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Research Article

The Effect of Drying Methods on Chemical Profiling of Zingiberaceae Herbs Production

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ABSTRACT

Background: Drying method is one of the important steps in post-harvesting in herbal production in order to prolong the shelf-life of herbal medicine and to ensure the good quality. **Objective:** This research aimed to profile and quantify the metabolites changing using metabolomic based on ^{1}H-NMR Spectroscopy on *Curcuma* and *Zingiber* genus due to the drying process. **Material and Methods:** Five species of the Curcuma and Zingiber genus were *Curcuma longa* L., *Curcuma aeruginosa* Roxb., *Curcuma xanthorrhiza* Roxb., *Zingiber officinale* Roscoe. and *Zingiber amaricans* Bl. The drying method applied were freeze drying and oven drying method at -108^{0}C and 50^{0}C, respectively. Samples were extracted using buffer phosphate and deuterated methanol (3:7). The analysis was performed using ^{1}H-NMR spectroscopy 400 MHz with TSP as an internal standard. The ^{1}H-NMR spectra were analysed with multivariate (Principal Component Analysis). **Results:** The relative concentrations of sucrose and fructose at 5 samples in freeze drying method showed significant higher content than those in oven drying. On the other hands, glucose was mostly significant higher concentration in oven drying at 5 samples. Fructose and sucrose were significantly higher in freeze drying method than those in oven drying in Curcuma and Zingiber genera. Relative glucose content was higher in oven drying at Curcuma genus **Conclusion:** Therefore, oven drying method which applied heating at 50^{0}C is an appropriate method for herbal medicine production, especially in *Curcuma* and *Zingiber* genus, for maintaining the major secondary metabolites.

Key words: Freeze drying, Oven drying, Multivariate, Curcuma Genus, Zingiber Genus.

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INTRODUCTION

Zingiberaceae plants have been applied widely as food additives and medicine throughout Southeast Asia Countries. Two famous genera *Curcuma* and *Zingiber* genus has been reported to possess antibacterial,[1] antioxidants[2] and anti-inflammatory activities.[3] Those activities are related to metabolites profile both primary and secondary. Primary metabolites such as amino acids, organic acids, sugars and secondary metabolites such as terpenoids, flavonoids, alkaloids are present in the Zingiberaceae family.[4,5] However, the abundance of those metabolites are affected by the post-harvesting method particularly at drying process.[6]

Drying is a critical step in preserving raw materials in which the process is necessary in order to minimize microorganism growth causing spoilage and decay. Heating is one of the oldest methods in preserving raw materials and mostly applied in small herbal industries. However, it dehydrate sample up to 60%.[7] If so, the study to investigate what is changing inside the material during the drying process needs to be done. As comparison is non-heating drying method which is able to minimize metabolites degradation. It is a freeze-drying technique which applied freeze stage to dry material under a vacuum condition. However, literatures concerning metabolomic of two genus Zingiberaceae due to drying-method using metabolomic based on^{1}H-NMR spectroscopy is rare.

Metabolomics is a chemical profiling method to analyse primary and secondary metabolites simultaneously in large-scale analysis. It is widely applied due to its unbiased, rapid, reproducible and simple sample preparation. Nuclear magnetic resonance (NMR) is one of the platforms has been used as a fingerprinting tool with multivariate techniques such as the principal component analysis (PCA). However, the major drawbacks of NMR spectroscopy are spectral resolution and sensitivity.[8] Its application has proved to be a powerful tool to chemical profiling in many studies in grapes,[9] in tomato,[10] in zingiber species[4] and in curcuma species.[11]

In this study, we aimed to investigate the influence of drying methods i.e. oven drying and freeze drying to Zingiber and Curcuma genus. We identified the major metabolites contributing to the discrimination due to drying method in the *Curcuma* and *Zingiber* genus (Zingiberaceae). Additionally, the metabolites which discriminate the Zingiberaceae species on the basis of their capacity to the drying method were also analyzed and their relative quantities were also determined.

MATERIALS AND METHODS

Plant material

Five species of the Curcuma and Zingiber genus consisting of *Curcuma longa* L., *Curcuma aeruginosa* Roxb., *Curcuma xanthorrhiza* Roxb., *Zingiber officinale* Roscoe. and *Zingiber amaricans* Bl. from Tanjungrejo, Sukoharjo, Indonesia. The rhizomes harvested at 8 mounth. The rhizomes washed, chopped into small pieces, blended and dried by heating at 50^{0}C for 72 hours and freeze drying at -108^{0}C for 72 hours. Each sample were three times replicates.

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Chemicals and plant materials

Methanol *d*-4 (CD_3OD) (99,95%), deuterium oxide (D_2O) (99,96%), (Merck, Darmstatd, Germany), Sodium salt of 3(trimethylsilyl)-propionate acid-d4 (TSP) 0,01% (Chem Cruz), kalium dihydrogen phosphat (KH_2PO_4), sodium deuteroxide (NaOD) 1% (Santa Cruz Biotecnology).

Samples of 30 mg plant material were weighed and extracted with 1.5 ml of a mixture of phosphate buffer (pH 6.0) in deuterium oxide containing 0.05% trimethylsilylproprionic acid sodium salt-*d*4 (TMSP) and methanol-d_4 (1:1). Samples were vortexed at room temperature for 1 min, ultrasonicated for 20 min and centrifuged at 13000 rpm for 10 min. An aliquot of 0.6 ml of the supernatant was transferred to 5 mm ^{1}H-NMR tubes for ^{1}H-NMR measurement.

Extraction for NMR analysis

For the extraction of polar metabolites, 270 µL of phosphate buffer mixture pH 6.0 and 630 µL CD_3OD were added to 30 mg of plant material. Samples were vortexed at room temperature for 1 min, ultrasonicated for 20 min and centrifuged at 13.300 rpm for 10 min. An aliquot of 600 µl of the supernatant was transferred to 5 mm ^{1}H-NMR tubes for ^{1}H-NMR measurement.[12]

NMR measurements

^{1}H-NMR spectra were recorded on 400 MHz Agilent spectrometer. TSP (sodium salt of 3(trimethylsilyl)-propionate acid-d4) was used as the internal standard. Each ^{1}H-NMR spectrum consisted of 128 scans with the following parameter: presaturation delay of 2 sec, acquisition time 3.408 sec, relaxation delay of 2 sec, observe pulse=6.8 µs (90^0). Two-dimensional J-resolved ^{1}H-NMR spectra were acquired using eight scans per 64 increments. J-resolved spectra were tilted by 45°, symmetrized and then calibrated to TSP. 1H-1H correlated COSY spectra were acquired with a 1.0 s relaxation delay and spectral width of 512 Hz about F1 and 4807.7 Hz about F2. Both dimensions were multiplied by sine-bell functions (SSB = 0).

Data analysis and statistics

^{1}H-NMR spectra were converted to an ASCII file used AMIX software (version 3.5) for futher multivariate analysis. The peak was integrated into bucket the size of 0.04 ppm (bucketing). PCA analysis was performed using *software* SIMCA-p (version 15,0) with pareto scaling. Metabolites were identified using MestRenova version 11.0. Identified metabolites do statistically using software SPSS (version 21.0.). The differences were tested on a 95% probability level (P≤0,05) using ANOVA.

RESULTS AND DISCUSSION

Metabolite identification

Metabolite identification has been performed using ^{1}H-NMR spectra and 2D-NMR technique such as J-resolved and COSY (Correlated Spectroscopy). ^{1}H-NMR spectrum is classified into three parts namely aliphatic compounds, amino acids and few organic peaks were placed at δH 0.5-2.5 ppm. Besides, organic acids, anomeric protons of carbohydrate are found in the region of δH 2.5-5.5 ppm (Figure 1) and aromatic, phenolic region are placed at the area of δH 5.5-8.0 ppm The metabolites identified cover amino acids, carbohydrates, organic acids, phenolic and sesquiterpenoids. All assignments were done by comparing the spectra with the literature under similar condition.

The metabolites were identified on the Curcuma and Zingiber genus shown in Table 1. The signals in the amino acids region were useful to identify a number of amino acid. Amino acids like alanine δ_H 3,76 ppm (1H, *d*, *J*=6,42 Hz, H-4); δ_H 1,49 ppm (3H, *d*, *J*=7,06 Hz, H-6). Valine was shown ar δ_H 1,07 ppm (3H, *d*, *J*=7,04 Hz, H-7); δ_H 1,02 ppm (3H, *d*, *J*=7,02 Hz, H-8) and threonine was shown at δ_H 3,53 ppm (1H, *d*, *J*=5,00 Hz, H-4); δ_H 4,04 ppm (1H, m, H-6); δ_H 1,33 ppm (3H, *d*, *J*=6,77 Hz, H-8). Carbohydrate region exhebited anomeric proton of sucrose, glucose and frucroce. Sucrose is a disaccharide molecule formed by glucose and fructose monosaccharides were associated with 1.2 α bonds (Jung *et al*., 2012). Sucrose was detected at δH 5.41 ppm (1H, *d*, *J*=3.65 Hz, H-7), δH 3.42 ppm (1H, *t*, *J*=9.44 Hz, H-10), δH 3, 74 ppm (1H, *t*, *J*=9.52 Hz, H-11), δH 3.49 ppm (1H, *dd*, *J*=3.76; 10.06 Hz, H-12), δH 3.65 (2H, s, H-13). In the COSY spectrum, the signal at δH 5.41 ppm (H-7) was correlated with the proton at δH 3.49 ppm (H-12). A-glucosa was shown at δ_H 5,17 ppm (*d*, *J*=4,06 Hz-, H-2); δ_H 3,49 ppm (1H, *dd*, *J*= 3,76, 10,06 Hz, H-3), while β-glucosa was shown at δ_H 4.56 ppm (*d*, *J*=8,08 Hz, H-2); 3.19 ppm (*t*, *J*=8,48 Hz, H-3). In the COSY spectrum, the signal at δH 5.17 ppm (H-2) was correlated with the proton at δH 3.49 ppm (H-3), while δH 4.56 ppm (H-2) was correlated with the proton at δH 3.19 ppm (H-3). Fructose was detected at δ_H 3,99 ppm (1H, *dd*, *J*=3,11; 12,27 Hz, H-4) and δ_H 4.16 ppm (1H, *d*, *J*=8,43 Hz, H-3). In the COSY spectrum, the signal at δ_H 3,99 ppm (H-4) was correlated with the proton at δ_H 4.16 ppm (H-3). Formic acid was shown at δ_H 8,49 ppm (1H, s, H-2), malic acid was detected only on Zingiber genus at δ_H 4,28 ppm (1H, *dd*, *J*=3,47; 9,19 Hz, H-2); 2,42 ppm (1H, *dd*, *J*=9,54; 15,21 Hz, H-5) and 2,73 ppm (1H, *dd*, *J*=3,58; 15,47 Hz, H-5). In the COSY spectrum, the signal at δ_H 4.28 ppm (H-2) was correlated with the proton at δ_H 2,42 ppm (H-5) and δ_H 2,73 ppm (H-5).

Curcumin and xanthorrhizol is a specific metabolite of the Curcuma genus. Curcumin was detected at δH 6.67 ppm (2H, *d*, *J*=15.52 Hz, H-3/3'), 7.57 ppm (2H, *d*, *J*=15.58 Hz, H-4/4'), 6.89 ppm (2H, *d*, *J*=8.38 Hz, H-9/9'), 7.15 ppm (2H, *dd*, *J*=3.15; 8.38 Hz, H-10/10'). In the COSY spectrum, the signal at δH 6.89 ppm (H-9/9') was correlated with the proton at δH 7.15 ppm (H-10/10'+..). Xanthorrhizol was detected at δH 7,23 ppm (*d*, *J*=3,05 Hz,H-2), 6.89 ppm (*d*, *J*=8.59 Hz, H-5) and 7.57 ppm (*dd*, *J*=8.21; 3.16 Hz, H-6). In the COSY spectrum, the signal at δH 6.89 ppm (H-5) was correlated with the proton at δH of 7.57 ppm ppm (H-6). Shogaol was detected at δH 2.61 ppm (4H, *q*, *J*=7.89 Hz, H-1,2), 6.10 ppm (1H, *d*, *J*=16.20 Hz, H-4), 6.90 ppm (1H, *d*, *J*=15.84 Hz, H-5), 6.57 ppm (1H, *d,* *J*=2.69 Hz, H-2'), 6.74 ppm (1H, *d,* *J*=8.24 Hz, H-5') and 6.65 ppm (1H, *dd,* *J*=2.13; 8.21 Hz, H-6'). In the COSY spectrum, the signal at δH 6.10 ppm (H-4) was correlated with the proton at δH of 6.90 ppm (H-5).

Multivariate data analysis (MvDA)

Multivariate analysis used to detect all differences and to determine the significances of the difference drying methods. Generated score plot in PCA was applied to identify the metabolite profile responsibility for different methods of drying process. PCA data analysis produced a significant separation of PC5 by 90.06% and PC4 by 5.26% of the variables contained in the sample. A PCA score plot (Figure 2) showed metabolite profiling of two different drying method were clearly separated, freeze and oven drying group in which the rhizome dried using a freeze drying were in the negative quadrant, while the heating was in the positive quadrant. A PCA colomn plot of PC5 (Figure 3) shown the metabolite responsibility for discrimination on different drying methods. The higher graph of the colomn plot showed the metabolites contributed more to the separation. Metabolites with are responsible for between two drying methods based on colomn plot PCA are in region of organic acids and anomeric protons of carbohydrate (δH 2.5-5.5 ppm) i.e. sucrose, glucose and fructose.

Metabolite quantification

Metabolite quantification was shown based on the intensity of the metabolite signal compared to the internal standard intensity (TSP). The differences intensity signal will be correlated to the metabolite

Table 1: ^{1}**H-NMR analysis of metabolites in the *Curcuma* and *Zingiber* genus with 1D and 2D NMR spectra (CD_3OD-KH_2PO_4 in D_2O, pH 6.0).[a] Abbreviations, s = singlet, d = doublet, t = triplet, dd = double-doublet.**

Metabolite	Chemical shifts (δ_H) (ppm)	
	Curcuma genus	Zingiber genus
Alanine	3.76 (1H, q, *J*=6.42 Hz)	3.76 (1H, d, *J*=6.35 Hz)
	1.49 (3H, d, *J*=7.06 Hz)	1.49 (3H, d, *J*=7.53 Hz)
Valine	1.07 (3H, d, *J*=7.04 Hz)	1.07 (3H, d, *J*=6.97 Hz)
	1.02 (3H, d, *J*=7.02 Hz)	1.02 (3H, d, *J*=6.99 Hz)
	3.53 (1H, d, *J*=5.00 Hz)	3.53 (1H, d, *J*=4.83 Hz)
Threonine	4,04 (1H, m)	4.04 (1H, m)
	1.33 (3H, d, *J*=6.77 Hz)	1.33 (3H, d, *J*=6.47 Hz)
	5.41 (1H, d, *J*=3.65 Hz)	5.41 (1H, d, *J*= 4.09 Hz)
	3.42 (1H, t, *J*=9.44 Hz)	3.42 (1H, t, *J*= 9.37 Hz)
Sucrose	3.74 (1H, t, *J*=9.52 Hz)	3.74 (1H, t, *J*=9.21 Hz)
	3.49 (1H, dd, *J*=3.76; 10.06 Hz)	3.49 (1H, dd, *J*= 3.89; 9.66 Hz)
	3.65 (2H, s)	3.65 (2H, s)
α-glucose	5.17 (1H, d, *J*= 4.06 Hz)	5.17 (1H, d, *J*= 3.85 Hz)
	3.49 (1H, dd, *J*=3.76; 10.06 Hz)	3.49 (1H, dd, *J*= 3.89; 9.66 Hz)
β-glucose	4.56 (1H, d, *J*=8.08 Hz)	4.55 (1H, d, *J*=8.27 Hz)
	3.19 (1H, t, *J*=8.48 Hz)	3.19 (1H, t, *J*=8.34 Hz)
Fructose	4.16 (1H, d, *J*= 8.43 Hz)	4.16 (1H, d, *J*= 8.47 Hz)
	3.99 (1H, dd, *J*=3.11; 12.27 Hz)	3.99 (1H, dd, *J*= 3.49; 11.96 Hz)
Formic acid	8.49 (1H, s)	8.49 (1H, s)
Malic acid	-	4.28 (1H, dd, *J*= 3.47; 9.19 Hz)
	-	2.42 (1H, dd, *J*= 9.54; 15.27 Hz)
	-	2.73 (1H, dd, *J*= 3.58; 15.47 Hz)
Curcumin	6.67 (2H, d, *J*=15.52 Hz)	-
	7.57 (2H, d, *J*= 15.58 Hz)	-
	6.89 (2H, d, *J*= 8.38 Hz)	-
	7.15 (2H, dd, *J*= 3.15; 8.38 Hz)	-
Xanthorrhizol	7,23 ppm (d, *J*=3,05 Hz)	-
	6.89 ppm (d, *J*=8.59 Hz)	-
	7.57 ppm (dd, *J*=8.21; 3.16 Hz	-
Shogaol	-	2.61 (4H, q, *J*=7.89 Hz)
	-	6.10 (1H, d, *J*=16.20 Hz)
	-	6.90 (1H, d, *J*=15.84 Hz)
	-	6.57 (1H, d, *J*=2.69 Hz)
	-	6.74 (1H, d, *J*=8.24 Hz)
	-	6.65 (1H, dd, *J*=2.13; 8.21 Hz)

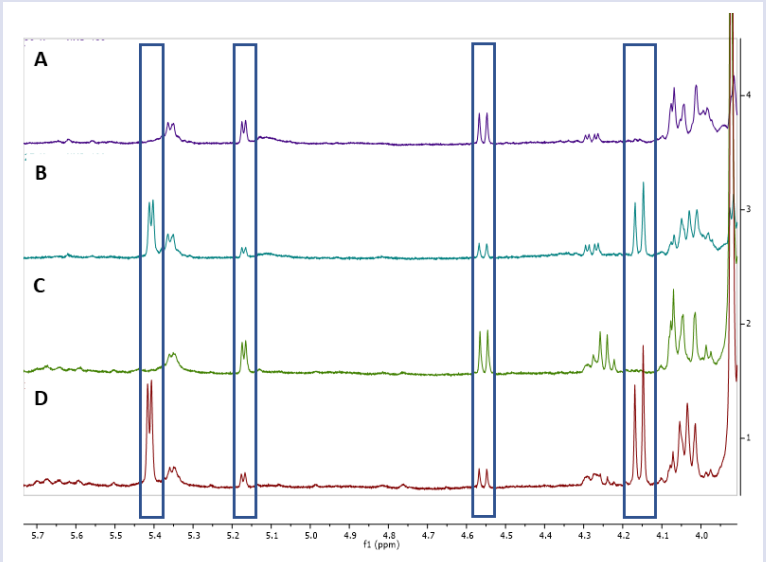

Figure 1: Representative ^{1}H-NMR spectra of rhizome *Zingiber. officinale* Roscoe at (A) heating and (B) freeze drying method; *Curcuma longa* L. at (C) heating and (D) freeze drying method.

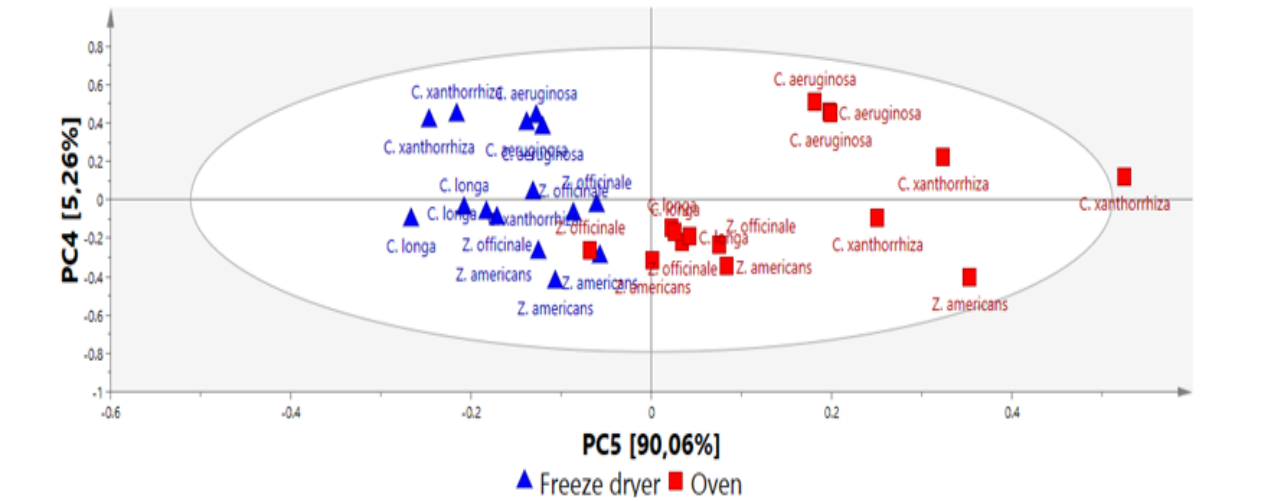

Figure 2: PCA Score plot of ^{1}H-NMR spectra in the extract of Curcuma and Zingiber for two drying methods.

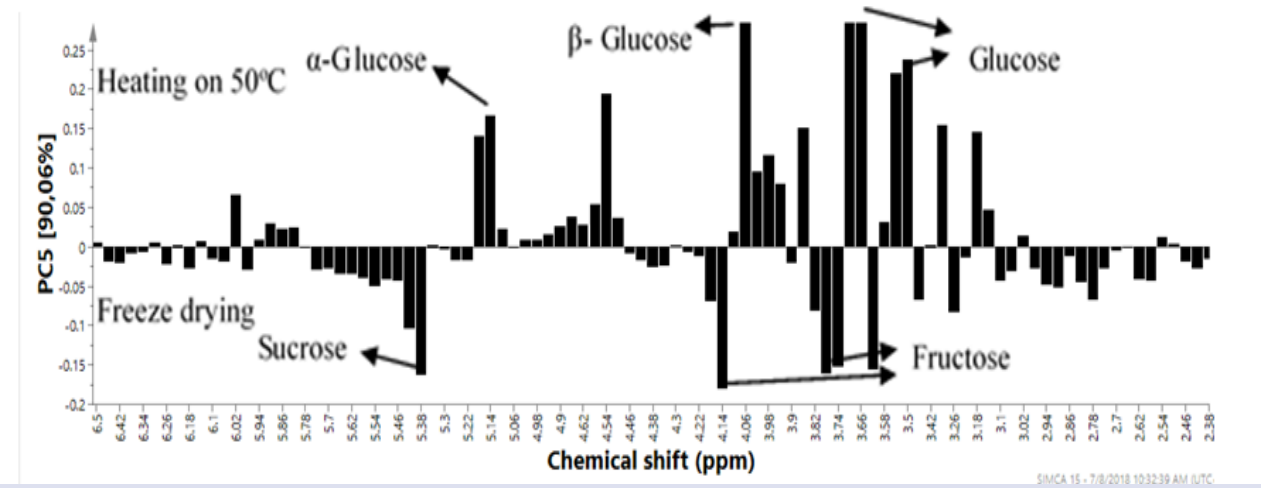

Figure 3: Column plot PCA of PC5 ^{1}H-NMR spectra in the extract of *Curcuma* and *Zingiber* involving oven and freeze-drying methods.

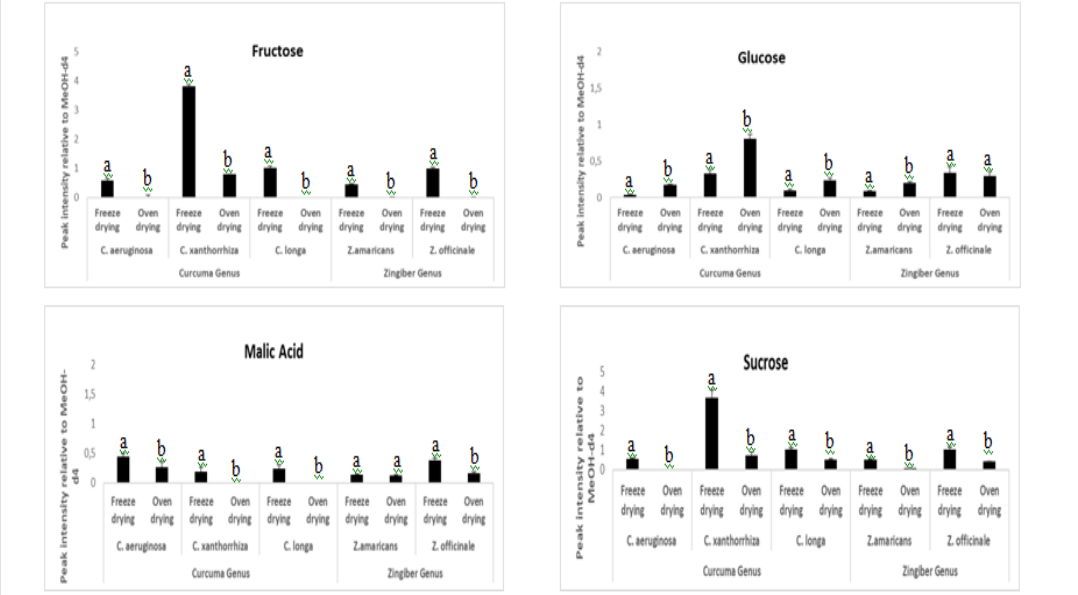

Figure 4: Quantification of identified metabolites responding to column plot in five species of the Curcuma and Zingiber genus were Curcuma longa L., Curcuma aeruginosa Roxb., Curcuma xanthorrhiza Roxb., Zingiber officinale Roscoe. and Zingiber amaricans Bl analyzed due to drying treatment by 1H-NMR. Different letters indicate the significant differences at $p<0.05$ for each sample as measured by independent T-test.

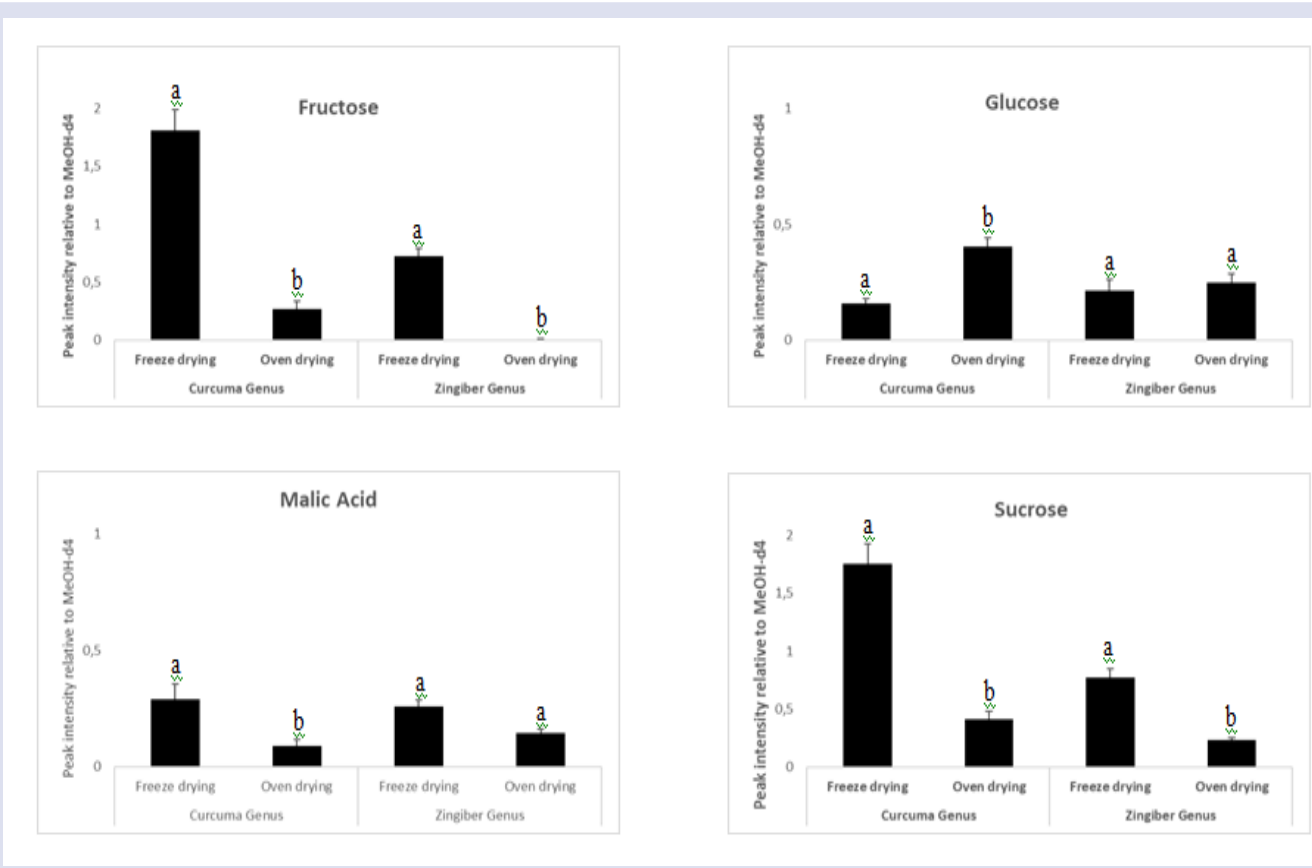

Figure 5: Quantification of identified metabolites responding to column plot in five species of the *Curcuma* and *Zingiber* genus analyzed due to drying treatment by ^{1}H-NMR. Different letters indicate the significant differences at $p<0.05$ for each genus as measured by independent T-test.

concentration. The major compound of the Curcuma genus is curcumin and xanthorrhizol,[13] while the bioactive compound of the Zingiber genus is shogaol.[14] The study suggested that secondary metabolites were not significantly changed in freeze and oven drying at 50^{0}C. Quantification of curcumin (δH 7.15 ppm), xanthorizol (δH 7,22 ppm) and shogaol (δH 6.10 ppm) were not significantly change due to drying methods. Decreasing concentration of curcumin and xanthorrhizol occured from 70^{0}C.[13] Shogaol contained in *Z. officinale* Roscoe. Shogaol is a derivative of gingerol which is more stable of heating. Volatile compounds i.e. 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol were not significantly differed between freeze drying method and heating drying at 60^{0}C on Chinese ginger (*Zingiber officinale* Roscoe.[15] Besides, Mahayothee *et al*[16] reported that hot air drying at 50^{0}C preserved curcumin content in *Zingiber montanum*.

Quantification of sucrose (δH 5,41 ppm), *α*-glucose (*δ*H 5.17 ppm), *β*-glucose (*δ*H 4.56 ppm), fructose (*δ*H 4.16 ppm) and malic acid (*δ*H 4,28 ppm) showed a significantly difference between drying methods. The relative concentrations of sucrose and fructose at 5 samples in freeze drying method showed significant higher content than those in oven drying. On the other hands, glucose was mostly significant higher concentration in oven drying at 5 samples (Figure 4). It is in line with the average of those metabolites in genus level. Fructose and sucrose were significantly higher in freeze drying method than those in oven drying in both genera. Relative glucose content was higher in oven drying at Curcuma genus (Figure 5). The fact that sucrose at temperature of 50^{o}C can undergo hydrolyzation to produce glucose might have correlation to the higher glucose concentration due to hydrolysis sucrose whereas fructose might be decreased due to hydrated by heating. Malic acid was significantly decreased at oven drying method. It was reported by increasing temperature might reduce malate concentration up to 50% when temperature increased from 15 to 25^{0}C.[17]

Overall, the present study has shown a comparative information on the chemical composition of one of the commercial herbal medicine preparations using oven drying. This method is common applied to produce herbal medicines in small industries. Having this drying method seems that the secondary metabolites which are known to have pharmacological activities remain stable inside.

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CONFLICTS OF INTEREST

None

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