

Generation of Sesame Flavor by the Thermal Reaction Technique

Seung Seok Yoo*

Department of Culinary and Foodservice Management, Sejong University, Seoul 143-747, Korea

Abstract In this study we investigated the volatile compounds that are generated in sesame and contribute to its characteristic flavor. Different reaction systems were used to examine how certain amino acids influenced flavor profiles, and also to evaluate the effects of sugar types on the distribution of those volatile compounds. The volatiles that were generated in each reaction system were selectively isolated and analyzed by gas chromatography and gas chromatography-mass spectrometry, respectively. Among the 20 identified compounds, nitrogen-containing alkylpyrazines were found to be the predominant volatiles. The alkylpyrazine amounts varied across the different model systems, with the total yield being highest in the arginine reaction mixture, followed by the alanine, serine, and lysine mixtures. In general, fructose generated the most extensive amount of volatiles compared to glucose and sucrose. However, the yield of specific flavor compounds varied according to the type of sugar used. Finally, the results clearly showed that a reaction temperature of 135°C and a reaction time of 20 min generated the highest amount of volatile compounds.

Keywords: sesame flavor, volatile, system composition, reaction condition, alkylpyrazine

Introduction

Due to their characteristic flavors sesame seed and sesame oil are two highly important sources of food additives (1-3). Although sesame has a variety of functional effects (4-6), its unique flavor and sensory characteristics are regarded as the key qualities for the development of food flavor. The intrinsic volatile compounds found in sesame are formed by the interaction of sugars and proteins mainly through the roasting process (7). The generation of these compounds is greatly affected by the surrounding system conditions such as the reaction temperature and time (8, 9). Among the volatiles that sesame generates, the pyrazines have been reported as the key heterocyclic compounds for producing its typical odor (10, 11).

Many studies have reported the optimal roasting and/or extraction conditions for sesame, including the best reaction times and temperatures. The favorability of these conditions is affected by various interconnected factors. The source or origin of sesame flavor has been examined by measuring the chemical changes that occur during the roasting process (12, 13). Other research has described the sensory qualities of the individual volatile components (7, 14). However, despite the reports on the optimal reaction conditions for generating sesame flavor, there is a lack of research addressing the generation and enhancement of sesame flavor using the roasting process (15, 16).

There are three main routes to preparing food flavors. The first route is by activating flavor chemical synthesis via the direct condensation of reactants and then purifying the target compounds. The second route simply involves mixing two or more known compounds from the already prepared flavor. The final route is related to generating flavors using heat or a thermal reaction process (17). The formation of flavor volatiles in cooked or processed foods

typically occurs by this route due to the degradation and/or reaction of proteins, carbohydrates, and lipids during the thermal treatment (18). The thermal reaction or thermal processing technique refers to a cooking or processing method that produces food flavors using various thermal treatments and food components in a natural food system (19). The typical devices used for this technique are the reaction bottle, the reaction bomb, and the controlled pressure reactor. The following steps are strongly recommended for successfully producing desired food flavors with the thermal reaction technique: 1) knowing the typical volatiles in the target food, 2) selecting the proper natural precursors for the reactants, 3) producing the volatiles in the optimal reaction conditions. Strecker degradation and the Maillard reaction are often reported as the keys to producing sesame notes during thermal processing (7, 20). Heterocycles containing nitrogen such as pyrazines are also known to shape the flavor of sesame and disseminate the roasted sesame odor (21).

The objectives of this study were tri-fold. The first objective was to investigate the flavor that is produced from the major flavor precursors of sesame seed using the thermal reaction technique. Secondly, to analyze the volatiles those were generated in the individual model systems. The last objective was to examine the factors that affect the generation of the volatile compounds. The results of this study may suggest possible ways sesame flavors can be generated through a controlled reaction.

Materials and Methods

Chemicals Reagent-grade sodium sulfate was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). HPLC-grade dichloromethane and propylene glycol were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI, USA). Aliphatic hydrocarbons (Kit 18) and 2-ethoxy-3-isopropylpyrazine were purchased as internal standards from Supelco Inc. (Bellefonte, PA, USA). GC-grade and ultra pure amino acids and sugars were purchased as

*Corresponding author: Tel: 82-2-3408-3824; Fax: 82-2-3408-3913
E-mail: yss2@sejong.ac.kr
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precursors from the Sigma Chemical Co.

Preparation of the reaction products for sesame flavor

In the model systems each reaction mixture was prepared from 0.2 mol of glucose and 0.2 mol of the selected amino acid (L-alanine, L-glycine, L-arginine, L-lysine, L-glutamic acid, L-cysteine, and L-serine) purchased from Sigma Chemical Co. in 100 mL of propylene glycol. Another set of reaction mixtures consisted of 0.2 mol of L-arginine and an equal molar amount of sugar (glucose, fructose, or sucrose) in the same medium. These reaction mixtures were transferred to a Parr reactor vessel (4520 Bench Top; Parr Instrument Co., Moline, IL, USA), and heated at 135°C for 20 min. The speed of the agitator was maintained at 150 rpm. The internal pressure and temperature of the reactor were monitored and controlled. The operating conditions of the reactor are shown in Table 1.

Isolation of the volatile components A selective purge-and-trap method was used to effectively fractionate and isolate the volatile compounds from the reaction products (22). The sample vessel was maintained at 60°C with a water bath during the volatile isolation. Thirty g of each product with 45 g of NaCl were diluted in 360 mL of distilled water and placed into the purging vessel. 2-Ethoxy-3-isopropylpyrazine was added as an internal standard for quantitation of the volatiles. The suspension was purged by nitrogen gas at a flow rate of 200 mL/min for 2 hr and trapped in 10 mL of 11.7%(w/v) hydrochloric acid solution. The acid solution was washed several times with methylene chloride and titrated with 30% NaOH solution to pH 13. The alkaline solution was then extracted by a liquid-liquid extraction, treated with anhydrous sodium sulfate, and further concentrated by the stream of nitrogen. The samples were kept at 4°C for further analysis.

Gas chromatographic analysis An HP-6890 gas chromatograph (Agilent Co., Wilmington, DE, USA) equipped with a flame ionization detector (FID) and a fused silica capillary column (25 m × 0.2 mm i.d., 0.32 μm thickness, Ultra-2; Agilent Co., Avondale, PA, USA) was used to analyze the volatiles isolated from the thermal reaction systems. The operating conditions were as follows: an injector temperature of 250°C; a detector temperature of 280°C; and helium as a carrier gas with a flow rate of 1.0 mL/min. The GC oven temperature was

initially held at 35°C for 4 min and then ramped at 4°C/min to 80°C, and 6°C/min to 260°C. Quantitative determinations were made without considering response factors. The data processing was carried out by using an HP-6890 GC Workstation (Agilent Co.).

Gas chromatography-mass spectrometric analysis

Volatiles isolated by the selective purge-and-trap method were analyzed by gas chromatography-mass spectrometry (GC/MS) using a Fisons HRGC 8000 gas chromatograph (Fisons Instruments Co., Milan, Italy) directly coupled with a VG Platform II (Micromass Ltd., Altrincham, UK). Mass spectra were obtained by electron ionization at 70 eV. The temperature of the ion source was 180°C. The spectra were recorded on a MassLynx data system (Micromass Ltd.). The GC column was the same as described in the previous section. All mass spectra obtained were identified by comparisons with the Wiley or NIST library (Micromass Ltd.).

Quantitation of the volatile compounds The quantity of each identified volatile compound was calculated using the relative ratio between the integrated peak areas of the identified compounds and the peak area of the internal standard. To determine references for the identified compounds, retention indices for each peak were calculated against the *n*-paraffin standard (C₁₀-C₂₈) and determined using the method by Majlat *et al.* (23).

Results and Discussion

Generation of the reaction for sesame flavor To produce the sesame flavor by the thermal reaction technique, each reaction mixture containing select amino acids and sugars was transferred to a reactor. The volatile compounds generated from each mixture were isolated by the modified purge-and-trap fractionation. As a result of acid-base interactions, most heterocycles, including alkylpyrazines and thiazoles, were effectively trapped and extracted (22). To obtain the flavor reaction the appropriate amino acids were selected based on the amino acid profile of sesame. Glutamic acid and arginine have been found to be the primary amino acids in sesame followed by glycine and leucine (24); lysine, serine, and cysteine are shown to greatly reduce in quantity during the roasting process. Based on these two criteria and the specific characteristics of each amino acid residue, alanine, arginine, glycine, glutamic acid, lysine, cysteine, and serine were chosen to examine the effects of the amino acid systems. Glucose, fructose, and sucrose, which also greatly reduce in quantity during roasting, were used to confirm the effects of the sugar systems.

Amino acids as precursors Figure 1 shows a typical gas chromatogram and the peak identification results for each amino acid reaction system that was analyzed by GC/MS. It also shows that the selectively identified volatile compounds included heterocycles such as alkylpyrazines; the quantified results are summarized in Table 2. Among the seven amino acids used as precursors, arginine was predominant followed by alanine and serine. The systems using arginine, alanine, and serine provided volatile

Table 1. The reactor operating conditions for the generation of sesame flavor

Type	Conditions
Reactor	Parr 4560 bench top
Impeller	Four blade
Agitation	150 rpm
Temperature	135°C
Time	20 min
Pressure	<160 psi

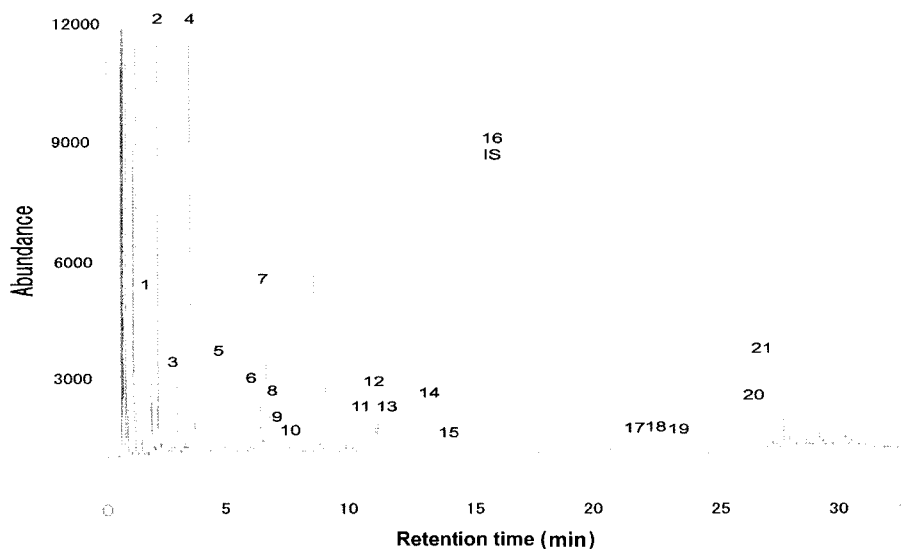


Fig. 1. The gas chromatogram of roasted sesame flavor by the purge-and-trap method; identified with GC/MS for comparing with other sesame flavor reactions. Peak names: 1, hexene; 2, pyrazine; 3, 4-methylthiazole; 4, methylpyrazine; 5, trimethyloxazole; 6, 2,5-dimethylpyrazine; 7, 2,6-dimethylpyrazine; 8, ethylpyrazine; 9, 2,3-dimethylpyrazine; 10, 2-vinylpyrazine; 11, 2-ethyl-6-methylpyrazine; 12, trimethylpyrazine; 13, 2-ethyl-3-methylpyrazine; 14, 2-methyl-6-vinylpyrazine; 15, isopropenyl pyrazine; 16, 2-ethoxy-3-isopropylpyrazine (IS); 17, 3-ethyl-2,5-dimethylpyrazine; 18, 3-ethyl-2,6-dimethylpyrazine; 19, 3-ethyl-2,3-dimethylpyrazine; 20, 2,3-diethyl-5-methylpyrazine; 21, 3,5-diethyl-2-methylpyrazine.

Table 2. The quantitation results of volatile compounds from the glucose reaction system with various amino acid precursors at 135°C for 20 min

No.	Compounds	RI ¹⁾	Amino acids ⁵⁾							
			Control	Arg	Ala	Gly	Glu	Lys	Cys	Ser
1	Hexene	717	- ²⁾	-	-	-	-	-	-	-
2	Pyrazine	740	-	8.6 ³⁾	-	2.7	3.0	-	5.9	2.3
3	4-Methylthiazole	789	-	-	-	-	-	-	2.2	-
4	Methylpyrazine	797	-	187.4	16.8	5.4	15.0	19.6	5.8	30.5
5	Trimethyloxazole	838	-	1.6	-	-	-	-	-	-
6	2,5-Dimethylpyrazine	889	-	60.7	46.9	5.6	2.1	32.1	3.3	4.6
7	2,6-Dimethylpyrazine	892	-	137.1	28.4	10.0	3.0	11.7	8.5	6.9
8	Ethylpyrazine	898	-	21.7	-	-	12.0	-	-	11.6
9	2,3-Dimethylpyrazine	910	-	24.3	4.3	2.4	-	2.6	1.8	3.1
10	2-Vinylpyrazine	924	-	11.8	1.5	-	-	-	-	-
11	2-Ethyl-6-methylpyrazine	969	-	21.2	23.2	2.8	-	-	-	6.1
12	Trimethylpyrazine	974	-	27.6	19.2	24.1	1.9	4.2	2.0	1.5
13	2-Ethyl-3-methylpyrazine	986	-	9.2	17.8	-	1.6	4.6	-	3.0
14	2-Methyl-6-vinylpyrazine	993	-	3.9	3.1	-	-	-	3.1	-
15	Isopropenylpyrazine	997	-	2.2	2.1	-	-	-	-	-
16	I.S. ⁴⁾	1018	200	200	200	200	200	200	200	200
17	3-Ethyl-2,5-dimethylpyrazine	1068	-	5.1	210.0	2.5	-	-	-	4.3
18	3-Ethyl-2,6-dimethylpyrazine	1074	-	4.8	28.7	3.4	-	-	-	2.1
19	3-Ethyl-2,3-dimethylpyrazine	1082	-	1.7	4.8	-	-	-	-	2.8
20	2,3-Diethyl-5-methylpyrazine	1097	-	-	9.7	-	-	-	-	-
21	3,5-Diethyl-2-methylpyrazine	1105	-	1.0	26.5	-	-	-	-	-

¹⁾Retention indices. ²⁾Trace amount. ³⁾Relative value. ⁴⁾Internal standard. ⁵⁾All samples were analyzed in triplicate.

compounds with greater yields and more diverse side groups.

A total of 21 components were identified with alkylpyrazines being the major heterocyclic compounds. In general, each amino acid system contained varying amounts of the same alkylpyrazines (25). However, some compounds including ethylpyrazine, 2-methyl-6-vinylpyrazine, and 3-ethyl-2,3-dimethylpyrazine were detected in only a few of the amino acid systems. A thiazole and an oxazole were only found in two specific amino acid systems (7). 4-Methylthiazole and trimethyloxazole were identified in the reaction mixtures containing cysteine and arginine, respectively. 2,3-Dimethyl-5-methylpyrazine was found in the alanine system.

Among the 20 volatile compounds identified methylpyrazine and 2,6-dimethylpyrazine occurred across all the amino acid systems; trimethylpyrazine and 2,5-dimethylpyrazine were also commonly found in all systems. Methylpyrazine, however, was predominant in the arginine system, trimethylpyrazine in the glycine system, 3-ethyl-2,5-dimethylpyrazine in the alanine system, and 2,6-dimethylpyrazine in the cysteine system. Fourteen alkylpyrazines and three alkenylpyrazines were the nitrogen-containing heterocycles that were identified.

Evaluations of some alkylpyrazines, lactones, and aldehydes showed that these compounds possess important sensory properties and play crucial roles in developing roasted sesame or sesame oil-like odors (7, 24). This characteristic was used to compare the yields and relative distributions of the volatiles formed in the amino acid systems. The results showed that arginine was the primary amino acid to generate the reaction for sesame flavor, followed by alanine and serine when methylpyrazine, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and trimethylpyrazine were selected for this comparison. These results could be explained by the reactivity of the side chains in the amino acid system. Heterocycles, including most alkylpyrazines, are formed by the interaction of an amino group with either reductones or dehydroreductones, which are the intermediates of the non-enzymatic browning reaction (19). Furthermore, alkylpyrazines are formed through the cyclization of acetoin with Strecker degraded amino acids that contain nitrogen (21). Here, the reactivity of the amino acid system may greatly affect the initiation of the process.

Free sugars as precursors Within the sugar systems, fructose precursor followed by glucose produced the greatest amount of volatiles (Table 2). Sucrose generated only a few volatile compounds at notably low quantities. Since sucrose is a non-reducing sugar it has a low reactivity for condensation during the initiation reaction (7). Although the sucrose content of sesame was reduced during roasting, this occurrence may be due to its incorporation into the reaction in different ways such as degradation or polymerization, which are not related to the generation of volatiles.

Among the 21 volatiles identified, methylpyrazine was the primary compound in the glucose system, followed by 2,6-dimethyl- and 2,5-dimethylpyrazine. In the fructose system, 2,5-dimethylpyrazine was the highest yielding compound, followed by 2,6-dimethyl-, trimethyl- and

methylpyrazine. The major volatiles produced were approximately 2-fold to 8-fold higher for the fructose system, and 10-fold to 60-fold lower in the sucrose system than in the glucose system. These results suggest that the reactivity derived from the sugar precursor can greatly affect the condensation of the reactants for the entire volatile formation process (19).

Reaction temperature The reaction temperature and reaction time are essential factors in each system for generating sesame flavor (8, 9). Each mixture containing equal molar amounts of glucose and arginine was reacted at 115, 135, or 155°C for 20 min to examine the effects of the reaction temperature. Table 4 shows the quantity of volatiles that were formed for each mixture at various temperatures during thermal processing. For most volatiles the yield increased dramatically as the reaction temperature increased from 115 to 135°C. For each reaction mixture most heterocycles had a yield that increased 3- to 7-fold. Specifically, higher yields of methylpyrazine, 2,6-dimethylpyrazine, and 2-ethyl-6-methylpyrazine were generated when the temperature was increased. The compounds 2-vinylpyrazine and 3-ethyl-

Table 3. The quantitation results of volatile compounds from the arginine reaction system with various sugar precursors at 135°C for 20 min

No.	Compounds	RI ¹⁾	Sugars ⁵⁾		
			Glucose	Fructose	Sucrose
1	Hexene	717	²⁾	-	-
2	Pyrazine	740	8.6 ³⁾	8.9	-
3	4-Methylthiazole	789	-	-	-
4	Methylpyrazine	797	187.4	209.8	5.6
5	Trimethyloxazole	838	1.6	3.1	-
6	2,5-Dimethylpyrazine	889	60.7	424.3	-
7	2,6-Dimethylpyrazine	892	137.1	281.0	13.9
8	Ethylpyrazine	898	21.7	18.5	-
9	2,3-Dimethylpyrazine	910	24.3	42.6	-
10	2-Vinylpyrazine	924	11.8	-	-
11	2-Ethyl-6-methylpyrazine	969	21.2	24.7	1.5
12	Trimethylpyrazine	974	27.6	231.5	1.5
13	2-Ethyl-3-methylpyrazine	986	9.2	13.3	-
14	2-Methyl-6-vinylpyrazine	993	3.9	8.6	-
15	Isopropenylpyrazine	997	2.2	5.0	-
16	I.S. ⁴⁾	1018	200	200	200
17	3-Ethyl-2,5-dimethylpyrazine	1068	5.1	26.2	1.6
18	3-Ethyl-2,6-dimethylpyrazine	1074	4.8	5.2	-
19	3-Ethyl-2,3-dimethylpyrazine	1082	1.7	6.9	-
20	2,3-Diethyl-5-methylpyrazine	1097	-	-	-
21	3,5-Diethyl-2-methylpyrazine	1105	1.0	3.9	-

¹⁾Retention indices. ²⁾Trace amount. ³⁾Relative value. ⁴⁾Internal standard. ⁵⁾All samples were analyzed in triplicate.

Table 4. The quantitation results of volatile compounds from the glucose and arginine reaction systems with various reaction temperatures for 20 min

No.	Compounds	RI ¹⁾	Temperature ⁵⁾ (°C)		
			115	135	155
1	Hexene	717	- ²⁾	-	-
2	Pyrazine	740	1.5 ³⁾	8.6	10.6
3	4-Methylthiazole	789	-	-	-
4	Methylpyrazine	797	27.7	187.4	128.9
5	Trimethyloxazole	838	1.2	1.6	-
6	2,5-Dimethylpyrazine	889	18.4	60.7	-
7	2,6-Dimethylpyrazine	892	22.3	137.1	87.5
8	Ethylpyrazine	898	4.4	21.7	14.7
9	2,3-Dimethylpyrazine	910	4.8	24.3	11.2
10	2-Vinylpyrazine	924	-	11.8	-
11	2-Ethyl-6-methylpyrazine	969	2.9	21.2	18.1
12	Trimethylpyrazine	974	5.7	27.6	8.2
13	2-Ethyl-3-methylpyrazine	986	3.5	9.2	7.0
14	2-Methyl-6-vinylpyrazine	993	-	3.9	-
15	Isopropenylpyrazine	997	-	2.2	-
16	I.S. ⁴⁾	1018	200	200	200
17	3-Ethyl-2,5-dimethylpyrazine	1068	-	5.1	3.5
18	3-Ethyl-2,6-dimethylpyrazine	1074	1.3	4.8	3.0
19	3-Ethyl-2,3-dimethylpyrazine	1082	-	1.7	-
20	2,3-Diethyl-5-methylpyrazine	1097	-	-	-
21	3,5-Diethyl-2-methylpyrazine	1105	-	1.0	-

¹⁾Retention indices. ²⁾Trace amount. ³⁾Relative value. ⁴⁾Internal standard. ⁵⁾All samples were analyzed in triplicate

Table 5. The quantitation results of volatile compounds from the glucose and arginine reaction systems with various reaction times at 135°C

No.	Compounds	RI ¹⁾	Time ⁵⁾ (min)		
			10	20	40
1	Hexene	717	- ²⁾	-	-
2	Pyrazine	740	4.3 ³⁾	8.6	5.7
3	4-Methylthiazole	789	-	-	-
4	Methylpyrazine	797	83.7	187.4	99.1
5	Trimethyloxazole	838	-	1.6	-
6	2,5-Dimethylpyrazine	889	25.8	60.7	20.5
7	2,6-Dimethylpyrazine	892	53.1	137.1	62.9
8	Ethylpyrazine	898	10.8	21.7	10.7
9	2,3-Dimethylpyrazine	910	10.1	24.3	8.7
10	2-Vinylpyrazine	924	-	11.8	-
11	2-Ethyl-6-methylpyrazine	969	8.2	21.2	11.3
12	Trimethylpyrazine	974	9.4	27.6	8.1
13	2-Ethyl-3-methylpyrazine	986	4.5	9.2	4.5
14	2-Methyl-6-vinylpyrazine	993	2.1	3.9	1.6
15	Isopropenylpyrazine	997	1.3	2.2	-
16	I.S. ⁴⁾	1018	200	200	200
17	3-Ethyl-2,5-dimethylpyrazine	1068	2.1	5.1	2.1
18	3-Ethyl-2,6-dimethylpyrazine	1074	-	4.8	-
19	3-Ethyl-2,3-dimethylpyrazine	1082	-	1.7	-
20	2,3-Diethyl-5-methylpyrazine	1097	-	-	-
21	3,5-Diethyl-2-methylpyrazine	1105	-	1.0	-

¹⁾Retention indices. ²⁾Trace amount. ³⁾Relative value. ⁴⁾Internal standard. ⁵⁾All samples were analyzed in triplicate.

2,3-dimethylpyrazine could be quantified only at 135°C.

In general, the higher the reaction temperature of the system the higher the yield of the volatile compounds (9). However, the yield of most volatiles decreased at the highest reaction temperature of 155°C compared to 135°C. It seems that at higher temperatures, greater amounts of polymerization products were generated, which did not necessarily correspond to the volatile yield since higher energy was required to produce more complicated compounds (26).

When comparing the relative concentrations of the generated volatiles, the total yields as well as the individual yields were highest at 135°C, making this the optimal reaction temperature. The results also showed that some alkylpyrazines such as methylpyrazine, 2,6-dimethylpyrazine, and 2-ethyl-6-methylpyrazine were generated more efficiently at higher temperatures.

Reaction time Each reaction mixture was heated either for 10, 20, or 40 min at 135°C to evaluate the effects of the reaction time on flavor generation (27). Table 5 shows the relative amounts of the major volatile compounds that were generated. The pattern here was the same as that

found for the reaction temperature, and an intermediate time (20 min) provided the greatest yields. All of the generated compounds increased as the reaction time extended up to 20 min.

The results of this study indicate that flavor generation reactions require adequate time and appropriate temperatures to obtain optimal concentrations of the target compounds. Also, the system composition, which consists of the proper amino-sugar reactants and the optimal reaction temperature and time, influences the generation of sesame flavor by affecting each flavor constituent and the flavor balance.

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