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Characterizing diversity based on nutritional and bioactive compositions of yam germplasm (*Dioscorea* spp.) commonly cultivated in China

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ABSTRACT

Yams (*Dioscorea* spp.) are widely cultivated as edible resources and medical materials in China. Characterizing chemical compositions in yam germplasm is crucial to determine their diversity and suitability for food and medicine applications. In this study, a core germplasm containing 25 yam landraces was used to create an effective classification of usage by characterizing their nutritive and medicinal compositions. All studied landraces exhibited high contents of starch from 60.7% to 80.6% dry weight (DW), protein (6.3–12.2% DW), minerals (especially Mg 326.8–544.7 mg/kg DW), and essential amino acids. Allantoin and dioscin varied considerably, with values of 0.62–1.49% DW and 0.032–0.092% DW, respectively. The quality variability of 25 yam landraces was clearly separated in light of UPGMA clustering and principal component analysis (PCA). Using an eigenvalue $\geq$1 as the cutoff, the first three principal components accounted for most of the total variability (62.33%). Classification was achieved based on the results of the measured parameters and principal component analysis scores. The results are of great help in determining appropriate application strategies for yam germplasm in China.

1. Introduction

Yams (*Dioscorea* spp) are extremely widespread in most tropical and subtropical regions [1]. They are principally grown for food and have organoleptic qualities that make them the preferred carbohydrate food [2]. Apart from food, some yam species are commonly utilized in many pharmaceutical preparations owing to their special bioactive constituents or

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precursors [3]. The Food and Agriculture Organization (FAO) [4] recorded that an annual production of 52 million tons of yam is produced in 50 tropical and subtropical countries. However, the production of yam from China was not included under the FAO statistics. In fact, China is an important and isolated yam domestication center [1]. An estimated 5–6 million tons of yam is produced annually by planting various landraces in this region [5]. In practice, these yams are not only widely consumed as fresh vegetables, but also processed into flour, flakes, chips, and dry-roasted slices in food industries. Within traditional Chinese medicine, medical decoction of pieces of yam is also a popular method of consumption [6].

Despite their popular consumption and economic importance, there is limited knowledge on the chemical characterization of Chinese yams. In particular, an obscure knowledge of their bioactive constituents has provided insufficient bases for pharmacological action. As a result, the risk of ineffective treatment has greatly increased in traditional Chinese medicine clinics. To date, the yam accessions used in medicine have always been disordered [6]. Previous studies on yams have mostly focused on componential investigations based on species grown in Africa and North America [7–9], and wild species from Nepal [10]. As for Chinese yams, most studies highlighted the physicochemical and functional properties of starch [11]. Based on chemical characterization related to amino acids, minerals, allantoin, and dioscin, the extent of diversity in yam accessions and their relationships are yet to be investigated in detail. In particular, some constituents are gradually gaining attention due to their nutritive and medicinal properties [12]. For example, allantoin and dioscin are well-known active constituents from *Dioscorea* plants, and present multiple important pharmacological effects including promoting cell proliferation and lowering plasma glucose, and antifungal, antithrombotic, anticancer, and hepatoprotective properties [13–15]. Currently, various yam landraces have been developed by Chinese farmers through long domestication processes [5]. It is difficult to decide the most appropriate classification of usage, given the fact that landraces/species differ in chemical characteristics due to their diverse agronomic traits such as tuber shape, tuber flesh color, and time to maturity [16]. Thus, it is crucial to characterize the chemical compositions of these individuals not only to determine their edible and potential medicinal properties for widespread commercial utilization, but also to facilitate conserving and improving yam germplasm.

Considering all previous studies, the aim of this work was to quantify the nutritional and bioactive compositions of core samples containing 25 yam landraces. Furthermore, multivariate data analysis techniques were employed to establish quality differentiation among these landraces as a functional classification of food or medicine.

2. Materials and methods

2.1. Sample collection and preparation

Twenty-five yam landraces from four species (*Dioscorea alata* L., *Dioscorea opposita* Thunb., *Dioscorea persimilis* Prain et Burkill, and *Dioscorea fordii* Prain et Burkill) were collected from the North to the South of the Yangtze River in China. For each landrace, three to five mature tubers from different plants were obtained by the local producer during the 2011/2012 cropping season [17]. Tubers were washed and the skin was peeled off and cut into slices. Slices were taken equally from the distal, middle, and proximal regions of the tubers, and were stored overnight in a −20°C refrigerator to avoid oxidation. The slices were further dried and ground into powder.

2.2. Proximate composition analysis

The content of starch was determined using the ferricyanide (acid hydrolysis) method described by the Association of Official Analytical Chemists (AOAC) [18]. The contents of protein and fiber were detected in accordance with the AOAC standard methods 976.05 and 962.09 [19]. All determinations were performed in triplicate and the results were expressed as g/100 g of dry weight (DW).

2.3. Mineral analysis

Quantifications of Fe, Zn, Mg, Ca, and Cu were performed using atomic absorption spectrometry (model NovAA400; Analytik Jena AG, Jena, Germany) after the sample was treated according to the AOAC method 923.03 [19]. The absorption of each mineral was measured at a specific wavelength. The concentration of the mineral was obtained from calibration curves constructed (according to each element) using external standard solutions (AccuStandard, Inc., New Haven, CT, USA). The result was expressed as mg/kg DW.

2.4. Amino acids analysis

After the powder samples were treated according to the reported method [10], the amino acid concentration was assayed using an automatic amino acid analyzer (Model L-8900; Hitachi, Tokyo, Japan) with postcolumn ninhydrin derivation and spectrophotometrical detection (wavelength 570 nm). An aliquot of 20 μL was injected into the amino acid analyzer equipped with an Hitachi custom column filled with 3-μm ion exchange resin (Hitachi). The amino acids were identified by comparing with the retention time of standard amino acids (Sigma-Aldrich Co, St. Louis, MO, USA) using norvaline as an internal standard. The content of the tested samples was quantified by comparing peak areas of samples with standard amino acid profiles. The amino acid content was expressed as g/100 g yam protein.

2.5. Allantoin determination

One gram of each sample was extracted using ultrasonication with a 20-mL mixture of ethanol and water (80:20 v:v) for 40 minutes and then filtered through a filter paper disk. Analysis of allantoin was performed using an Agilent 1200 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (G1315D). A Hypesil OD C18 column (200 mm × 4.6 mm, 5 μm; Elite Analytical Instruments Co. Ltd., Dalian, China) was used to separate allantoin, operating at 30°C with a flow rate of 0.5 mL/min. The

mobile phases consisted of methanol (Merck; Darmstadt; Germany) (A) and water (B) in the ratio of 10% A and 90% B. The runs were set at 224 nm and maintained for 10 minutes.

The allantoin was identified by comparing the retention time of a standard substance (National Institutes for Food and Drug Control, China; Figure S1). Ten microliters of serial allantoin concentrations (0.4 μg/mL, 1.2 μg/mL, 2.0 μg/mL, 2.8 μg/mL, 4.0 μg/mL, and 6.0 μg/mL) were injected to construct a calibration curve. A good linearity ($y = 171.078x + 16.712$, $r^2 = 0.9994$) was obtained for quantification of allantoin.

2.6. Dioscin determination

Two grams of each sample was extracted twice using a refluxing process with 50-mL ethanol:water (95:5 v:v; 1.5 h/extraction). The mixture was filtered through a filter paper disk and the clean filtrate was evaporated to dryness in a 60°C water bath. The residue was gradually dissolved using 25 mL deionized water and the solution was subsequently separated using water saturated N-butanol three times (30 mL/time). The solution of N-butanol was collected and dried and the residue was dissolved using 25 mL methanol. The solution of methanol was further filtered through a 0.45-μL millipore filter (Automatic science instrument CO., LTD, TIANJIN, China) for analysis.

The analysis of dioscin was performed in an Agilent 1200 HPLC system (Agilent Technologies). Dioscin was separated in a Shim-pack VP-ODS C18 column (200 mm × 4.6 mm, 5 μm; Shimadzu, Kyoto, Japan). The elution consisted of methanol (A) and water (B) in the ratio of 88% A and 12% B with a flow rate of 1.0 mL/min. The runs were set at 210 nm and maintained for 15 minutes. The dioscin was identified by comparison with the retention time of a standard substance (National Institutes for Food and Drug Control, China; Figure S1). Under these conditions, a good linearity ($y = 5.139x + 16.815$, $r^2 = 0.9994$) was achieved in the concentration ranges from 10.4 μg/mL to 156.0 μg/mL to quantify the dioscin content.

2.7. Statistical analysis

All assays were carried out in triplicate and the results are expressed as means and standard deviations. The statistical differences between yam species were obtained through one-way analysis of variance followed by Tukey's test at 95% confidence level ($p < 0.05$).

A matrix of samples ($n = 25$) and variables ($n = 8$; totaling 200 data points) was built in terms of the importance of all detected variables. Clustering analysis was used to highlight landrace similarities based on unweighted pair group method arithmetic averages (UPGMA) and Euclidean distance by using the computer program NTSYS-pc, version 2.1 (State University of New York; New York, USA). Additionally, to simplify the presentation and interpretations of quality variables, principal component analysis (PCA) was also used to reduce the multidimensional data set to lower dimensions. Furthermore, a comprehensive PCA score model for each landrace was used to assess their quality, and developed as follows:

$$PC = w_1 \times PC1 + w_2 \times PC2 + w_s \times PCs \quad (1)$$

where w is a weight and is equal to the ratio of variance for PCs and total variance. All computations were performed using the SPSS software, version 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Nutritional compositions

The contents of proximate composition and minerals are shown in Table 1. Considerable variability was detected among the 25 yam landraces with respect to tuber nutritional compositions. Starch constituted between 60.7% and 80.6% DW with a mean of 69.5%. This finding confirmed the frequently reported values (50–80%) for the major cultivated yam species [20,21]. Notably, a significant variation ($p < 0.05$) in starch content was found among species. Compared with *D. opposita*, the species from *D. fordii*, *D. alata*, and *D. persimilis* presented higher starch content.

In addition, the protein content (6.3–12.2%) exhibited mostly high variability, which was higher than values reported for *D. alata* varieties [22]. This variability may be the result of improving the yam protein supply through the selection of landraces. For example, the landraces HZS, QYS, and TIE maintained by local farmers had the highest protein content (10.6–12.2%), implying that they may be appropriate protein sources for consumption. The mean fiber content was 1.06%, with individual values ranging from 0.67% to 1.50%, and significant differences ($p < 0.05$) were also found among different species (Table 1).

Trace minerals are very important in terms of nutritional value in yam tubers. The yam landraces had high contents of Mg (326.8–544.7 mg/kg) and Ca (295.8–558.2 mg/kg). By contrast, yam landraces had low contents of Zn (8.2–25.9 mg/kg), Fe (8.3–25.8 mg/kg), and Cu (3.5–5.4 mg/kg; Table 1). The content of all minerals were investigated and Zn and Cu showed significant variability ($p < 0.05$) among species. Generally, the landraces from *D. opposita* and *D. persimilis* had significantly higher Mg and Ca content than those from the other two species. In comparison with previous reports, the Mg and Ca contents of Chinese yams were higher than the values reported [23,24]. The difference of minerals observed for yams is likely a substantial consequence, such as species, elemental composition and pH of local soil, and mineral fertilization [25]. Overall, the highlight of Chinese yams was the higher Mg content. The Mg values obtained, particularly in landraces from *D. opposita*, *D. alata*, and *D. persimilis*, were higher than the FAO recommended dietary allowance of 420 mg for men and 320 mg for women [26]. Taking into account the prevailing deficiencies of Mg intake in many underdeveloped regions, these yams may be a valuable source to offset the deficits.

The individual contents of essential amino acids (EAAs) and nonessential amino acids (NEAAs) in yam samples are showed in Table 2. Among 16 amino acids detected, NEAAs dominated the protein content in yam tubers, in particular, Arg (7.96–14.45 g/100 g protein), Glu (7.70–12.70 g/100 g protein), and Ser (3.10–9.48 g/100 g protein) showed higher amounts compared with the other amino acids. With regard to EAAs, the yam landraces predominantly contained Thr

Table 1 – The contents of proximate compositions, minerals, and bioactive compounds determined for 25 yam landraces in China.

Species	Landraces	Proximate compositions (% DW)			Minerals (mg/kg DW)					Bioactive components (% DW)	
		Starch	Protein	Fiber	Ca	Mg	Zn	Fe	Cu	Allantoin	Dioscin
Dioscorea alata	HSY	64.4	8.7	0.76	376.9	478.3	14.07	13.5	4.6	1.49	0.088
	SS1	71.2	9.7	0.90	422.0	436.1	10.3	8.3	4.2	0.99	0.081
	SS2	80.6	7.6	0.67	453.3	411.0	12.43	16.2	4.3	0.75	0.055
	SS3	76.8	9.5	1.10	316.5	390.2	9.9	10.3	4.4	0.78	0.061
	SS4	73.1	8.8	1.11	316.4	431.7	25.9	22.2	4.8	0.72	0.053
	SS5	71.2	7.2	0.84	358.9	386.9	10.6	12.7	4.5	1.17	0.049
	SS6	72.9	6.6	1.19	389.8	458.8	15.1	11.1	4.3	0.81	0.057
	SS7	70.6	6.4	1.04	364.6	326.8	9.8	12.1	4.3	0.73	0.066
	SS8	73.9	6.9	1.05	404.3	420.8	8.2	9.6	4.7	0.75	0.057
		72.7 ± 4.5^{a}	7.9 ± 1.2^{a}	0.96 ± 0.18^{b}	378.1 ± 45.5^{b}	$415.6 \pm 44.6b^{c}$	12.9 ± 5.3^{a}	12.8 ± 4.2^{b}	4.4 ± 0.2^{a}	0.91 ± 0.26^{a}	0.063 ± 0.013^{b}
Dioscorea opposita	TIE	65.4	10.6	1.12	542.8	530.5	14.0	17.0	4.2	0.80	0.072
	TGS	66.9	9.9	1.07	472.5	501.1	11.2	15.2	4.5	1.23	0.092
	HZS	62.9	12.2	1.19	419.9	438.9	13.5	16.7	4.4	0.71	0.073
	BYS	72.5	6.3	1.50	558.2	544.7	16.3	17.8	5.3	0.79	0.081
	SJS	60.7	7.9	1.38	397.3	332.6	9.9	14.3	3.5	0.67	0.077
	QYS	65.6	11.8	1.06	452.9	469.7	12.8	16.7	4.4	0.76	0.082
	HNS	70.2	7.2	1.22	500.7	518.3	19.6	22.7	4.5	0.87	0.081
	CTS	62.2	6.7	1.12	453.0	478.5	22.7	25.8	5.4	0.79	0.074
	NPS	68.8	9.6	0.99	472.9	486.9	18.6	24.1	5.0	0.90	0.081
	LNS	62.2	7.5	1.00	451.4	440.5	12.5	14.8	4.1	0.71	0.057
		65.7 ± 3.9^{b}	9.0 ± 2.1^{a}	1.17 ± 0.16^{a}	472.2 ± 50.3^{a}	474.2 ± 61.0^{a}	15.1 ± 4.1^{a}	18.5 ± 4.1^{a}	4.5 ± 0.6^{a}	$0.82 \pm 0.16^{a,b}$	0.077 ± 0.009^{a}
Dioscorea fordii	GDS1	77.1	9.9	1.03	295.7	352.9	18.2	19.2	4.6	0.90	0.034
	GDS2	75.9	9.8	0.92	300.5	345.8	18.5	20.2	4.5	0.86	0.032
	GDS3	76.7	10.2	1.14	285.6	356.3	17.9	18.2	5.1	0.83	0.033
		76.5 ± 0.62^{a}	9.9 ± 0.22^{a}	$1.03 \pm 0.11^{a,b}$	$293.9 \pm 7.6^{b,c}$	351.7 ± 5.4^{c}	18.2 ± 0.4^{a}	19.2 ± 1.0^{a}	4.7 ± 0.3^{a}	$0.86 \pm 0.03^{a,b}$	0.033 ± 0.001^{d}
Dioscorea persimilis	GXS1	71.0	8.3	0.89	476.4	467.0	14.5	17.3	4.23	0.62	0.041
	GXS2	68.2	7.7	0.92	465.5	474.2	13.2	19.3	3.82	0.67	0.044
	GXS3	72.2	8.2	0.88	469.5	467.0	14.2	18.7	4.15	0.71	0.044
		70.5 ± 2.0^{a}	8.1 ± 0.3^{a}	0.90 ± 0.02^{b}	470.4 ± 5.5^{a}	$469.4 \pm 4.4^{a,b}$	14.0 ± 0.6^{a}	$18.4 \pm 1.0^{a,b}$	4.1 ± 0.2^{a}	0.67 ± 0.05^{b}	0.043 ± 0.001^{c}
Mean		69.5	8.5	1.06	423.6	443.0	14.3	16.1	4.5	0.85	0.067
CV (%)		7.8	20.3	17.9	16.8	13.8	31.9	29.7	9.2	24.6	0.002

Values are expressed as the mean ($n = 3$).
^{a,b,c,d} Different letters in each column represent significant differences ($p < 0.05$) as assessed by analysis of variance followed by Tukey's test.
CV = coefficient of variation; DW = dry weight.

Table 2 – The amino acid contents determined for 25 yam landraces in China.

Species	Landraces	Essential amino acids (% protein)							Nonessential amino acids (g 100/g protein)								
		Thr	Val	Met	Iso	Leu	Phe	Lys	Asp	Ser	Glu	Pro	Gly	Ala	Tyr	His	Arg
Dioscorea alata	HSY	6.29	2.67	0.95	3.93	4.23	4.19	4.78	4.29	8.33	11.98	3.21	4.22	6.23	1.42	4.27	12.44
	SS1	7.81	2.01	1.42	3.40	4.71	5.45	7.89	2.51	8.37	12.70	2.22	1.60	6.56	1.47	3.21	10.65
	SS2	5.25	2.98	2.97	4.47	2.92	3.75	5.86	3.55	6.89	9.22	2.98	2.87	4.89	2.15	4.00	12.90
	SS3	4.55	2.25	2.50	1.64	3.66	1.77	7.20	2.98	6.21	7.95	4.15	3.47	5.24	0.75	3.66	10.79
	SS4	3.88	1.28	0.40	4.06	2.16	4.05	3.92	9.93	7.97	7.76	2.09	2.18	4.20	0.38	2.76	7.96
	SS5	5.72	1.32	1.23	3.48	2.56	3.98	8.11	5.98	3.10	7.70	3.02	4.58	3.29	0.67	1.87	12.24
	SS6	4.41	1.76	1.86	2.95	4.02	3.00	6.06	5.27	5.54	8.72	1.99	4.64	7.22	0.41	4.25	13.43
	SS7	7.64	2.70	0.97	3.57	5.05	4.47	4.19	6.24	7.46	7.79	2.20	4.49	6.22	0.80	4.80	8.75
	SS8	6.73	1.32	1.43	2.51	2.88	4.98	4.25	3.44	7.35	9.54	2.88	4.72	4.86	1.81	3.95	11.43
		5.81	2.03	1.53	3.33	3.58	3.96	5.81	4.91	6.80	9.26	2.75	3.64	5.41	1.10	3.64	11.18
		± 1.42^{b}	± 0.66^{a}	± 0.80^{a}	± 0.86^{a}	± 1.00^{a,b}	± 1.08^{a}	± 1.63^{a}	± 2.29^{a}	± 1.68^{b}	± 1.88^{a}	± 0.7^{a}	± 1.17^{b}	± 1.25^{a}	± 0.64^{a,b}	± 0.9^{b}	± 1.85^{b}
Dioscorea opposita	TIE	8.58	1.54	1.03	4.43	4.92	4.23	3.35	6.49	8.56	7.89	2.25	2.64	3.33	0.72	2.63	9.71
	TGS	9.14	3.01	1.11	5.05	2.33	5.28	4.02	7.64	7.37	9.55	3.33	3.48	5.23	1.02	5.69	11.22
	HZS	6.54	5.02	2.46	3.33	4.76	2.68	4.78	4.31	8.03	11.60	1.22	1.89	3.28	1.19	2.99	13.33
	BYS	5.39	1.95	2.26	3.98	3.61	4.26	5.29	6.82	4.89	9.11	1.37	3.01	4.43	0.93	3.97	11.26
	SJS	7.15	3.92	0.73	2.59	5.24	3.56	3.83	5.28	6.53	10.28	3.29	4.22	5.96	0.82	1.78	9.24
	QYS	5.76	3.59	1.79	3.19	4.61	2.88	4.65	6.73	4.28	10.13	2.18	5.33	5.72	1.71	4.04	9.74
	HNS	6.42	3.72	1.08	2.54	3.81	2.25	3.40	4.50	5.46	10.24	3.27	1.59	6.27	1.14	3.80	11.97
	CTS	8.00	2.01	0.86	3.33	4.99	4.25	3.15	4.73	7.55	9.62	1.80	2.53	6.78	1.79	3.00	10.22
	NPS	6.74	1.89	1.98	4.47	4.96	4.68	2.48	6.37	8.53	12.60	1.08	1.84	7.20	1.64	4.84	8.02
	LNS	7.30	1.72	1.67	1.97	4.36	2.05	5.64	4.72	4.58	8.50	2.21	3.90	3.78	1.29	4.25	14.45
		7.10	2.84	1.50	3.49	4.36	3.61	4.06	5.76	6.58	9.95	2.20	3.04	5.20	1.23	3.70	10.92
		± 1.19^{a}	± 1.18^{a}	± 0.61^{a}	± 0.98^{a}	± 0.89^{a}	± 1.10^{a}	± 1.01^{b}	± 1.18^{a}	± 1.66^{b}	± 1.39^{a}	± 0.86^{a}	± 1.20^{b}	± 1.43^{a}	± 0.38^{a,b}	± 1.13^{a,b}	± 1.95^{b}
Dioscorea fordii	GDS1	8.14	2.40	0.6	2.64	3.18	4.23	6.63	4.22	9.48	8.89	2.1	3.33	5.68	0.68	4.95	14.13
	GDS2	8.58	2.12	0.52	2.82	2.94	4.15	6.85	4.08	9.25	9.02	1.75	3.25	5.21	0.72	5.06	13.85
	GDS3	8.69	2.35	0.55	2.75	2.89	4.52	6.57	4.15	9.14	9.13	1.84	2.76	5.15	0.63	4.82	13.76
		8.47	2.29	0.56	2.74	3.00	4.30	6.68	4.15	9.29	9.01	1.90	3.11	5.35	0.68	4.94	13.91
		± 0.29^{a}	± 0.15^{a}	± 0.04	± 0.09^{a}	± 0.15^{b}	± 0.19^{a}	± 0.15^{a}	± 0.07	± 0.17^{a}	± 0.12^{a}	± 0.18^{b}	± 0.31^{b}	± 0.29^{a}	± 0.04^{b}	± 0.12^{a}	± 0.19^{a}
Dioscorea persimilis	GXS1	8.13	2.19	0.68	3.45	3.67	4.91	3.88	4.63	6.93	10.54	2.73	5.56	5.62	1.59	3.77	12.98
	GXS2	7.72	2.42	0.71	3.21	3.82	4.65	3.57	4.31	7.39	9.64	2.56	6.02	5.85	1.68	4.26	12.58
	GXS3	7.45	1.89	0.67	3.25	3.51	5.04	3.76	4.38	7.25	9.78	2.28	5.72	5.47	1.72	4.58	12.36
		7.73	2.17	0.69	3.30	3.67	4.89	3.74	4.43	7.19	9.98	2.52	5.77	5.65	1.66	4.20	12.64
		± 0.35$_{a}$	± 0.27^{a}	± 0.02^{a,b}	± 0.13^{a}	± 0.16^{a,b}	± 0.19^{a}	± 0.16^{b}	± 0.15	± 0.24^{b}	± 0.48^{a}	± 0.23^{a}	± 0.23^{a}	± 0.19^{a}	± 0.07^{a}	± 0.41^{a}	± 0.31^{a,b}
Mean		6.64	2.44	1.43	3.38	3.93	3.85	4.92	5.27	6.83	9.63	2.46	3.43	5.33	1.16	3.74	11.28
CV (%)		21.7	40.2	49.4	25.9	24.6	27.3	31.8	32.8	24.3	16.3	31.8	35.9	23.4	43.2	26.5	17.1

Values are expressed as the mean ($n = 3$).

[a,b] Different letters in each column indicate significant differences ($p < 0.05$) as assessed by analysis of variance followed by Tukey's test.

CV = coefficient of variation.

(3.88–9.14 g/100 g protein), Lys (2.48–8.11 g/100 g protein), and Leu (2.16–5.24g g/100 g protein) followed by Phe, Iso, Val, and Met. Similarly, considerable variability existed between amino acids among species. Namely, *D. fordii* and *D. persimilis* had higher contents of Thr, His, and Arg, while *D. alata* and *D. opposita* showed lower levels but had relatively high amounts of Met and Leu.

The ratio of EAAs to NEAAs in this study was found to be 0.44–0.66 (not shown). The proportion of the predominant EAAs in the total protein amount was comparable with the required amino acids guidelines by the FAO/World Health Organization [27]. This indicated that tubers from the yams analyzed are well balanced in amino acids. For instance, 1 g of yam tuber protein in this study can provide substantial quantities of Thr (38.8–91.4 mg), Lys (24.8–81.1 mg), Phe (17.7–54.5 mg), Leu (21.6–52.4 mg), and Iso (16.4–50.5 mg; Table 2). These investigations provided useful information on amino acid compositions, which indicated that these yam landraces are reasonably good sources of dietary amino acids and for the preparation of protein supplements.

3.2. Bioactive constituents

The contents of bioactive constituents in 25 yam landraces are presented in Table 1. The amounts of allantoin and dioscin varied considerably with values of 0.62–1.49% DW and 0.032–0.092% DW, respectively, and showed significant difference ($p < 0.05$) among species. Of the studied species, we found that *D. opposita* and *D. alata* possessed higher average contents of dioscin and allantoin (0.077% and 0.063% DW) than the other species investigated. A significant variability was also revealed among landraces within single species. For example, out of nine landraces from *D. alata*, HSY and SS1 showed significantly higher contents of dioscin and allantoin. Similarly, amongst 10 landraces from *D. opposita*, the extent of allantoin (0.67–1.23% DW) and dioscin (0.057–0.092% DW) also varied greatly depending on the type of landrace. In comparison with earlier studies for other regional yams, the studied landraces presented higher allantoin values [28,29]. This variability can probably be attributed to different planting zones, and alterable extraction and determination methods [30]. Notably, all yams tested in this study were from the section Enantiophyllum of the *Dioscorea* genus; with very few literatures reporting on dioscin content available for comparison. For yams from the Enantiophyllum section, it is very difficult to create an analytical approach which is effective in detecting dioscin, given the fact that the content is extremely low in the tuber of these yams. Therefore, the approach developed in this study is worth proposing to assess medicinal properties in yams.

3.3. UPGMA clustering and PCA analysis

In terms of the importance of nutritive and bioactive compositions, eight important parameters (starch, protein, fiber, total minerals, total EAAs, total NEAAs, allantoin, and dioscin) were used as variables after standardization to carry out statistical analysis. UPGMA clustering clearly separated the 25 yam landraces into three major clusters (Figure 1). Cluster I included three landraces of TGS, HSY, and SS1 with the

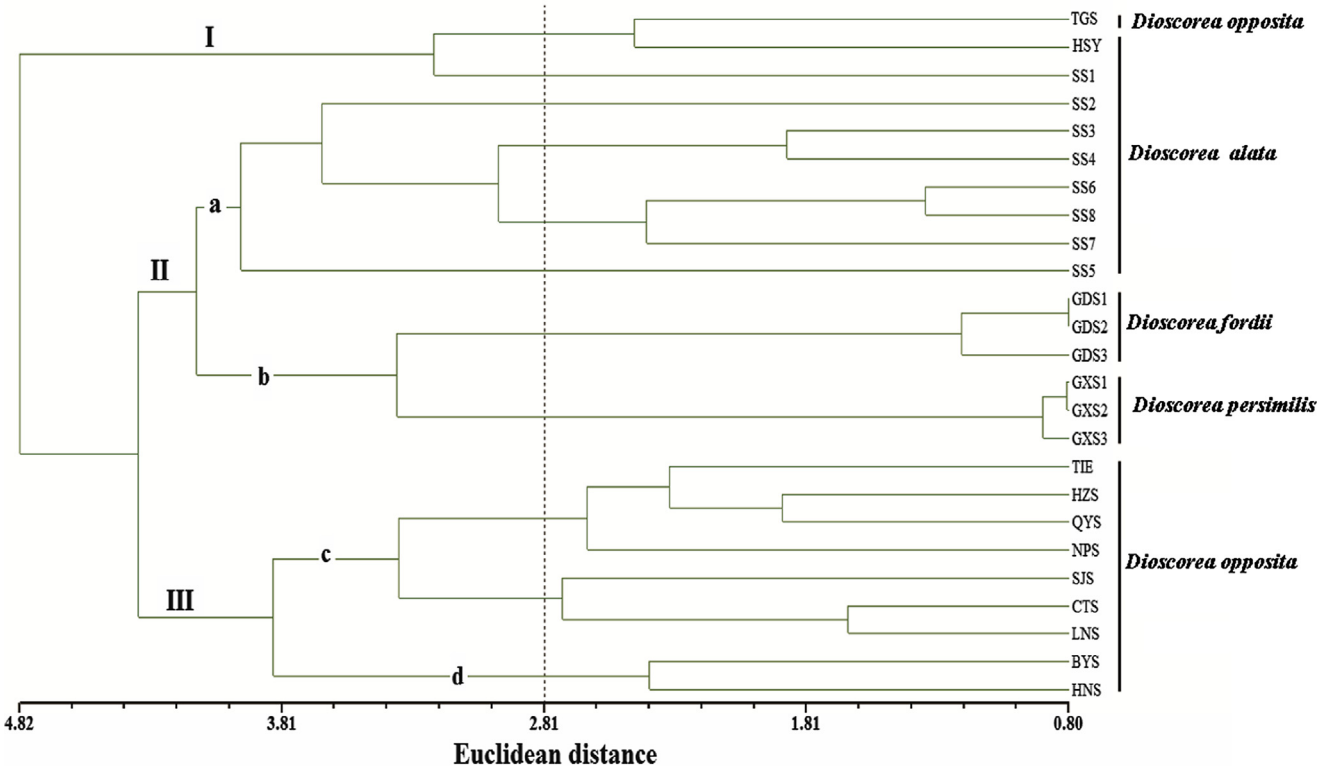

Figure 1 – Dendrogram based on unweighted pair group method with arithmetic mean clustering and Euclidean distance for 25 yam landraces using eight nutritive and bioactive parameters of yams (see Table 1).

Table 3 – Eigenvalue, variance, and factor loadings of the first eight PC factors for the tested variables.

	Factor loadings							
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Eigenvalue	2.31	1.60	1.07	0.92	0.84	0.62	0.42	0.15
Cumulative variance (%)	28.93	48.90	62.33	74.69	85.19	92.88	98.12	100.00
Starch	**−0.77**	−0.03	0.13	−0.21	0.33	0.33	0.35	0.12
Protein	0.04	0.37	0.47	**0.73**	−0.24	0.21	0.13	0.00
Fiber	0.51	−0.41	**0.55**	−0.30	−0.13	−0.22	0.33	−0.09
Total minerals	**0.72**	−0.14	−0.35	−0.05	0.02	0.55	0.10	−0.14
Total EAA	0.48	−0.18	0.33	0.22	**0.75**	−0.01	−0.17	0.02
Total NEAA	0.15	**−0.65**	−0.47	0.48	0.02	−0.20	0.24	0.12
Allantoin	0.12	**0.80**	−0.29	0.05	0.30	−0.28	0.28	−0.12
Dioscin	**0.82**	0.43	0.00	−0.22	−0.10	0.03	0.04	0.28

Bold numbers indicate the higher weight of each composition in each PC factor.
EAA = essential amino acids; NEAA = nonessential amino acids; PC = principal components.

highest contents of dioscin and allantoin. Cluster II comprised 13 landraces, and was further divided into two subgroups containing seven landraces from *D. alata* (a), and six landraces from *D. fordii* and *D. persimilis* (b). This cluster was mainly characterized by landraces with relatively high contents of starch and allantoin. The remaining landraces, which displayed similar characteristics of relatively higher fiber, total minerals, total EAAs, and dioscin content, formed Cluster III with two subgroups (c and d); all landraces included in this group originated from the species *D. opposita*.

The eigenvalue, variance, and loadings of all PC factors for the tested variables are shown in Table 3. The PCA provided eight principal components, which accounted for the total variability. Next, by using an eigenvalue ≥1 as the cutoff to define the main PC factors, we found that the first three PCs accounted for most of the total variability (62.33%). Namely, PC1, PC2, PC3 explained 28.93%, 19.97%, and 13.43% of the total variance in the variables set, respectively. The sample score plots for PC1 versus PC2 and PC1 versus PC3 are shown in Figure 2. A good separation of yam samples was achieved in both figures. Firstly, PC1 allowed separation of the landraces from *D. opposita* (Figures 2A and 2B) due to their highest PC1 values. This is closely related to the fact that the samples from this group showed relatively high levels of dioscin and minerals, moderate levels total EAAs, and low levels of starch. By contrast, the samples in Group b (Figure 2A) and Group ii (Figure 2B) showed lower PC1 values, which could be characterized by the highest levels of starch, and low levels of dioscin. Secondly, in light of the relatively high PC2 values, the group (Figure 2A) comprising TGS, SS1, and TGS was well separated by PC2. This separation was explained by the fact that the three yam individuals presented the highest level of allantoin, and a low level of total NEAAs. Overall, the chemical profiles were feasible to classify the yam accessions.

As shown in Figures 1 and 2, there appears to be a species pattern of variation in chemical compositions; with the exception of the three landraces TGS, HSY, and SS1, which were grouped into a cluster (Figure 2A). The rest of the landraces from identical species generally shared a closer relationship in terms of their chemical characteristics. It is believed that chemotypes appear to be genetically controlled by some alleles and are probably generated through segregating the physicochemical characteristics in yam breeding programs [31]. In our previous study, the studied yam species/

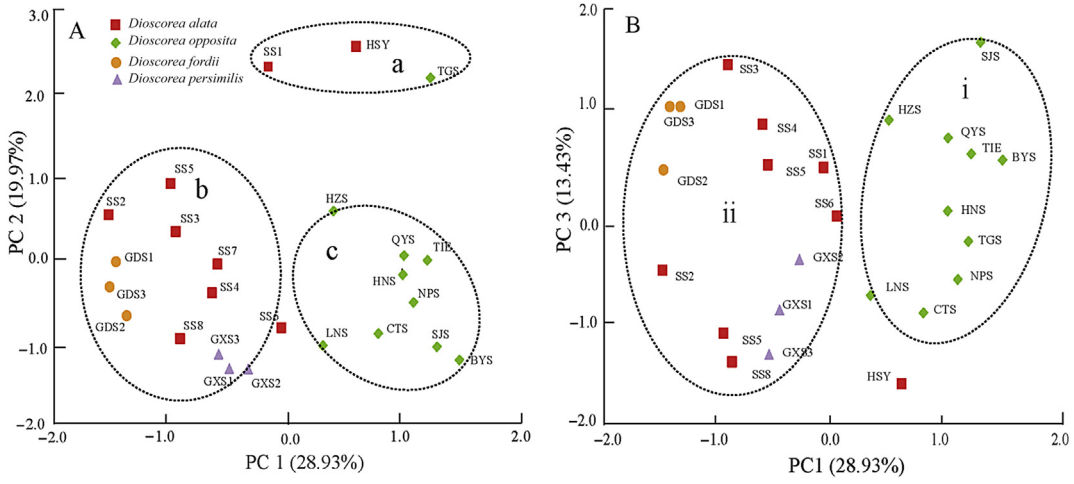

Figure 2 – Score plots of principal component (PC) analysis for 25 yam landraces based on eight nutritive and bioactive parameters: (A) PC1 versus PC2; and (B) PC1 versus PC3.

landraces were confirmed to have rich allelic diversity [17]. An explanation for compositional variability observed in the present study may be, therefore, attributed to genetic difference.

However, knowledge about the nutritive and medicinal quality of yams is essential to determine appropriate application strategies. In an effort to establish yam quality and classification, Tamiru et al [20] demonstrated that UPGMA clustering and PCA can distinguish aerial yams from those accessions with underground tubers based on the compositional and pasting properties of yam flour. Lebot et al [31] also evaluated quality, and managed to cluster 48 *D. alata* varieties from Vanuatu into three groupings by determining physicochemical characteristics. In this study, the classification of the 25 studied yam landraces was achieved by applying multivariate statistical analysis. The comprehensive PCA scores for all samples were further calculated using the model of Eq. (1), implying that TGS, HSY, and SS1 in Group a (Figure 2A) have higher PCA scores due to their nature of holding high levels of dioscin, allantoin, and minerals, of the values 1.32, 1.10, and 0.71, respectively (not shown). This result robustly supports that these landraces can be recommended as a medicinal cluster. However, the remaining landraces may be recommended for consumption as food owing to their low content of bioactive components and high content of starch, protein, and beneficial amino acids, and this result is in accordance with the current custom of yam utilization in China [5]. Overall, this classification may result in yams being more readily selected as an appropriate source of food or for use in medicinal products.

4. Conclusion

In this study, considerable variations were found among 25 yam landraces in terms of major tuber nutritional compositions and bioactive constituents. The discriminating technologies of UPGMA clustering and PCA analysis enabled visualization of this complex dataset and underlying relationships among investigated samples. The spatial distribution (Figures 1 and 2) of these samples could be clearly separated and showed distinct quality differences. The landraces of TGS, HSY, and SS1 exhibited high levels of bioactive constituents which gave them the highest PCA scores in terms of dioscin and allantoin, representing superior medicinal properties. By contrast, the rest of the landraces contained low levels of bioactive constituents and high levels of starch, protein, and amino acids, which constitute preferential sources of food. The combination of chemical characterization and multivariate data analysis provides a feasible classification of usage in yams. The classification is of great benefit in determining appropriate application strategies for yam germplasm in China.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jfda.2015.12.003.

REFERENCES

[1] Alexander J, Coursey DG. The origins of yam cultivation. In: Peter UJ, Dimbleby GW, editors. The domestication and exploitation of plants and animals. London: Cerald Duckworth; 1969. p. 405–25.
[2] Arnau G, Abraham K, Sheela MN, Chair H, Sartie A, Asiedu R. Yams. In: Bradshaw JE, editor. Root and Tuber Crops. New York: Springer-Verlag Inc; 2010. p. 127–48.
[3] Sautour M, Mitaine-Offer AC, Lacaille-Dubois MA. The *Dioscorea* genus: a review of bioactive steroid saponins. J Nat Med 2007;61:91–101.
[4] Food and Agriculture Organization (FAO). FAO STAT agriculture data. Rome, Italy: Food and Agriculture Organization of the United Nations; 2007.
[5] Huang WH. Nonpollution and standardization cultivation for yam. Beijing: China Agriculture Press; 2005.
[6] Xu GJ, Xu LS. Species systematization and quality evaluation of commonly used Chinese traditional drugs, vol. II. Fuzhou: Fujian Science and Technology Press; 1997.
[7] Wanasundera JPD, Ravindran G. Nutritional assessment of yam (*Dioscorea alata*) tubers. Plant Food Hum Nutr 1994;46:33–9.
[8] Ogbuagu MN. Nutritive and antinutritive composition of the wild (in-edible) species of *Dioscorea bulbifera* (Potato Yam) and *Dioscorea dumentorum* (Bitter Yam). J Food Technol 2008;6:224–6.
[9] Contreras-Pacheco MDL, Santacruz-Ruvalcaba F, García-Fajardo JA, Sánchez GJDJ, Ruíz LMA, Estarrón-Espinosa M, Castro-Castro A. Diosgenin quantification, characterisation and chemical composition in a tuber collection of *Dioscorea* spp. in the state of Jalisco, Mexico. Int J Food Sci Technol 2013;48:2111–8.
[10] Bhandari MR, Kasai T, Kawabata J. Nutritional evaluation of wild yam (*Dioscorea* spp.) tubers of Nepal. Food Chem 2003;82:619–23.
[11] Jiang QQ, Gao WY, Li X, Xia YZ, Wang HY, Wu SS, Huang LQ, Liu CX, Xiao PG. Characterization of starches isolated from five different *Dioscorea* L. species. Food Hydrocoll 2012;29:35–41.
[12] Lee SC, Tsai CC, Chen JC, Lin JG, Lin CC, Hu ML, Lu S. Effects of Chinese yam on hepato-nephrotoxicity of acetaminophen in rats. Acta Pharmacol Sin 2002;23:503–8.
[13] Niu CS, Chen W, Wu HT, Cheng KC, Wen YJ, Lin KC, Cheng JT. Decrease of plasma glucose by allantoin, an active principle of yam (*Dioscorea* spp.), in streptozotocin-induced diabetic rats. J Agr Food Chem 2010;58:12031–5.
[14] Lee MY, Lee NH, Jung D, Lee JA, Seo CS, Lee H, Kim JM, Shin HK. Protective effects of allantoin against ovalbumin

(OVA)-induced lung inflammation in a murine model of asthma. Int Immunopharmacol 2010;10:474–80.
[15] Wei YL, Xu YS, HanX Qi Y, Xu LN, Xu YW, Yin LH, Sun HS, Liu KX, Peng JY. Anticancer effects of dioscin on three kinds of human lung cancer cell lines through inducing DNA damage and activating mitochondrial signal pathway. Food Chem Toxicol 2013;59:118–28.
[16] Ding Z, Gilbert MG. Flora of China, Volume 24 (Dioscoreacea). In: Wu Z, Raven PH, editors. *Flagellariaceae* through *Marantaceae*. USA: Missouri Botanical Garden; 2000. p. 276–96.
[17] Wu ZG, Li XX, Lin XC, Jiang W, Tao ZM, Nitin M, Fan CY, Bao XQ. Genetic diversity analysis of yams (*Dioscorea* spp.) cultivated in China using ISSR and SRAP markers. Genet Resour Crop Evol 2014;61:639–50.
[18] Association of Official Analytical Chemists (AOAC). Official methods of analysis. 11th ed. Washington, DC: AOAC; 1984.
[19] Association of Official Analytical Chemists (AOAC). Official methods of analysis of AOAC International. 16th ed. Arlington, VA: AOAC International; 1995.
[20] Tamiru M, Maass BL, Pawelzik E. Characterizing diversity in composition and pasting properties of tuber flour in yam germplasm (*Dioscorea* spp.) from Southern Ethiopia. J Sci Food Agric 2008;88:1675–85.
[21] Bhattacharjee R, Gedil M, Sartie A, Otoo E, Dumet D, Kikuno H, Kumar PL, Asiedu R. Dioscorea. In: Kole C, editor. Wild Crop Relatives, Genomic, and Breeding Resources, Industrial Crops. Berlin: Springer-Verlag; 2011. p. 71–96.
[22] Udensi EA, Oselebe HO, Lweala OO. The investigation of chemical composition and functional properties of water yam (*Dioscorea alata*): effect of varietal differences. Pakistan J Nutr 2008;7:342–4.
[23] Abiodun OA, Akinoso R. Effect of harvesting periods on the chemical and pasting properties of trifoliate yam flour. Food Chem 2014;142:159–65.
[24] Huang CH, Chiang PY, Chen YY, Wang CCR. Chemical compositions and enzyme activity changes occurring in yam (*Dioscorea alata* L.) tubers during growth. LWT-Food Sci Technol 2007;40:1498–506.
[25] Bhandari MR, Kawabata J. Assessment of antinutritional factors and bioavailability of calcium and zinc in wild yam (*Dioscorea* spp.) tubers of Nepal. Food Chem 2004;85:281–7.
[26] Food and Agriculture Organization (FAO). Human vitamin and mineral requirement. Rome, Italy: Food and Nutrition Division; 2001.
[27] FAO/WHO. Food and Agriculture Organization/World Health Organization report energy and protein requirements. WHO Technical Report Series No. 724. Geneva: WHO; 1985.
[28] Fu YC, Ferng LH, Huang PY. Quantitative analysis of allantoin and allantoic acid in yam tuber, mucilage, skin, and bulbil of the *Dioscorea* species. Food Chem 2006;94:541–9.
[29] Yoon KD, Chin YW, Kim JW. Determination of allantoin in *Dioscorea* rhizoma by high performance liquid chromatography using cyano columns. Nat Prod Sci 2008;14:254–9.
[30] Lee SY, Ganesan P, Ahn J, Kwak HS. *Lactobacillus acidophilus* fermented yam (*Dioscorea opposita* Thunb.) and its preventive effects on gastric lesion. Food Sci Biotechnol 2011;20:927–32.
[31] Lebot V, Malapa R, Molisale T, Marchand JL. Physicochemical characterization of yam *Dioscorea alata* L. tubers from Vanuatu. Genet Resour Crop Evol 2006;53:1199–208.