
Effect of thermal processing on physical, chemical properties and volatile compounds of coconut (*Cocos nucifera* L.) sugar

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Abstract:

The objectives of this research were to study the physical, chemical properties and volatile compounds of both coconut sap and sugar and investigate changes in the physical, chemical properties and volatile compounds of coconut sugar during heating processes. The physical, chemical properties including moisture content (MC), water activity (a_w), total soluble solids (TSS), color parameters, intimidated browning product (IBP) and browning index (BI) values and volatile compounds were determined. The coconut sap was heated at 110 and 120°C for 6 and 4.5 hours, respectively. The physical, chemical properties and volatile compounds of coconut sugar were investigated. The results found that the MC and a_w decreased with increasing heating times while TSS increased with the times for both of heating temperatures of 110 and 120°C. The color parameters for both heating temperatures of 110 and 120°C fell in the same trend which was the L^* decreased, a^* , b^* , IBP and BI values increased with heating times. The major volatile compounds found in coconut sugar for both temperatures were 2, 3-dimethyl pyrazine, 2, 5-dimethyl pyrazine, 2-methyl pyrazine and 5-methyl furfural. Most of volatile compounds increased with heating times. Long heating time could be concerned to develop the aroma of coconut sugar.

Keywords: Coconut sugar, Heating conditions, Physical and chemical properties, Volatile compounds.

Introduction

Cocos nucifera L. is a scientific name of coconut. It belongs to Areaceae family. The coconuts are applied by many traditional uses, ranging from food to cosmetics. For example, the fruit contains a number of water (also called “juice”) which provides health benefits and also gives oil and milk that are used in cooking and frying as well as in soaps and cosmetics. The fluid, chiefly water with dissolved sugars and mineral salts derived from coconut, it is called “sap”. The coconut sap is commonly used to produce palm sugar.

Palm sugar is one of the local sweeteners which are widely consumed in Thailand or Asian countries. Normally, the sugar is used for making cakes, desserts, food or drinks because it provides better taste and aroma when compare with sugar cane. So, palm sugar is alternative sugar in the present. The sugar is made by heating the sap obtained from the coconut tree (*Coconut nucifera* L.) or palm tree (*Arenga pinnata*) [1]. For the traditional production, the sap is filtered and then transferred into a big wok. The filtrated is heated at 100-120°C for a few hours to obtain a concentrated sap which provides a typical aroma. The sap is poured into the bamboo moulds to form a solid palm sugar.

Normally, quality attributes of food products including color, flavor and texture are very important for consumers. Then both color and flavor play a vital role in customer perceptions. In the case of palm sugar production, the color and flavor is developed during heating processes. It means heating conditions have an effect on the product qualities. The major reaction occurs during heating process that is Maillard reaction and caramelisation. Both reactions are caused of color and aroma formation [2]. The degree of aroma formation and color intensity depend on the structure of sugar present in the sap and the degree of interaction between the amino groups [3]. Ho and others [4] had studied the changes in volatile compounds of palm sap during heating process and found that the important volatiles compound were 2, 3-diethyl-5-methyl pyrazine (2,3 D-5MP) and 2-ethyl-3,5-dimethyl pyrazine (2E-3,5 DP). It was found that most of volatile compounds in N-Heterocyclic, O-Heterocyclic, aldehyde and ketones groups increased with heating times.

Nowadays, the study of volatile compounds in coconut sap or coconut sugar is still limited. Therefore, the aims of this research were to study the effect of heating conditions and observed changes during heating processes on the physical, chemical properties as well as volatile compounds of coconut sugar.

Materials and methods

Materials

Coconut saps obtained from the Ampawa-Chaiputtananuruk Conservation Project which located in Ampawa District, Samut Songkram Province, Thailand. Each 20 L of the coconut sap was collected in plastic bottles and kept under -18°C during transportation. The frozen samples were stored at 5°C for 3 days before analysis. Physical and chemical properties including moisture content [2] water activity, pH [5], total soluble solid, color, intimidated browning product (IBP) [2] and browning index (BI) [2], as well as volatile compounds [4] of the coconut sap were determined.

The chemical standards were purchased from Sigma-Aldrich, Switzerland. The standards used in the experiment were 2-methyl pyrazine, 2, 5-dimethyl pyrazine, 2, 3-dimethyl pyrazine, ethyl pyrazine, 2-ethyl-3, 5-dimethyl pyrazine, 2, 3-diethyl-5-methyl pyrazine, furfural, 5-methyl furfural and tridecane.

Coconut sugar production

The methods of Ho and others [5] and Naknean [2] were modified by using 2.5 L of coconut sap and 2 heating temperatures (110°C and 120°C). Each coconut sap samples was heated at 110°C and 120°C and stirred with a rotational speed of 150 rpm. Approximately 60 mL of the heated samples were collected at intervals of 30 min. The samples temperatures were determined. Heating process was terminated when the total soluble solid of the samples was equal to 80°Brix . The samples were cooled immediately and store at -18°C until the time of analysis.

Moisture content

Moisture contents of coconut sugars were determined by a modified method of Naknean [2]. Approximately 2-5 g of sample were weighted and placed into the pre-dried empty moisture can. The moisture can containing samples was dried in the vacuum oven (VD53, Binder, Germany) at 60°C under pressure lower than 9.33 kPa for 6 hr. The moisture cans were cooled down by transferring to a desiccator for 30 min. The moisture cans were weighed and the moisture contents of the samples were calculated.

Water activity

Water activity of coconut sugars was determined at room temperature (30°C) by using a water activity meter (Series 3 TE, Aqualab, USA). The sample was placed into a sample cup and inserted into a water activity meter. The water activity values obtained recorded.

Total soluble solids

Total soluble solids of each coconut sugars for 10 µL was measured using refractometer (0 – 82%) (Atago, Japan).

Color

Color of coconut sugars was measured using a colorimeter (HunterLab, Reston, VA, USA) following the CIELAB space. Seven milliliter of each sample was applied and transferred to the colorimeter. The colorimeter was adjusted for reflectance, illuminant D 65, and angle of 10°. Color parameters obtained were in terms of L* (lightness or darkness), a* (redness or greenness), and b* (yellowness or blueness) values.

Intimidated and Browning index

The IBP and BI of coconut sugar were investigated at the absorbance of 280 and 420 nm, respectively following the method of Naknean [2]. The sample was diluted with distilled water. The appropriate dilution was 8 and 4-fold for both IBP and BI, respectively to obtain reliable absorbance readings. The spectrophotometer (Lampda 25, PerkinElmer, USA) was used to measure the absorbance of samples.

Volatile compounds

The method of Ho and others [4] was modified to apply in both sample preparation and volatile compounds analysis. Solid phase micro extraction (SPME) was used to extract the volatile compounds from coconut sugars. Two gram of each sample was taken into a 12 mL vial and mixed with 1 ppm of Tridecane. The vial was covered with PTFE/silicone septum. Then, the vial was equilibrated in a water bath at 60°C for 20 min. A 50/30 µm Divinylbenzene/ Carboxen/ Polydimethysioxane SPME fiber was inserted into the vial to trap *N*- and *O*-heterocyclic group volatile compounds and was exposed to the sample headspace at 60°C for 10 min. The fiber was transferred directly to the injector port of gas chromatography-mass spectrophotometry (GC-MS) system.

The volatile compounds of coconut sugars were identified using GC-MS (Scion 436-GC, Bruker, Germany) with DB-625 capillary column (30 m x 0.25 mm) (Agilent Technologies, USA). The injector and detector temperature were 240 and 280°C, respectively. The helium gas was used to be a carrier gas. The flow rate of 2 mL/min and constant pressure of 117.5 kPa were applied. The column temperature was programmed from 50°C (held for 2 min), at 20°C/ min to 80°C (held for 1 min), at 20°C/ min to 100°C (held for 1 min), then at 30°C/ min to 230°C (held for 2 min). Other conditions were as follows: scanning mass range (m/z) 50 – 550 a.m.u at a rate of 1.5 scan/ s; electron ionization energy at 70eV.

Results and discussion

Physical and chemical properties of coconut sugar during heating process

Heating was applied to concentrate the coconut sap at 110 and 120°C, for 6 and 4.5 hours, respectively. Changes during heating process in physical and chemical properties of coconut sugar for 110 and 120°C are presented in Table 1 and 2, respectively. The results revealed that heating time had a significant effect on MC and a_w ($p < 0.05$). Heating temperature at 120°C had a shorter heating time than those of 110°C because performing at higher temperature led to increasing driving force that accelerated moisture movement faster. The MC of coconut sugars for both 110 and 120°C decreased from 83.07 to 6.85% w.b. and 83.07 to 11.17% w.b., respectively, a_w decreased from 0.986 to 0.576 and 0.677, respectively with increasing of heating time. Increasing sample temperature led to removal of water from coconut sap by evaporation. According to the decreasing of MC and a_w of both heating temperatures with heating times, the TSS increased with increasing heating time. The results were supported by Akochi-K and others [6] and Rao and others [7] who exhibited that the increasing of TSS and decreasing of MC and a_w could be related with rapid evaporation of water from coconut sap during heating process.

For color parameters, both of heating temperatures for 110°C and 120°C were in the same trend. L^* values of both of heating temperature for 110°C and 120°C were in the ranges of 82.25 - 50.12 and 82.25 – 67.73, respectively. Increasing heating times led to decrease lightness. The results indicated that the coconut became darker with heating time when compared with the control at 0 hr. The a^* values of both heating temperature increased from -0.01 – 6.21 for heating temperature of 110°C and 0.01 – 6.99 for 120°C with increasing

heating time. In addition, the b^* , IBP and BI values increased with heating time for both temperature. Ibarz and others [8], Naknean [2] and Rattanathanalerk and others [9] had studied the effect of thermal processing on the color changes of food products. The results demonstrated that increasing heating times had an effect to decrease L^* and increase a^* , b^* IBP and BI value. The changes of color were described by Martins and others [10] who reported that cooking temperature and heating time were important to both Maillard and caramelisation reaction that affected color changing.

Changes in volatile compounds of coconut sugar during heating process

The volatile compounds can be divided into 2 groups which were *N*-heterocyclic including 2, 3-diethyl-5-methyl pyrazine (2,3 D-5MP), 2,3-dimethyl pyrazine (2,3 DP), 2,5-dimethyl pyrazine (2,5 DP), 2-ethyl-3,5-dimethyl pyrazine (2E-3,5 DP), 2-methyl pyrazine (2MP) and ethyl pyrazine (EP) and *O*-heterocyclic such as 5-methyl furfural (5MF) and furfural (F). The volatile compounds of coconut sugars for both 110°C and 120°C heating temperatures are demonstrated in the Table 3 and 4. The results found that 2,3 DP, 2,5 DP, 2MP and 5MF were the major compounds of coconut sugar for both 110 and 120°C heating temperatures. At the end of heating times of 6 and 4.5 hours for heating temperatures of 110 and 120°C, respectively, most of volatile compounds increased after the heating process in both heating temperatures. The results were agreed with Ho and others [4] who revealed that high temperature was necessary for the formation of various volatile compounds and heating time also required for the aroma developments.

Conclusions

The results of this research revealed that coconut sap contained 83.07% w.b. of moisture content, 0.986 of water activity, 15.25 °Brix of total soluble solids, 0.580 of IBP and 0.039 of BI values. The content of 2,3 D5MP was found to be the highest (0.390 ppb) followed by 5MF, 2,3 DP, 2E-3,5 DP, 2,5 DP, 2MP, EP and F, respectively. The moisture content and water activity of coconut sugar during heating process decreased with increasing heating time while the total soluble solids increased with the times. In the end of heating process of 6 and 4.5 hours for heating temperature of 110 and 120°C, respectively, the L^* value decreased, a^* , b^* , IBP and BI values increased with heating times. The major volatile compounds were 2,3

DP, 2,5 DP, 2MP and 5MF. Most of volatile compounds increased with heating times. Long heating time could be required to produce aromatic coconut sugar.

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Table 1: Physical, chemical properties of coconut sugar during heating process at 110°C

Heating times (hr.)	Sample temperatures (°C)	Moisture Content (% w.b.)	a_w	TSS (°Brix)	Color parameters			IBP	BI
					L*	a*	b*		
0	30.88	83.07±0.37 ^a	0.986±0.06 ^a	15.25±0.10 ^m	82.25±0.81 ^a	0.01±0.21 ^d	12.03±0.78 ^l	0.58±0.32 ^{ef}	0.039±0.01 ^g
0.5	72.00	82.64±0.22 ^a	0.986±0.01 ^a	16.07±0.10 ^l	85.03±0.84 ^a	-0.10±0.14 ^d	11.86±0.33 ^j	0.438±0.04 ^f	0.027±0.00 ⁱ
1.0	72.50	81.35±0.15 ^b	0.984±0.00 ^a	17.43±0.08 ^k	84.63±0.70 ^a	-0.08±0.09 ^d	12.57±0.49 ^{ij}	0.391±0.07 ^f	0.021±0.00 ^m
1.5	72.50	79.42±0.96 ^c	0.987±0.00 ^a	18.83±0.34 ^j	83.58±0.95 ^{ab}	-0.05±0.10 ^d	13.80±0.76 ⁱ	0.507±0.04 ^f	0.023±0.00 ^k
2.0	73.50	77.69±0.51 ^d	0.986±0.00 ^a	21.10±0.41 ⁱ	82.46±0.27 ^{ab}	-0.04±0.03 ^d	15.16±0.20 ^h	0.608±0.05 ^{ef}	0.021±0.00 ^l
2.5	73.50	74.44±0.86 ^e	0.980±0.00 ^b	23.53±0.59 ^h	80.86±1.46 ^{ab}	-0.06±0.09 ^d	16.10±0.58 ^h	0.684±0.03 ^{ef}	0.025±0.00 ^j
3.0	73.00	71.24±0.77 ^f	0.973±0.00 ^c	26.70±0.93 ^g	79.37±1.19 ^{ab}	0.08±0.10 ^d	18.65±0.82 ^g	0.884±0.01 ^{de}	0.031±0.00 ^h
3.5	73.50	66.65±1.23 ⁱ	0.970±0.00 ^c	30.57±0.48 ^f	78.24±1.51 ^{ab}	0.05±0.12 ^d	20.49±0.91 ^f	1.122±0.04 ^d	0.069±0.03 ^e
4.0	73.25	60.15±2.50 ^j	0.957±0.01 ^d	36.03±2.23 ^e	74.36±1.51 ^{ab}	0.38±0.16 ^d	24.98±1.36 ^e	1.880±0.46 ^{bc}	0.053±0.00 ^f
4.5	73.00	51.69±1.46 ^k	0.936±0.00 ^e	43.30±0.85 ^d	73.42±1.44 ^{ab}	0.47±0.19 ^d	28.33±0.89 ^d	1.650±0.05 ^c	0.071±0.01 ^d
5.0	73.50	41.79±2.39 ^l	0.897±0.01 ^f	52.57±1.72 ^c	71.75±0.18 ^b	1.14±0.07 ^c	33.41±0.93 ^c	2.063±0.29 ^b	0.083±0.01 ^c
5.5	73.50	19.72±0.95 ^m	0.755±0.01 ^g	73.80±0.52 ^b	74.48±0.04 ^{ab}	2.56±0.41 ^b	45.77±1.97 ^a	2.411±0.00 ^b	0.103±0.01 ^b
6.0	76.50	6.85±1.34 ⁿ	0.576±0.05 ^h	80.57±0.48 ^a	50.12±9.98 ^c	6.21±1.96 ^a	40.20±3.72 ^b	2.414±0.01 ^a	0.223±0.03 ^a

Table 2: Physical, chemical properties of coconut sugar during heating process at 120°C

Heating times (hr.)	Sample temperatures (°C)	Moisture Content (% w.b.)	a_w	TSS (°Brix)	Color parameters			IBP	BI
					L*	a*	b*		
0	30.88	83.07±0.37 ^a	0.986±0.06 ^a	15.25±0.10 ^j	82.25±0.81 ^a	0.01±0.21 ^h	12.03±0.78 ^j	0.58±0.32 ^e	0.039±0.01 ⁱ
0.5	75.5	81.86±0.30 ^b	0.986±0.00 ^a	17.03±0.15 ⁱ	81.34±2.01 ^b	0.23±0.32 ^g	12.73±1.89 ⁱ	0.518±0.03 ^e	0.022±0.00 ^j
1.0	76.25	79.15±0.19 ^c	0.985±0.00 ^a	19.23±0.23 ^h	80.37±2.59 ^c	0.21±0.27 ^g	15.15±0.59 ^h	0.547±0.07 ^e	0.023±0.00 ^j
1.5	75.75	75.98±1.06 ^d	0.983±0.00 ^a	21.63±0.50 ^g	77.49±3.52 ^d	0.30±0.25 ^f	16.25±0.31 ^g	0.678±0.03 ^e	0.039±0.01 ⁱ
2.0	76.25	72.58±0.56 ^e	0.975±0.00 ^b	25.67±0.68 ^f	76.48±2.95 ^e	0.28±0.29 ^f	18.98±0.28 ^f	1.069±0.12 ^d	0.063±0.03 ^e
2.5	76.75	66.43±0.95 ^f	0.968±0.00 ^c	30.13±0.16 ^e	73.19±2.80 ^f	0.48±0.28 ^e	21.98±0.11 ^e	1.387±0.04 ^{cd}	0.051±0.01 ^f
3.0	77.00	59.37±1.10 ^g	0.955±0.00 ^d	36.10±0.99 ^d	71.15±3.96 ^g	0.65±0.39 ^d	25.43±0.06 ^d	1.822±0.20 ^b	0.075±0.01 ^d
3.5	76.50	47.66±2.80 ^h	0.933±0.01 ^e	47.13±5.55 ^c	69.51±3.43 ^h	1.04±0.26 ^c	29.79±1.43 ^c	2.075±0.28 ^{bc}	0.099±0.03 ^c
4.0	77.25	30.02±7.23 ⁱ	0.842±0.05 ^f	65.40±5.92 ^b	68.66±4.53 ⁱ	2.56±0.22 ^b	39.42±3.92 ^b	2.379±0.03 ^a	0.186±0.08 ^b
4.5	78.00	11.17±0.97 ^j	0.677±0.03 ^g	80.37±0.34 ^a	67.73±0.11 ^j	6.99±1.45 ^a	58.00±5.74 ^a	2.387±0.01 ^a	0.297±0.06 ^a

a_w : water activity, TSS: total soluble solids (°Brix)

IBP: Intermediate Browning Product

BI: Browning Intensity

Mean values in the same column with different superscripts are significantly different at $P \leq 0.05$ by Duncan's Multiple Range Test.

Table 3: Volatile compounds of coconut sugar during heating process at 110°C

Heating time (hr.)	Sample temperatures (°C)	Volatile compounds content (ppb)							
		N-heterocyclic					O-heterocyclic		
		2,3 D-5MP	2,3 DP	2,5 DP	2E-3,5 DP	2MP	EP	5MF	F
0	30.88	0.390±0.78 ^{ns}	0.023±0.04 ^{ns}	0.014±0.00 ^{ns}	0.015±0.01 ^{ns}	0.014±0.00 ^{bc}	0.003±0.01 ^{ns}	0.024±0.01 ^{ns}	0.002±0.00 ^a
0.5	72.00	0.144±0.20 ^{ns}	0.005±0.01 ^{ns}	0.019±0.01 ^{ns}	0.015±0.00 ^{ns}	0.013±0.00 ^c	0.006±0.01 ^{ns}	0.023±0.00 ^{ns}	0.002±0.00 ^a
1.0	72.50	0.084±0.12 ^{ns}	0.008±0.00 ^{ns}	0.018±0.01 ^{ns}	0.015±0.00 ^{ns}	0.015±0.00 ^{bc}	0.006±0.01 ^{ns}	0.024±0.01 ^{ns}	0.002±0.00 ^a
1.5	72.50	0.000±0.00 ^{*ns}	0.008±0.00 ^{ns}	0.020±0.01 ^{ns}	0.013±0.00 ^{ns}	0.012±0.00 ^c	0.006±0.01 ^{ns}	0.022±0.00 ^{ns}	0.002±0.00 ^a
2.0	73.50	0.000±0.00 ^{*ns}	0.008±0.00 ^{ns}	0.017±0.01 ^{ns}	0.012±0.00 ^{ns}	0.012±0.00 ^c	0.006±0.01 ^{ns}	0.021±0.00 ^{ns}	0.002±0.00 ^a
2.5	73.50	0.000±0.00 ^{*ns}	0.004±0.01 ^{ns}	0.012±0.00 ^{ns}	0.013±0.00 ^{ns}	0.013±0.00 ^c	0.000±0.00 ^{*ns}	0.022±0.00 ^{ns}	0.002±0.00 ^a
3.0	73.00	0.000±0.00 ^{*ns}	0.004±0.01 ^{ns}	0.012±0.00 ^{ns}	0.011±0.00 ^{ns}	0.013±0.00 ^c	0.006±0.01 ^{ns}	0.022±0.00 ^{ns}	0.002±0.00 ^a
3.5	73.50	0.000±0.00 ^{*ns}	0.008±0.00 ^{ns}	0.032±0.03 ^{ns}	0.016±0.01 ^{ns}	0.012±0.00 ^c	0.000±0.00 ^{*ns}	0.021±0.00 ^{ns}	0.002±0.00 ^a
4.0	73.25	0.000±0.00 ^{*ns}	0.004±0.01 ^{ns}	0.012±0.00 ^{ns}	0.014±0.00 ^{ns}	0.013±0.00 ^c	0.000±0.00 ^{*ns}	0.022±0.00 ^{ns}	0.002±0.00 ^a
4.5	73.00	0.000±0.00 ^{*ns}	0.004±0.01 ^{ns}	0.022±0.01 ^{ns}	0.012±0.00 ^{ns}	0.013±0.00 ^c	0.000±0.00 ^{*ns}	0.021±0.00 ^{ns}	0.002±0.00 ^a
5.0	73.50	0.000±0.00 ^{*ns}	0.000±0.00 ^{*ns}	0.022±0.00 ^{ns}	0.011±0.00 ^{ns}	0.013±0.00 ^c	0.000±0.00 ^{*ns}	0.022±0.00 ^{ns}	0.000±0.00 ^{*b}
5.5	73.50	0.000±0.00 ^{*ns}	0.009±0.00 ^{ns}	0.016±0.00 ^{ns}	0.010±0.00 ^{ns}	0.019±0.01 ^b	0.011±0.00 ^{ns}	0.022±0.00 ^{ns}	0.000±0.00 ^{*b}
6.0	76.50	0.000±0.00 ^{*ns}	0.008±0.00 ^{ns}	0.025±0.00 ^{ns}	0.010±0.00 ^{ns}	0.031±0.00 ^a	0.011±0.00 ^{ns}	0.022±0.00 ^{ns}	0.000±0.00 ^{*b}

Table4: Volatile compounds of coconut sugar during heating process at 120°C

Heating time (hr.)	Sample temperatures (°C)	Volatile compounds content (ppb)							
		N-heterocyclic					O-heterocyclic		
		2,3 D-5MP	2,3 DP	2,5 DP	2E-3,5 DP	2MP	EP	5MF	F
0	30.88	0.390±0.78 ^{ns}	0.023±0.04 ^{ns}	0.014±0.00 ^b	0.015±0.01 ^{ab}	0.014±0.00 ^b	0.003±0.01 ^{bc}	0.024±0.01 ^{ns}	0.002±0.00 ^a
0.5	72.00	0.000±0.00 ^{*ns}	0.040±0.06 ^{ns}	0.040±0.01 ^a	0.022±0.01 ^a	0.014±0.00 ^b	0.000±0.00 ^{*c}	0.040±0.03 ^{ns}	0.001±0.00 ^{ab}
1.0	72.50	0.000±0.00 ^{*ns}	0.040±0.06 ^{ns}	0.019±0.01 ^b	0.012±0.00 ^b	0.013±0.00 ^b	0.000±0.00 ^{*c}	0.023±0.00 ^{ns}	0.002±0.00 ^a
1.5	72.50	0.000±0.00 ^{*ns}	0.000±0.00 ^{*ns}	0.018±0.00 ^b	0.011±0.00 ^b	0.012±0.00 ^b	0.000±0.00 ^{*c}	0.021±0.00 ^{ns}	0.001±0.00 ^{ab}
2.0	73.50	0.000±0.00 ^{*ns}	0.040±0.06 ^{ns}	0.017±0.01 ^b	0.011±0.00 ^b	0.013±0.00 ^b	0.007±0.01 ^{abc}	0.022±0.00 ^{ns}	0.002±0.00 ^a
2.5	73.50	0.595±0.29 ^{ns}	0.000±0.00 ^{*ns}	0.016±0.00 ^b	0.011±0.00 ^b	0.016±0.00 ^b	0.006±0.01 ^{abc}	0.021±0.00 ^{ns}	0.002±0.00 ^a
3.0	73.00	0.600±0.00 ^{ns}	0.040±0.06 ^{ns}	0.016±0.01 ^b	0.011±0.00 ^b	0.017±0.00 ^b	0.000±0.00 ^{*c}	0.021±0.00 ^{ns}	0.002±0.00 ^a
3.5	73.50	0.000±0.00 ^{*ns}	0.080±0.00 ^{ns}	0.016±0.00 ^b	0.011±0.001 ^b	0.016±0.00 ^b	0.006±0.01 ^{abc}	0.021±0.00 ^{ns}	0.002±0.00 ^a
4.0	73.25	0.053±0.08 ^{ns}	0.080±0.00 ^{ns}	0.014±0.00 ^b	0.010±0.00 ^b	0.021±0.001 ^b	0.014±0.00 ^{ab}	0.020±0.00 ^{ns}	0.000±0.00 ^{*b}
4.5	73.00	0.000±0.00 ^{ns}	0.047±0.05 ^{ns}	0.048±0.03 ^a	0.010±0.00 ^b	0.051±0.02 ^a	0.016±0.00 ^a	0.021±0.00 ^{ns}	0.000±0.00 ^{*b}

2,3 D-5MP: 2, 3-diethyl-5-methyl pyrazine, 2,3 DP: 2,3-dimethyl pyrazine, 2,5 DP: 2,5-dimethyl pyrazine, 2E-3,5 DP: 2-ethyl-3,5-dimethyl pyrazine,

2MP: 2-methyl pyrazine, 5MF: 5-methyl furfural, EP: ethyl pyrazine, F: furfural.

Mean values in the same column with different superscripts are significantly different at $P \leq 0.05$ by Duncan's Multiple Range Test. ^{ns} is not significant different.

*The a number of the compounds cannot detect but the peak was found in GC-MS chromatogram