the drift gas, expressed as Equation (2) [9].

$$K = v_d/E = L/(t_d \times E) \quad (1)$$

where L is the length of the drift zone, and t_d is the drift time.

$$K = \frac{3}{16} \frac{q}{N} \sqrt{\frac{1}{m} + \frac{1}{M}} \sqrt{\frac{2\pi}{kT}} \frac{1+\Delta}{\pi r^2 \Omega} \quad (2)$$

where q is the ion charge of analyte, N is the density of drift gas molecules, m is the mass of analyte ion, M is the mass of drift gas molecular, k is the Boltzmann constant, T is the temperature, Ω is the collision cross section, r is the minimum in the potential curve, and Δ is the correction term for approximations.

Since the operation of IMS was under atmospheric pressure, IMS was characterized by limited selectivity due to induce ion-molecule and ion-ion competitive reactions in the ionization chamber. The selectivity of IMS can be improved in terms of effective pre-separation method [14] [15]. It has been reported that the coupling of a GC column as a previous separation step to IMS improved the results when this platform is applied to olive oil and Iberian ham authentication [7] [14]. Based on the examples mentioned above, GC-IMS possesses high sensitivity and efficiency, low detection limit (ppbv level), rapid detection time (5 - 20 mins), simple operation and inexpensive, effectively used for metabolic profiling of human breath, process and quality control analysis, as well as food quality and safety control [16] [17]. Though the volatile components of can be detected by traditional GC-MS method, the application of GC-IMS method could remove the pre-treatment of down samples and improve the efficiency, that's why GC-IMS was selected for the detection of WDD and WGD samples.

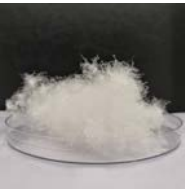

In this work, GC-IMS was employed to develop a simple and rapid detection method to distinguish between WDD and WGD through complete spectral fingerprint analysis of volatile compounds. It can be avoid the adulteration in WGD products by using GC-IMS and chemometric analysis. The detection parameters of GC-IMS for down samples were established in this paper. Specifically, the reason of generated unpleasant odor from WDD was investigated through the determination of volatile compounds with potential use for developing novel deodorant in manufacturing process. The GC-IMS technique used for identification of oil, wine and ham was already reported, however, this is the first time for determination of volatile compounds by GC-IMS to assign the quality of down materials.

2. Materials and Methods

2.1. Reagents and Samples

A total of 4 down samples (2 WDD and 2 WGD samples) were selected. Down samples were obtained from *ZHANJIANG ZIKING EIDERDOWN PRODUCTION COMPANY*. WDD and WGD samples were confirmed by two groups of parallel tests. In addition, down samples were cut and divided into small piece around 1 cm of length. The sample was washed by pure water and dried naturally. Subsequently, samples were stored at cool and dry place. The detail of down samples was listed in **Table 1**. The data of down content was supplied by

Table 1. Detail of down samples used in this study.

No.	Name	Down	Down content (%)	Oil and fat content (3 h, %)	Figure
1	BYR-1	WDD	91.32 ± 0.15	0.73 ± 0.11	
2	BYR-2				
3	BER-1	WGD	94.83 ± 0.08	0.42 ± 0.07	
4	BER-2				

ZIKING EIDERDOWN PRODUCTION COMPANY. The oil and fat content were collected and averaged among three repeated samples by Soxhlet extraction method, according to GB/T 10288-2016—Test of Down and Feather. Other reagents used in this work were of analytical grade, pursued from Sigma-Aldrich (*St. Louis, MO, USA*).

2.2. Instrumentation and Software

The Analyses of WDD and WGD samples were performed on an GC-IMS commercial instrument (*FlavourSpec*[®]) from Gesellschaft für Analytische Sensorysysteme mbH (*G.A.S., Dortmund, Germany*), equipped with a radioactive ionisation source (3H) of 6.5 keV and a heated splitless injector with 2 mm ID, 6.5 mm OD × 78.5 mm fused quartz glass, which enables direct sampling of the headspace by using a 2.5 mL Hamilton syringe furnished with a 51 mm needle. IMS was fitted with GC columns, a weak-polar column with 15 m of length, an internal diameter of 0.53 mm (FS-SE-54-CB-1 of CS-Chromatographie Service GmbH, Düren, Germany). The GC-IMS method employed for the in-line vapor phase generation and mobility measurements is depicted in **Figure 1**. The remaining instrumental parameters used are summarized in **Table 2**. For analysis, 1 g of WDD or WGD sample with 2 ml pure water was placed in a 20 ml headspace sampling vial closed by a magnetic cap. After 30 mins of incubation at 50 °C, 500 µL of sample headspace was automatically injected by means of a heated syringe (85 °C) into the heated injector (80 °C) of the GC-IMS equipment. After injection, N₂ of 99.99% purity as carrier gas passed through the injector inserting the sample into the gas column, which was heated at 40 °C for timely separation. The N₂ flow rate was increased from 2 to 100 ml·min⁻¹ in 20 mins based on linear flow control. Following the GC separation, the volatile compounds were eluted in the isothermal mode and driven into the ionization chamber for ionization, prior to spectrometric detection. Molecules were ionized under Tritium atmosphere (6.5 keV) and the resulting ions driven to the drift region

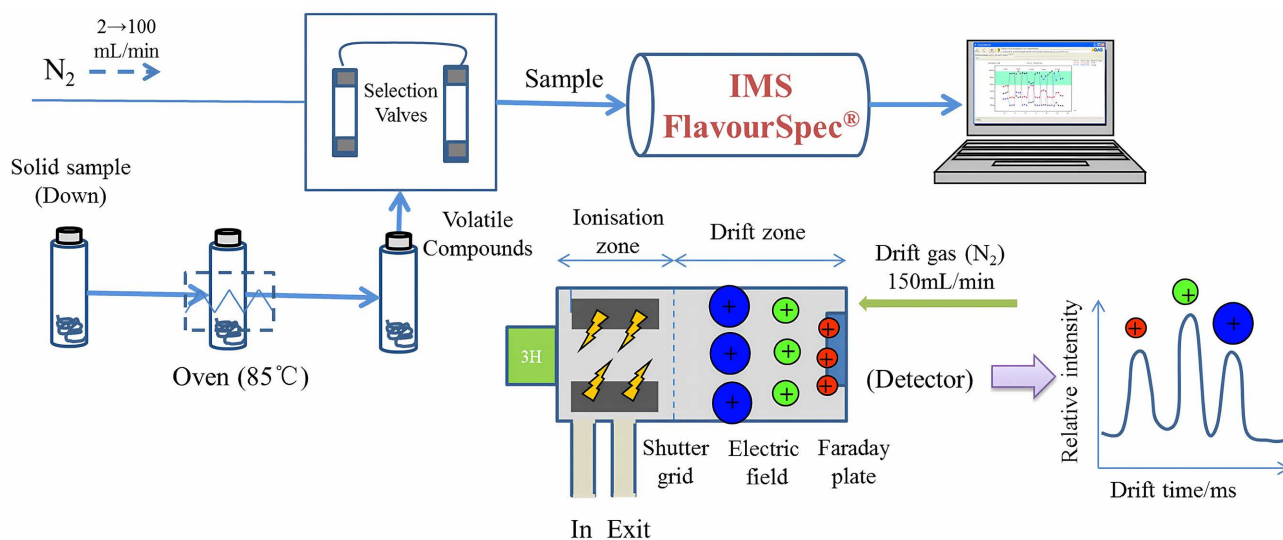


Figure 1. Overview of working principles of GC-IMS for down samples.

Table 2. Instrumental parameters used for GC-IMS.

Parameters	Value and unit
Drift tube temperature	45 °C
Drift tube length	5 cm
Drift tube diameter	10 mm
Drift gas flow	150 ml·min ⁻¹
Sample inlet flow	100 ml·min ⁻¹
Grid pulse width	100 μs
Sampling frequency	150 kHz
Repetition rate	30 ms
Voltage	400 V

via a shutter grid. The drift tube was 5 cm long and operated at a constant voltage of 400 V, a temperature of 45 °C and a drift N₂ gas flow rate of 150 ml·min⁻¹. Each spectra had an average of 12 scans, obtained using a grid pulse width of 100 μs, a repetition rate of 30 ms and a sampling frequency of 150 kHz.

2.3. Data Analysis

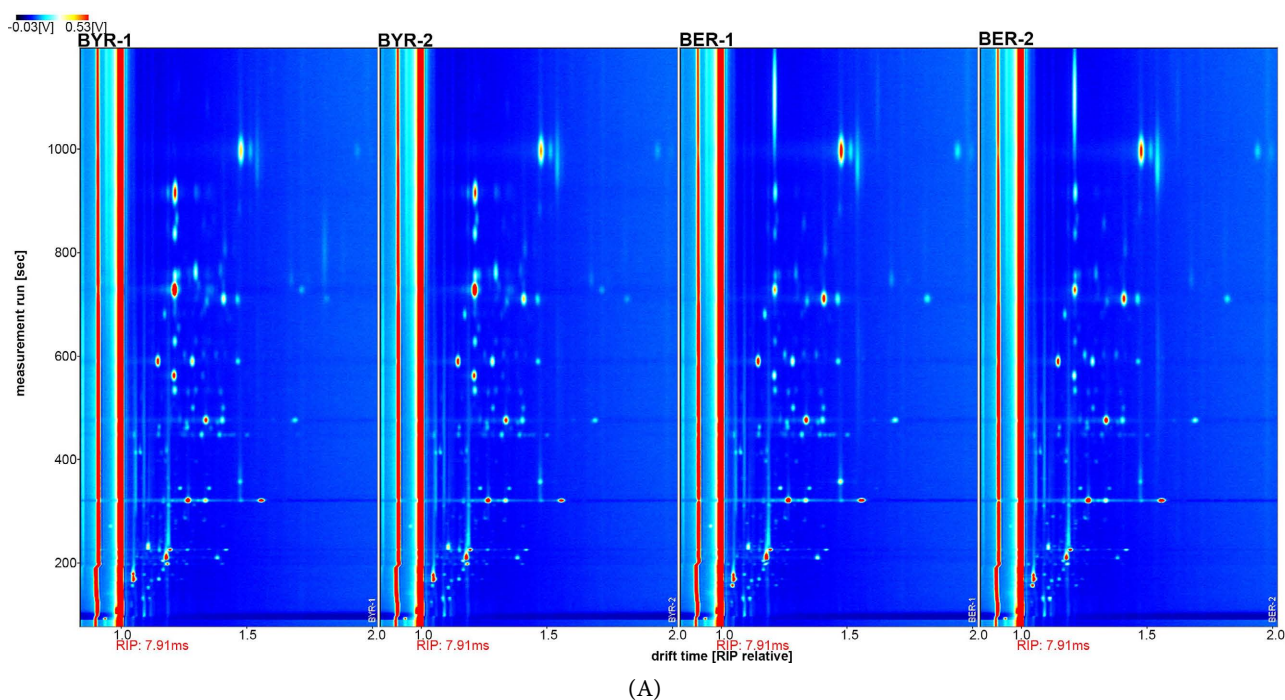
Data were acquired in the positive ion mode using laboratory analytical viewer (LAV) software (version 2.0.0) from *G.A.S.* Moreover, GC-IMS Library Search software supplied by *G.A.S.* was employed to identify unknown compounds. The GC-IMS data of one sample was a two-dimensional matrix (retention time and drift time). Since the very large data sets obtained by GC-IMS, an appropriate pretreatment procedure is necessary in order to avoid variation and misclassification in the multivariate statistical analysis. First, peak alignment was applied to each dataset with regard to RIP taken as a reference. Next, a smoothing proce-

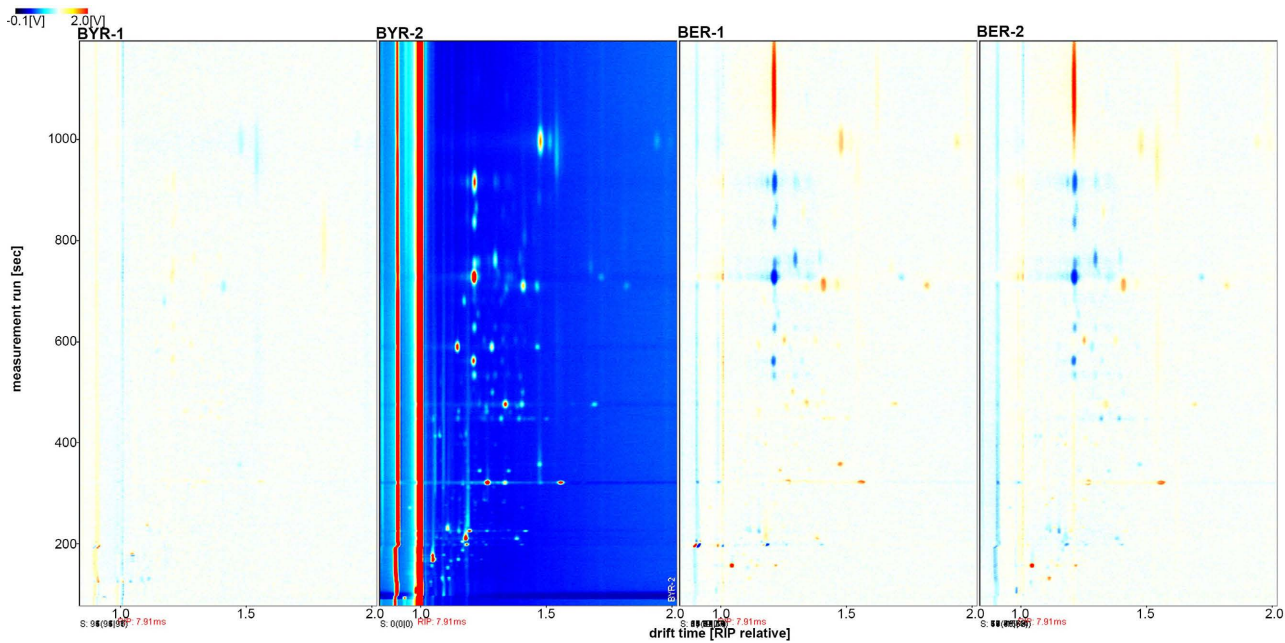
cedure based on second order Savitzky-Golay filtering and a slight baseline correction was needed. The zone of each topographic plot, which contains the majority of the data obtained with both devices, was selected (WDD: retention time from 85.0 to 1040.0 s and drift time from 7.0 to 15.6 ms. WGD: retention time from 85.0 to 1195.0 s and drift time from 7.0 to 16.1 ms.). Principal Components Analysis (PCA) was used to reduce the dimensionality and maximise class separability. The IMS fingerprints of down samples were created by summing the intensities at the same drift time and normalized by dividing the maximum value. All the calculations and preprocessing were assessed by using in-house MATLAB routines (*version R2016a, Mathworks, Natick, MA, USA*). The PCA-model was built by applying the MATLAB Statistical Toolbox (*version 9.1*). Before importing the GC-IMS data into MATLAB, all raw data files were first converted to csv text files using the csv export tool implemented in the LAV software from *G.A.S.*

3. Results and Discussion

3.1. GC-IMS Spectra to Stablish Differences between WDD and WGD Samples

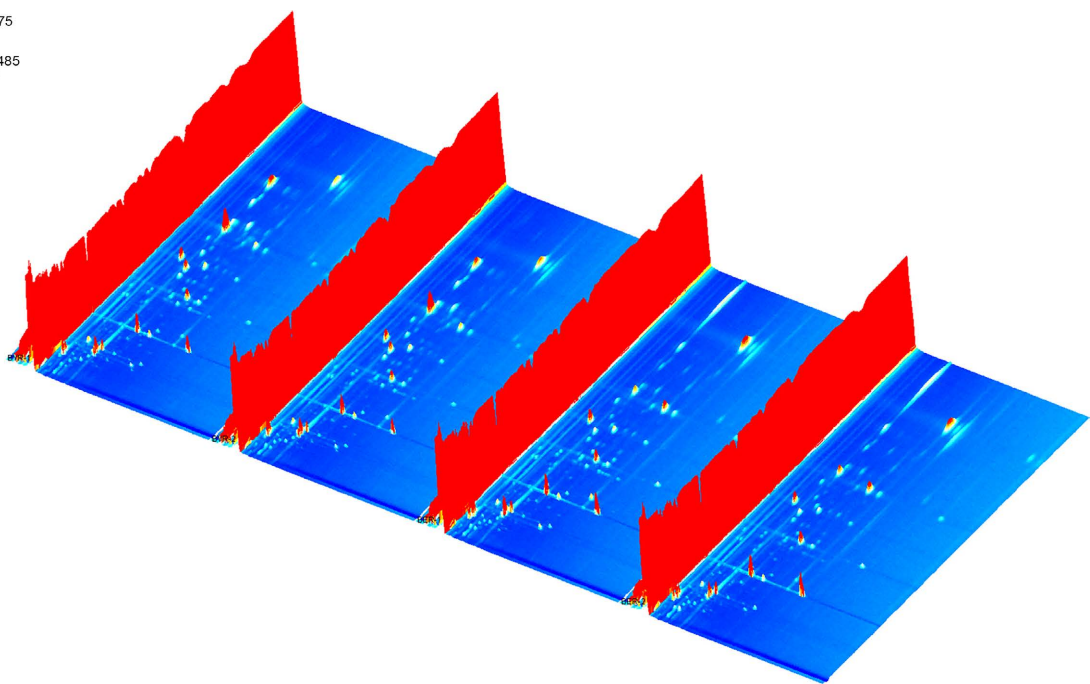
A GC-IMS analysis results in a topographic plot and tri-dimensional spectrum as shown in **Figure 2**. It is a tri-dimensional map in which Y axis represents the retention time in the chromatographic column (in seconds), X axis represents the drift time in the drift tube (in milliseconds), and Z axis the intensity value (in V) of each compound. Especially, one compound can generate more than one signal or spot (monomer and dimer or even trimer) depending on their concentration, and the brightness of spots related to their concentration. In order to





(B)

ALPHA: -0.7504382618522075
 BETA: 2.666868364711636
 GAMMA: 3.5217
 FACTOR: 0.8864097112623485
 zFak: 0.05085570471696191
 xPlus: 20
 yPlus: 161
 zPlus: 0



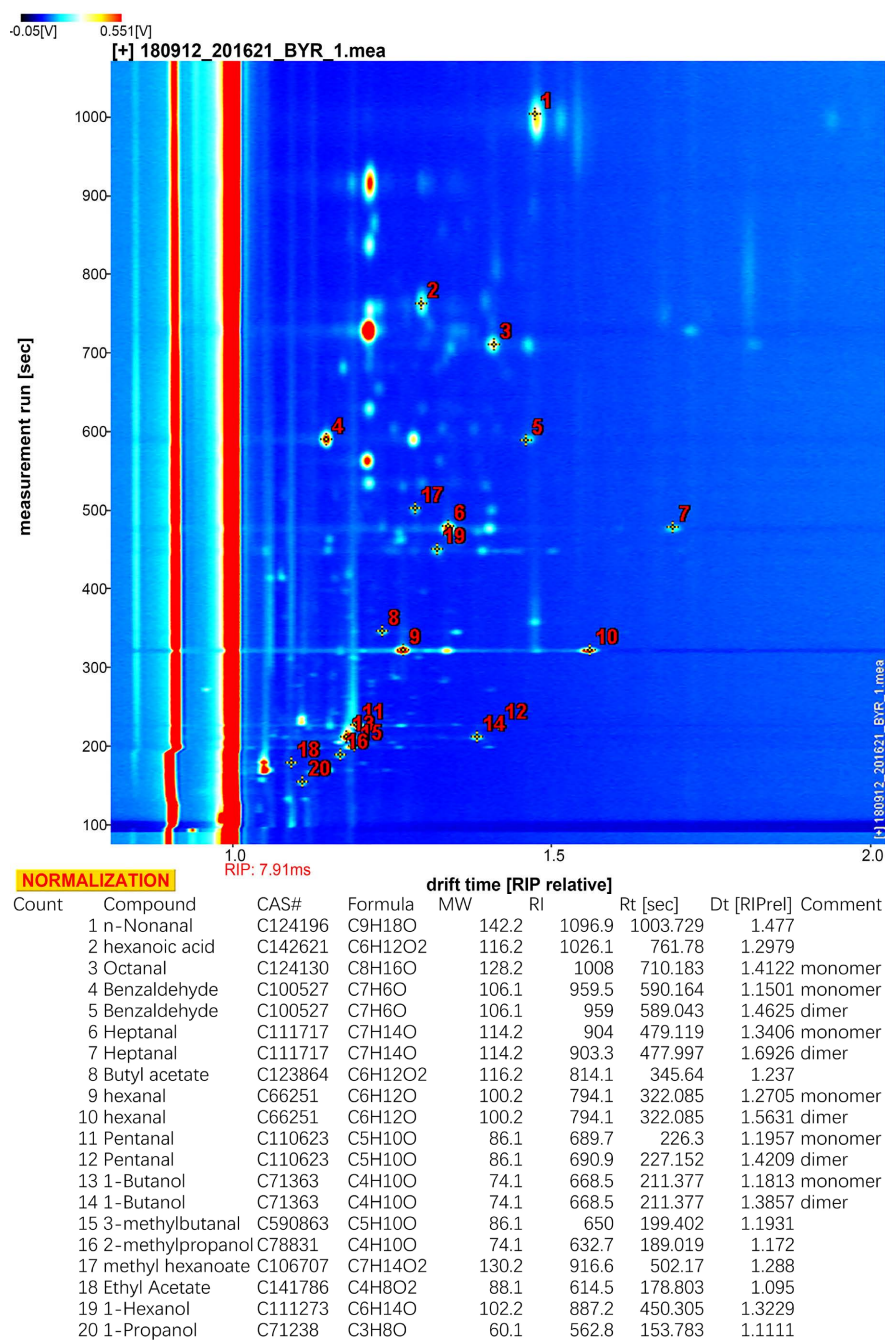
3D-View

(C)

Figure 2. Topographic plot of GC-IMS spectra originating from WDD and WGD ((A) Original spectrum; (B) Spectrum with BYR-2 as background subtracted; (C) Tri-dimensional spectrum).

compare the spectra of WDD and WGD obviously, the BYR-2’s spectrum was set as background, and the original spectrum (**Figure 2(A)**) was treated with background subtracted. Thus, the different region of characteristic volatile compounds between WDD and WGD can be easily observed in **Figure 2(B)** due to the compounds and their concentration. The red spots indicate the volatile

compounds' concentration of WGD was higher than that of WDD, and the blue spots means contrary. From the tri-dimensional map shown in **Figure 3(C)**, the difference between WDD and WGD can be differed through featuring peaks analysis at different drift times at the same retention time. Many compounds indicate the formation of different product ions with the highest ion mobility for the monomer and the lowest for the dimer or trimer in the 3D array. Therefore, a great variety in the spectra profiles of WDD samples was observed due to the higher oil and fat content.



(A)

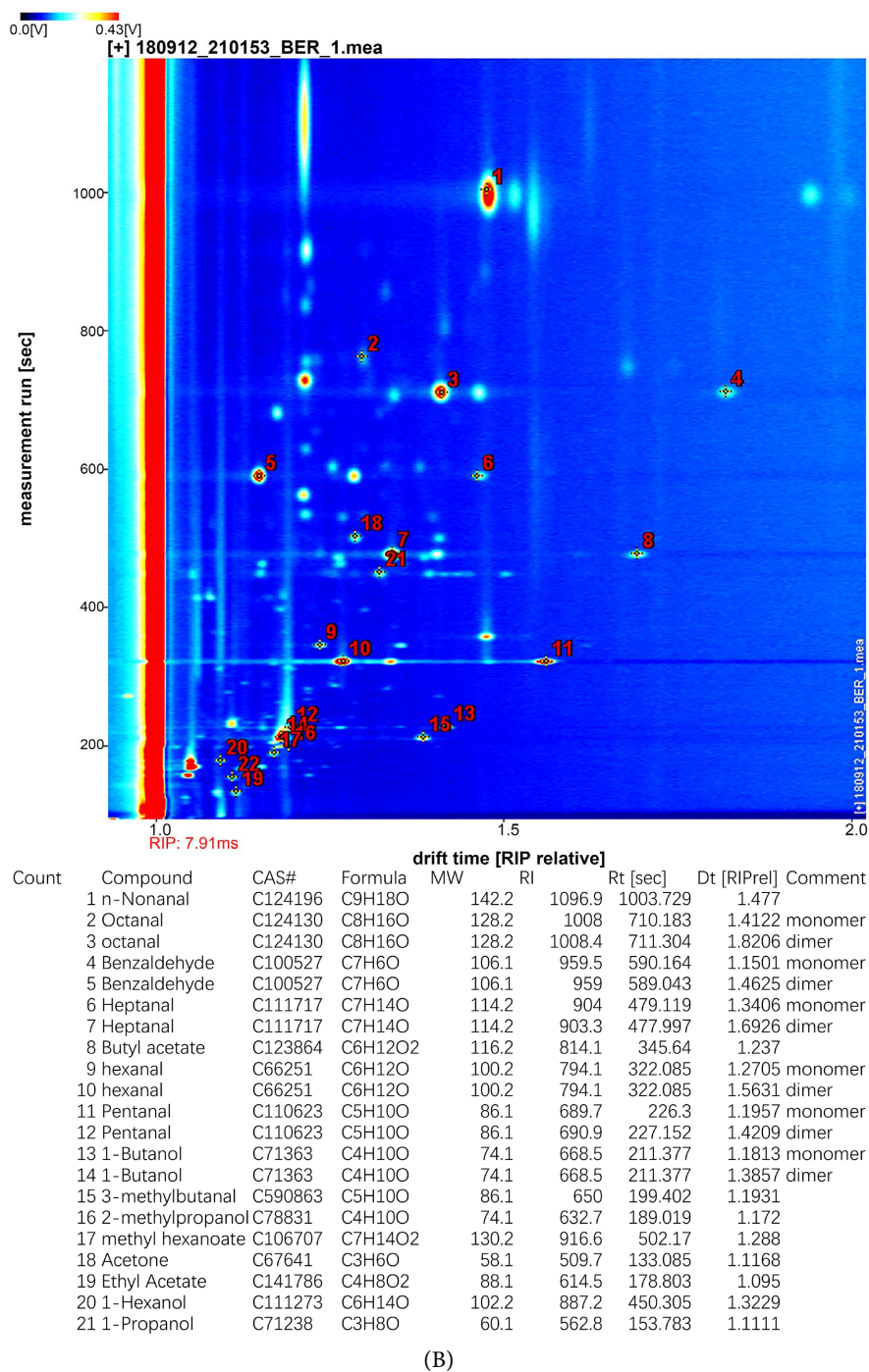


Figure 3. Typical GC-IMS chromatogram-mobility plot and identified signals of WDD and WGD ((A) BYR-1; (B) BER-1).

3.2. GC-IMS Spectra: Identification of Volatile Compounds

In order to identify the selected markers, a commercial GC-IMS Library was used and the compounds tentatively identified using this library was confirmed by comparison of retention time and drift time with their real reference standards. Initially, a visual inspection of the GC-IMS spectra of the two types of

down samples was carried out in order to identify potential useful signals to differentiate WDD and WGD based on different volatile compounds profiles in WDD and WGD samples, so as to establish a fast and reliable method for authenticity screenings. The spectra and the determined volatile compounds of the two different groups between WDD and WGD were shown in the corresponding PCA loadings plots (**Figure 3**). As can be seen, most of the signals appeared between 200 and 800 s of retention time had the strongest influence on class separation, although there is a signal at the retention time of 1003 ([1] n-Nonanal) in both samples. It can be seen that volatile compounds with low molecular weight are present at the highest concentration levels and elute in a time range between 200 and 400 s. A total of 20 and 21 volatile compounds were individually identified in WDD and WGD samples respectively through GC-IMS analysis. The signals generally are typically found in both WDD and WDD analyzed, for example, n-Nonanal, Octanal (monomer), Benzaldehyde (monomer and dimer), Heptanal (monomer and dimer), Butyl acetate, Hexanal (monomer and dimer), Pentanal (monomer and dimer), 1-Butanol (monomer and dimer), 3-methylbutanal, 2-methylpropanol, Methyl hexanoate, Ethyl Acetate, 1-Hexanol, 1-Propanol, just with variations in their intensities, were observed. The significant differences between volatile compounds profiles of WDD and WGD, which characterize WDD from WGD, were observed on variation in their intensities at later retention times. As an example, in the case of WDD, the characteristic signal of Hexanoic acid was found, however, the spectrum of WGD possessed the identified characteristic signal of Octanal (dimer) and Acetone.

The complex volatile fingerprints obtained from GC-IMS have been analysed using a non-supervised multivariate data analysis technique known as PCA. It describes the systemic patterns of variation between the samples. PCA was conducted to confirm the possibility of differentiating between WDD and WGD based on the IMS signals associate to the type of volatile compounds. In **Figure 4**, it can be observed that the two clusters formed can effectively distinguish between WDD and WGD. WDD samples show very similar patterns, trending to standardization, probably due to its volatile compounds composition. Furthermore, based on the analysis of volatile compounds, the possibility of the reason for generated unpleasant odor from WDD was estimate as the existence of hexanoic acid, which was not found in the WGD samples. Meanwhile, hexanoic acid was a kind of fatty acid, with unpleasant odor. In order to compare the volatile compounds of WDD and WGD, the fingerprint map was established through the selection of the peaks in the spectrum by using LAV-Gallery Plot software.

3.3. Analysis of Fingerprint map between WDD and WGD

The fingerprint information (**Figure 5**) was obtained from the peak intensity for all the compounds resolved in the GC-IMS topographic plot (**Figure 3**). The total volatiles compounds were divided into three zones, in green, yellow and red boxes. The compounds in green zone existed in both WDD and WGD samples

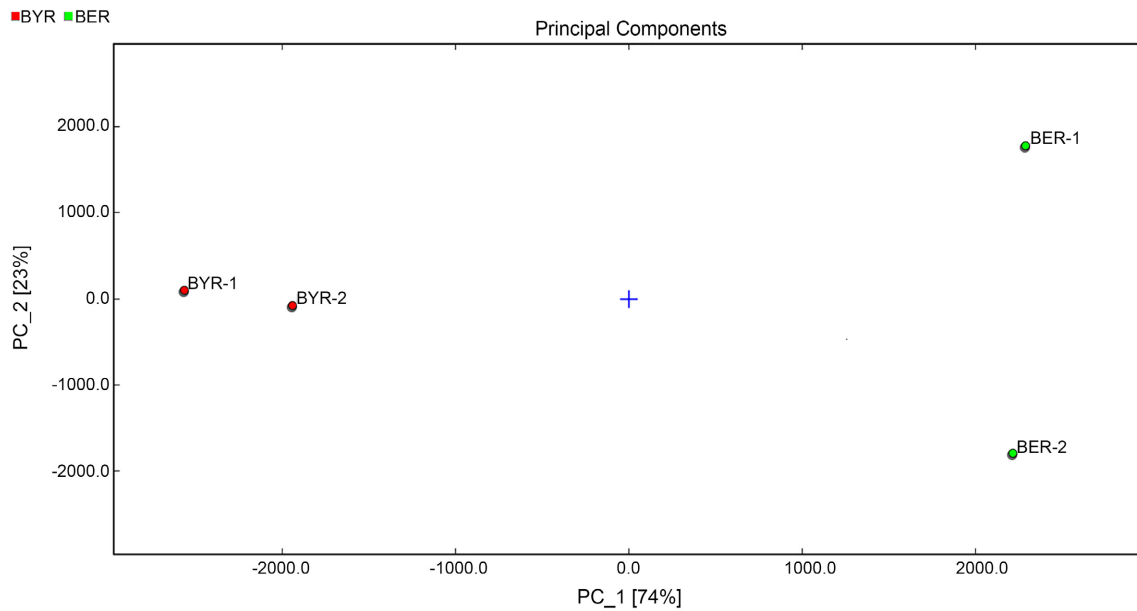


Figure 4. Clusters obtained for down (WDD and WGD) samples by PCA strategy.

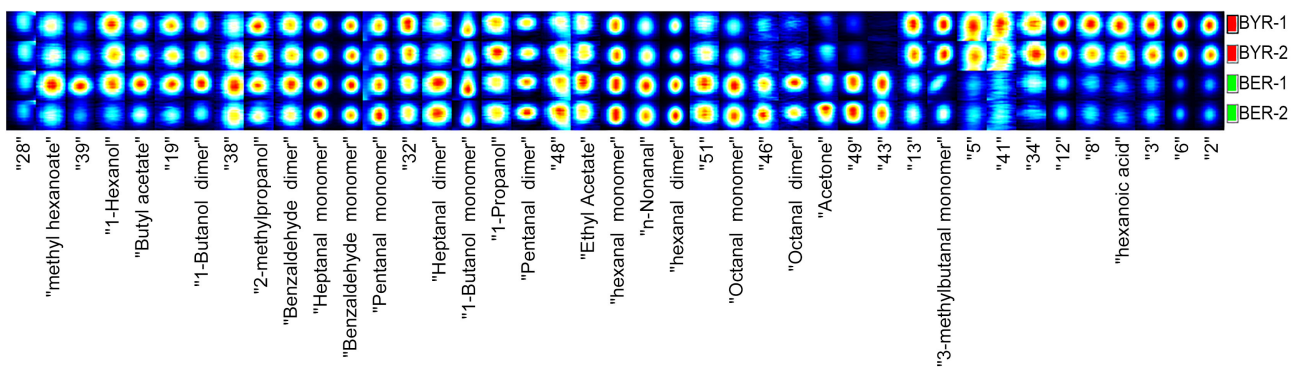


Figure 5. Fingerprint spectra between WDD and WGD samples.

with minor variations in their intensities related to concentrations. For example, Hexanal, Ethyl Acetate, Pentanal, 1-Propanol, 1-Butanol, Heptanal, Benzaldehyde, 2-methylpropanol, Butyl acetate, 1-Hexanol, Methyl hexanoate with similar concentration were found in both WDD and WGD fingerprint map. In order to distinguish WDD and WGD samples, yellow and red zone was necessary to investigate for compounds in detail. First, only hexanoic acid and 3-methylbutanal were identified in the case of red zone, in which the volatile compound concentration of WDD was much higher than that of WGD. Based on the estimation of the unidentified signals by GC-IMS analysis, the signals of [2] [3] related to the volatile compounds was inferred as Heptanoic acid, 3-mercaptopropionic acid, and the signals of [5] and [6] were Heptanoic acid methyl esters. According to the result of Oil and fat content, the oil and fat turned to be fatty acid was a typical reaction of autoxidation. Thus, through the analysis of identified signals, the volatile compounds of WDD contained more organic acid, especially fatty acid, than that of WGD, which can be explained that WDD samples

generated obviously unpleasant odor due to the decomposition of higher oil and fat content.

3.4. Discussion

Based on the GC-IMS result for the volatile compounds of down samples (WDD and WGD) is presented as an adequate alternative to the traditional techniques for down identification and quality control. It can be highlighted that GC-IMS does not require individual information about volatile organic compound presented in the down samples analyzed to achieve a correct classification. Although, some compounds responsible of flavor and odor of the down were identified using the same methodology. The total analysis time is reduced and neither sample treatment nor solvents are needed.

In a specific perspective, the application of GC-IMS analysis will be helpful for solving the unpleasant odor of WDD based on the identification of WDD's characteristic volatile compounds. According the fingerprint spectra established under the same GC-IMS conditions, Ethyl Acetate, 1-Propanol, 1-Butanol, 2-methylpropanol, Butyl acetate, 1-Hexanol etc. were detected in both samples with minor concentration difference. But the most characteristic volatile compounds of WGD were belong to aldehydes (octanal, hexanal and n-Nonanal), whereas WDD possessed high concentration of carboxylic acids (hexanoic, heptanoic acid, 3-mercaptopropionic acid) which represent a group of important autoxidation reaction products. The WDD and WGD samples can be distinguished fast and easily due to the characteristic volatile compounds, even the ratio of WDD. In addition, related to the result of odors in sensory evaluation between WDD and WGD, the main reason of unpleasant odor was possibly attributed to the high concentration of volatile carboxylic acids. Therefore, the deodorant contained suitable weak-alkaline compounds were the potential selection for WDD processing.

4. Conclusions

In this paper, the potential of a GC column coupled to an IMS detector to distinguish WDD from WGD samples and the reason of unpleasant odor generated from WDD sample have been successfully demonstrated. Preliminary results suggest the GC-IMS method in order to differentiate WDD with WGD through PCA. This fact is important because the type of down employed in down products has a great influence on its appreciation by consumers and issues related to quality control. Another outcome from the application of this method was the detection of some compounds that contribute to volatile compounds profile in WDD and WGD samples. Furthermore, a total of 22 compounds were successfully separated and identified through GC-IMS method. Meanwhile, the characteristic compounds of WDD and WDD were also identified so as to obtain the visible difference. The content of volatile organic acid, especially fatty acid in WDD was determined to be much higher than that in WGD, according to inten-

sities difference analysis from fingerprint spectra. Based on the identification of volatile compounds, the generation of unpleasant odor in down products can be attributed to the high content of organic acid, for example, hexanoic acid. Those aspects indicate that it could be highly recommended to select appropriate deodorant for down processing.

Therefore, the GC-IMS method proposed proved to be fast, easy and applicable to the on-line processing control of down product. Moreover, its relative low cost compared with other methodologies and portability working at atmospheric temperature and pressure makes it easy to implant in laboratories or factories. Although down has a solid and complex matrix, the IMS application only requires the generation of the volatile compounds in the headspace prior to the analysis. From the view of practical point, the variation of volatile compounds information was relevant to check the consistency of down product and quality differences between batches, as is the case for the detection of signals responsible of off-odors.

Acknowledgements

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Conflicts of Interest

The authors have no competing interests to declare.

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