



# Differentiation of fatty acid, amino acid, and volatile composition in waxy and non-waxy proso millet

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## Abstract

The unique flavor of the proso millet (*Panicum miliaceum* L.) is one of the key reasons why consumers prefer it. Fatty acids and amino acids have an important influence on the nutrition and flavor of proso millet. In this study, we identified fatty acids, amino acids, and volatile compositions in waxy and non-waxy proso millet porridge by a headspace solid phase microextraction (HS-SPME) method in conjunction with gas chromatography-mass spectrometry (GC-MS). We identified 8 fatty acids, 7 essential amino acids, and 59 volatile compounds from the proso millet. The results of principal component analysis (PCA) clearly demonstrated the profiles of waxy and non-waxy samples and it was observed that better clustering of waxy proso millet porridge can be achieved. There were also some correlations between fatty acids, amino acids and aroma substances. This study will provide the basis for the research of volatile components of proso millet and promote the application of proso millet in food industry.

**Keywords:** *Panicum miliaceum* L.; volatile composition; HS-SPME; GC-MS.

**Practical Application:** Compared the differentiation of fatty acid, amino acid, and volatile composition in waxy and non-waxy proso millet and promote the application of proso millet in food industry.

## 1 Introduction

Proso millet (*Panicum miliaceum* L.) is one of the important crop in Northern China and also has nutritional and healthy function (Chandrasekara & Shahidi, 2011). With the improvement of people's living and health level, proso millet attracted more attention due to its nutritional benefits and medicinal value (Wadikar et al., 2006). The unique flavor of the proso millet is also one of the key reasons consumers like it. In addition, fatty acids and amino acids, the components of proso millet, have an important influence on the nutrition and flavor of proso millet. Therefore, the research about the fatty acids, amino acids, and volatile compositions in proso millet porridge is very important.

Flavor is a critical quality trait in cereals affecting consumer acceptance. Some researches indicated that amylose content has an effect on cereals' volatile compositions. Genetic background, growing conditions, and post-harvest handling are factors which have been shown to affect the flavor of rice (Champagne, 2008) and the different content of amylose also lead to the discrepancies of flavor in cooked rice (Champagne et al., 2004; Fukuda et al., 2014). The research showed that under the same heat treatment conditions, odor-active compounds is more easily produced in waxy wheat flour (Xu et al., 2017). Although the proso millet has two types, including waxy proso millet (low amylose content) and non-waxy proso millet (high amylose content) according to the amylose content (Hunt et al., 2013), little information is concerning volatile composition in waxy and non-waxy proso millet.

In recent years, headspace solid phase microextraction (HS-SPME) in combination with gas chromatography-mass spectrometry (GC-MS) is an effective method (Arthur &

Pawliszyn, 1990) to analysis the volatile compositions in many crops, including rice (Givianrad, 2012), foxtail millet (Liu et al., 2015), wheat (Mattiolo et al., 2017), Chinese rice wine (Liu et al., 2012b), bread (Raffo et al., 2015) and so on. But till now, no study had shown this method used to research the volatile compositions in proso millet porridge. Therefore, the research was to identify and compare the fatty acids, amino acids, and volatile components of waxy and non-waxy proso millet.

## 2 Materials and methods

### 2.1 Proso millet samples

Five waxy and five non-waxy proso millet cultivars (Table 1) were used for the experiments. The amylose contents of waxy and non-waxy proso millet were 2.63-3.48% and 18.52-38.67%. Some studies indicated that waxy and non-waxy proso millet had some differences in physicochemical properties and cooking edibility (Yang et al., 2018). This is also the basic of our research.

### 2.2 Amylose content

The amylose contents of these ten cultivars were determined according to GB/T 15683-2008/ISO 6647-1 (International Standards Organisation, 2008).

### 2.3 Fatty acids

The fatty acids from the grains of proso millet were analyzed by GC-MS using GCMS-QP 2010Ultra (Shimadzu

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**Table 1.** The information and amylose content of samples.

	No.	Varieties	Amylose content (%)
Non-waxy	1	Longmi5	38.67 ± 1.10a
	2	Longmi11	33.45 ± 2.00b
	3	Ningmi10	33.83 ± 1.80b
	4	Gumi21	31.86 ± 0.90c
	5	Yumi2	18.52 ± 0.80d
Waxy	6	Chishu2	2.63 ± 0.10f
	7	Jinshu3	3.11 ± 0.40e
	8	Jinshu9	2.24 ± 0.10f
	9	Qishu1	3.48 ± 0.30e
	10	Yushu1	2.97 ± 0.10ef

Difference in letter in the same column denotes significant difference at  $p < 0.05$ .

Corporation, Kyoto, Japan). The chromatograph was fitted with a 30 m × 0.25 mm × 0.25 μm DB-23 column from Supelco (Agilent Technologies, Palo Alto, CA, USA). The oven temperature was kept at 50 °C for 1 min and programmed at 25 °C/min to 180 °C, and then programmed at 2 °C/min to 230 °C, held there for 5 min. The carrier gas (helium) flow was kept constant at 1.9 mL/min and the split ratio value was of 1:30. Pulsed splitless injection (1 μL) was performed at an injection port temperature of 250 °C. The MS temperatures were as follows: ion source: 220 °C.

#### 2.4 Amino acid

The determination of amino acids was performed by precolumn derivatization HPLC method. Analysis was carried out on a Rigol L3220 HPLC system (RIGOL Technologies, Beijing, China). Separation was performed on a Kromasil C18 reversed phase column (250mm × 4.6mm, 5 μm; Akzo Nobel N.V Technologies, Amsterdam, Netherlands). The injection volume was 10 μL. The column temperature was 40 °C and the flow rate was 1.0 mL/min. 1 L mobile phase A consisted of 7.6 g sodium acetate anhydrous, 70 mL acetonitrile. PH values of the mobile phase A were adjusted to 6.5 by 2% acetic acid solution. Mobile phase B: 80% acetonitrile solution. The gradient program used for separation was as follows: 0-15 min, 0% B; 15-25 min, 10% B; 25-33 min, 30% B; and 33-33.1 min, 45% B, 33.1-38.1 min, 100% B; 38.1-45 min, 0% B.

#### 2.5 Modified HS-SPME sampling

Proso millet porridge was cooked by traditional method. Three grams of proso millet and 30 mL of distilled water were added into a 50 mL glass beaker with cup, placed at room temperature for 15 min, boiled at 100 °C for 30 min by microwave oven. Extraction and concentration of the volatiles of porridge were performed using the method of Ceva-Antunes et al. (2006), Chin et al. (2007) and Mondello et al. (2005).

#### 2.6 GC-MS

For the GC-MS analysis, a GC-MS spectrometer (TRACE-ISQ, Thermo Fisher Scientific, America) was used. The desorption time was 5 min in the injection port at 250 °C and the transfer

line was maintained at 230 °C. A column, HP-INNOWAX, 30 m × 0.25 mm × 0.25 μm (Agilent, America) was applied. It was temperature programmed at 40 °C for 2.5 min, then increased to 200 °C at a rate of 5 °C/min, subsequently increased to 240 °C at a rate of 10 °C/min, maintained at 240 °C for 4.5 min. The carrier gas was helium, which was delivered at a linear velocity of 1 mL/min, and the splitless mode was used. The mass selective detector was operated in an electron impact ionization mode at 70 eV, in a scan range of 35-450 amu. The interface temperature was 250 °C and the source kept at 200 °C (Zeng et al., 2009). Retention time of each volatile was converted to the linear retention index (RI) using n-alkanes (Supelco) as the references. The results from the volatile analyses were provided in chromatographic peak area counts (Zeng et al., 2009). All experiments were performed in triplicate.

#### 2.7 Statistics analysis

Data were analysed using SPSS 20.0 statistical software program. Analysis of variance and Principal component analysis (PCA) were carried out using SPSS 20.0 statistical software to assess differences in volatile compositions between waxy and non-waxy proso millet porridge.

### 3 Results and discussion

#### 3.1 Fatty acids of proso millet

The total saturated, monounsaturated, polyunsaturated, and unsaturated of proso millet were 11.61-13.89%, 22.23-27.30%, 59.88-64.96%, and 86.77-88.40%, respectively. Proso millet oil is a healthy product and it is possible to develop an extraction process for proso millet oil in the future due to its high content of unsaturated fatty acids (Jiménez et al., 2009). Eight fatty acids were tested in this study, of which linolenic acid (59.05-63.73%) and oleic acid (20.73-26.40%) were the two dominant fatty acids (Table 2). However, Shen et al. (2018) and Zhang et al. (2015) identified 5 and 6 fatty acids, respectively, which may be due to differences in identification methods.

The scatter plot for the two first PC (PC1, 43.51%; PC2, 25.75%) indicates the differences in fatty acids of proso millet varieties (Figure 1A, 1B). The waxy proso millet varieties were all distributed in the area of PC1 positive values. Except for sample 5, the non-waxy proso millet varieties were distributed in the area of PC1 negative values. The main contributors corresponding to waxy samples (except for sample 5) were cis-13-octadecenoic, linoleic acid, and linolenic acid. Samples 10 was characterized with oleic acid and arachidic.

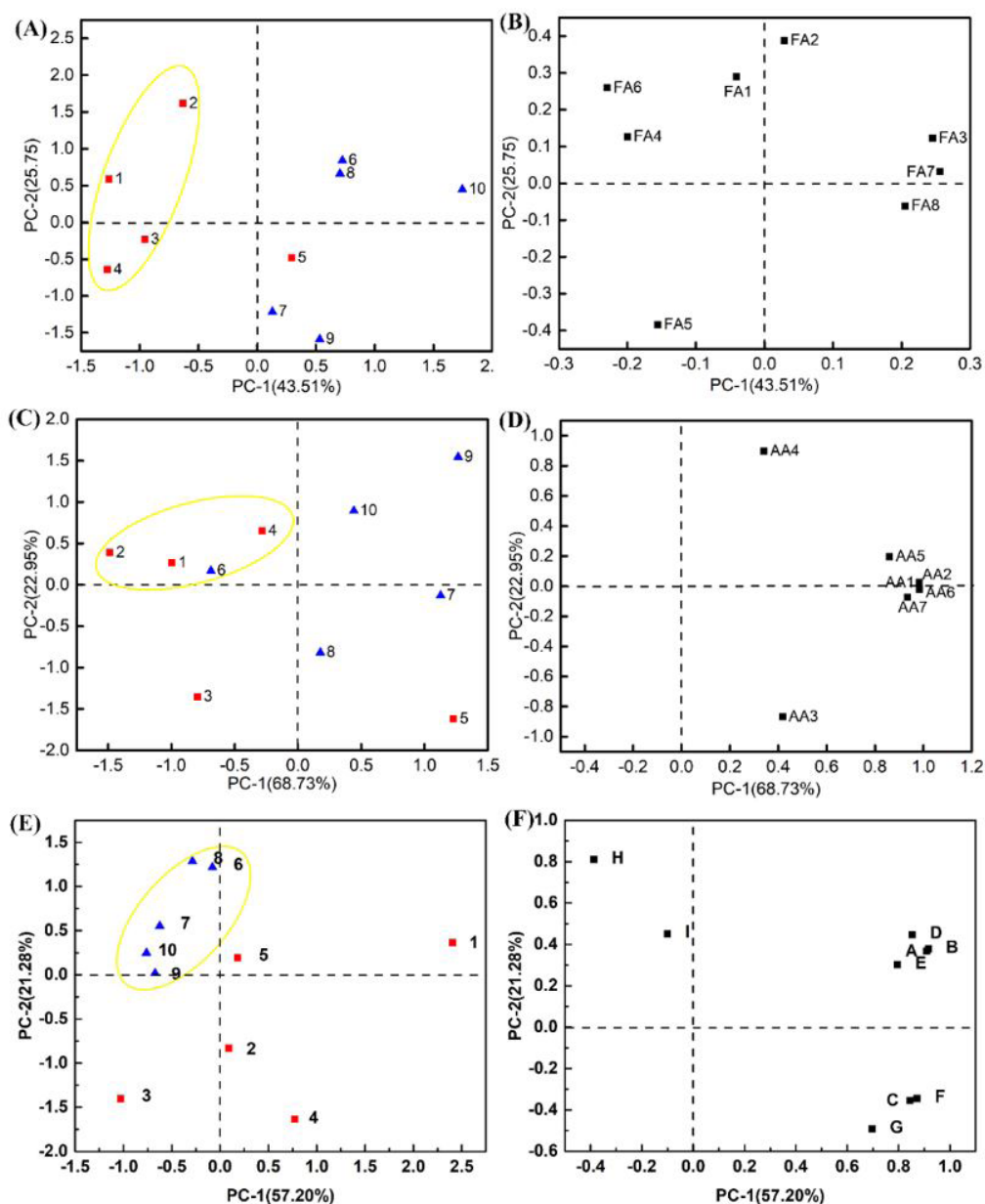
#### 3.2 Amino acids of proso millet

Essential amino acids are important for human health and need to be obtained through food. The total essential amino acid and non-essential amino acid of proso millet were 6.92-13.47% and 14.24-23.18%, respectively. Seven amino acids were tested in this study, of which Leu (2.85-5.17%) and Phe (1.94-4.73%) were the two dominant amino acids (Table 3). Shen et al. (2018) found eight amino acids in proso millet, of which Trp was not found in our study.

Table 2. The fatty acid of waxy and non-waxy proso millet.

	waxy									
	01	02	03	04	05	06	07	08	09	10
Palmitic acid(16:0)	9.67 ± 0.06c	9.82 ± 0.11c	9.38 ± 0.08d	10.17 ± 0.16b	9.43 ± 0.27d	10.21 ± 0.03b	9.27 ± 0.06d	10.57 ± 0.57a	8.77 ± 0.15e	9.29 ± 0.14d
Stearic acid(18:0)	2.13 ± 0.13b	2.38 ± 0.01a	1.91 ± 0.01c	1.65 ± 0.26d	1.68 ± 0.06d	2.15 ± 0.04b	1.95 ± 0.01c	1.90 ± 0.17c	1.71 ± 0.03d	2.17 ± 0.10b
Oleic acid(18:1)	21.95 ± 0.16e	23.46 ± 0.03d	22.51 ± 0.23e	20.73 ± 0.13f	24.97 ± 0.23b	24.92 ± 0.10c	22.08 ± 0.12e	23.47 ± 1.12d	23.94 ± 0.19d	26.40 ± 0.08a
Cis-13-Octadecenoic(18:1)	1.24 ± 0.01cd	1.26 ± 0.01b	1.32 ± 0.02b	1.50 ± 0.01a	1.09 ± 0.03d	0.90 ± 0.01e	0.91 ± 0.01e	1.36 ± 0.22ab	0.89 ± 0.01e	0.92 ± 0.04e
Linoleic acid(18:2)	61.70 ± 0.36b	59.82 ± 0.01c	62.18 ± 0.32b	63.41 ± 0.92a	60.76 ± 0.64c	59.68 ± 0.09c	63.73 ± 0.19a	60.10 ± 2.57c	62.73 ± 0.11b	59.05 ± 0.48c
Linolenic acid(18:3)	2.27 ± 0.25a	2.24 ± 0.05a	1.66 ± 0.16b	1.55 ± 0.33b	1.03 ± 0.04c	1.03 ± 0.02c	0.93 ± 0.02c	1.19 ± 0.24c	0.85 ± 0.01d	0.83 ± 0.06d
Arachidic(20:0)	0.47 ± 0.04c	0.57 ± 0.01bc	0.51 ± 0.01c	0.55 ± 0.02c	0.64 ± 0.02b	0.71 ± 0.01b	0.70 ± 0.01b	0.86 ± 0.16a	0.65 ± 0.02b	0.81 ± 0.04a
Docosanoic(22:0)	0.39 ± 0.08b	0.40 ± 0.04b	0.39 ± 0.07b	0.45 ± 0.01ab	0.41 ± 0.01b	0.42 ± 0.05b	0.46 ± 0.01ab	0.56 ± 0.10a	0.49 ± 0.02ab	0.55 ± 0.04a
TS	12.66 ± 0.32a	13.16 ± 0.35a	12.19 ± 1.04ab	12.81 ± 0.94a	12.16 ± 0.16ab	13.49 ± 0.35a	12.37 ± 0.68a	13.89 ± 1.22a	11.61 ± 0.69b	12.81 ± 0.42a
TMUS	23.18 ± 0.57d	24.72 ± 0.41c	23.82 ± 0.23d	22.23 ± 0.33e	26.06 ± 0.13b	25.82 ± 0.86b	22.98 ± 0.18e	24.83 ± 1.08c	24.82 ± 0.42c	27.31 ± 0.42a
TPUS	63.97 ± 0.34a	62.05 ± 0.69b	63.83 ± 0.31a	64.96 ± 0.23a	61.79 ± 1.07b	60.70 ± 0.58c	64.65 ± 0.47a	61.29 ± 0.57b	63.58 ± 1.05a	59.88 ± 0.62c
TU	87.15 ± 0.25a	86.77 ± 1.06ab	87.65 ± 0.54a	87.18 ± 0.86a	87.85 ± 0.75a	86.52 ± 0.66ab	87.63 ± 0.69a	86.12 ± 0.64a	88.40 ± 0.68a	87.19 ± 1.17a
TU/TS	6.89 ± 0.04ab	6.60 ± 0.06b	7.19 ± 0.31a	6.81 ± 0.17ab	7.23 ± 0.30a	6.42 ± 0.27b	7.08 ± 0.23b	6.20 ± 0.17b	7.62 ± 0.14b	6.81 ± 0.30b

Difference in letter in the same column denotes significant difference at  $p < 0.05$ . TS: total saturated; TMUS: total monounsaturated; TPUS: total polyunsaturated; TU: total unsaturated (total monounsaturated + total polyunsaturated); TU/TS: total unsaturated/total saturated.



**Figure 1.** PCA score (A) and loading plot (B) of fatty acids for samples. PCA score (C) and loading plot (D) of amino acids for samples. PCA score (E) and loading plot (F) of GC-MS data for samples. 1-10: number of proso millet; FA1-FA8: palmitic acid, stearic acid, oleic acid, cis-13-octadecenoic, linoleic acid, linolenic acid, arachidic, and docosanoic; AA1-AA6: Ile, Leu, Phe, Lys, Thr, Val, and Met; A-I: alcohols, aldehydes, alkanes, ketones, benzenes, acids and esters, amines, heterocyclics, and olefins.

The scatter plot for the two first principal components (PC1, 68.73%; PC2, 22.95%) indicates the differences in amino acids of proso millet varieties (Figure 1C, 1D). Except for sample 6, the waxy proso millet samples were all distributed in the area of PC1 positive values. Except for sample 5, the non-waxy proso millet varieties were distributed in the area of PC1 negative values and samples 1, 3, 4, 6 were well integrated into one class.

### 3.3 Volatile compositions in proso millet porridge

Table S1 and Table 4 showed that altogether 59 volatile compounds were identified, including 6 alcohols, 14 aldehydes, 22 alkanes, 4 ketones, 1 benzenes, 8 acids and esters, 2 amines,

1 heterocyclics, and 1 olefins. Previous studies reported that alcohols, aldehydes, ketones, and heterocyclics existed in rice (Ahmed et al., 2016), wheat (Xu et al., 2017), buckwheat (Janeš et al., 2009) and foxtail millet, and these were consistent with our results.

Generally, alcohols are formed by the decomposition of the secondary hydroperoxides of fatty acids (Liu et al., 2012a). The total peak area of alcohols was ranged from  $2.46 \times 10^6$  to  $17.28 \times 10^6$  in samples. The 1-pentanol, 1-hexanol and 1-octen-3-ol were present in all samples. 1-pentanol, 1-hexanol, 1-octen-3-ol have been identified as the odour-active compounds and have fruity and plastic, green, mushroom and citrus aromatic characteristics,

**Table 3.** The essential amino acid of waxy and non-waxy proso millet.

Samples	Amino acid (%)										
	Ile	Leu	Phe	Lys	Thr	Val	Met	EAA	NAA	EAA/TAA	
Non-waxy	01	0.84 ± 0.06de	3.31 ± 0.04e	2.26 ± 0.06f	0.07 ± 0.01bc	0.20 ± 0.03bcd	1.03 ± 0.03f	0.25 ± 0.03cd	7.96 ± 0.23f	14.24 ± 0.24g	35.87 ± 0.20c
	02	0.74 ± 0.04e	2.85 ± 0.04f	1.94 ± 0.04g	0.07 ± 0.01bc	0.17 ± 0.04cd	0.91 ± 0.03g	0.23 ± 0.01d	6.92 ± 0.03g	12.51 ± 0.16h	35.61 ± 0.23c
	03	0.95 ± 0.06cd	3.38 ± 0.04e	4.17 ± 0.06b	0.05 ± 0.03c	0.15 ± 0.01d	1.13 ± 0.03e	0.29 ± 0.03cd	10.12 ± 0.23d	18.04 ± 0.41e	35.92 ± 0.13c
	04	0.96 ± 0.03cd	3.98 ± 0.08d	2.25 ± 0.03f	0.09 ± 0.01bc	0.23 ± 0.01abc	1.22 ± 0.01d	0.32 ± 0.01c	9.04 ± 0.14e	16.66 ± 0.33f	35.18 ± 0.23d
waxy	05	1.31 ± 0.06a	5.05 ± 0.08ab	4.73 ± 0.11a	0.04 ± 0.03c	0.28 ± 0.06a	1.55 ± 0.06ab	0.52 ± 0.04a	13.47 ± 0.44a	23.18 ± 0.08a	36.75 ± 0.17ab
	06	0.86 ± 0.07de	3.35 ± 0.04e	2.32 ± 0.08f	0.07 ± 0.00bc	0.20 ± 0.01bcd	1.09 ± 0.01ef	0.39 ± 0.01b	8.28 ± 0.10f	14.40 ± 0.16g	36.52 ± 0.25b
	07	1.35 ± 0.06a	5.17 ± 0.07a	3.07 ± 0.07d	0.08 ± 0.01bc	0.23 ± 0.01abc	1.61 ± 0.03a	0.55 ± 0.03a	12.06 ± 0.08b	22.60 ± 0.24b	34.79 ± 0.13de
	08	1.04 ± 0.10c	3.87 ± 0.04d	3.57 ± 0.06c	0.05 ± 0.00c	0.25 ± 0.01ab	1.25 ± 0.04d	0.49 ± 0.03a	10.53 ± 0.20d	17.96 ± 0.04e	36.95 ± 0.17a
	09	1.33 ± 0.03a	5.00 ± 0.06b	2.51 ± 0.06e	0.15 ± 0.04a	0.28 ± 0.04a	1.51 ± 0.06b	0.54 ± 0.03a	11.32 ± 0.11c	22.03 ± 0.13c	33.94 ± 0.13f
	10	1.17 ± 0.06b	4.34 ± 0.03c	2.47 ± 0.06e	0.11 ± 0.03ab	0.26 ± 0.03ab	1.37 ± 0.04c	0.40 ± 0.06b	10.12 ± 0.24d	19.28 ± 0.04d	34.43 ± 0.11e

Difference in letter in the same column denotes significant difference at  $p < 0.05$ . EAA: essential amino acid; NAA: non-essential amino acid; TAA: Total amino acids.

**Table 4.** The total content of volatile compounds from different varieties of proso millet porridge.

Species	No.	Peak area (area counts $\times 10^6$ )								
		A	B	C	D	E	F	G	H	I
		Alcohols	Aldehydes	Alkanes	Ketones	Benzenes	Acids and esters	Amines	Heterocyclics	Olefins
Non-waxy	1	12.28	126.38	127.00	10.07	0.88	193.74	11.57	--	--
	2	7.26	48.33	100.72	3.86	--	92.06	9.40	1.34	--
	3	2.56	16.59	34.42	--	--	40.71	5.88	--	--
	4	10.39	61.63	139.08	2.76	--	185.29	11.94	--	--
	5	9.10	59.25	97.59	5.73	--	134.84	1.13	2.38	--
Waxy	6	9.99	65.06	53.47	4.69	0.39	36.82	3.53	4.27	--
	7	7.68	55.52	40.39	2.89	--	56.13	--	3.16	--
	8	9.69	64.89	51.48	4.13	--	42.02	5.39	3.25	2.19
	9	7.74	42.17	29.53	2.95	--	18.94	3.02	1.64	--
	10	7.67	39.36	31.09	3.24	--	23.31	--	1.90	--

Difference in letter in the same column denotes significant difference at  $p < 0.05$ .

respectively (Liu et al., 2012a). Xu et al. reported that 1-octen-3-ol was found higher in waxy wheat flour than normal wheat flour (Xu et al., 2017), which was consistent with our results.

The aldehyde, has a great influence on the aroma of cereal products due to its lower threshold of odor, and it is obtained by the self-oxidation and enzymatic oxidation of the double carbon-carbon bond of unsaturated fatty acids in the cereal (Varlet et al., 2007). The total peak area of aldehydes were ranged from  $16.59 \times 10^6$  to  $126.38 \times 10^6$  in samples. The (Z)-2-heptenal, nonanal, hexanal, and isovanillin, TBDMS derivative were presented in all samples, and provided grassy, citrus and green, green and fatty, grassy characteristics odor, respectively (Liu et al., 2012a). Heptanal can provide fatty and citrus characteristics. Octanal can provide soapy and green characteristics. They were presented in all but sample 2 and 3. In proso millet porridge, (E,E)-2,4-nonadienal was absent from only two samples (1,4); (E,E)-2,4-decadienal was detected in only two samples (1,6), and these two compounds have “nutty and fatty” and “fatty” odor (Ahmed et al., 2016).

Ketones are formed by the self-oxidation of fatty acids, especially unsaturated fatty acids, which can provide soap and fruity odor to food. The total peak area of ketones were ranged from  $2.71 \times 10^6$  to  $10.07 \times 10^6$  in samples and the ketones were not detected in the sample 3. 6-methyl-5-hepten-2-one has been also identified as odour-active compounds in buckwheat (Janeš et al., 2009). The total peak area of acids and esters were ranged between  $18.94 \times 10^6$  and  $193.74 \times 10^6$  in samples. The ethyl, 4-hydroxymandelate, 2TMS derivative and 4-ethylbenzoic acid, cyclopentyl ester were presented in all samples.

### 3.4 PCA of volatile compounds in proso millet porridge

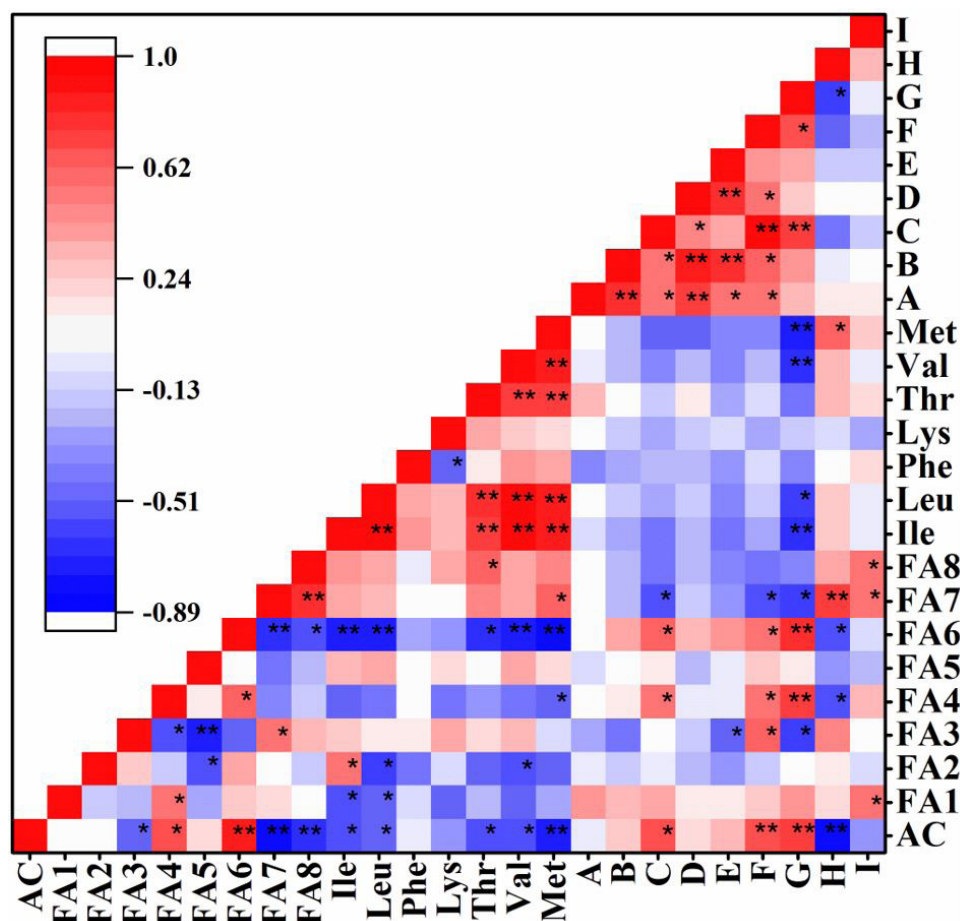
Figure 1C and 1D indicated that PC1 and PC2 accounted for 57.20% and 21.28% of the total variances of volatile peak areas, respectively. The score plots (Figure 1E) clearly demonstrated the profiles of waxy and non-waxy samples and it was observed that better clustering of waxy proso millet porridge can be achieved. PC1 showed the difference between the volatiles of waxy samples and most of the non-waxy samples (1, 2, 4 and 5),

which displayed negative and positive values on PC1, respectively. PC2 could distinguish the waxy samples from the sample 3. The loading plot (Figure 1F) could show the information between the different varieties. Samples 1 and 5 characterized with higher alcohols, aldehydes, ketones, benzenes. What's more, samples 2 and 4 were predominantly affected by alkanes, acids and esters, amines.

### 3.5 Correlations between amylose content, fatty acids, amino acids, and volatile compositions

The correlations among amylose content, fatty acids, amino acids, and volatile compositions were analyzed using PCA and are given in Figure 2. The result indicated that amylose content was positive correlated with cis-13-octadecenoic and linolenic acid and negative correlated with oleic acid, arachidic, and docosanoic. Interestingly, there were correlations between amylose and Ile, Leu, Thr, Val, and Met and they are all negatively correlated. Shen et al. (2018) found that waxy proso millet had higher Thr, Val, Ile, and Leu than non-waxy proso millet, which was agreement with this results. The results showed that there were also some correlations between fatty acids, amino acids and aroma substances. This may be because volatile components are a complex process involving genotypes, growth, storage and processing.

The amylose content was positive correlated with alkanes, acids and esters, and amines and negative correlated with heterocyclics. Volatile components of cereals are affected by many factors including amylose content (Fukuda et al., 2014; Xu et al., 2017). Some research reported that amylose is a kind of a long linear polymer and it can form complexes with volatiles, and consequently the retention of the volatiles increased. In addition, waxy cereals might also have a glutinous-like flavor due to the low amylose content (Claver et al., 2010; Fukuda et al., 2014). Though our results were inconsistent with these studies, the waxy and non-waxy proso millet can be distinguished better by PCA and amylose content was related to some aroma components. The aldehydes, alkanes, ketones, benzenes, and acids and esters showed significant positive correlations with



**Figure 2.** Pearson correlation matrices between volatile compounds and amylose content. \* and \*\* indicated significant correlation at p level < 0.05 and p level < 0.01, respectively. AC: amylose content; FA1-FA8: palmitic acid, stearic acid, oleic acid, cis-13-octadecenoic, linoleic acid, linolenic acid, arachidic, and docosanoic, respectively; A-I: alcohols, aldehydes, alkanes, ketones, benzenes, acids and esters, amines, heterocyclics, and olefins.

alcohols. The aldehydes was positive correlated with alkanes, ketones, benzenes, and acids and esters. The ketones, acids and esters, and amines were highly correlated to alkanes. There was a significant positive correlation between acids and esters and amines. Some studies reported that aldehydes, alcohols and ketones usually were derived from oxidation of fatty acids present in cereals. Therefore, there is some connection between these volatile compounds.

#### 4 Conclusion

We identified 8 fatty acids and 7 essential amino acids were identified from the proso millet. 6 alcohols, 14 aldehydes, 22 alkanes, 4 ketones, 1 benzenes, 8 acids and esters, 2 amines, 1 heterocyclics, 1 olefins. Here, 15 constituents were found to be common to all samples. PCA clearly demonstrated the profiles of waxy and non-waxy samples and it was observed that better clustering of waxy proso millet porridge can be achieved. The result indicated that amylose content was positive correlated with cis-13-octadecenoic, linolenic acid, alkanes, acids and esters, and amines and negative correlated with oleic acid, arachidic, docosanoic, Ile, Leu, Thr, Val, Met, and heterocyclics. The results

showed that there were also some correlations between fatty acids, amino acids and aroma substances.

#### References

- Ahmed, A. M., Zhang, C., & Liu, Q. (2016). Comparison of physicochemical characteristics of starch isolated from sweet and grain sorghum. *Journal of Chemistry*, 2016, 1-15. <http://dx.doi.org/10.1155/2016/7648639>.
- Arthur, C. L., & Pawliszyn, J. (1990). Solid phase microextraction with thermal desorption using fused silica optical fibers. *Nalytical Chemistry*, 62(19), 2145-2148. <http://dx.doi.org/10.1021/ac00218a019>.
- Ceva-Antunes, P. M. N., Bizzo, H. R., Silva, A. S., Carvalho, C. P. S., & Antunes, O. A. C. (2006). Analysis of volatile composition of siriguela (*Spondias purpurea* L.) by solid phase microextraction (SPME). *Lebensmittel-Wissenschaft + Technologie*, 39(4), 437-443. <http://dx.doi.org/10.1016/j.lwt.2005.02.007>.
- Champagne, E. T. (2008). Rice aroma and flavor: a literature review. *Cereal Chemistry*, 85(4), 445-454. <http://dx.doi.org/10.1094/CCHEM-85-4-0445>.
- Champagne, E. T., Bett-Garber, K. L., McClung, A. M., & Bergman, C. (2004). Sensory characteristics of diverse rice cultivars as influenced by genetic and environmental factors. *Cereal Chemistry*, 81(2), 237-243. <http://dx.doi.org/10.1094/CCHEM.2004.81.2.237>.

- Chandrasekara, A., & Shahidi, F. (2011). Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods*, 3(3), 144-158. <http://dx.doi.org/10.1016/j.jff.2011.03.007>.
- Chin, S. T., Nazimah, S. A. H., Quek, S. Y., Man, Y. B. C., Rahman, R. A., & Hashim, D. M. (2007). Analysis of volatile compounds from Malaysian durians (*Durio zibethinus*) using headspace SPME coupled to fast GC-MS. *Journal of Food Composition and Analysis*, 20(1), 31-44. <http://dx.doi.org/10.1016/j.jfca.2006.04.011>.
- Claver, I. P., Zhang, H., Li, Q., Zhu, K., & Zhou, H. (2010). Impact of the soak and the malt on the physicochemical properties of the sorghum starches. *International Journal of Molecular Sciences*, 11(8), 3002-3015. <http://dx.doi.org/10.3390/ijms11083002>. PMID:21152287.
- Fukuda, T., Takeda, T., & Yoshida, S. (2014). Comparison of volatiles in cooked rice with various amylose contents. *Food Science and Technology Research*, 20(6), 1251-1259. <http://dx.doi.org/10.3136/fstr.20.1251>.
- Givianrad, M. H. (2012). Characterization and assessment of flavor compounds and some allergens in three iranian rice cultivars during gelatinization process by HS-SPME/GC-MS. *E-Journal of Chemistry*, 9(2), 716-728. <http://dx.doi.org/10.1155/2012/396836>.
- Hunt, H. V., Moots, H. M., Graybosch, R. A., Jones, H., Parker, M., Romanova, O., Jones, M. K., Howe, C. J., & Trafford, K. (2013). Waxy phenotype evolution in the allotetraploid cereal broomcorn millet: mutations at the GBSSI locus in their functional and phylogenetic context. *Molecular Biology and Evolution*, 30(1), 109-122. <http://dx.doi.org/10.1093/molbev/mss209>. PMID:22936718.
- International Standards Organisation – ISO. (2008). Rice- determination of amylose content. Geneva: ISO.
- Janeš, D., Kantar, D., Kreft, S., & Prosen, H. (2009). Identification of buckwheat (*Fagopyrum esculentum* Moench) aroma compounds with GC-MS. *Food Chemistry*, 112(1), 120-124. <http://dx.doi.org/10.1016/j.foodchem.2008.05.048>.
- Jiménez, J. J., Bernal, J. L., Nozal, M. J., Toribio, L., & Bernal, J. (2009). Profile and relative concentrations of fatty acids in corn and soybean seeds from transgenic and isogenic crops. *Journal of Chromatography A*, 1216(43), 7288-7295. <http://dx.doi.org/10.1016/j.chroma.2009.08.015>. PMID:19716136.
- Liu, J., Tang, X., Zhang, Y., & Zhao, W. (2012a). Determination of the volatile composition in brown millet, milled millet and millet bran by gas chromatography/mass spectrometry. *Molecules*, 17(3), 2271-2282. <http://dx.doi.org/10.3390/molecules17032271>. PMID:22367023.
- Liu, J., Xu, Y., & Zhao, G. (2012b). Rapid determination of ethyl carbamate in Chinese rice wine using headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of the Institute of Brewing*, 118(2), 217-222. <http://dx.doi.org/10.1002/jib.33>.
- Liu, J., Zhao, W., Li, S., Zhang, A., Zhang, Y., & Liu, S. (2015). Determination of volatile compounds in foxtail millet sake using headspace solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Chemistry*, 2015, 1-9.
- Mattiolo, E., Licciardello, F., Lombardo, G. M., Muratore, G., & Anastasi, U. (2017). Volatile profiling of durum wheat kernels by HS-SPME/GC-MS. *European Food Research and Technology*, 243(1), 147-155. <http://dx.doi.org/10.1007/s00217-016-2731-z>.
- Mondello, L., Costa, R., Tranchida, P. Q., Chiofalo, B., Zumbo, A., Dugo, P., & Dugo, G. (2005). Determination of flavor components in Sicilian goat cheese by automated HS-SPME-GC. *Flavour and Fragrance Journal*, 20(6), 659-665. <http://dx.doi.org/10.1002/ffj.1529>.
- Raffo, A., Carcea, M., Castagna, C., & Magri, A. (2015). Improvement of a headspace solid phase microextraction-gas chromatography/mass spectrometry method for the analysis of wheat bread volatile compounds. *Journal of Chromatography. A*, 1406, 266-278. <http://dx.doi.org/10.1016/j.chroma.2015.06.009>. PMID:26118802.
- Shen, R., Ma, Y., Jiang, L., Dong, J., Zhu, Y., & Ren, G. (2018). Chemical composition, antioxidant, and antiproliferative activities of nine Chinese proso millet varieties. *Food and Agricultural Immunology*, 29(1), 625-637. <http://dx.doi.org/10.1080/09540105.2018.1428283>.
- Varlet, V., Prost, C., & Serot, T. (2007). Volatile aldehydes in smoked fish: analysis methods, occurrence and mechanisms of formation. *Food Chemistry*, 105(4), 1536-1556. <http://dx.doi.org/10.1016/j.foodchem.2007.03.041>.
- Wadikar, D., Vasudish, C. R., Premavalli, K. S., & Bawa, A. S. (2006). Effect of variety and processing on antinutrients in finger millet. *Journal of Food Science and Technology*, 43(4), 370-373.
- Xu, J., Zhang, W., Adhikari, K., & Shi, Y.-C. (2017). Determination of volatile compounds in heat-treated straight-grade flours from normal and waxy wheats. *Journal of Cereal Science*, 75, 77-83. <http://dx.doi.org/10.1016/j.jcs.2017.03.018>.
- Yang, Q., Zhang, P., Qu, Y., Gao, X., Liang, J., Yang, P., & Feng, B. (2018). Comparison of physicochemical properties and cooking edibility of waxy and non-waxy proso millet (*Panicum miliaceum* L.). *Food Chemistry*, 257, 271-278. <http://dx.doi.org/10.1016/j.foodchem.2018.03.009>. PMID:29622210.
- Zeng, Z., Zhang, H., Zhang, T., Tamogami, S., & Chen, J. Y. (2009). Analysis of flavor volatiles of glutinous rice during cooking by combined gas chromatography-mass spectrometry with modified headspace solid-phase microextraction method. *Journal of Food Composition and Analysis*, 22(4), 347-353. <http://dx.doi.org/10.1016/j.jfca.2008.11.020>.
- Zhang, A., Liu, X., Wang, G., Wang, H., Liu, J., Zhao, W., & Zhang, Y. (2015). Crude fat content and fatty acid profile and their correlations in foxtail millet. *Cereal Chemistry Journal*, 92(5), 455-459. <http://dx.doi.org/10.1094/CCHEM-12-14-0252-R>.



## Supplementary Material

Supplementary material accompanies this paper.

Table S1: Volatile compounds in proso millet porridge.

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