Determination of Main Bitter Compounds in Soaked and Germinated Sesame Pastes

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Abstract: The flavor and taste of the foods play an important or even a decisive role in the acceptance and preference of the consumers. It was found that the sesame paste prepared with the germinated sesame seeds was bitter in our previous experiment. In the study, the volatile and non-volatile bitter-taste components of the sesame paste samples were comprehensively analyzed. 2-methylbutanal, hexanal, acetic acid, and butyric acid were the predominant volatile compounds in the soaked and germinated sesame pastes. Oxalate was significantly reduced by the germination ($\rho < 0.05$). The contents of sesaminoltriglucoside in sesame pastes ranged from 129.04 to 217.57 $\mu$g/g. Both total and individual free amino acid contents increased with the prolongation of the germinating time. The bitter-taste amino acid Arg had the highest score of Taste Activity Value for the bitterest sample made from the seeds germinated for 36 hours. The bitter-tasting Arg was first reported to impart a bitter taste to the germinated sesame paste.

Key words: sesame pastes, non-volatile, bitterness, volatile compounds, germination

1 Introduction

Sesame (Sesamum indicum L.) has long been consumed because of its reputation as a health food¹ and its characteristic aroma and taste², in the form of the sesame seeds and sesame oils and sesame paste. And sesame has been widely cultivated in the tropical and subtropical regions of the world, including Asia, Africa, and South America. The worldwide annual production of sesame is estimated at $5.47 \times 10^6$ tons³.

Germination is a convenient, natural, and effective technique to improve the antioxidative activities, nutritional quality while minimizing the content of undesirable anti-nutritional components of the food materials⁴⁻⁶. It has been reported that the contents of the sesamol and the $\alpha$-tocopherol in the sesame seeds increased through germination⁷,⁸. The sesame sprouts contained the higher content of total phenolic compounds, $\gamma$-aminobutyric acid, and free amino acids, and the more abundant unsaturated fatty acids⁹. Soaking facilitated the germinating process of the sesame seeds and improved the proximate composition⁴. Besides, soaking for a short time is a common procedure in processing the sesame seeds to remove the dust and impurities. Our previous study found that the sesame paste prepared with the soaked and germinated sesame seeds were richer in Vitamin E²⁰.

The flavor and taste of the foods play an important or even decisive role in the acceptance and preference of the consumers. Generally, both the volatile flavor substance and soluble compounds present the consumers the sensory response, including olfactory and gustatory perceptions¹¹. However, there is scarce literature about the flavor or taste of germinated sesame products. Olagunju and Ifesan (2013) reported the decreased sensory scores of wheat-sesame cookies with increased addition of germinated sesame seed flour¹². We found in our previous experiment that the sesame paste prepared with the germinated sesame seeds became bitter, and the bitter taste was stronger with the longer duration of germination (unpublished work). So far, the bitter components in the germinated sesame seeds haven't been explored and identified. In the present study, the volatile flavors and the soluble taste components including oxalic acid, free amino acids, and sesaminoltriglucoside (STG) were thoroughly studied, and the bitter component of the germinated sesame pastes was identified for the first time.

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2 Materials and Methods

2.1 Materials

The white sesame seeds (cultivar: Yuzhi No.11) of about 50 kg were kindly provided by Henan Academy of Agricultural Sciences (Zhengzhou, China). The year of harvest was 2014.

2.2 Preparation of the sesame pastes from the soaked and germinated seeds

Sesame seeds (1 kg) were cleaned, soaked in distilled water in the ratio of 1:2 (w/v) at room temperature for 2 h. The excess water was drained and the soaked sesame seeds were evenly put on the wet tray in the incubator (Model WS250Ⅱ, Shanghai Shuli instrument and Meter Co., Ltd., China) for 0 h, 9 h, 19 h, 29 h, and 36 h, respectively at 28°C and 95% relative humidity. The germination process was monitored to make humidity constant and prevent mould growth. The seeds without any treatment served as a control.

The sesame paste samples were prepared from the seeds after different germination duration. Germinated sesame seeds were baked to 20% - 25% moisture, and then roasted at 130°C using Automatic Roasting Machine (Zhucheng Hong-nuo Mechanical Technology Co. Ltd., Weifang, China) for about 40 minutes, until the sesame seeds were yellow and crisp. Sesame seeds were cooled to approximately 45°C, then milled to paste using the colloid mill (Model JM-L80, Wenzhou Longwan Huawei Machinery Factory, Wenzhou, China), and stored at 4°C until use.

2.3 Analysis of the volatile compounds (solid-phase microextraction GC-MS)

The volatiles of the sesame pastes was extracted using the solid-phase microextraction (SPME) and subjected to GC-MS analysis[13-15]. Briefly, each sesame paste sample of 5 ± 0.1 g was put in a 10 mL headspace vial, and extracted with DVB/CAR/PDMS solid microextraction fiber (2 cm/50 μm, Supelco, Bellefonte) at 40°C in a thermostatic water bath for 60 min.

The extracted samples were desorbed at 250°C for 0.5 h and analyzed on a GC-MS (QP2010, Shimadzu, Japan) with an Rtx-5MS 30 m × 0.25 mm × 0.25 μm chromatographic column (Agilent Technologies, USA).

The helium gas (purity ≥ 99.99%) as a carrier was used at a flow rate of 1.0 mL/min. The temperature was programmed at initial 40°C for 1 min, then increased at 3°C/min to 100°C and held for 5 min, increased at 10°C/min to 230°C, finally increased at 15°C/min to 290°C, held for 5 min.

Mass spectrometry was performed in the ionizing mode, with an electron energy of 70 eV; ion source temperature of 230°C; a port temperature of 250°C; and a scanning range of 40~500 m/z.

The volatiles was identified by comparison with NIST14.lib and NIST14s.lib Mass Spectral Database (Hewlett-Packard Co., Palo Alto, CA USA) and their retention index. The retention index (RI) was calculated using a series of linear hydrocarbons C6-C30 (Sigma-Aldrich trading Co., LTD, Shanghai, China) run at the same chromatographic conditions.

2.4 Measurement of oxalic acid

The content of oxalic acid was performed according to Sá et al.[17], and Ran and Zhou[18]. Briefly, 1.5 g of sesame paste was weighed, transferred into 250 mL flask, and hydrolyzed with 50 mL of 0.1 N hydrochloric acid. The mixture was filtered through Whatman No. 4 filter paper, and the filtrate was precipitated with adding 25% calcium chloride (w/v). The precipitant was dissolved using 1 N sulfuric acid, and titrated by 0.02 N potassium permanganate solution until the color of the solution turned to be pink and kept for more than 30 s.

2.5 Sesaminoltriglucoside analysis

The sesaminoltriglucoside (STG) was extracted and analyzed with HPLC and further identified with LC-MS according to Moazami et al.[19]. Briefly, the sesame pastes were defatted and extracted with 85% ethanol for 5 h followed by 70% ethanol. The supernatants obtained after centrifugation were filtered with the 0.45 μm filter membrane. The extract was analyzed using a Waters LC-MS (E2695, Waters Co., Ltd., Milford, USA) equipped with the AQ-C18 column (46 mm × 150 mm, 5 μm). The chromatographic condition was as follows: mobile phase A was methanol, and water as mobile phase B, linear-gradient elution of 30%~60% methanol for 45 min, a flow rate of 1.0 mL/min, detection wavelength of 290 nm; column temperature of 30°C; and injection volume of 20 μL. MS was performed in the ESI ionizing mode, the drying gas was 500 L/h, the drying gas temperature was 400°C, capillary voltage: 3.0 kV and the scanning range was 100~1500 m/z.

Naringenin (4’,5,7-trihydroxyflavone, purity ≥ 98%) was bought from Nanjing Guangrun Biotechnology Co. Ltd. (Nanning, China). The solution (80 μg/mL) was added to the samples for internal calibration. According to the corresponding chromatographic peak area and relative correction factor (0.345)[20], the contents of STG in the germinated sesame pastes were calculated.

2.6 Determination of free amino acids

The measurement of free amino acids was performed according to Song et al.[20]. Briefly, the samples were treated with 1% sulfosalicylic acid, and the supernatant was filtered with a 0.22 μm membrane. Free amino acids were analyzed using the Automatic Amino Acid Analyzer (Model L-8900, Hitachi Co., Ltd., Tokyo, Japan) equipped with an-
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2.7 Taste activity value (TAV)

TAV was determined according to Rotzoll, Dunkel and Hofmann, and calculated by dividing the determined concentration of the flavor compound by its threshold value. The compounds with TAV greater than one were considered as active taste ingredients in the food.

2.8 Statistical analysis

All measurements were carried out in triplicate. The data were evaluated by one-way analysis of variance (ANOVA) and Fisher’s least significant differences (LSD) at \( p < 0.05 \). ANOVA and LSD tests were carried out using statistical software SPSS (version 16.0, SPSS, Chicago, IL).

### 3 Results and Discussion

3.1 The volatiles of the sesame pastes prepared from the soaked and germinated sesame seeds

The volatile components were analyzed using solid-phase microextraction GC-MS and their relative contents were listed in Table 1. A total of 66 volatile compounds

| Volatile compounds | RI | IDEN | SG2-0 | SG2-9 | SG2-19 | SG2-29 | SG2-36 | CK |
|--------------------|----|------|-------|-------|--------|--------|--------|----|
| 1 2-Methylpyrazine  | 754 | MS, RI | 2.51 ± 0.01 | 0.90 ± 0.01 | 0.77 ± 0.01 | – | 1.20 ± 0.0 | 1.40 ± 0.01 |
| 2 2,5-Dimethylpyrazine | 886 | MS, RI | 20.05 ± 0.22 | 5.73 ± 0.08 | 7.88 ± 0.10 | 2.53 ± 0.01 | 6.00 ± 0.14 | 6.79 ± 0.02 |
| 3 2,6-Dimethylpyrazine | 896 | MS, RI | – | – | – | – | – | – |
| 4 2,3-Dimethylpyrazine | 902 | MS, RI | 1.48 ± 0.01 | – | – | – | – | – |
| 5 Pyrazine,2-ethyl-6-methyl | 974 | MS, RI | 3.03 ± 0.02 | 0.32 ± 0.00 | 0.48 ± 0.01 | – | 1.49 ± 0.02 | – |
| 6 2-Ethyl-5-methylpyrazine | 976 | MS, RI | 6.83 ± 0.04 | 1.16 ± 0.01 | 1.80 ± 0.01 | 0.57 ± 0.01 | 1.64 ± 0.03 | 1.57 ± 0.01 |
| 7 2,3,5-Trimethylpyrazine | 983 | MS, RI | 8.19 ± 0.05 | 1.18 ± 0.01 | – | – | 0.60 ± 0.00 | – |
| 8 3-Ethyl-2,5-dimethylpyrazine | 1030 | MS, RI | – | 1.24 ± 0.01 | 2.28 ± 0.01 | 0.37 ± 0.00 | 0.68 ± 0.01 | – |
| 9 2-Ethyl-3,5-dimethylpyrazine | 1030 | MS, RI | 8.91 ± 0.14 | – | – | – | – | 0.77 ± 0.01 |
| 10 2,3-Dimethyl-5-ethylpyrazine | 1030 | MS, RI | 1.00 ± 0.01 | – | 0.21 ± 0.00 | – | – | – |
| Total pyrazines | 52.00 ± 0.35 | 10.53 ± 0.76 | 12.65 ± 0.16 | 3.47 ± 0.46 | 11.61 ± 0.17 | 10.53 ± 0.09 |
| 11 Phenylethylene | 874 | MS, RI | 0.17 ± 0.01 | – | – | – | – | – |
| 12 Cyclohexene | 978 | MS, RI | – | – | – | – | – | – |
| 13 3,7-Dimethyl-1,3,6-octatriene | 1000 | MS, RI | – | – | – | – | – | 0.51 ± 0.01 |
| 14 1-Methyl-1-(1-methylethylidene)-cyclohexene | 1060 | MS, RI | – | – | – | – | – | – |
| 15 Terpinolene | 1080 | MS, RI | – | – | – | 0.68 ± 0.02 | 0.29 ± 0.00 | – |
| Total alkenes | 0.17 ± 0.01 | – | – | 0.68 ± 0.02 | 0.29 ± 0.00 | 0.51 ± 0.01 |
| 16 Hexane | < 600 | MS | – | – | – | 21.45 ± 3.76 | – | – |
| 17 1,4-Dichloro-2-methylbenzen | 1070 | MS, RI | – | – | – | – | 0.92 ± 0.06 |
| 18 Naphthalene | 1120 | MS, RI | 0.46 ± 0.01 | 0.28 ± 0.01 | 0.69 ± 0.01 | – | – | – |
| Total alkenes | 0.46 ± 0.01 | 0.28 ± 0.01 | 0.69 ± 0.01 | 21.45 ± 3.76 | – | 0.92 ± 0.06 |
| 19 Isobutyraldehyde | < 600 | MS | 9.57 ± 1.32 | 9.66 ± 0.09 | – | – | – | – |
| 20 3-Methylbutanal | < 600 | MS | 14.22 ± 2.24 | 16.42 ± 2.09 | 17.21 ± 2.05 | 16.67 ± 1.84 | 31.96 ± 4.12 |
| 21 2-Methylbutanal | < 600 | MS | – | 24.29 ± 1.98 | 18.26 ± 1.24 | 12.43 ± 1.51 | 21.50 ± 1.85 | – |
| 22 Hexanal | 759 | MS, RI | – | 5.15 ± 0.07 | – | 10.39 ± 0.09 | 3.47 ± 0.06 | – |
| 23 Furfural | 810 | MS, RI | – | – | – | – | – | – |
| 24 Heptaldehyde | 881 | MS, RI | 0.42 ± 0.01 | 0.82 ± 0.01 | 0.55 ± 0.01 | 1.92 ± 0.04 | 1.03 ± 0.08 | – |
| 25 3-(Methylthio)propionaldehyde | 891 | MS, RI | – | 0.26 | – | – | 0.84 ± 0.05 | – |
| 26 2-Heptenal | 937 | MS, RI | – | – | 0.38 ± 0.01 | – | 1.13 ± 0.02 | – |
| 27 Benzaldehyde | 938 | MS, RI | 1.48 ± 0.08 | 2.16 ± 0.02 | 1.82 ± 0.07 | 2.41 ± 0.03 | 2.04 ± 0.02 | 4.61 ± 0.03 |
| 28 5-Methylfurfural | 949 | MS, RI | – | – | – | – | – | – |
| 29 Phenylacetaldheyde | 1014 | MS, RI | – | 1.27 ± 0.02 | 1.38 ± 0.01 | – | 1.64 ± 0.01 | 6.61 ± 0.04 |
| 30 1-Nonanal | 1039 | MS, RI | – | 0.93 ± 0.01 | – | 2.88 ± 0.03 | 1.98 ± 0.01 | 2.91 ± 0.02 |
| 31 Benzenecacetaldehyde, α-ethylidene | 1130 | MS, RI | – | – | 0.25 ± 0.00 | – | – | – |
### Table 1

Continued.

| Volatile compounds                          | RI   | IDEN         | SG2-0   | SG2-9   | SG2-19  | SG2-29  | SG2-36  | CK       |
|---------------------------------------------|------|--------------|---------|---------|---------|---------|---------|----------|
| 32 3-Phenyl-1,2-butanal                    | 1130 | MS, RI       | 0.37 ± 0.01 | –       | –       | –       | –       | –        |
| Total aldehydes                             |      |              | 37.35 ± 0.24 | 71.83 ± 0.56 | 39.05 ± 0.37 | 47.2 ± 0.29 | 49.54 ± 0.59 | 47.72 ± 1.35 |
| 33 2-Octanone                               | 870  | MS, RI       | 1.16 ± 0.01 | 0.60 ± 0.01 | 0.70 ± 0.01 | 1.40 ± 0.01 | –       | –        |
| 34 1,3-Diazapane-2,4-dione                  | 1024 | MS, RI       | –       | –       | –       | –       | 0.69 ± 0.01 | –        |
| 35 4H-Pyrano-4-one,2,3-dihydro-acetophenone | 1040 | MS, RI       | 0.63 ± 0.01 | –       | –       | –       | –       | –        |
| 36 3,5-Dihydroxy-6-methyl-pyranone          | 1096 | MS, RI       | –       | –       | –       | 1.31 ± 0.01 | –       | –        |
| Total ketones                               |      |              | 1.79 ± 0.05 | 0.60 ± 0.01 | 0.70 ± 0.01 | 1.40 ± 0.01 | 2.00 ± 0.06 | –        |
| 37 Ethyl butyrate                           | 780  | MS, RI       | –       | –       | –       | –       | –       | –        |
| 38 γ-Butyrolactone                          | 899  | MS, RI       | –       | –       | –       | –       | –       | –        |
| 39 Methyl nicotinate                        | 1094 | MS, RI       | –       | –       | –       | –       | 0.21 ± 0.00 | –        |
| 40 4-Methoxy-cinnamate Octyl               | 1855 | MS, RI       | –       | –       | 0.49 ± 0.01 | –       | –       | –        |
| 41 Tetradecanoic acid, ethyl ester          | 1966 | MS, RI       | –       | –       | 0.80 ± 0.01 | –       | –       | –        |
| 42 Bis(2-ethylhexyl) adipate                | 2080 | MS, RI       | –       | –       | 4.47 ± 0.04 | 6.14 ± 0.05 | 3.37 ± 0.05 | –        |
| Total alcohols                              |      |              | –       | 5.76 ± 0.03 | 6.35 ± 0.08 | 3.37 ± 0.05 | –       | –        |
| 43 2,3-Butanediol                           | 732  | MS, RI       | 0.95 ± 0.05 | –       | –       | –       | –       | –        |
| 44 Furfuryl alcohol                         | 831  | MS, RI       | –       | –       | –       | –       | 0.89 ± 0.01 | –        |
| 45 Benzyl alcohol                           | 1006 | MS, RI       | –       | –       | –       | –       | 0.56 ± 0.01 | –        |
| 46 Phenethyl alcohol                        | 1073 | MS, RI       | –       | –       | –       | –       | 0.19 ± 0.00 | –        |
| Total ketones                               |      |              | –       | 0.95 ± 0.05 | –       | 1.64 ± 0.04 | –       | –        |
| 47 Acetic acid                              | < 600 | MS           | 8.40 ± 0.09 | 9.92 ± 1.05 | –       | –       | –       | –        |
| 48 Butyric acid                             | 730  | MS, RI       | –       | –       | 2.65 ± 0.03 | –       | –       | –        |
| 49 Propanoic acid                           | 1455 | MS, RI       | 0.66 ± 0.01 | –       | –       | –       | –       | –        |
| 50 Myristic acid                            | 1462 | MS, RI       | –       | 2.37 ± 0.03 | –       | –       | –       | –        |
| 51 Pentadecanoic acid                       | 1806 | MS, RI       | –       | 0.60 ± 0.01 | –       | –       | –       | –        |
| 52 9-Hexadecanoic acid                      | 1904 | MS, RI       | –       | 2.32 ± 0.03 | –       | –       | –       | –        |
| 53 Palmitic acid                            | 1940 | MS, RI       | –       | 10.18 ± 1.42 | 0.78 ± 0.02 | 2.97 ± 0.03 | –       | –        |
| 54 6-Octadecenoic acid                      | 1953 | MS, RI       | –       | 0.72 ± 0.02 | –       | –       | –       | –        |
| 55 Stearic acid                             | 1971 | MS, RI       | –       | 1.71 ± 0.04 | –       | –       | –       | –        |
| Total acids                                 |      |              | 0.66 ± 0.01 | 8.40 ± 0.09 | 17.90 ± 0.86 | 12.57 ± 0.92 | 0.78 ± 0.02 | 2.97 ± 0.03 |
| 56 Dimethyl sulfide                         | < 600 | MS           | –       | –       | –       | –       | –       | –        |
| 57 Dimethyl sulfide                         | 757  | MS, RI       | 0.52 ± 0.02 | 0.44 ± 0.01 | –       | 16.80 ± 0.02 | –       | –        |
| 58 Dimethyl sulfide                         | 952  | MS, RI       | 0.43 ± 0.01 | –       | –       | –       | –       | –        |
| 59 2-Pentyl furan                           | 969  | MS, RI       | 1.67 ± 0.04 | 1.54 ± 0.02 | 3.95 ± 0.07 | 2.57 ± 0.02 | 4.86 ± 0.05 | –        |
| 60 4-Dimethylaminopyridine                  | 977  | MS, RI       | –       | 2.56 ± 0.03 | –       | –       | –       | –        |
| 61 2-Acetylpyrrole                          | 1028 | MS, RI       | –       | –       | –       | 1.98 ± 0.01 | –       | –        |
| 62 1,4-Dichloro-2-methyl benzene            | 1030 | MS, RI       | –       | 0.13 ± 0.00 | –       | –       | –       | –        |
| 63 N,N-Dimethylhexadecylamine               | 1670 | MS, RI       | –       | 4.61 ± 0.04 | –       | –       | –       | –        |
| 64 4-Octadeyl-Morpholine                    | 1802 | MS, RI       | –       | 2.78 ± 0.02 | –       | –       | –       | –        |
| 65 p-Cresol                                 | 1030 | MS, RI       | –       | –       | –       | –       | –       | –        |
| 66 4-Vinyl-2-methoxy phenol                 | 1233 | MS, RI       | –       | –       | –       | 0.45 ± 0.01 | –       | –        |
| Total others                                |      |              | 0.95 ± 0.05 | 2.11 ± 0.03 | 11.62 ± 0.10 | 3.95 ± 0.07 | 21.35 ± 0.07 | 5.31 ± 0.02 |

RI: Retention index; MS: Mass spectra; IDEN: Identified method.

SG2-0: the sesame paste prepared from the seed subjected to soaking of 2h and germination of 0h; SG2-9: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 9h; SG2-19: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 19h; SG2-29: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 29h; SG2-36: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 36h; CK: the control, the sesame paste prepared from the seeds subjected to nor soaking or germination.
were identified by comparing their spectra and retention indices with those in mass spectra database and authentic standards. The main classes of the volatile compounds included pyrazines, aldehydes, and acids.

The contents of pyrazines showed a vast variation among the samples and were significantly increased by solely soaking the sesame seeds for 2 hours \((p<0.05)\). The results may be ascribed to that during the soaking process, the sesame seeds absorbed the water. With the increasing activities of the amylase and protease, polysaccharides and proteins decomposed; reducing sugars and amino acids increased; and thus, more pyrazines can be formed from the Maillard reaction.

2-methylbutanal and hexanal were the most abundant aldehydes in the soaked and germinated seeds sesame pastes, and they were also reported to be the important aroma ingredients in the roasted almonds, 2-methylbutanal is formed via Strecker degradation of isoleucine\(^{25}\), while hexanal by the lipids autoxidation\(^{24}\).

The relative contents of the acids increased through germination. The most abundant acid in the germinated samples was acetic acid and butyric acid, which are formed by carbohydrate degradation\(^{23}\).

### 3.1.1 Evaluation of the contents of oxalic acids

Oxalic acid is a major bitter ingredient in the sesame pastes. We found that it was significantly reduced by the germination treatment, and reached the lowest level at the germination for 29 h (0.65%), which was 51.13% lower than that of the control sample \((p<0.05)\), while the content of oxalic acid just after soaking was as high as that in the control (Table 2). The results indicated that soaking did not reduce the level of the oxalic acid, and the oxalate in the sesame might be present mainly in the form of the insoluble oxalic acid-mineral complex.

The oxalate content for wheat bran was 0.46%\(^{24}\). The total oxalate contents were in the range of 5.45-5.92% in the leaflets of *Averrhoa bilimbi* and *Averrhoa carambola*, and the oxalates were present in the form of the calcium oxalate crystals which may impair the health of the patients with reduced renal activity\(^{24}\). Our results indicated that germination could reduce the oxalate content, and thus reduce the astringency of the sesame paste and decrease its risk to individuals with renal disorders.

### 3.2 The effect of soaking and germinating on the contents of STG

Sesaminoltriglucosides (STG) are the main lignan-glycosides in the sesame seeds\(^{25, 26}\). Although possessing the strong antioxidant activity, the glycosides usually have a bitter taste\(^{27}\), and thus the content of STG was determined (Table 2).

The contents of STG in sesame pastes ranged from 129.04 to 217.57 μg/g. Samples SG2-0 and SG2-9 had higher content than the control group \((p<0.05)\). The measured values of the STG content were lower than those reported by Moazzami and Kamal-Eldin\(^{25}\). The variation may

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**Table 2** Influence of soaking and germination process on the contents of oxalic acid and sesaminoltriglucoside in the sesame pastes.

| Sesame pastes | Oxalic acid (%) | Sesaminoltriglucoside (μg/g) |
|--------------|----------------|----------------------------|
| SG2-0        | 1.33 ± 0.22\(^{a}\) | 217.57 ± 12.35\(^{b}\) |
| SG2-9        | 1.04 ± 0.23\(^{ab}\) | 206.91 ± 10.31\(^{a}\) |
| SG2-19       | 0.76 ± 0.21\(^{b}\)  | 199.89 ± 19.37\(^{b}\) |
| SG2-29       | 0.65 ± 0.11\(^{b}\)  | 129.04 ± 13.88\(^{b}\) |
| SG2-36       | 0.83 ± 0.30\(^{b}\)  | 178.93 ± 19.25\(^{b}\) |
| CK           | 1.33 ± 0.17\(^{a}\)  | 150.62 ± 16.59\(^{b}\) |

Data were expressed as the means ± SD (n=3). The values with the different letters are significantly different \((p<0.05)\).

SG2-0: the sesame paste prepared from the seed subjected to soaking of 2h and germination of 0h; SG2-9: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 9h; SG2-19: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 19h; SG2-29: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 29h; SG2-36: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 36h; CK: the control, the sesame paste prepared from the seeds subjected to no soaking or germination.
be due to the different sesame material used as well as the partial loss of the seed coat during the roasting of the sesame seeds.

### 3.3 The profiling of the free amino acids

Free amino acids contribute to the taste directly and participate in the Maillard reaction and Strecker degradation to form the volatile flavor compounds. Phe, Val, Met, Ile, Leu, and Arg are bitter, Glu, Asp, and His have sour taste. Thr, Ser, Gly, Ala, and Pro are sweet. Free amino acids of the sesame pastes prepared from the treated sesame seeds were shown in Table 3. The results showed that total and individual free amino acid contents were increased by germinating, which was in accord with those reported by Liu et al. 

The sample SG2-36 had the highest content of both the total and the individual free amino acids except for Phe, Met, Glu, Asp, His, Gly, and Cys. The contents of free amino acids positively correlated with the activities of the protease and the endopeptidase. This may be due to the fact that during the process of the germination, the proteases including the endopeptidase, carboxypeptidase and aminopeptidase have enhanced activity, and hydrolyzed the proteins to the small peptides and free amino acids.

In Table 4, free amino acids of the sesame paste samples were classified into four taste groups, i.e., bitter, acid, sweet, and aged. And TAVs were calculated to reveal the main amino acids contributing to the taste of the sesame paste samples. The sweet-taste amino acid Ala had the TAV of 1.33 for the sample SG2-36. The bitter-taste amino acid Arg had the highest score of TAV, i.e. 1.82, for the sample SG2-36 which tasted bitterest. The compounds with TAV greater than 1 were considered as active taste ingredients. The larger the TAVs, the greater impacts on the taste.

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**Table 3** The levels of free amino acids in the sesame pastes prepared from the soaked and germinated sesame seeds.

| Taste attribute | Amino acids | SG2-0   | SG2-9   | SG2-19  | SG2-29  | SG2-36  | CK     |
|-----------------|------------|--------|--------|--------|--------|--------|--------|
| Bitter          | Phe        | 8.20 ± 0.64 | 16.88 ± 2.24 | 16.54 ± 1.96 | 20.09 ± 1.88 | 15.95 ± 1.46 | 19.50 ± 2.46 |
|                 | Val        | 7.03 ± 0.86 | 11.06 ± 2.74 | 12.90 ± 1.52 | 16.18 ± 2.11 | 29.41 ± 3.04 | 10.21 ± 1.98 |
|                 | Met        | 1.85 ± 0.24 | 2.63 ± 0.19 | 3.24 ± 0.02 | 4.51 ± 0.03 | 2.54 ± 0.01 | 2.18 ± 0.13 |
|                 | Ile        | 5.40 ± 0.63 | 8.48 ± 0.57 | 9.45 ± 1.74 | 12.16 ± 1.58 | 21.93 ± 1.48 | 8.51 ± 0.92 |
|                 | Leu        | 4.61 ± 0.52 | 10.97 ± 1.63 | 13.02 ± 0.98 | 16.87 ± 2.45 | 26.13 ± 2.52 | 10.52 ± 1.26 |
|                 | Arg        | 25.06 ± 1.67 | 43.35 ± 3.19 | 54.13 ± 3.62 | 49.25 ± 5.02 | 90.82 ± 6.87 | 42.07 ± 2.98 |
|                 | Total      | 52.15 ± 1.25 | 93.37 ± 4.37 | 109.28 ± 3.94 | 119.06 ± 1.29 | 186.78 ± 6.72 | 92.99 ± 6.58 |
| Acid            | Glu        | 6.94 ± 0.83 | 46.71 ± 5.24 | 43.08 ± 4.98 | 70.47 ± 7.58 | 37.84 ± 3.05 | 44.93 ± 4.65 |
|                 | Asp        | 16.71 ± 1.29 | 22.76 ± 2.39 | 26.97 ± 3.83 | 14.96 ± 2.55 | 19.24 ± 1.37 | 14.19 ± 1.55 |
|                 | His        | 6.13 ± 1.84 | 9.14 ± 1.02 | 9.62 ± 1.16 | 12.07 ± 0.86 | 6.72 ± 0.05 | 8.69 ± 1.01 |
|                 | Total      | 29.78 ± 2.16 | 78.61 ± 4.69 | 79.67 ± 5.73 | 97.5 ± 4.65 | 63.8 ± 4.39 | 67.81 ± 7.01 |
| Sweet           | Thr        | 4.05 ± 0.51 | 6.22 ± 0.74 | 8.68 ± 0.95 | 9.77 ± 1.36 | 150.77 ± 10.83 | 5.16 ± 0.78 |
|                 | Ser        | 14.70 ± 1.28 | 30.76 ± 3.95 | 30.01 ± 3.56 | 35.88 ± 4.13 | 37.84 ± 4.36 | 39.46 ± 4.12 |
|                 | Gly        | 4.46 ± 0.56 | 6.14 ± 0.79 | 7.17 ± 1.23 | 9.60 ± 1.63 | 8.79 ± 0.55 | 4.94 ± 0.38 |
|                 | Ala        | 17.69 ± 1.96 | 32.10 ± 3.68 | 41.25 ± 5.46 | 41.35 ± 5.27 | 79.75 ± 5.36 | 20.89 ± 2.47 |
|                 | Pro        | 6.31 ± 0.04 | 15.04 ± 1.09 | 13.73 ± 1.12 | 16.47 ± 1.25 | 26.34 ± 1.81 | 11.52 ± 1.47 |
|                 | Total      | 47.21 ± 3.46 | 90.26 ± 2.15 | 100.84 ± 7.36 | 113.07 ± 4.27 | 303.49 ± 6.83 | 81.97 ± 3.28 |
| Aged            | His        | 4.58 ± 0.02 | 7.44 ± 0.05 | 8.03 ± 0.06 | 9.75 ± 0.06 | 60.63 ± 4.48 | 6.98 ± 1.01 |
|                 | Tyr        | 6.74 ± 0.03 | 13.91 ± 1.18 | 16.16 ± 0.09 | 18.31 ± 2.57 | 25.28 ± 2.01 | 12.08 ± 1.63 |
|                 | Total      | 11.32 ± 0.01 | 21.35 ± 0.97 | 24.19 ± 1.34 | 28.06 ± 1.89 | 85.91 ± 5.39 | 19.06 ± 3.46 |
| Undefined       | Cys        | 3.04 ± 0.35 | 3.01 ± 0.22 | 3.07 ± 0.19 | 2.91 ± 0.02 | 2.31 ± 0.01 | 3.54 ± 0.22 |
|                 | Total      | 143.50 ± 5.34 | 286.60 ± 7.59 | 317.05 ± 10.41 | 360.60 ± 5.19 | 642.29 ± 8.62 | 265.37 ± 15.35 |

Data were expressed as the means ± SD (n=3). SG2-0: the sesame paste prepared from the seed subjected to soaking of 2h and germination of 0h; SG2-9: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 9h; SG2-19: the sesame paste prepared from the seed subjected to soaking of 2h and germination of 19h; SG2-29: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 29h; SG2-36: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 36h; CK: the control, the sesame paste prepared from the seeds subjected to nor soaking or germination.

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Table 4  The Taste Activity Values of free amino acids in the sesame pastes.

| Taste attribute | Amino acids | Taste threshold (mg/100 g) | SG2-0 | SG2-9 | SG2-19 | SG2-29 | SG2-36 | CK |
|-----------------|------------|-----------------------------|-------|-------|--------|--------|--------|-----|
| Bitter          | Phe        | 0.09 ± 0.01                 | 0.19 ± 0.01 | 0.18 ± 0.01 | 0.22 ± 0.01 | 0.18 ± 0.01 | 0.22 ± 0.01 |
|                 | Val        | 0.18 ± 0.01                 | 0.28 ± 0.03 | 0.32 ± 0.02 | 0.40 ± 0.02 | 0.74 ± 0.03 | 0.26 ± 0.01 |
|                 | Met        | 0.06 ± 0.01                 | 0.09 ± 0.01 | 0.11 ± 0.00 | 0.15 ± 0.00 | 0.08 ± 0.00 | 0.07 ± 0.01 |
|                 | Ile        | 0.06 ± 0.01                 | 0.09 ± 0.01 | 0.11 ± 0.02 | 0.14 ± 0.02 | 0.24 ± 0.02 | 0.09 ± 0.01 |
|                 | Leu        | 0.02 ± 0.00                 | 0.06 ± 0.01 | 0.07 ± 0.00 | 0.09 ± 0.00 | 0.14 ± 0.01 | 0.06 ± 0.01 |
|                 | Arg        | 0.50 ± 0.02                 | 0.87 ± 0.04 | 1.08 ± 0.04 | 0.99 ± 0.08 | 1.82 ± 0.11 | 0.84 ± 0.02 |
| Umami           | Glu        | 0.23 ± 0.01                 | 1.56 ± 0.11 | 1.44 ± 0.16 | 2.35 ± 0.12 | 1.26 ± 0.23 | 1.50 ± 0.17 |
|                 | Asp        | 0.17 ± 0.00                 | 0.23 ± 0.01 | 0.27 ± 0.02 | 0.15 ± 0.01 | 0.19 ± 0.01 | 0.14 ± 0.01 |
|                 | His        | 0.31 ± 0.01                 | 0.46 ± 0.09 | 0.48 ± 0.02 | 0.60 ± 0.01 | 0.34 ± 0.00 | 0.43 ± 0.02 |
| Sweet           | Thr        | 0.02 ± 0.00                 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.01 | 0.58 ± 0.02 | 0.02 ± 0.00 |
|                 | Ser        | 0.10 ± 0.01                 | 0.21 ± 0.02 | 0.20 ± 0.02 | 0.24 ± 0.03 | 0.25 ± 0.03 | 0.26 ± 0.03 |
|                 | Gly        | 0.03 ± 0.00                 | 0.05 ± 0.00 | 0.06 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.00 | 0.04 ± 0.00 |
|                 | Ala        | 0.29 ± 0.01                 | 0.54 ± 0.02 | 0.69 ± 0.03 | 0.69 ± 0.03 | 1.33 ± 0.03 | 0.35 ± 0.01 |
|                 | Pro        | 0.02 ± 0.00                 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.09 ± 0.00 | 0.04 ± 0.00 |
| Aged            | Lys        | 0.09 ± 0.00                 | 0.15 ± 0.01 | 0.16 ± 0.01 | 0.20 ± 0.00 | 1.21 ± 0.01 | 0.14 ± 0.00 |

SG2-0: the sesame paste prepared from the seed subjected to soaking of 2h and germination of 0h; SG2-9: the sesame paste prepared from the seeds subjected to soaking of 2h and germination of 9h; SG2-19: the sesame paste Prepared from the seeds subjected to soaking of 2h and germination of 19h; SG2-29: the sesame paste Prepared from the seeds subjected to soaking of 2h and germination of 29h; SG2-36: the sesame paste Prepared from the seeds subjected to soaking of 2h and germination of 36h; CK: the control, the sesame paste Prepared from the seeds subjected to no soaking or germination.

Thus, Arg was considered to be the main compound contributing to the bitter taste of the soaked and germinated sesame pastes.

4 Conclusions
This study provides the first comprehensive characterization of the volatile and non-volatile bitter-taste components of the soaked and germinated sesame paste. Strecker degradation product 2-methylbutanal, the lipids autooxidation product hexanal, and acids from carbohydrate degradation were the predominant volatile compounds after germination. The bitter-taste amino acid Arg had the highest score of TAV for the sample SG2-36 which tasted bitterest, which imparted a bitter taste to the germinated sesame paste.

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Compliance with ethical standards
Conflicts of Interest
The authors declare that they have no conflict of interest.

References
1) Elleuch, M.; Bedigian, D.; Zitoun, A. Sesame (Sesamum indicum L.) seeds in food, nutrition, and health. in Nuts & Seeds in Health & Disease Prevention. Elsevier, pp. 1029-1036 (2011).
2) Ryu, S.N.; Kim, M.K.; Xi, J.; Ho, C.T. Influence of seed roasting process on the changes in volatile compounds of the sesame (Sesamum indicum L.) oil. in Flavor Chemistry of Ethnic Foods. Springer, Boston, MA. pp. 229-237 (1999).
3) Phumichai, C.; Matthayatthaworn, W.; Chuenpom, N.; Wongkaew, A.; Somsaeng, P.; Yodyingyong, T.; Pan-klang, P.; Jenweerawat, C.; Keawsaard, Y.; Phumichai, T.; Sreewong, T.; Kaveeta, R. Identification of a scar marker linked to a shattering resistance trait in sesame. Turk. J. Field Crops 22, 258-265 (2017).
4) Bau, H.M.; Villaume, C.; Nicolas, J.P.; Mejean, L. Effect of germination on chemical composition, biochemical

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constituents and antinutritional factors of soya bean 
(Glycine max) seeds. J. Sci. Food Agr. 73, 1-9 (1997).
5) Bolívara, C.C.; Luis, C.Z. Impact of germination on phenolic content and antinflammatory activity of 13 edible seed species. Food Chem. 119, 1485-1490 (2010).
6) Murugkar, D.A.; Krishna, J. Effect of sprouting on nutritional and functional characteristics of soybean. J. Food Sci. Tech. 46, 240-243 (2009).
7) Hahn, T.S.; Park, S.J.; Martin, L.Y. Effects of germination on chemical composition and functional properties of sesame (Sesamum indicum L.) seeds. Bioresource. Technol. 99, 1643-1647 (2009).
8) Liu, B.; Guo, X.; Zhu, X.; Liu, Y. Nutritional evaluation and antioxidative activity of sesame sprouts. Food Chem. 129, 799-803 (2011).
9) Kajihausa, O.E.; Fasasi, R.A.; Atolagbe, Y.M. Effect of different soaking time and boiling on the proximate composition and functional properties of sprouted sesame seed flour. Nigerian Food J. 32, 8-15 (2014).
10) Qiu, J.; Hou, L.; Zhang, Z.; Wang, X.; Zeng, G. Effect of soaking and germinating on the quality of sesame seeds and sesame butter. Food Sci. 39, 39-44 (2018).
11) Wu, F.; Yang, N.; Chen, H.; Jin, Z.; Xu, X. Effect of germination on flavor volatiles of cooked brown rice. Cereal Chem. 88, 497-503 (2011).
12) Olagunju, A.I.; Iesan, B.O.T. Nutritional composition and acceptability of cookies made from wheat flour and germinated sesame (Sesamum indicum) flour blends. Curr. J. Applied Sci. Tech. 3, 702-713 (2013).
13) Hou, L.; Zhang, Y.; Wang, X. Characterization of the volatile compounds and taste attributes of sesame pastes processed at different temperatures. J. Oleo Sci. 68, 551-558 (2019).
14) Wei, C.K.; Ni, Z.J.; Thakur, K.; Liao, A.M.; Huang, J.H.; Wei, Z.J. Aromatic effects of immobilized enzymatic oxidation of chicken fat on flaxseed (Linum usitatissimum L.) derived Maillard reaction products. Food Chem. 306, 125560 (2020).
15) Shang, Y.F.; Cao, H.; Wei, C.K.; Thakur, K.; Liao, A.M.; Huang, J.H.; Wei, Z.J. Effect of sugar types on structural and flavor properties of peony seed Maillard reaction products. J. Food Process Res. 44, e14341 (2020).
16) Topi, D. Volatile and chemical compositions of freshly squeezed sweet lime (Citrus limetta) juices. J. Raw Mater. Process Foods 1, 22-27 (2020).
17) Sá, R.D.; Vasconcelos, A.L.; Santos, A.V.; Padilha, R.J.; Alves, L.C.; Soares, L.A.; Randau, K.P. Anatomy, histochecmistry and oxalid acid content of the leaflets of Averrhoa bilimbi and Averrhoa carambola. Brazil. J. Pharma 29, 11-16 (2019).
18) Ran, Q.; Zhou, X. Determination of the content of the oxalic acid in sesame seeds. Sichuan Grain Oil Tech. 13, 34-36 (1992).
19) Moazzami, A.A.; Andersson, R.E.; Kamal-El Din, A. HPLC Analysis of sesaminolglycosides in sesame seeds. J. Agric. Food Chem. 54, 633-638 (2006).
20) Song, J.; Bi, J.; Chen, Q.; Wu, X.; Lyu, Y.; Meng, X. Assessment of sugar content, fatty acids, free amino acids, and volatile profiles in jujube fruits at different ripening stages. J. Food Chem. 270, 344-352 (2019).
21) Rotzoll, N.; Dunkel, A.; Hofmann, T. Quantitative studies, taste reconstitution, and omission experiments on the key taste compounds in morel mushrooms (Morchella delicosa Fr.). J. Agric. Food Chem. 54, 2705-2711 (2006).
22) Whitfield, F.B.; Mottram, D.S. Volatiles from interactions of Maillard reactions and lipids. Crit. Rev. Food Tech. 31, 1-58 (1992).
23) Erten, E.S.; Cadwallader, K.R. Identification of predominant aroma components of raw, dry roasted and oil roasted almonds. Food Chem. 217, 244-253 (2017).
24) Siener, R.; Honow, R.; Voss, S.; Seidler, A.; Hesse, A. Oxalate content of cereals and cereal products. J. Agric. Food Chem. 54, 3008-3011 (2006).
25) Moazzami, A.A.; Kamal-El Din, A. Sesame seed is a rich source of dietary lignans. J. Am. Oil Chem. Soc. 83, 719-723 (2006).
26) Ulyatu, F.; Pudji, H.; Tyas, U.; Umar, S. The changes of sesaminolrighocoside and antioxidant properties during fermentation of sesame milk by Lactobacillus plantarum. Int. J. Food Res. J. 22, 1945-1952 (2015).
27) Drewnowska, A.; Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: A review. Am. J. Clin. Nutr. 72, 1424-1435 (2000).
28) Zou, Y.; Kang, D.; Liu, R.; Qi, J.; Zhou, G.; Zhang, W. Effects of ultrasonic assisted cooking on the chemical profiles of taste and flavor of spiced beef. Ultrason. Sonochem. 46, 36-45 (2018).
29) Zhang, H.; Zhang, Y.; Ding, Y.; Li, G.; Chen, Y.; Han, Y. Changes of physiological indicators and protein metabolism during peanut germination. Food Sci. 34, 311-316 (2013).