Pulverizing processes affect the chemical quality and thermal property of black, white, and green pepper (*Piper nigrum* L.)

Hong Liu¹ · Jie Zheng² · Pengzhan Liu³ · Fankui Zeng⁴

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Abstract In this study, the effects of different pulverizing methods on the chemical attributes and thermal properties of black, white and green pepper were evaluated. Cryogenic grinding minimally damaged the lipid, moisture, crude protein, starch, non-volatile ether extract, piperine, essential oil and the typical pepper essential oil compounds of the spices. The pulverizing methods and storage significantly affected the compositions of the fatty acid in the peppers, except for palmitic acid and lignoceric acid. The amino acid contents and the thermo-gravimetric analysis curve were hardly influenced by the grinding techniques. The use of cryogenic grinding to prepare pepper ensured the highest quality of pepper products. Regardless of grinding technique, the values of moisture, piperine, unsaturated fatty acids, essential oil, monoterpenes, and the absolute concentrations of typical pepper essential oil constituents (except caryophyllene oxide) decreased, whereas the amino acid, lipid, protein, starch, and non-volatile ether extract content as well as the thermal

properties were insignificantly changed after storage at 4 °C for 6 months.

Keywords Pulverizing processes · Essential oil composition · Amino acid · Fatty acid composition · Thermo-gravimetric analysis

Abbreviations

BPCM	Black pepper ground by cryogenic grinding
BPHM	Black pepper ground by hammer milling
WPCM	White pepper ground by cryogenic grinding
WPHM	White pepper ground by hammer milling
GPCM	Green pepper ground by cryogenic grinding
GPHM	Green pepper ground by hammer milling

Introduction

Black, white, and green peppers are derived from the fruits of *Piper nigrum* L., which belongs to the Piperaceae family. The main constituents of pepper are volatile oils and piperine, which contribute to the antioxidant, antimutagenic, antitumor, antibacterial, antifungal, analgesic, and anti-inflammatory activities, and offers immense pharmacological benefits (Butt et al. 2013; Zarai et al. 2013; Tasleem et al. 2014; Nikolić et al. 2015; Zou et al. 2015; Luz et al. 2016; Tang et al. 2017; Chandran et al. 2017; Wang et al. 2017). The effective substances and most chemical components of pepper are heat-sensitive substances. Different pulverizing processes have damaged these effective substances at varying degrees because of the mechanical forces and temperature applied during the grinding processes. Cryogenic grinding is widely used to obtain the best quality of spice powders (Ghodki and Goswami 2017). The cryogenic ground spice also dispensed more uniformly in spice formulations, resulting in a

✉ Fankui Zeng
zengfk@licp.cas.cn

Hong Liu
liuhong201602@licp.cas.cn

- ¹ CAS Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China
- ² Department of Food Science and Engineering, Jinan University, Guangzhou 510632, China
- ³ School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China
- ⁴ Research and Development Center for Eco-Material and Eco-Chemistry, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China

minimized loss of the essential oils. The treatment of black pepper with cryogenic grinding resulted in higher levels of essential oil and monoterpenes (Murthy and Bhattacharya 2008). In addition, grinding at cryogenic temperatures could prevent the mechanical degradation of the molecular structure during grinding, which commonly occurred in heat-based approaches. In our previous study on the flavor attributes of peppers (Liu et al. 2013), cryogenic grinding was better than hammer milling in preserving the sensory properties and flavor attributes of black, white, and green peppers without significantly affecting its quality. However, the flavor quality of the ground pepper was diminished during storage (Liu et al. 2013).

Although several studies have described the cryogenic grinding of spices, such as cumin (Goswami and Singh 2003) and pepper (Murthy and Bhattacharya 2008; Liu et al. 2013), the effects of cryogenic grinding on the chemical profile and thermal property of green and white pepper have yet to be fully explored. In this study, the effects of cryogenic grinding and conventional hammer milling on the contents of moisture, lipid, protein, starch, non-volatile ether extract (NVEE), piperine, essential oils, fatty acids, and amino acids in black, white, and green pepper were analyzed comprehensively. The thermo-gravimetric analysis (TGA) was conducted to investigate the effects of different pulverizing methods on the thermal properties of the samples. In addition, the effect of storage on the physicochemical quality of each spice was also assessed.

In our previous study (Liu et al. 2013), the volatile oils were extracted through headspace solid-phase micro-extraction (HP-SPME), which identified the flavor characteristics of the pepper samples. In the current study, the essential oils were extracted through hydro-distillation, which characterizes the chemical profile of essential oils in pepper. By also considering the information on other components, such as moisture, lipids, starch, fatty acids, amino acids, and piperine as well as the thermal properties, a throughout physicochemical profile was generated for the pepper composition treated with different processing techniques. The results of the two combined analyses offered a comprehensive physicochemical, nutritional, and sensory profile of peppers with respect to the influences of the pulverizing techniques.

Materials and methods

Materials

Fresh berries of the pepper variety Panniyur-1 were collected in May 2016 at the state-operated Dongchang farm in Hainan, China. Black pepper was produced from fully mature orange-to-red fruits that were dried in a cross-flow dryer at 50 °C, resulting in fruits with a brownish-black

color and a shriveled surface. White pepper was produced from ripe orange-to-red fruits that were dried in a cross-flow dryer at 50 °C after the outer skin was removed. Green pepper was produced from unripe green fruits by blanching them in boiling water for 10 min to inactivate the browning enzymes and then drying them in a cross-flow dryer at 50 °C (Gopinathan and Manilal 2004; Liu et al. 2013). Broken and immature seeds as well as foreign matter were removed from the samples prior to all treatments (Liu et al. 2013). The sizes of the peppercorns were 3.5–4.5 mm, and the initial moisture contents in the peppers were 9.0–14.0%.

Samples of black, white, and green peppercorns were ground separately by using a cryogenic grinder (SPEX 6870; SPEX Metuchen, New Jersey, USA). First, the peppercorns were pre-frozen in a liquid nitrogen bath (− 210 to − 196 °C) for 10 min. The samples were then subjected to five pulverization cycles with a magnetically driven impactor at 10 s^{−1} in a liquid nitrogen bath. Each pulverization cycle consisted of a 2 min grinding period followed by a refreezing period. The refreezing step was included to maintain the appropriate cryogenic temperature of the pepper samples during the entire process because grinding can increase the temperature of the spice. The comparison samples of the black, white, and green pepper powder were prepared with a hammer mill (IKM-310 system, China) at ambient temperature in accordance with standard protocols. The temperatures of the sample after hammer milling were 37–40 °C. The ground samples were passed through 250 µm sieves and stored separately in opaque bags at 4 °C for 6 months for the storage experiments (Liu et al. 2013).

Standards

The chemical standards of α -pinene, β -pinene, β -myrcene, 3-carene, limonene, and γ -terpinene were purchased from Dr. Ehrenstorfer (GmbH, Germany). The standards of β -caryophyllene, α -caryophyllene, and caryophyllene oxide were purchased from Aldrich (Steinheim, Germany). The standards of all amino acids were purchased from Fluka, Sigma-Aldrich (GmbH, Germany). The standards for saturated fatty acid methyl esters (No: ME10-1KT) were purchased from Supelco, Sigma-Aldrich (GmbH, Germany). The standards for unsaturated fatty acid methyl esters (No: 18919-1AMP) were purchased from Supelco, Sigma-Aldrich (GmbH, Germany). The purity of all the standards was > 90%.

Moisture content

Approximately 2.0 g of each sample was weighed to determine the moisture content (%) by using an infrared moisture analyzer (MB45, Ohaus, America).

Lipid, crude protein, and starch

Lipid was determined by using the standard methods of the Association of Official Analytical Chemists (AOAC 2003). The nitrogen content of the sample was determined by using the Kjeldahl instrument. The crude protein content was calculated with the conversion equation of crude protein = $6.25 \times N$ (AOAC 2001). Starch content was determined by using the standard methods of the Association of Official Analytical Chemists Official Method 996.11-Starch (Total) in Cereal Products: Amyloglucosidase- α - Amylase Method (AOAC-AACC AOAC-AACC Method 1996).

Non-volatile ether extract

The Shaikh method (Shaikh et al. 2006) was used to measure the NVEE content as $\% \text{ NVEE} = Re/S \times 100$, where S is the weight of the sample and Re is the weight of the ether-soluble residue.

Piperine and essential oil

The piperine content of the ground pepper was determined by conducting high-performance liquid chromatography (Agilent Technologies, USA) (Wood et al. 1988).

The ground pepper samples were subjected to hydro-distillation in a Clevenger-type apparatus for 6 h in accordance with a European Pharmacopoeia procedure (Kapoor et al. 2009). A colorless essential oil with a characteristic odor and a sharp taste was obtained for each sample. This oil was then dried over an anhydrous sodium sulfate, transferred in a glass bottle, and stored in a refrigerator at 4 °C until analysis.

The essential oil was diluted at 1:50 with *n*-hexane. 1 μL of the sample was injected by using an automatic injector (CTC 6500, America) into an Agilent 7890A gas chromatograph (Agilent Technologies, USA) coupled with an Agilent 5975C mass spectrometer (Agilent Technologies, USA) equipped with a HP-5MS capillary column (5% phenyl methyl siloxane). An electron ionization system with ionization energy of 70 eV was applied. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and ion source temperatures were 250 and 280 °C, respectively. The oven temperature for the analysis of the essential oil was programmed as follows: 60 °C (2 min), 60–210 °C (5 °C/min), 210 °C (0 min), 210–280 °C (10 °C/min), and 280 °C (1 min). The compounds were identified and quantified with the same process as in a previous work (Liu et al. 2013).

Analysis of amino acid constituents

The amino acid composition was determined by using an automated amino acid analyzer (A3000-IC, membrapure GmbH, Germany) after the samples were hydrolyzed with 6 mol/L HCl in vacuum at 110 °C for 24 h (Latif et al. 2013). The amino acid standards were used as the external standards for the quantitative analysis.

Analysis of fatty acid compositions

The ground pepper samples (20.0 g) were extracted with diethyl ether in a Soxhlet apparatus for 8 h to isolate the oil in accordance with the GB/T 5009.6-2003. The fatty acid compositions of the oils were determined by converting them into fatty acid methyl esters (FAMES), followed by gas chromatograph (GC) analysis (Yang et al. 2011; Zeng et al. 2011).

Exactly 20.0 mg of the sample was thoroughly mixed with KOH-methanol solution (2.0 mL, 0.5 M, 70 °C) for 10 min in a 50 mL round bottom flask, and BF₃-methanol solution (3.0 mL) was then added. The reaction was kept at 70 °C for another 5 min. The reaction mixture was cooled to room temperature. The FAMES were extracted into hexane (3 $\times$ 1.0 mL) and then dried over anhydrous sodium sulfate. 10 μL of the samples was used for GC analysis.

A Hewlett-Packard 5890 GC was used to analyze the fatty acid composition. The FAMES were separated on a FFFAP column (PERMABOND-FFFAP DF-0.25, 25 m $\times$ 0.25 mm i.d., Macherey-Nagel, Düren, Germany) by using nitrogen as the carrier gas. The following temperature program was applied: 150 °C (2 min), raised to 230 °C at 10 °C/min and held at 230 °C for 8 min. The split ratio was 20:1. The injector and flame ionization detector temperatures were set at 250 and 300 °C, respectively. The fatty acid components of the oil were identified by comparison of the retention time with fatty acid methyl ester standards, and the relative percentage content of fatty acids was quantified by the peak area normalization method.

Thermo-gravimetric analysis (TGA)

The thermo-gravimetric analysis was performed by using a Netzsch STA 449F3 instrument under TGA configuration, with the sample and reference crucibles made of $\alpha\text{-Al}_2\text{O}_3$. Prior to the experiments, the weight, temperature, and sensitivity of the instrument were calibrated in accordance with the calibration sets provided by Netzsch. An empty $\alpha\text{-Al}_2\text{O}_3$ crucible served as the reference. Approximately 5 mg of the pepper powder sample was placed in the sample crucible, which was covered by a lid with a pinhole. The sample was heated from room temperature to 500 °C

Table 1 The contents^a of chemical components in black, white, and green pepper samples produced by cryogenic-grinding and hammer-milling stored at 4 °C for 0 and 6 months (values on dry weight basis)

Parameter	Months	BPCM	BPHM	WPCM	WPHM	GPCM	GPHM
Moisture content (%)	0	9.30 ± 0.05bx	9.07 ± 0.19ax	13.74 ± 0.10ex	12.37 ± 0.16dx	10.10 ± 0.13cx	9.92 ± 0.03cx
	6	8.05 ± 0.10by	8.04 ± 0.21by	12.62 ± 0.29dx	10.48 ± 1.78cy	6.62 ± 0.44aby	6.06 ± 0.34ay
Lipid content (%)	0	17.96 ± 0.07fx	15.32 ± 0.03ex	13.88 ± 0.04bx	12.11 ± 0.03ax	14.22 ± 0.03dx	13.97 ± 0.03cx
	6	17.77 ± 0.24ex	15.17 ± 0.29dx	13.68 ± 0.20bx	12.10 ± 0.10ax	14.20 ± 0.05cx	13.77 ± 0.24bx
Crude protein content (%)	0	12.59 ± 0.06ex	12.50 ± 0.05dex	11.35 ± 0.05bx	11.18 ± 0.14ax	12.45 ± 0.01dx	12.31 ± 0.04cx
	6	12.52 ± 0.03ex	12.51 ± 0.01ex	11.33 ± 0.03bx	11.21 ± 0.04ax	12.43 ± 0.03dx	12.32 ± 0.03cx
Starch content (%)	0	45.73 ± 0.68ex	44.53 ± 0.06cx	42.43 ± 0.12bx	41.53 ± 0.06ax	46.60 ± 0.10fx	45.17 ± 0.29dx
	6	45.48 ± 0.42dx	44.67 ± 0.18cx	42.45 ± 0.05bx	41.35 ± 0.30ax	46.55 ± 0.05ex	45.10 ± 0.10dx
NVEE content (%)	0	15.47 ± 0.06bcx	13.73 ± 0.40ax	21.23 ± 0.64ex	18.13 ± 0.06dx	15.70 ± 0.10cx	14.55 ± 1.11abx
	6	15.33 ± 0.12cx	13.57 ± 0.12ax	21.10 ± 0.10ex	18.10 ± 0.10dx	15.40 ± 0.17cx	14.33 ± 0.29bx
Piperine content (g/100 g)	0	4.15 ± 0.01fx	4.01 ± 0.02ex	3.44 ± 0.01bx	3.32 ± 0.06ax	3.87 ± 0.06dx	3.69 ± 0.02cx
	6	2.92 ± 0.03ay	2.90 ± 0.05ay	2.85 ± 0.10ay	2.93 ± 0.03ay	2.92 ± 0.06ay	2.93 ± 0.07ay
Essential oil content (mL/100 g)	0	3.81 ± 0.16cx	3.07 ± 0.14bx	2.36 ± 0.08ax	2.32 ± 0.31ax	3.03 ± 0.05bx	2.97 ± 0.46bx
	6	2.17 ± 0.06cy	2.13 ± 0.08cy	1.53 ± 0.05ay	1.52 ± 0.03ay	1.63 ± 0.03by	1.62 ± 0.03by

^aValues represent means ± S. D. (n = 3). Means followed by the same letters within the row (a–f) and within the column per parameter (x and y) are not significantly different ($p < 0.05$)

at 10 °C/min under a streaming nitrogen atmosphere (purge: 50 mL/min; protective: 20 mL/min) to suppress oxidation.

Statistical analysis

Statistical analyses were performed by using one-way analysis of variance (ANOVA) on the SPSS software (Version 13.0; SPSS Inc, Chicago, IL, USA). All data were expressed as mean ± standard deviation (SD) for triplicate measurements. A p value of 0.05 or less was considered statistically significant.

Results and discussion

Chemical components

Evident differences were observed in the piperine and essential oil contents of the black, white, and green pepper samples (Table 1). The essential oil contents of black, white, and green peppers prepared through cryogenic grinding and stored for 0 month in our experiments were 3.81, 2.36 and 3.03 mL/100 g, respectively. The values were reduced correspondingly in the hammer-milled treatment. However, the significant difference was detected only in black pepper. The effects of cryogenic grinding on the essential oil content in black pepper have been previously reported, and their results are congruent with our findings (Murthy and Bhattacharya 2008). The piperine

contents of the cryogenically ground samples derived from the black, white, and green pepper stored for 0 month were 4.15, 3.44, and 3.87 g/100 g, respectively. The values were significantly lower in the hammer-milled samples. Previous studies have shown that heat processing significantly reduced the piperine content of pepper because of the heat-induced chemical alteration in the pungency component of the spice (Suresh et al. 2007). In the current study, the hammer-milled treatments might damage and reduce the essential oil and piperine contents by increasing the temperature during the process. During cryogenic grinding, the vaporization of essential oil was minimized, as most of the essential oil compounds were retained within the powder under solid status because of the low sample temperature. Thus, our data similar to previous studies, suggested that the use of cryogenic grinding improves the essential oil and piperine contents of the final product when compared to those prepared through traditional grinding methods. However, the contents of essential oil and piperine were significantly reduced in all samples after storage at 4 °C for 6 months.

Table 1 shows the mean values and standard deviations of the moisture, lipid, protein, starch, and NVEE of the pepper samples produced through different pulverizing methods. Significant differences in the chemical composition were found for all three pepper types when the cryogenically ground samples were compared with the hammer-milled samples. The contents of moisture, lipid, protein, starch and NVEE were all significantly lower in the hammer-milled than in the cryogenic-grinding pepper

Table 2 The percentages^a (%) of the essential oils in black, white, and green pepper samples produced by cryogenic-grinding and hammer-milling under storage at 4 °C for 0 month (a); and 6 months (b)

Components	<i>RI</i> ^b	BPCM	BPHM	WPCM	WPHM	GPCM	GPHM
(a)							
α -Thujene	923	0.23 ± 0.00	0.15 ± 0.01	0.27 ± 0.00	0.29 ± 0.00	0.24 ± 0.01	0.25 ± 0.00
α -Pinene	932	5.20 ± 0.01	5.20 ± 0.23	5.75 ± 0.01	4.33 ± 0.02	5.59 ± 0.02	3.42 ± 0.02
Camphene	945	0.22 ± 0.00	0.18 ± 0.00	0.32 ± 0.00	0.34 ± 0.01	0.21 ± 0.01	0.20 ± 0.01
Sabinene	969	1.84 ± 0.01	0.47 ± 0.06	1.51 ± 0.01	0.58 ± 0.01	1.58 ± 0.01	0.25 ± 0.01
β -Pinene	973	6.57 ± 0.61	8.90 ± 0.13	9.32 ± 0.91	10.99 ± 0.03	8.24 ± 0.03	7.69 ± 0.04
β -Myrcene	984	2.57 ± 0.01	1.96 ± 0.08	2.98 ± 0.01	0.34 ± 0.02	2.45 ± 0.01	0.91 ± 0.01
α -Phellandrene	999	0.19 ± 0.00	6.22 ± 0.09	0.11 ± 0.00	0.13 ± 0.00	0.20 ± 0.01	0.17 ± 0.01
3-Carene	1009	19.37 ± 0.11	12.29 ± 0.47	22.13 ± 0.51	15.96 ± 0.95	18.85 ± 0.13	14.52 ± 0.19
α -Terpinene	1012	0.14 ± 0.01	0.15 ± 0.04	0.11 ± 0.00	0.13 ± 0.00	0.14 ± 0.01	0.12 ± 0.01
ρ -Cymene	1015	1.31 ± 0.02	0.84 ± 0.02	5.23 ± 0.01	4.59 ± 0.02	2.31 ± 0.10	1.70 ± 0.21
Limonene	1026	13.27 ± 0.83	7.18 ± 0.29	14.94 ± 0.93	12.88 ± 0.35	11.73 ± 0.24	9.69 ± 0.28
γ -Terpinene	1051	1.38 ± 0.00	0.38 ± 0.06	1.25 ± 0.01	0.28 ± 0.00	1.04 ± 0.01	0.34 ± 0.02
Terpinolene	1080	0.74 ± 0.01	0.98 ± 0.04	0.85 ± 0.01	0.27 ± 0.02	0.67 ± 0.04	0.48 ± 0.06
Linalool	1089	1.37 ± 0.01	1.46 ± 0.00	1.74 ± 0.01	1.81 ± 0.00	1.51 ± 0.01	1.83 ± 0.01
Terpinen-4-ol	1166	ND	ND	0.39 ± 0.00	0.49 ± 0.01	0.11 ± 0.01	0.10 ± 0.00
α -Terpineol	1176	0.20 ± 0.00	0.30 ± 0.00	0.27 ± 0.00	0.31 ± 0.01	0.18 ± 0.00	0.26 ± 0.01
Myrtenol	1185	0.15 ± 0.00	0.15 ± 0.00	0.34 ± 0.00	0.41 ± 0.00	0.12 ± 0.00	0.20 ± 0.01
Eugenol	1336	0.13 ± 0.01	0.23 ± 0.01	0.11 ± 0.00	0.42 ± 0.01	ND	ND
δ -Elemene		7.33 ± 0.03	8.26 ± 0.07	4.21 ± 0.01	5.42 ± 0.01	7.99 ± 0.13	8.43 ± 0.27
α -Cubebene	1346	0.25 ± 0.00	0.24 ± 0.01	0.19 ± 0.00	0.10 ± 0.00	0.44 ± 0.01	0.22 ± 0.01
α -Copaene	1375	3.98 ± 0.01	3.81 ± 0.01	1.56 ± 0.01	1.59 ± 0.01	4.48 ± 0.02	3.47 ± 0.01
β -Elemene	1388	1.35 ± 0.01	1.41 ± 0.01	0.69 ± 0.01	0.75 ± 0.01	1.69 ± 0.01	0.94 ± 0.01
β -Caryophyllene	1418	16.25 ± 0.04	15.13 ± 0.59	8.86 ± 0.03	15.92 ± 0.07	12.93 ± 0.11	18.44 ± 1.08
α -Caryophyllene		2.34 ± 0.00	1.32 ± 0.01	1.73 ± 0.14	4.85 ± 0.32	2.62 ± 0.12	5.87 ± 0.51
β -Farnesene	1450	0.17 ± 0.01	0.10 ± 0.00	0.14 ± 0.01	0.12 ± 0.00	0.11 ± 0.00	0.14 ± 0.01
γ -Gurjunene		0.53 ± 0.01	0.52 ± 0.01	0.24 ± 0.00	0.28 ± 0.01	0.80 ± 0.01	0.43 ± 0.02
Germacrene D	1478	0.64 ± 0.01	0.62 ± 0.01	0.11 ± 0.00	0.13 ± 0.00	0.82 ± 0.01	0.48 ± 0.01
β -Selinene	1484	0.75 ± 0.06	0.64 ± 0.01	0.15 ± 0.01	0.17 ± 0.00	1.02 ± 0.01	0.54 ± 0.01
α -Selinene	1492	0.10 ± 0.00	0.10 ± 0.01	0.10 ± 0.00	0.10 ± 0.01	0.12 ± 0.00	0.15 ± 0.00
α -Farnesene	1503	0.16 ± 0.00	0.20 ± 0.00	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01	0.11 ± 0.01
β -Bisabolene	1512	1.58 ± 0.00	2.51 ± 0.01	1.10 ± 0.00	2.15 ± 0.04	1.47 ± 0.01	2.32 ± 0.01
δ -Cadinene	1517	1.48 ± 0.00	1.53 ± 0.01	1.65 ± 0.00	1.70 ± 0.01	2.24 ± 0.02	2.35 ± 0.01
(<i>E</i>)-nerolidol	1550	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.00	0.11 ± 0.00	0.11 ± 0.01	0.15 ± 0.01
Caryophyllene oxide	1575	1.32 ± 0.01	9.31 ± 0.02	4.14 ± 0.01	6.68 ± 0.12	1.50 ± 0.11	4.54 ± 0.14
δ -Cadinol	1626	0.16 ± 0.00	0.16 ± 0.01	0.18 ± 0.00	0.16 ± 0.00	0.12 ± 0.00	0.10 ± 0.00
α -Cadinol	1643	0.11 ± 0.01	0.10 ± 0.00	0.16 ± 0.00	0.18 ± 0.00	0.24 ± 0.01	0.10 ± 0.00
α -Farnesol	1667	0.50 ± 0.06	0.42 ± 0.01	0.12 ± 0.00	0.14 ± 0.01	0.14 ± 0.04	0.15 ± 0.02
α -Bisabolol	1675	0.16 ± 0.01	0.71 ± 0.01	0.12 ± 0.01	0.24 ± 0.04	0.10 ± 0.00	0.65 ± 0.00
Monoterpenes		53.03	44.90	64.77	51.11	53.25	39.74
Oxygenated monoterpenes		1.85	2.14	2.85	3.44	1.92	2.39
Sesquiterpenes		36.91	36.39	20.87	33.43	36.90	43.89
Oxygenated sesquiterpenes		2.36	10.81	4.84	7.51	2.21	5.69
Total		94.15	94.24	93.33	95.49	94.28	91.71
Ratio of monoterpenes and sesquiterpenes		1.40	1.00	2.63	1.33	1.41	0.85
(b)							
α -Thujene	923	0.41 ± 0.04	0.23 ± 0.01	0.43 ± 0.02	0.10 ± 0.00	0.33 ± 0.01	0.25 ± 0.02
α -Pinene	932	3.82 ± 0.06	3.50 ± 0.03	5.64 ± 0.13	3.56 ± 0.12	3.85 ± 0.06	3.06 ± 0.04

Table 2 continued

Components	<i>R</i> ^b	BPCM	BPHM	WPCM	WPHM	GPCM	GPHM
Camphene	945	0.69 ± 0.12	0.23 ± 0.01	0.50 ± 0.06	0.32 ± 0.06	0.29 ± 0.04	0.18 ± 0.01
Sabinene	969	0.14 ± 0.00	0.73 ± 0.01	0.98 ± 0.08	0.55 ± 0.04	0.86 ± 0.07	0.24 ± 0.06
β-Pinene	973	6.89 ± 0.21	4.42 ± 0.22	8.54 ± 0.63	3.92 ± 0.10	6.16 ± 0.09	4.96 ± 0.08
β-Myrcene	984	2.86 ± 0.24	0.79 ± 0.01	3.84 ± 0.04	0.15 ± 0.01	2.66 ± 0.32	1.32 ± 0.00
α-Phellandrene	999	0.31 ± 0.02	0.21 ± 0.01	0.10 ± 0.01	0.51 ± 0.08	0.15 ± 0.01	0.15 ± 0.01
3-Carene	1009	10.78 ± 0.42	12.26 ± 0.34	14.18 ± 0.66	19.63 ± 1.87	11.45 ± 0.87	7.73 ± 0.09
α-Terpinene	1012	0.22 ± 0.01	0.15 ± 0.00	0.55 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.10 ± 0.00
ρ-Cymene	1015	1.45 ± 0.06	1.15 ± 0.05	0.13 ± 0.01	1.71 ± 0.04	0.36 ± 0.07	1.56 ± 0.06
Limonene	1026	9.74 ± 0.17	8.63 ± 0.42	15.44 ± 0.85	9.23 ± 0.19	10.66 ± 0.35	9.93 ± 0.04
γ-Terpinene	1051	0.42 ± 0.04	0.43 ± 0.01	0.21 ± 0.02	0.36 ± 0.02	0.30 ± 0.06	0.28 ± 0.01
Terpinolene	1080	0.86 ± 0.02	0.26 ± 0.03	1.99 ± 0.13	0.83 ± 0.04	2.23 ± 0.06	0.35 ± 0.02
Linalool	1089	2.62 ± 0.16	2.89 ± 0.12	2.51 ± 0.06	1.89 ± 0.06	3.65 ± 0.09	2.65 ± 0.07
Terpinen-4-ol	1166	0.34 ± 0.08	0.45 ± 0.01	0.86 ± 0.02	1.92 ± 0.10	0.32 ± 0.00	1.10 ± 0.06
α-Terpineol	1176	1.15 ± 0.13	1.56 ± 0.03	1.44 ± 0.04	0.55 ± 0.02	1.10 ± 0.05	0.76 ± 0.04
Myrtenol	1185	1.19 ± 0.01	1.21 ± 0.04	0.67 ± 0.02	0.84 ± 0.08	0.28 ± 0.04	0.44 ± 0.03
Eugenol	1336	0.24 ± 0.06	0.68 ± 0.06	1.18 ± 0.00	0.48 ± 0.01	0.14 ± 0.01	0.10 ± 0.01
δ-Elemene		5.51 ± 0.32	8.99 ± 0.12	3.85 ± 0.56	10.26 ± 0.64	6.64 ± 0.06	6.74 ± 0.33
α-Cubebene	1346	0.46 ± 0.04	3.10 ± 0.06	0.20 ± 0.01	0.50 ± 0.06	0.49 ± 0.01	0.43 ± 0.02
α-Copaene	1375	3.78 ± 0.09	1.77 ± 0.10	1.99 ± 0.06	2.98 ± 0.02	4.65 ± 0.47	2.78 ± 0.01
β-Elemene	1388	2.18 ± 0.10	2.21 ± 0.13	1.29 ± 0.09	0.65 ± 0.02	2.56 ± 0.02	1.28 ± 0.06
β-Caryophyllene	1418	18.58 ± 0.76	18.92 ± 0.69	14.06 ± 0.13	17.10 ± 0.87	15.86 ± 1.10	25.99 ± 0.92
α-Caryophyllene		2.40 ± 0.08	5.15 ± 0.18	2.07 ± 0.28	1.83 ± 0.16	2.74 ± 0.24	1.39 ± 0.08
β-Farnesene	1450	0.32 ± 0.02	0.18 ± 0.02	0.26 ± 0.01	0.12 ± 0.01	0.50 ± 0.06	0.10 ± 0.01
γ-Gurjunene		0.14 ± 0.00	0.54 ± 0.06	0.17 ± 0.04	0.80 ± 0.01	0.15 ± 0.01	0.34 ± 0.07
Germacrene D	1478	0.43 ± 0.06	0.39 ± 0.01	0.19 ± 0.02	0.29 ± 0.02	0.49 ± 0.02	0.25 ± 0.02
β-Selinene	1484	1.06 ± 0.04	0.75 ± 0.01	0.11 ± 0.04	0.15 ± 0.01	1.23 ± 0.06	2.89 ± 0.07
α-Selinene	1492	0.81 ± 0.02	0.10 ± 0.00	0.10 ± 0.01	0.23 ± 0.04	0.28 ± 0.04	1.01 ± 0.06
α-Farnesene	1503	0.16 ± 0.01	0.29 ± 0.04	0.14 ± 0.00	0.28 ± 0.02	0.23 ± 0.01	0.11 ± 0.01
β-Bisabolene	1512	2.80 ± 0.06	0.99 ± 0.05	0.19 ± 0.02	2.03 ± 0.06	0.61 ± 0.06	1.73 ± 0.08
δ-Cadinene	1517	1.65 ± 0.01	0.44 ± 0.02	0.86 ± 0.06	2.22 ± 0.01	2.32 ± 0.09	2.42 ± 0.10
(E)-nerolidol	1550	0.16 ± 0.01	0.13 ± 0.01	0.12 ± 0.02	0.11 ± 0.00	0.14 ± 0.01	0.13 ± 0.01
Caryophyllene oxide	1575	4.53 ± 0.09	10.76 ± 0.00	8.19 ± 0.62	11.89 ± 0.07	5.41 ± 0.05	10.83 ± 0.08
δ-Cadinol	1626	0.31 ± 0.01	0.16 ± 0.00	0.13 ± 0.01	0.15 ± 0.01	0.34 ± 0.02	0.30 ± 0.04
α-Cadinol	1643	0.10 ± 0.03	0.20 ± 0.00	0.16 ± 0.00	0.20 ± 0.02	0.24 ± 0.01	0.13 ± 0.02
α-Farnesol	1667	0.60 ± 0.04	0.49 ± 0.01	0.37 ± 0.02	0.12 ± 0.01	0.56 ± 0.06	0.38 ± 0.10
α-Bisabolol	1675	0.43 ± 0.02	0.72 ± 0.04	0.21 ± 0.06	0.23 ± 0.01	0.39 ± 0.01	0.98 ± 0.01
Monoterpenes		38.59	32.99	52.53	40.97	39.42	30.11
Oxygenated monoterpenes		5.54	6.79	6.66	5.68	5.49	5.05
Sesquiterpenes		40.28	43.82	25.48	39.44	38.75	47.46
Oxygenated sesquiterpenes		6.13	12.46	9.18	12.70	7.08	12.75
Total		90.54	96.06	93.85	98.79	90.74	95.37
Ratio of monoterpenes and sesquiterpenes		0.95	0.71	1.71	0.89	0.98	0.58

^aThe percentage of the essential oils was calculated in peak areas using a normalization method without using correction factors. Values represent mean ± S.D. (n = 3)

^b*RI* calculate was according to the retention time of components on non-polar HP-5MS column

ND not detected

Fig. 1 The absolute concentrations (ppm) of the typical essential oils in cryogenically ground and hammer-milled black, white and green pepper under storage at 4 °C for 0 month (a); and 6 months (b)

samples. Except for moisture, no significant difference was detected in the contents of lipid, protein, starch, and NVEE of the corresponding samples after storage at 4 °C for 6 months.

Cryogenic grinding was superior to hammer milling because it well preserved the moisture, piperine, essential oil, lipid, protein, starch, NVEE, and other chemical constituents in the samples. These phenomena may be due to the lower extents of water loss, chemical composition decay, and losses of functional compounds, such as

piperine and essential oil, under lower temperatures in cryogenic grinding. High temperatures could cause heat-induced chemical alterations, such as browning and oxidative decomposition, in spices (Schweiggert et al. 2007).

Essential oil components

The relative percentages of the essential oil compounds in cryogenically ground and hammer-milled samples stored

Table 3 The contents^a of amino acids in black, white, and green pepper samples produced by cryogenic-grinding and hammer-milling under storage at 4 °C for 0 month and 6 months

	Months	BPCM	BPHM	WPCM	WPHM	GPCM	GPHM
Essential amino acid composition (g/100 g crude protein)							
Threonine	0	0.36 ± 0.01ay	0.34 ± 0.02axy	0.36 ± 0.02ax	0.36 ± 0.02ax	0.34 ± 0.01ax	0.33 ± 0.02axy
	6	0.34 ± 0.01cdxy	0.32 ± 0.01abx	0.35 ± 0.01dx	0.33 ± 0.01bcx	0.32 ± 0.01abxy	0.31 ± 0.01ax
Phenylalanine	0	0.60 ± 0.01by	0.56 ± 0.01ax	0.67 ± 0.01cx	0.66 ± 0.01cx	0.57 ± 0.01ax	0.57 ± 0.01ax
	6	0.58 ± 0.03axy	0.55 ± 0.01ax	0.67 ± 0.01bx	0.65 ± 0.01bx	0.56 ± 0.02ax	0.55 ± 0.03ax
Methionine	0	0.08 ± 0.02ax	0.08 ± 0.02ax	0.06 ± 0.02ax	0.06 ± 0.03ax	0.09 ± 0.03ax	0.05 ± 0.02ax
	6	0.08 ± 0.01ax	0.08 ± 0.00ax	0.06 ± 0.01abx	0.05 ± 0.01bx	0.08 ± 0.03ax	0.05 ± 0.01bx
Isoleucine	0	0.38 ± 0.03ax	0.38 ± 0.01ax	0.41 ± 0.01ax	0.38 ± 0.03ax	0.39 ± 0.01ay	0.38 ± 0.01axy
	6	0.35 ± 0.05ax	0.33 ± 0.05ax	0.37 ± 0.04ax	0.36 ± 0.02ax	0.36 ± 0.03axy	0.33 ± 0.04ax
Lysine	0	0.25 ± 0.01bx	0.24 ± 0.01bx	0.20 ± 0.02ay	0.18 ± 0.01axy	0.29 ± 0.01cy	0.28 ± 0.01cxy
	6	0.24 ± 0.03bx	0.23 ± 0.03bx	0.17 ± 0.02axy	0.16 ± 0.02ax	0.26 ± 0.01bx	0.26 ± 0.02bx
Tyrosine	0	0.51 ± 0.05cy	0.39 ± 0.01ax	0.57 ± 0.04dx	0.56 ± 0.01cdx	0.44 ± 0.03abx	0.45 ± 0.01bx
	6	0.51 ± 0.08cy	0.32 ± 0.02ax	0.56 ± 0.02cx	0.56 ± 0.02cx	0.43 ± 0.03bx	0.43 ± 0.04bx
Leucine	0	1.12 ± 0.06ax	1.11 ± 0.15ax	1.31 ± 0.07bx	1.25 ± 0.06abx	1.12 ± 0.03ax	1.12 ± 0.02ax
	6	1.04 ± 0.10ax	1.03 ± 0.10ax	1.24 ± 0.01bx	1.22 ± 0.07bx	1.06 ± 0.08ax	1.10 ± 0.10abx
Valine	0	0.56 ± 0.01aby	0.44 ± 0.10axy	0.57 ± 0.02bx	0.52 ± 0.08abx	0.55 ± 0.01aby	0.49 ± 0.08abxy
	6	0.51 ± 0.08bcxy	0.39 ± 0.01ax	0.53 ± 0.06cx	0.48 ± 0.08abcx	0.53 ± 0.03abcx	0.42 ± 0.03abx
Histidine	0	0.40 ± 0.02bx	0.33 ± 0.01ay	0.40 ± 0.01bx	0.38 ± 0.05abx	0.39 ± 0.01bx	0.33 ± 0.07ax
	6	0.40 ± 0.01bx	0.29 ± 0.01ax	0.39 ± 0.01bx	0.37 ± 0.02bx	0.32 ± 0.01ax	0.29 ± 0.01ax
Total of essential amino acid	0	4.26	3.87	4.55	4.35	4.18	4.00
	6	4.05	3.54	4.34	4.18	3.92	3.74
Non-essential amino acid composition (g/100 g crude protein)							
Alanine	0	0.84 ± 0.01by	0.79 ± 0.01axy	0.91 ± 0.01dx	0.89 ± 0.00cx	0.79 ± 0.00ax	0.79 ± 0.01ax
	6	0.83 ± 0.03aby	0.72 ± 0.10ax	0.89 ± 0.03bx	0.84 ± 0.09abx	0.78 ± 0.02abx	0.72 ± 0.11ax
Serine	0	0.56 ± 0.03by	0.54 ± 0.01aby	0.62 ± 0.02cy	0.60 ± 0.01cy	0.54 ± 0.02aby	0.52 ± 0.01axy
	6	0.53 ± 0.01bx	0.50 ± 0.01ax	0.56 ± 0.01cx	0.56 ± 0.01cx	0.51 ± 0.01ax	0.50 ± 0.01ax
Aspartic acid	0	1.34 ± 0.02bcy	1.30 ± 0.09bxy	1.22 ± 0.03ax	1.21 ± 0.01ax	1.56 ± 0.01dy	1.39 ± 0.02cx
	6	1.31 ± 0.01cxy	1.23 ± 0.03bx	1.14 ± 0.03ay	1.14 ± 0.02ay	1.55 ± 0.01ey	1.37 ± 0.01dx
Glycine	0	0.45 ± 0.02cx	0.44 ± 0.02cx	0.40 ± 0.00ax	0.39 ± 0.01ax	0.43 ± 0.01bcx	0.41 ± 0.01abx
	6	0.44 ± 0.01dx	0.42 ± 0.03bcdx	0.40 ± 0.00abx	0.38 ± 0.01ax	0.43 ± 0.01cdx	0.41 ± 0.01bcx
Glutamic acid	0	1.56 ± 0.06ax	1.51 ± 0.08ax	1.80 ± 0.01by	1.76 ± 0.01bx	1.53 ± 0.02ax	1.51 ± 0.06ax
	6	1.51 ± 0.07ax	1.46 ± 0.07ax	1.80 ± 0.01by	1.74 ± 0.03bx	1.52 ± 0.03ax	1.46 ± 0.14ax
Arginine	0	0.32 ± 0.07bx	0.28 ± 0.01abx	0.25 ± 0.01ax	0.25 ± 0.01ax	0.27 ± 0.01abx	0.27 ± 0.01abx
	6	0.28 ± 0.01cx	0.28 ± 0.02cx	0.25 ± 0.01abx	0.24 ± 0.01ax	0.27 ± 0.01bcx	0.26 ± 0.01abcx
Proline	0	0.86 ± 0.03aby	0.80 ± 0.01axy	1.03 ± 0.02cx	1.02 ± 0.03cx	0.90 ± 0.06by	0.83 ± 0.03axy
	6	0.80 ± 0.01axy	0.78 ± 0.06ax	0.97 ± 0.01bx	0.97 ± 0.05bx	0.88 ± 0.06ax	0.77 ± 0.02ax
Total of non-essential amino acid	0	5.93	5.66	6.23	6.12	6.02	5.72
	6	5.70	5.39	6.01	5.87	5.94	5.49

^aValues represent means ± S. D. (n = 3). Means followed by the same letters within the row (a-e) and within the column per parameter (x and y) are not significantly different ($p < 0.05$)

for 0 month are presented in Table 2a. A total of thirty-eight compounds were identified, which accounted for more than 91.71% of the total compounds. The primary compounds of all samples were α -pinene, β -pinene, β -

myrcene, 3-carene, limonene, linalool, δ -elemene, α -copaene, α -caryophyllene, β -caryophyllene, β -bisabolene, δ -cadinene and caryophyllene oxide. The relative percentages of monoterpenes in the essential oil of black, white,

and green pepper processed by cryogenic grinding and stored for 0 month were 53.03, 64.77, and 53.25%, respectively, which were higher than those in the hammer-milled samples. The reduced amounts of monoterpenes in the hammer-milled samples might be attributed to the higher temperature that the peppercorns experienced during hammer milling compared with that in cryogenic grinding (Murthy and Bhattacharya 2008). However, a marginal difference was observed in the distribution pattern of sesquiterpene compounds in both ambient and cryogenically ground black pepper samples, as the sesquiterpene compounds were hardly affected by the high temperature. These results agreed well with the cryogenic grinding of black pepper (Murthy and Bhattacharya 2008). The ratios of monoterpenes to sesquiterpenes were in the range of 1.40–2.63 in the cryogenically ground samples stored for 0 month, and 0.85–1.33 in the hammer-milled samples. This discrepancy indicated that cryogenic grinding was superior to hammer milling when the content of monoterpenes in the pepper essential oil samples was considered. After storage in refrigeration at 4 °C for 6 months (Table 2b), the values of oxygenated monoterpenes and oxygenated sesquiterpenes significantly

increased presumably because of the oxidation of these compounds. The ratios of monoterpenes to sesquiterpenes in the essential oil samples were markedly reduced during storage, causing the loss or degradation of certain compounds as a result of browning or oxidative decomposition of the spices during storage.

Figure 1a, b show the absolute concentration (ppm) changes of the typical essential oils in the black, white, and green pepper prepared by cryogenically grinding and hammer-milling under storage at 4 °C for 0 and 6 months. All of the analyzed compounds were significantly higher in the cryogenically ground samples than in the hammer-milled samples, and the most significant differences were observed in the green pepper samples. The values of α -pinene, β -pinene, β -myrcene, 3-carene, limonene, γ -terpinene, β -caryophyllene, α -caryophyllene and caryophyllene oxide in the cryogenically ground green pepper were 55.73, 61.46, 69.91, 47.91, 56.24, 346.23, 116.69, 183.31, and 107.68% higher than those in the hammer-milled green pepper, respectively. The values of these components in the cryogenically ground black and white pepper were 7.74–32.91% and 22.54–64.25% higher than those in the hammer-milled pepper, respectively. These results

Table 4 The percentages^a (%) of fatty acid compositions in black, white, and green pepper samples produced by cryogenic-grinding and hammer-milling under storage at 4 °C for 0 month and 6 months

Fatty acid	Months	BPCM	BPHM	WPCM	WPHM	GPCM	GPHM
Lauric acid	0	10.28 ± 0.10fx	9.41 ± 0.03ex	6.83 ± 0.05dx	5.92 ± 0.07cx	3.72 ± 0.18bx	2.77 ± 0.01ax
	6	13.21 ± 0.18fy	11.9 ± 0.08ey	8.95 ± 0.07dy	7.06 ± 0.06cy	5.21 ± 0.03by	4.15 ± 0.01ay
Miristic acid	0	15.68 ± 0.06ex	12.46 ± 0.17cx	17.66 ± 0.16fx	14.23 ± 0.00dx	6.26 ± 0.03bx	5.56 ± 0.05ax
	6	16.65 ± 0.03cy	15.34 ± 0.05by	19.93 ± 0.64dy	16.65 ± 0.10cy	7.85 ± 0.01ay	7.62 ± 0.05ay
Palmitic acid	0	18.45 ± 0.08ax	17.05 ± 0.00ax	17.12 ± 0.10ax	15.18 ± 0.01ax	16.99 ± 0.05ax	15.25 ± 0.05ax
	6	18.94 ± 0.01ax	21.52 ± 0.06ax	17.67 ± 0.03ax	15.84 ± 0.18ax	17.56 ± 0.05ax	18.68 ± 0.64ax
Stearic acid	0	1.09 ± 0.07ax	3.05 ± 0.01cx	3.15 ± 0.01cx	5.35 ± 0.08ex	2.14 ± 0.03bx	3.56 ± 0.08dx
	6	1.98 ± 0.10ax	2.47 ± 0.01bx	3.67 ± 0.03cx	5.93 ± 0.07dx	2.64 ± 0.06bx	3.62 ± 0.03cx
Lignoceric acid	0	2.45 ± 0.01bx	2.42 ± 0.18bx	6.25 ± 0.00dx	6.55 ± 0.10ex	2.25 ± 0.01ax	2.66 ± 0.01cx
	6	2.74 ± 0.01ax	2.69 ± 0.10ax	6.95 ± 0.06ex	5.75 ± 0.05dx	2.85 ± 0.00bx	2.98 ± 0.03cx
Oleic acid	0	8.89 ± 0.17bx	8.56 ± 0.03ax	25.68 ± 0.25fx	24.66 ± 0.10ex	11.05 ± 0.50dx	10.70 ± 0.15cx
	6	7.15 ± 0.10by	6.95 ± 0.16ay	23.85 ± 0.10dy	25.75 ± 1.11dy	8.39 ± 0.18cy	8.25 ± 0.10cy
Linoleic acid	0	28.65 ± 0.10dx	24.92 ± 0.03cx	23.18 ± 0.05bx	21.65 ± 0.25ax	32.99 ± 0.10ex	32.65 ± 0.64ex
	6	26.87 ± 0.05dy	21.35 ± 0.10cy	18.56 ± 0.06by	17.98 ± 0.18ay	32.04 ± 0.03fy	30.93 ± 0.25ey
α -Linoleic acid	0	12.63 ± 0.15bx	9.71 ± 0.03ax	ND	ND	24.58 ± 0.10dx	20.23 ± 0.09cx
	6	10.52 ± 0.10bx	8.82 ± 0.18ax	ND	ND	23.25 ± 0.15dx	18.67 ± 0.05cx
Saturated	0	47.95	44.39	51.01	47.23	31.36	29.8
	6	53.52	53.92	57.17	51.23	36.11	37.05
Unsaturated	0	50.17	43.19	48.86	46.31	68.62	68.58
	6	44.54	37.12	42.41	41.73	63.68	57.85

^a Values represent means ± S. D. (n = 3). Means followed by the same letters within the row (a–f) and within the column per parameter (x and y) are not significantly different ($p < 0.05$)

ND not detected

Fig. 2 TGA curves of black, white, and green pepper powder samples produced by cryogenic-grinding and hammer-milling under storage at 4 °C for **a** 0 month for temperature scales of 0–500 and 270–350 °C, respectively; and **b** 6 months for temperature scales of 0–500 and 270–350 °C, respectively

matched well with the findings of previous studies (Balasubramanian et al. 2012; Jacob et al. 2000). After storage at 4 °C for 6 months, the concentrations of α -pinene, β -pinene, β -myrcene, 3-carene, limonene, γ -terpinene, β -caryophyllene, and α -caryophyllene were significantly reduced in all samples. However, the concentration of caryophyllene oxide in the peppers increased significantly after storage for 6 months, which might be attributed to the oxidation that occurred during storage.

These data demonstrated that the cryogenic grinding technique utilized in our study was superior to ambient grinding in terms of the recovery of typical essential oils in the ground powder. This phenomenon likely occurred as a result of the heat-induced chemical alterations, including browning and oxidative decomposition, in spices during conventional ambient grinding techniques, which ultimately led to significant losses in the typical essential oil constituents of the pepper and diminished the quality of the product. After the samples were stored in a refrigerator at

4 °C for 6 months, the absolute concentrations of the eight typical essential oil constituents were drastically reduced, demonstrating that the essential oil constituents of the pepper suffered some degree of loss during storage regardless of grinding technique.

Amino acid constituents

To our knowledge, the amino acid constituents of pepper powders have never been reported. To evaluate the quality of the three pepper powders obtained through cryogenic grinding, the amino acid composition was determined and compared with those obtained by hammer milling. The effect of storage at 4 °C for 6 months was also assessed (Table 3). Leucine, aspartic acid, and glutamic acid are the major amino acids in the peppers having contents of more than 1 g/100 g crude protein. Significant differences were observed in the contents of some amino acids of the black, white, and green peppers probably because of the different processing methods and different plucking time. However, little difference could be observed in the contents of amino acids in the samples derived from different pulverizing methods. In white pepper, only the alanine content was 2.25% higher in the cryogenically ground than in the hammer-milled samples. In black pepper, in addition to alanine, the contents of phenylalanine, tyrosine, and histidine also showed significant differences in the samples derived through different pulverizing methods. Higher amounts of histidine, aspartic acid, and proline were detected in the cryogenically ground green pepper than in the hammer-milled samples. The decrease in these amino acids may be due to the higher temperature during hammer milling (Latif et al. 2013). Storage did not significantly affect the contents of amino acids in pepper.

Fatty acid compositions

Table 4 shows the fatty acid composition of the pepper samples prepared through different pulverizing processes. The saturated fatty acids detected in the samples were lauric acid (C_{12:0}), miristic acid (C_{14:0}), palmitic acid (C_{16:0}), stearic acid (C_{18:0}), and lignoceric acid (C_{24:0}), all of which accounted for 29.8–57.17%. The unsaturated fatty acids present in the sample mainly included oleic acid (C_{18:1}), linoleic acid (C_{18:2}), and α -linoleic acid (C_{18:3}), all of which accounted for 37.12–68.62%.

The fatty acid compositions in different pepper products were less studied and compared. In the present study, significant differences were detected in the fatty acid compositions of the black, white, and green peppers. α -linoleic acid was absent in white pepper, but it accounted for 8.82–12.63% and 18.67–24.58% in black and green peppers, respectively. The most abundant fatty acid in white pepper was

monounsaturated fatty acid oleic acid (23.85–25.75%), followed by linoleic acid (17.98–23.18%). In comparison, the most abundant fatty acid observed in black pepper was linoleic acid (21.35–28.65%), followed by palmitic acid (17.05–21.52%), miristic acid (12.46–16.65%), lauric acid (9.41–13.21%), and α -linoleic acid (8.82–12.63%). The principal fatty acids in the green pepper were linoleic acid at a level of 30.93–32.99%, followed by α -linoleic acid (18.67–24.58%). The percentages of unsaturated fatty acids in green pepper were distinctly higher than that in black and white pepper presumably because of the different processing methods and different plucking times. Green pepper is obtained only from green unripe fruit, whereas black and white pepper typically derived from red ripe fruit (Liu et al. 2013).

Significant differences were observed in the relative percentages of unsaturated fatty acid in the black, white, and green peppers prepared through different pulverizing methods. The relative percentages of oleic acid, linoleic acid, and α -linoleic acid were higher in the cryogenically ground than in the hammer-milled samples. This phenomenon might be explained by the accelerated oxidation of unsaturated fatty acids as a result of both the elevated temperature and the increased contact between lipid and oxygen in the conventional hammer-milling process. Moreover, significant differences were detected in the relative percentages of saturated fatty acid in the black, white, and green peppers prepared through different pulverizing methods, except for palmitic acid in the black, white, green peppers and lignoceric acid in black pepper. The relative percentages of miristic acid and lauric acid were significantly lower in the hammer-milled than in the cryogenically ground samples, whereas the opposite was observed for stearic acid was after storage at 4 °C for 0 month.

Saturated fatty acids were slightly increased (by 5.57–9.53%, 4.0–6.16%, and 4.75–7.25% in the black, white, and green peppers, respectively), and unsaturated fatty acids were decreased (by 5.63–6.07%, 4.58–6.45%, and 4.94–10.73% in the black, white, and green peppers, respectively) after storage at 4 °C for 6 months. The results confirmed the findings of a previous work by Nazemroaya et al. (2009) and indicated that lipid oxidations were active at a storage temperature of 4 °C.

Thermal properties

The gelatinization and retrogradation properties of the black pepper starch have been measured through differential scanning calorimetry (Zhu et al. 2017). However, the thermal properties of pepper powder have yet to be reported. The thermal properties of the three pepper powders obtained through cryogenic grinding and hammer

milling were measured by conducting TGA (Fig. 2a). The effects of storage at 4 °C for 6 months on the thermogravimetric properties were also assessed (Fig. 2b). As shown in Fig. 2, the black, white, and green peppers displayed different thermal properties.

The samples displayed different thermal profiles in the temperature range of 270–350 °C (expanded scale, Fig. 2a, b with mass losses of 38.62–42.82%, 48.07–51.16%, and 41.91–43.63% for the black, white, and green peppers, respectively. This might be attributed to the decomposition of crude protein, lipid, and starch at this temperature range. The decreases in the weight of the samples in the temperature range of 20–110 °C were not obvious between different samples, which had mass losses of 8.04–9.30%, 10.48–13.74%, and 6.06–10.10% for the black, white, and green peppers in the temperature range of 20–110 °C, respectively. The weight loss at this temperature was consistent with the moisture content of the corresponding sample. However, pulverizing processes and storage did not significantly affect the TGA curves of the pepper samples.

Conclusion

Cryogenic grinding improved the chemical quality of ground pepper but exerted no obvious effect on the thermal properties. The contents of moisture, lipid, crude protein, starch, NVEE, piperine, essential oil, and the absolute concentrations of typical essential oil constituents showed significantly higher values in the pepper powders prepared through cryogenic grinding than those through hammer milling. The fatty acid compositions in the peppers were significantly affected by the pulverizing methods, except for palmitic acid in the black, white, green peppers and lignoceric acid in black pepper. Among all of the parameters studied, the amino acid contents and the TGA curve were hardly influenced by the grinding techniques. The use of cryogenic grinding to prepare pepper ensured the highest quality of pepper products. After the samples were stored at 4 °C for 6 months, regardless of grinding technique, the moisture, piperine, essential oil, unsaturated fatty acid and monoterpenes of the samples suffered loss. The absolute concentrations of the typical pepper essential oil constituents (except caryophyllene oxide) were also reduced during storage at 4 °C. However, the thermal properties (TGA curve) and the contents of lipid, protein, starch, NVEE, and amino acids were hardly influenced after storage. Moreover, little differences were observed in the TGA curves of the black, white, and green peppers, whereas significant differences were detected in the contents of lipid, crude protein, starch, NVEE, piperine, essential oil, some amino acids, and some fatty acids of the

black, white, and green peppers. These findings might be due to the different processing methods and different plucking time.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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