THERMAL DECOMPOSITION OF TWO SYNTHETIC GLYCOSIDES BY TG, DSC AND SIMULTANEOUS Py-GC-MS ANALYSIS

W.-C. Xie[1], *Z.-C. Tan*[2*], *X.-H. Gu*[3], *J. Tang*[3], *G.-Y. Wang*[4], *C.-R. Luo*[4] *and L.-X. Sun*[2]

[1]School of Food Science and Technology, Guangdong Ocean University, Zhanjiang 524025, China
[2]Thermochemistry Laboratory, Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian 116023, China
[3]Key Laboratory of Food Science and Safety, Ministry of Education, Southern Yangtz University, Wuxi 214036, China
[4]Huabao Food Flavors and Fragrances Co. Ltd, Shanghai, 201821, China

To develop thermal stable flavor, two glycosidic bound flavor precursors, geranyl-tetraacetyl-β-*D*-glucopyranoside (GLY-A) and geranyl-β-*D*-glucopyranoside (GLY-B) were synthesized by the modified Koenigs–Knorr reaction. The thermal decomposition process and pyrolysis products of the two glycosides were extensively investigated by thermogravimetry (TG), differential scanning calorimeter (DSC) and on-line pyrolysis-gas chromatography mass spectroscopy (Py-GC-MS). TG showed the T_p of GLY-A and GLY-B were 254.6 and 275.7°C. The T_{peak} of GLY-A and GLY-B measured by DSC were 254.8 and 262.1°C respectively.

Py-GC-MS was used for the simply qualitative analysis of the pyrolysis products at 300 and 400°C. The results indicated that: 1) A large amount of geraniol and few by-products were produced at 300°C, the by-products were significantly increased at 400°C; 2) The characteristic pyrolysis product was geraniol; 3) The primary decomposition reaction was the cleavage of O-glycosidic bound of the two glycosides flavor precursors. The study on the thermal behavior and pyrolysis products of the two glycosides showed that this kind of flavor precursors could be used for providing the foodstuff with specific flavor during heating process.

Keywords: *DSC, flavor precursors, glycosides, Py-GC-MS analysis, pyrolysis, TG-DTG, thermal decomposition, thermal stability*

Introduction

Geraniol, because of its inherent rose flavor, has been widely used as additives in food stuffs, tobaccos, medicines, etc. But the volatility limited its wide application in the high temperature processing. Glycosides are sugar derivatives, an overwhelming number of them occurring in nature. Many studies on fruits and vegetables showed that a significant portion of volatile flavor compounds including geraniol may occur in many plants by enzymatic hydrolysis of glycosidic bound flavor precursors [1–3]. Many investigations also demonstrated that there was inherent relation between the flavor and glycosides as flavor precursors. Although these glycosides are non-volatile and flavorless, hydrolysis of them could release compounds that may contribute to flavor either directly or by subsequent rearrangement.

The cleavage of O-glycosidic bound could be carried out by enzymatic, chemical hydrolysis and pyrolysis [4]. But it was found that the initial studies on the flavor precursors mostly aimed at the natural extraction, structure identification and enzyme hydrolysis [5–7]. The thermal analysis and the application of these compounds were rarely reported. To study the possibility of glycosides used as flavor additives in high temperature processing, the common naturally occurring glycoside of geraniol, geranyl-β-*D*-glucopyranoside (GLY-B) and its full-acetylated compound, geranyl-tetraacetyl-β-*D*-glucopyranoside (GLY-A) were stereospecifically synthesized firstly. Then thermogravimetric analysis (TG) was employed to investigate the mass loss under different temperatures. Differential scanning calorimeter (DSC) was used to detect the caloric changes of the samples in pyrolysis process. And pyrolysis-gas chromatography-mass spectroscopy (Py-GC-MS) was used to investigate the decomposition process and pyrolysis products [8–11]. The thermal behavior and the thermal stability of the two flavor precursors were investigated in this paper.

Experimental

Materials

Glycosides preparation

The GLY-A and GLY-B were prepared by the modified Koenigs–Knorr method under strictly anhydrous condition (shown in Scheme 1) [12]. All reagents are purchased as analysis grade.

* Author for correspondence: tzc@dicp.ac.cn

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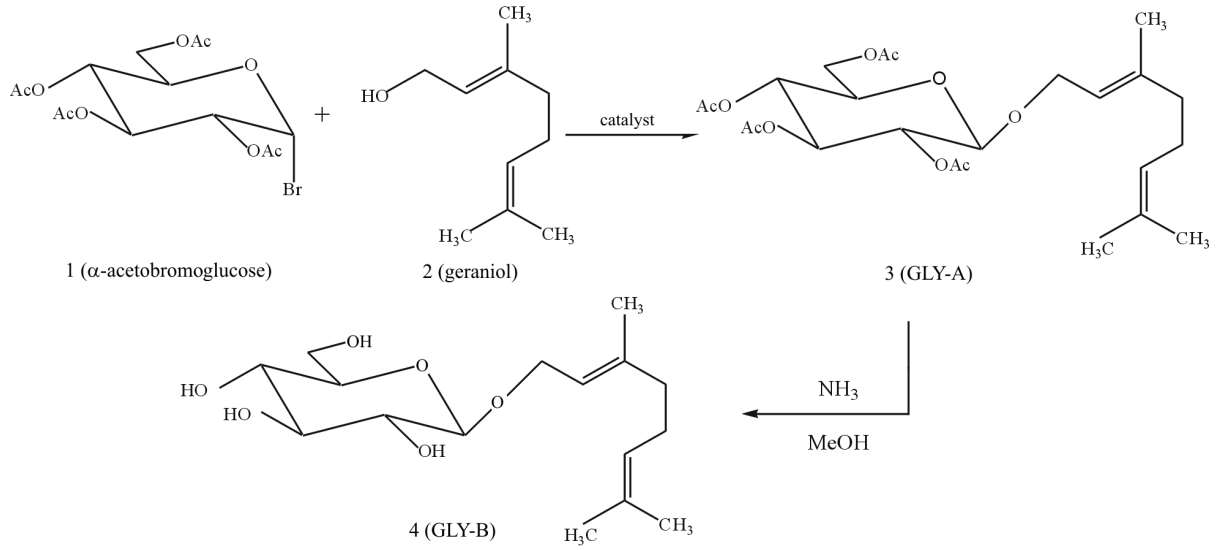

Scheme 1 General synthetic procedure for GLY-A and GLY-B

Preparation of GLY-A

Acetobromo-α-*D*-glucose 1(2,3,4,6-tetra-O-acetyl-α-*D*-glucopyranosyl bromide) was prepared from dry glucose, acetic anhydride, red phosphorus and bromine. Acetobromo-α-*D*-glucose 6.16 g (15 mmol) and geraniol 1.54 g (10 mmol) dissolved in dichloromethane, then 5.50 g freshly prepared, dried silver carbonate (20 mmol) was added as catalyst and 5 g 4Å molecular sieves was used for water binding. The reaction mixture was refluxed free of light. Progress of the reaction was monitored by thin layer chromatography (TLC). On completion of the reaction, the residue was separated on silica gel column chromatography and eluted with petroleum ether-ethyl acetate (20:1 v:v) to give 3 (GLY-A) as syrup.

Preparation of GLY-B

GLY-A 0.53g (1.1 mmol) was suspended in 50 mL methanol. The mixture was cooled to –5~0°C and ammonia [dried by NaOH(s)] was bubbled into. After GLY-A thoroughly dissolved, the treatment was continued for 30 min. The resulting solution was put in a refrigerator over night. A white solid 4 (GLY-B) was obtained after the solvent was evaporated to dryness.

Methods

^{1}H NMR and ^{13}C NMR spectra were recorded by using a Brucker 500 Hz spectrometer. Each sample was dissolved in $CDCl_3$ containing TMS as the internal standard. IR spectra were recorded with a Nicolet-5DX infrared spectrometer. The samples were analysed as KBr micropellets. LC-MS-MS experiments were performed on triple quadrupole mass spectrometer API 3000^{TM} system, Turbospray^{TM} source.

TG was measured by using a Perkin-Elmer TGA-50 analyzer. The measurements were carried out in air atmosphere in platinum crucible. The heating rate was 10°C min^{-1} and the sample masses were in the range of 5–10 mg. DSC analysis was conducted on a Netzsch DSC 200 PC with a heating rate of 10°C min^{-1} in nitrogen atmosphere. The sample masses were in the range of 5–10 mg in platinum crucible. Py-GC-MS analysis was tested on a combined system of an Australian SGE pyrolyzer and Agilent 6890N-5793N GC-MS. Pyrolysis temperature was set up at 300 and 400°C, respectively, heating time 1 min. GC qualitative analysis was conducted with a DB-Wax fused silica capillary column (30 m×0.25 mm i.d., film thickness 0.25 μm); flow rate of He was 1.1 mL min^{-1}; column temperature, held at 60°C for 4 min, then raised to 280°C at the rate of 4°C min^{-1}; injector temperature 250°C; EI-MS scan range 29–550 amu ; EI ionization energy 70 eV.

Results and discussion

Sample preparation

The procedure of the synthesis according to in this study was shown in Scheme 1. And some techniques such as ^{1}H NMR, ^{13}C NMR, IR spectra (Table 1) and LC-MS-MS mass spectrography (Figs 1 and 2) were adopted to identify the stereo-structure of the glycosides. Based on these data, the structures were deduced to be GLY-A and GLY-B, and the structural identifications were corresponded to the results of the two samples reported earlier [2]. The yield of GLY-A was 56% (purity 98.2%); The yield of GLY-B was 48% (purity 96%).

Table 1 NMR and IR analysis of the synthesized GLY-A and GLY-B

Compound	^{1}H NMR δ (in $CDCl_3$)	^{13}C NMR δ (in $CDCl_3$)	IR/cm^{-1} KBr micropollets
GLY-A	1.60–1.67 (9H,8,9,10-CH_3) 1.73–2.03 (12H,4CH_3)	16.39–26.28 (7C, CH_3) 39.46 (Ger, C-5),	2927, 1747
$C_{24}H_{36}O_{10}$	2.09 (4H, 2CH_2), 3.94 (1H, Glu-5'CH)	39.51 (Ger, C-4), 60.54 (Ger, C-1)	1668(W), 1225
MW: 484	4.03 (2H,Glu-6'CH), 4.19 (2H, CH_2) 4.32 (1H,Glu-4'CH), 4.90 (1H,Glu-3'CH_2) 5.07 (1H, =CH), 5.19 (1H, Glu-2'CH) 5.27 (1H,=CH), 5.70 (1H, Glu-1'CH)	63.09 (Glu-6'C), 66.96 (Glu-5'C) 68.27 (Glu-4'C), 70.18 (Glu-3'C) 73.10 (Glu-2'C), 96.93 (Glu-1'C) 119.91 (Ger, C-6), 123.66 (Ger,C-2) 131.67 (Ger, C-3), 140.31 (Ger, C-7) 169.12–170.66 (Glu, 4CO)	1040, 923
GLY-B	1.60 (3H, 10-CH_3), 1.69 (6H, 8,9-CH_3)	16.33 (Ger, C-10), 17.63 (Ger, C-9)	3392, 2924
$C_{16}H_{28}O_6$	2.05 (4H, 4,5-CH_2) 3.26–3.60 (4H,m,Glu-2'-4')	25.62 (Ger, C-8) 26.40 (Ger,C-5)	1653(w)
MW: 316	3.84 (2H, Glu-6') 4.20 (1H, *J*=7.0 Hz, Glu-1') 4.33 (2H, 1-CH_2) 5.41 (1H, 6-C=CH), 5.43 (1H, 2-C=CH)	39.61 (Ger, C-4), 61.29 (Ger, C-1) 61.34 (Glu,C-6'), 65.86 (Glu,C-5') 69.37 (Glu,C-4'), 73.28 (Glu,C-3') 75.65 (Glu,C-2'), 101.46 (Glu,C-1') 119.77 (Ger, C-2), 123.83 (Ger, C-6) 131.63 (Ger, C-7), 141.28(Ger, C-3)	907

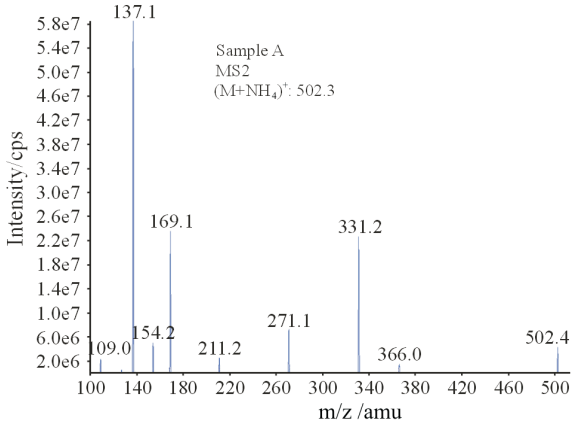

Fig. 1 LC-MS-MS spectrogram of GLY-A

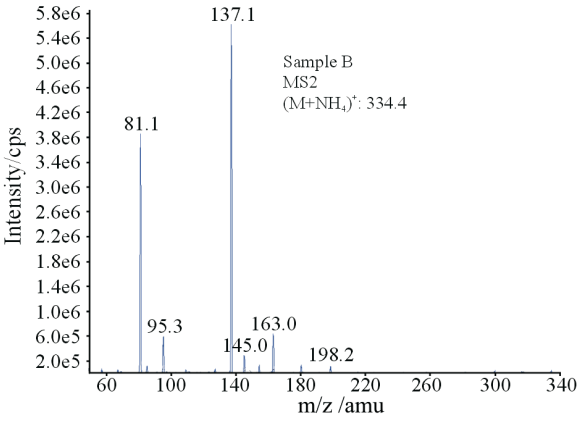

Fig. 2 LC-MS-MS spectrogram of GLY-B

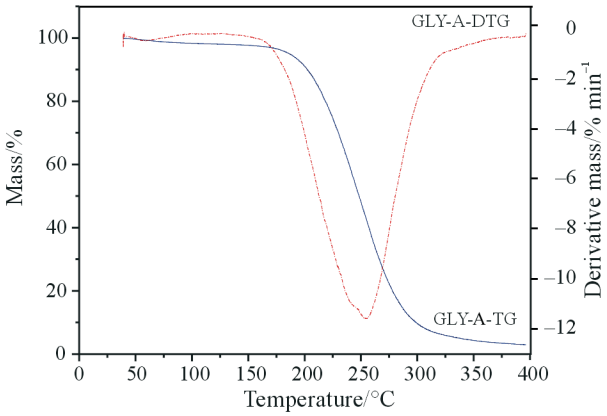

Fig. 3 TG-DTG curve of GLY-A

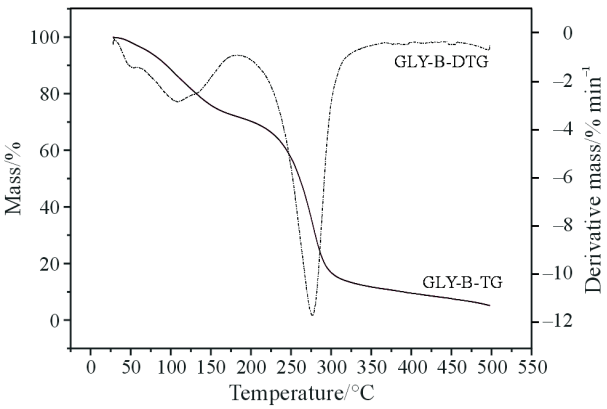

Fig. 4 TG-DTG curve of GLY-B

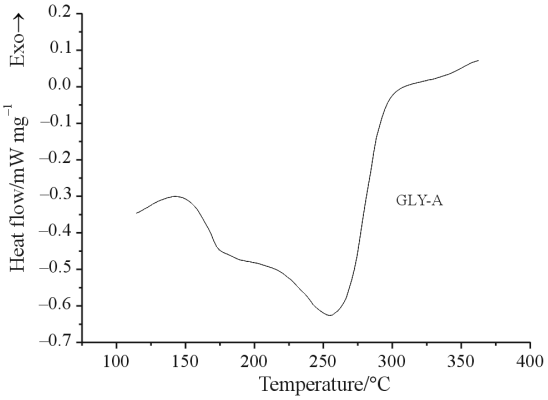

Fig. 5 DSC curve of GLY-A

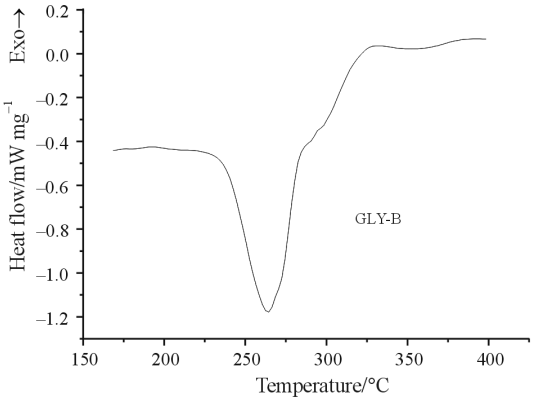

Fig. 6 DSC curve of GLY-B

Pyrolysis behavior of GLY-A and GLY-B

Figures 3 and 4 showed both TG and DTG curves were recorded for GLY-A and GLY-B *vs.* temperature from room temperature to 400°C. The curves indicated the relationship between temperature change and mass loss of the samples. It revealed that the pyrolysis process of GLY-A had a main decomposition stage in the temperature range of 200–300°C. In this stage, the mass loss was very fast and significant with the largest mass loss rate of 11.56% min^{-1} at the T_p of 254.6°C. The total mass loss was 96.91%. It was observed that pyrolysis process of GLY-B included three stages of loss mass. The initial stage was caused by ethyl acetate used in sample preparation; the second stage was caused by the by-product, acetamide, which was confirmed by Py-GC-MS. The mass loss was very low during first two stages. The third stage was the main one attributed to the total degradation of the GLY-B from 230–310°C. The largest mass loss rate of 11.75% min^{-1} appeared at T_p of 275.7°C. The total mass loss was 94.70%.

The DSC curves of the samples were shown in Figs 5 and 6. It presented the thermograph of the samples *vs*. the temperature. The measurements of the onset temperature of thermal decomposition allowed us to determine the thermal stability of samples from another point. T_{peak} of GLY-A was 254.8°C and T_{peak} of GLY-B was 262.1°C. It can be seen that there was a shoulder peak in the DSC curve of GLY-A between 170–200°C, which may be caused by the cleavage of acetyls from GLY-A. Because geraniol was released by the cleavage of O-glycosidic bound at 254.8°C, the acetyls were cleaved from the glycoside easier than O-glycosidic bound according to the molecular structure. Moreover there were four acetyls in GLY-A, which caused a broad peak as shown in the DSC curve of GLY-A. This conclusion should be confirmed by further experiment. In general, the results detected by TG and DSC were consistent. The data concerning the decomposition temperature and the enthalpy change were summarized in Table 2.

Study of pyrolysis products of GLY-A and GLY-B

TG and DSC seemed to be important means to study thermal properties of organic compounds. As noted above in TG and DSC analysis, the thermal stability of the samples was satisfactory. However, the pyrolysis products were not identified. The researches on components of pyrolysis products were important for the application of the glycosides as additives in food manufacture. The simultaneous Py-GC-MS analysis was used with a special pyrolyser connected directly with a gas chromatograph. The decomposition products were identified by MS after separated by GC. The MS spectrogram of geraniol was shown in Fig. 7, and four main ions of other each pyrolysis product were offered. The TIC curves of GLY-A and GLY-B were shown in Figs 8 and 9 and the most important characteristic compounds were summarized in Tables 3 and 4.

The identification of the pyrolysis products of GLY-A and GLY-B could be carried out by

Table 2 The degradation process of GLY-A and GLY-B

Sample name	Degradation process					
	DSC				DTG-TG	
	T_{onset}/°C	T_{peak}/°C	T_{end}/°C	ΔH/kJ mol^{-1}	T_p/°C	Decomposition stage/°C
GLY-A	219.7	254.8	293.1	64.25	254.6	200–300
GLY-B	236.3	262.1	279.3	56.60	275.7	230–310

Table 3 Py-GC-MS result of GLY-A at different temperatures

Compounds of pyrolysis	*m/z*[a]	*T*=300°C		*T*=400°C	
		peak #	area/%	peak.#	area/%
Geraniol	41 **69** 123 **154**	1	76.63	1	17.83
Acetamide	15 41 44 **59**[b]	2	12.45	2	2.08
Ethyl acetate	29 **43** 61 **88**			3	9.66
Beta citral	**41** 69 84 **152**	4	3.21	4	0.55
Myrcene	41 69 **93 136**			5	0.42
n-hexene	41 43 **57 86**			6	66.67
Butylated hydroxytoluene	81 145 **205 220**	7	2.18	7	0.24
Unknown	41 69 **109 139**			8	2.16
1-methylcyclopentanol	42 58 **71 100**	9	5.53	9	0.39

[a]The ion of highest abundance and molecular ion were bold; [b]The molecular ion was the highest abundance ion

Table 4 Py-GC-MS result of GLY-B at different temperatures

Compound of pyrolysis	*m/z*[a]	*T*=300°C		*T*=400°C	
		peak #	area/%	peak #	area/%
Geraniol	41 **69** 123 **154**	1	51.86	1	35.59
Nerol	41 **69** 123 **154**	2	19.16		
Acetamide	15 41 44 **59**[b]	3	6.33	3	5.95
Ethyl acetate	29 **43** 61 **88**	4	1.18	4	9.95
Myrcene	41 69 **93 136**			5	22.51
Trans ocimene	41 79 **93 136**			6	8.38
3-methylbutanoic acid, 3-methylbutyl ester	57 **70** 85 **172**			7	3.94
Allo-ocimene	79 105 **121 136**			8	2.48
1-methylcyclopentanol	42 58 **71 100**	9	21.47		
2,2'-oxibis-ethanol	31 **45** 75 **106**			10	11.20

[a]The ion of highest abundance and molecular ion were bold; [b]The molecular ion was the highest abundance ion

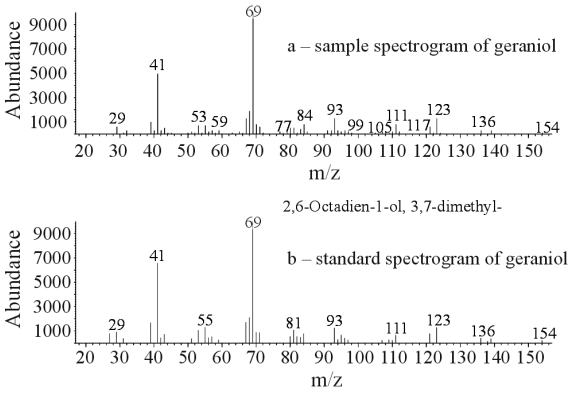

Fig. 7 MS spectrogram of geraniol

Py-GC-MS. The pyrolysis temperatures chosen for them were 300 and 400°C. Figure 8 showed that pyrolysis of GLY-A produced 76.63% geraniol at 300°C, but at 400°C produced 17.83% geraniol. Figure 9 showed that pyrolysis of GLY-B produced 51.86% geraniol at

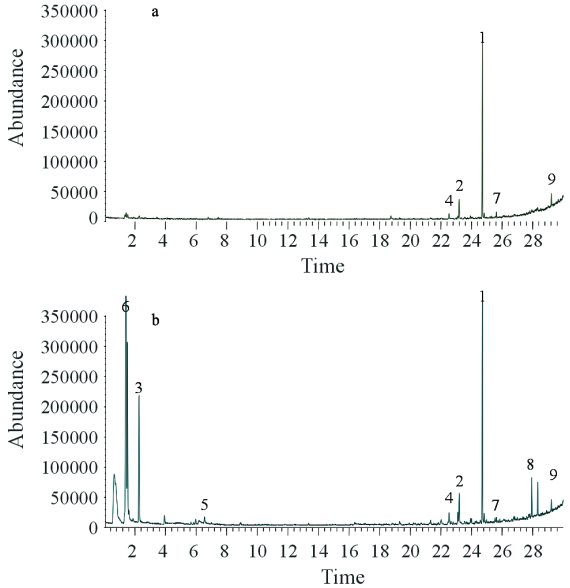

Fig. 8 Total ion chromatograms of GLY-A at different temperatures a – 300, b – 400°C

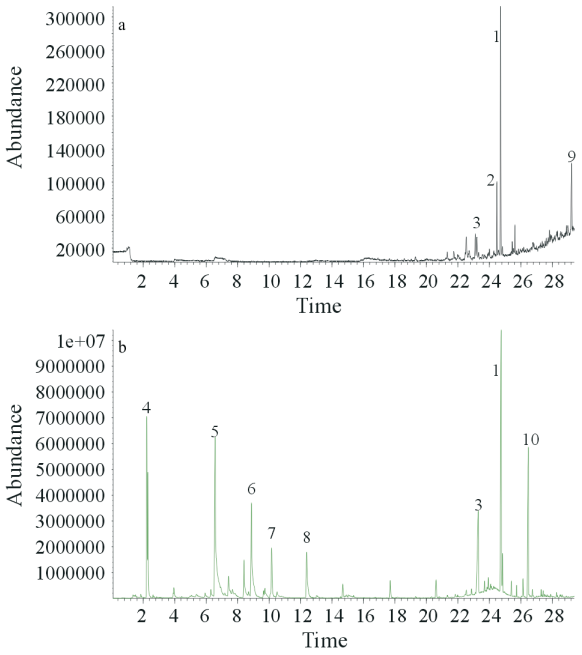

Fig. 9 Total ion chromatograms of GLY-B at different temperatures a – 300, b – 400°C

300°C, and at 400°C produced 35.59% geraniol. Among pyrolysis products, ethyl acetate was solvent residue and acetamide was by-product during sample preparation. Myrcene, beta citral, allo-ocimene, *trans*-ocimene etc. were derivates of geraniol. Others were glucose decomposition compounds or rearrangement compounds. The results indicated pyrolysis of glycosides at 300°C was better than at 400°C, because there was a significant amount of geraniol compound is produced, and fewer by-products. Geraniol was the main product and played a very important role in producing the specific flavor. So 300°C was preferable an application temperature. The Py-GC-MS indicated that the flavor precursors release characteristic flavor during high temperature treatment.

Furthermore, the possibility of GLY-A and GLY-B used as flavor precursors was compared below. Firstly, pyrolysis of GLY-A and GLY-B produced 76.63% and 51.86% geraniol at 300°C respectively. Secondly, the yield of GLY-A was 56% but GLY-B was 48%, and GLY-A was obtained easier than GLY-B. According to these results, GLY-A has more advantages than GLY-B in application.

Conclusions

The thermal behavior and the decomposition products of two glycosides were studied by TG, DSC and Py-GC-MS analysis. The main product of pyrolysis was geraniol, so it can be concluded that the thermolysis mechanism of these glycosides flavor precursors was the cleavage of O-glycosidic bound. The primary decomposition reaction was that the flavor precursors break down to regenerate the flavor and glucose on heating. Moreover, GLY-A has more advantages than GLY-B for using as flavor precursors.

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