Bangladesh J. Bot. **48**(4): 989-1000, 2019 (December)

DETECTION AND ANALYSIS OF FREE AMINO ACIDS IN WAXY MAIZE DH LINES BY HPLC IN NORTH-EAST CHINA

LONG JIANG, HAIYAN YU, DIANYUAN CHEN, XIAOMING YU, HUI XU,
XUE MA, BO SUN AND XIN MU FAN

College of Agronomy, Jilin Agricultural Science and Technology University,
Jilin, Jilin 132101, China

Keywords: Free amino acids, HPLC, Germplasm evaluation, Waxy maize DH lines

Abstract

Composition of free amino acid in waxy maize DH lines was analysed and the nutritional quality of waxy maize germplasm resources was evaluated by o-phthaladehyde (OPA) pre-column derivatization reversed-phase high performance liquid chromatography (HPLC). Results obtained showed that there were at least 14 kinds of free amino acids, DH57 line had the highest (25.185 mg/g), and DH21 line had the lowest (6.203 mg/g) and the average was 12.226 mg/g. The contents of essential amino acids varied from 2.59 to 14.09 mg/g, accounting for 29.55 - 75.64% of the total free amino acids. The quality of free amino acids was evaluated by comprehensive indices with principal component analysis and the first top three DH lines were DH57, DH59 and DH55. The contents of free amino acids in 60 waxy maize DH lines had obvious genetic diversity. As to the free amino acid content, the amino acids quality of DH57, DH59 and DH55 were the best, while DH21 was the worst. Results provide a theoretical guidance for the cultivation of new varieties of waxy maize with good quality.

Introduction

At present, seed companies in China regard haploid technology as the main breeding technology of maize inbred lines, which have established a special "DH production department" for the large-scale production of homozygous inbred lines in the commercial maize breeding system (Jiang *et al.* 2014, Dong *et al.* 2015). Fresh waxy maize not only tastes well but also has rich nutrition. With development of economy and improvement of living standards, the demand for fresh waxy maize grows rapidly in China, which has developed into a new industry (Shi *et al.* 2002). China is the origin of waxy maize and has rich germplasm resources (Huang and Rong 1998). In China, it had carried out the maize DH breeding in order to speed up the cultivation of excellent waxy inbred lines and applied to waxy maize breeding practice. Many studies had shown that it was likely possible that fresh waxy maize with good nutrition and health care became the staple cereal, resulting in a boom of waxy maize DH breeding (Gong *et al.* 2012). Waxy maize germplasm was the basic material to select new varieties and occupied a pivotal position in the waxy maize DH breeding process.

Free amino acids (FAA) contained various essential amino acids, which cannot be synthesized by human, and also flavour amino acids, which were important indicators to evaluate food nutritional quality. There were a lot of studies on free amino acids. Yang and Sun determined composition of free amino acids in litchi and apple and evaluated the fruit quality of different varieties (Yang *et al.* 2011, Sun *et al.* 2012). Li *et al.* (2011) analysed the composition and content of free amino acids in different types of tobacco and *Porphyra yezoensis*. It has become a public awareness that enhancement of essential amino acid contents and health benefit components in grain crops by genetic engineering and protein design can improve nutritional quality (Beauregard

---

*Author for correspondence:< jlnykjxyjl@163.com>.

and Hefford 2006, Wenefrida *et al.* 2009, Galili and Amir 2013). Enrichment of some essential amino acids, such as lysine (Lys), tryptophan (Trp), and methionine (Met) has been successful in maize (Galili and Amir 2013).

The free amino acids content in maize grain was determined by Wang *et al.* (2010) using HPLC method. Hao *et al.* (2008) measured the content of amino acid in fresh sweet and glutinous maize grains, and their results provided a reference for waxy maize breeding. There were some reports on the changes of free amino acids content in maize grains of hybrids (Caňas *et al.* 2009, Seebauer *et al.* 2004). Caňas *et al.* found that under low N fertilization conditions, maize Io line accumulated glutamine, asparagine and alanine preferentially in the developing kernels, whereas in maize F_2 line, glutamine and proline were the predominant amino acids (Caňas *et al.* 2009). Seebauer *et al.* found that the major amino acids in the cob of maize were glutamine (Gln), aspartic acid (Asp), asparagine (Asn), glutamate, and alanine. Gln concentrations dropped dramatically from 2 to 14 d after silking in both pollinated and unpollinated cobs, whereas all other measured amino acids accumulated over time in unpollinated spikelets and cobs, especially Asn (Seebauer *et al.* 2004). However, little research has been conducted on free amino acids in waxy maize inbred lines.

Currently, several new fresh waxy maize varieties are being bred in China. Although most of the waxy maize varieties have met certification requirements, a few had excellent quality. National and regional reports indicated that how to improve eating quality of waxy maize is the main task of the current waxy maize breeding (Zhang *et al.* 2008). Therefore, it is urgent for breeders to continue to find extra waxy maize germplasm resources, to evaluate and utilize existing inbred lines properly and to cultivate new waxy maize varieties. The present researchers used o-phthaladehyde (OPA) pre-column derivatization reversed phase high performance liquid chromatography (RP-HPLC) to detect the composition of free amino acids in 60 waxy maize inbred lines and screen waxy maize DH lines with excellent quality through analysis and evaluate their nutritional and eating quality. Results obtained would provide a theoretical guidance for the cultivation of new varieties of waxy maize with good quality.

Materials and Methods

Sixty waxy maize DH lines provided by Jilin Agricultural Science and Technology University special maize breeding research group were used for this experiment, which were planted in Jilin Maize Breeding Base of Jilin Agricultural Science and Technology University in May 2017. In August, 20 grains from middle ear of good grout, dried naturally and pest-free maize inbred lines were selected and put into oven at 40ºC for 24 hrs. The grains were crushed into powder by grinder and passed through a 60-mesh standard sieve and kept in sealed bags (Liu *et al.* 2007). Reagents used were ethanol, acetic acid, sodium hydroxide, sodium dihydrogen phosphate, sodium tetraborate, phthalaldehyde and mercaptoethanol of analytical grade. Acetonitrile and methanol (Sigma, USA) were of HPLC grade. Ultrapure water was prepared by Milli-Q ultrapure water system (Millipore, USA).

Dionex U3000 high performance liquid chromatography system including DGP-3600 pump, WPS-3000 auto sampler, TCC-3100 automatic temperature control oven, DAD-3000 detector and a chameleon chromatography workstation were used. AUW220 electronic balance (Daojin, Japan), HWS-26 electric heated water bath (Shanghai, China), KQ-100DE CNC ultrasonic cleaner (Kunshan Ultrasonic Instrument Co., Ltd, Kunshan, China) and Sigma3-30k high-speed refrigerated centrifuge (Sigma, USA) were used.

The column was Agilent Eclipse XDB-C18 column (5 µm, 250 mm × 4.6 mm), the column temperature was 40ºC, the detection wavelength was 338 nm and the flow rate was 1 ml/min.

Mobile phase Buffer A was methanol: acetonitrile: water = 46:46:10 (v/v/v); mobile phase buffer B was 10 mM sodium dehydrogenate phosphate (pH 7.5). The following elution program was applied:100% buffer B, 0 min; 82% buffer B, 10 min; 76% buffer B, 15 min; 59% buffer B, 21 min; 57.8% buffer B, 23 min; 42% buffer B, 25 min; 41% buffer B, 27min and 100% buffer B, 31 min.

Five microliter borate buffers (pH 9.5) were drawn by an automatic syringe, then 1.0 µl o-phthaladehyde (OPA) was drawn and the syringe was washed once. Two microliter sample was drawn, and the syringe was washed once. The sample was mixed eight times *in situ* and after 90 sec, the simple was injected.

The present workers accurately weighed 0.3 g sample and mixed it with same volume of distilled water and loaded it into test tube. The sample was subjected to sonication treatment for 30 min at room temperature, and centrifuged at 12000 r/min at 4ºC for 15 min. The residue was extracted once again. The two supernatants were combined and filtered through a 0.22 µm Millipore nylon. The sample was stored in −20℃ and analysed by o-phthaladehyde (OPA) pre-column derivatization reversed phase HPLC.

According to retention time of 16 kinds of amino acid standards, the types of amino acids were determined. Using a series of concentrations of amino acid standard as abscissa and peak area as vertical axis, linear equations of 16 kinds of amino acids were obtained and free amino acid contents of samples were calculated using area normalization method (Peter *et al.* 1979).

Principal amino acids components were analysed by SPSS20 software package (www.spss.com/statistics) according to the following formula:

$I = a_1 y_1 + a_2 y_2 ... + a_m y_m$,

where I represents comprehensive evaluation index of sample; a_m presents variance contribution of main component; y_m presents every main component value of each sample.

Results and Discussion

Under the chromatographic conditions described above, a series of different concentrations of mixed solution of 16 kinds of amino acids standards were measured and their standard chromatograms were obtained (Fig. 1). To obtain the linear equations of 16 kinds of amino acids, the peak area Y against the corresponding concentration X (µg/ml) to establish standard curves (Table 1). The results showed that the detected concentrations were in a good linear relationship and all the correlation coefficients (r) were greater than 0.9990.

Free amino acids in a sample through OPA *in situ* derivatization were separated and detected by RP-HPLC, and their chromatogram peaks were shown in Fig. 2. Qualitative analysis showed that 16 kinds of free amino acids were detected in the samples including seven kinds of essential amino acids and 9 kinds of non-essential amino acids. The results of free amino acids content showed that at least 14 kinds of amino acids were detected in 60 waxy maize DH lines (Table 2). DH57 line had the highest free amino acids content (25.185 mg/g), while DH21 line had the lowest free amino acids content (6.203 mg/g). The average free amino acids content for 60 waxy corn inbred lines was 12.226 mg/g. The free amino acids contents in 24 DH lines were higher than the average value. As to composition of free amino acids in the samples, Asp, Glu, Ser and Tyr were the top four amino acids. The total content of these four amino acids was between 3.205 and 19.603 mg/g, accounting for 30.88 - 79.84% of the total content of the free amino acids. Thr content was the lowest, and was not detected in the majority of samples.

Table 1. The regression analysis of 16 standard amino acids of waxy maize DH lines.

Amino acids	Linear equation	Correlation
Asp	y = 0.0068x + 0.0124	0.9993
Glu	y = 0.0125x + 0.0176	0.9989
Ser	y = 0.0373x − 0.0688	0.9992
His	y = 0.0215x − 0.0287	0.9994
Gly	y = 0.0733x − 0.1247	0.9993
Thr	y = 0.0348x − 0.0353	0.9992
Ala	y = 0.0375x − 0.1047	0.9990
Arg	y = 0.0288x − 0.0744	0.9995
Cys	y = 0.0307x − 0.0450	0.9994
Tyr	y = 0.0083x + 0.0667	0.9989
Val	y = 0.0166x − 0.0274	0.9991
Met	y = 0.0687x + 0.0330	0.9994
Phe	y = 0.0241x − 0.0389	0.9993
Iso	y = 0.0273x − 0.0159	0.9995
Leu	y = 0.0568x − 0.0159	0.9997
Lys	y = 0.0336x − 0.1640	0.9992

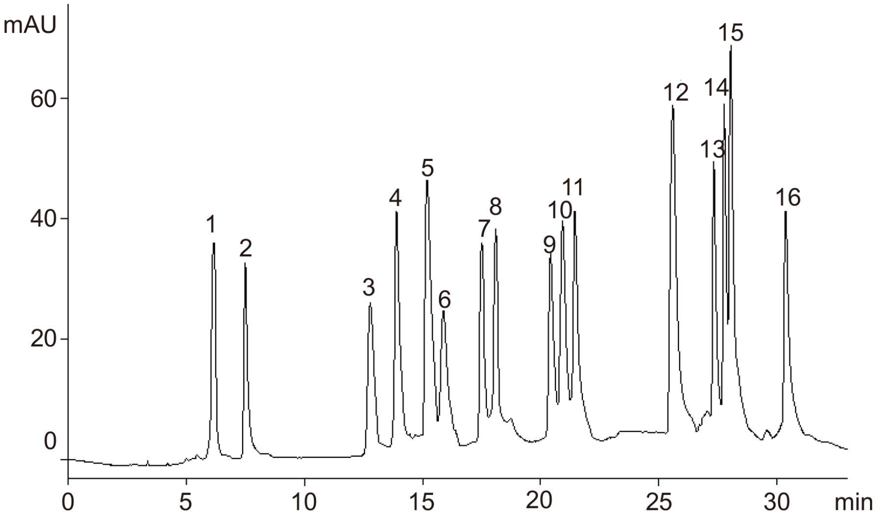

Fig. 1. A HPLC chromatogram of amino acid standards. 1. Asp 2. Glu 3. Ser 4. His 5. Gly 6. Thr 7. Ala 8. Arg 9. Cys 10. Tyr 11. Val 12. Met 13. Phe 14. Ile 15. Leu 16. Lys.

Free amino acids content varied largely in different waxy maize DH lines from 0.000 to 8.727 mg/g (Table 3). Thr and Ala had greater variability and the variation coefficient of them was greater than 100%. Arg, Cys, Val, Asp, Tyr, Ser, Met and Lys were in the middle and the variation coefficient of them ranged from 50 to 100%. Glu, Gly, His, Leu, Phe and Ile had lower variability and the variation coefficient of them was less than 50%. The types of free amino acids in waxy maize inbred lines were significantly different.

With the continuous improvement of people's living standard, people are increasingly concerned about the nutritional quality and eating quality of waxy maize. Improvement of eating quality of waxy maize is the main task of the current waxy maize DH breeding (Galili and Amir 2013). Previously, the inheritance of free amino acids in maize was studied and the breeders could select some inbred lines with high content of free amino acids as parents to nurture new varieties with high amino acid content. Ke *et al.* (2006) found that the genetic traits of maize amino acids showed the following characteristics: Isoleucine, arginine and glycine contents were mainly influenced by additive gene effect; Lysine, methionine and aspartic content were influenced by both additive effect and non-additive effect; The remaining ten kinds of amino acids content were mainly affected by non-additive effect; Proline, phenylalanine, valine, leucine and alanine had relatively high broad heritability, while isoleucine, arginine and glycine had relatively high narrow heritability. Qi *et al.* (2001) also found that to improve lysine content in maize grain, parents must have high lysine content. To increase methionine content, difference in methionine content between parent inbred lines should not be too large and high content in both parent lines was the best method.

Those amino acids which cannot be synthesized by human (or other invertebrates) or their synthetic speeds are far from body need and must be provided by food proteins are called essential amino acids including Ile, Leu, Lys, Thr, Val, Phe and Met. The content of essential amino acids (EAA) in waxy maize DH lines was between 2.71 mg/g and 14.21 mg/g, accounting for 29.67 - 75.76% of the total free amino acids. The average value of them was 6.09 mg/g (Table 2). The content of each essential amino acid was also different in different waxy maize DH lines. DH59 line had the highest Ile and Met contents. DH55 line had the highest Leu content. DH58 line had the highest Lys, Thr, Val and Phe contents. Although human body can synthesize His and Arg, their synthetic speeds usually can’t meet the normal need. Therefore, they were also called semi-essential amino acids or conditionally essential amino acids and were essential amino acids for the growth of children. In DH lines, the semi-essential amino acids content was between 0 and 2.21 mg/g, accounting for 0 to 14.03% of total free amino acids. The average content of them was 0.98 mg/g. DH36 line had the highest His content. DH16 line had the highest Arg content. The amino acids which can be synthesized from simple precursors by human (or other vertebrate) body do not need to be obtained from food are called non-essential amino acids. The content of non-essential amino acid was between 2.95 and 14.39 mg/g in waxy maize DH lines.

Foods contained some flavour amino acids, i.e. Glu, Asp, Gly and Ala. Those can interact with a variety of taste receptors in the mouth, so it can make food delicious. Umami amino acids included Glu and Asp. Sweet amino acids included Ala, Gly and Ser. Aromatic amino acids included Tyr and Phe. Waxy maize’s taste was very important, in which flavor amino acids played an important role. Analysis showed that of the total free amino acids, umami amino acid content in waxy maize DH lines was between 0.887 and 9.619 mg/g, accounting for 6.09 - 47.48% and the average content was 3.37 mg/g (Table 2), sweet amino acid content was between 0.736 and 4.240 mg/g, accounting for 4.97 - 30.84% and the average content was 1.97 mg/g (Table2), aromatic amino acid content was between 0.784 and 9.389 mg/g, accounting for 8.61 - 48.28% and the average content was 3.23 mg/g (Table 2).

Table 2. Free amino acids composition and relative quantity in waxy maize DH lines (mg/g).

No.	Asp$^{\triangle\blacksquare}$	Glu$^{\triangle\blacksquare}$	Ser$^{\triangle\blacklozenge}$	His$^{\triangle\bigstar}$	Gly$^{\triangle\blacklozenge}$	Thr$^{\blacktriangle}$	Ala$^{\triangle\blacklozenge}$	Arg$^{\triangle\bigstar}$	Cys$^{\triangle}$	Tyr$^{\triangle\bullet}$	Val$^{\blacktriangle}$	Met$^{\blacktriangle}$	Phe$^{\blacktriangle\bullet}$	Ile$^{\blacktriangle}$	Leu$^{\blacktriangle}$	Lys$^{\blacktriangle}$	TAA	E/T/%	CE/T/%	DAA/T/%
DH1	2.212	2.843	0.835	0.719	0.058	-	0.212	0.234	0.112	1.788	0.666	0.122	0.664	0.432	0.322	0.646	11.818	38.86	8.02	72.48
DH2	0.678	2.892	1.078	0.624	0.052	-	0.224	0.236	0.142	1.227	0.556	0.082	0.594	0.466	0.222	0.576	9.686	38.14	8.62	70.67
DH3	0.388	1.664	1.312	0.642	0.044	-	0.184	0.164	0.078	1.036	0.612	0.042	0.316	0.386	0.162	0.417	7.468	39.88	10.68	66.68
DH4	1.622	1.928	2.288	0.532	0.064	0.192	0.171	0.222	0.274	2.720	0.512	0.078	0.356	0.472	0.264	1.122	12.834	43.22	5.68	71.58
DH5	1.322	2.058	2.332	1.120	0.068	0.112	0.218	0.162	0.242	2.906	0.444	0.108	0.478	0.536	0.382	1.430	13.886	45.42	8.92	67.52
DH6	3.952	2.366	1.058	1.114	0.126	0.062	0.216	0.180	0.168	2.762	0.942	0.123	0.512	0.232	0.186	0.586	14.554	36.48	8.93	75.30
DH7	0.588	2.062	1.516	0.752	0.056	-	0.142	0.108	0.121	1.808	0.842	0.070	0.420	0.432	0.286	0.596	9.762	45.48	8.82	67.43
DH8	1.412	1.836	1.268	1.124	0.092	-	0.140	0.240	0.304	2.657	0.713	0.109	0.441	0.467	0.331	1.019	12.138	47.26	11.12	64.64
DH9	1.478	2.102	2.365	0.964	0.056	-	0.268	0.245	0.126	1.342	0.762	0.122	0.596	0.382	0.349	0.724	11.867	35.87	10.36	69.27
DH10	1.234	2.916	2.372	1.246	0.067	-	0.212	0.169	0.212	2.280	1.023	0.114	0.689	0.496	0.392	0.818	14.166	40.72	9.92	68.84
DH11	2.835	3.482	1.262	1.196	0.158	0.052	0.316	0.307	0.272	4.367	2.496	0.212	0.814	0.630	0.510	0.867	19.700	50.16	7.62	66.85
DH12	1.628	2.939	2.328	1.288	0.127	-	0.274	0.338	0.198	2.875	0.673	0.152	0.726	0.539	0.472	0.896	15.410	41.09	10.42	70.57
DH13	0.977	2.046	1.155	0.771	0.072	-	0.140	0.130	0.071	0.412	1.165	0.096	0.360	0.413	0.263	0.638	8.700	38.49	10.35	59.22
DH14	0.986	2.842	1.065	0.858	0.106	-	0.157	0.156	0.064	0.444	1.077	0.130	0.359	0.300	0.250	0.674	9.469	34.17	10.71	62.94
DH15	1.431	3.133	1.187	0.998	0.111	-	0.149	0.124	0.107	0.553	1.070	0.112	0.569	0.432	0.415	0.651	11.042	34.43	10.16	64.59
DH16	0.536	1.564	1.353	1.048	0.100	0.133	1.456	1.045	0.285	2.953	1.257	0.254	0.665	0.823	0.609	1.023	15.102	50.28	13.86	57.06
DH17	0.468	2.038	1.704	1.035	0.118	0.072	0.181	0.167	0.169	1.900	0.700	0.142	0.677	0.657	0.456	0.832	11.296	47.49	10.46	62.73
DH18	1.281	1.481	2.367	0.376	0.087	0.071	0.404	0.165	0.545	2.367	1.204	0.060	0.585	0.609	0.228	0.914	12.745	46.82	4.25	67.25
DH19	0.600	1.479	1.820	0.511	0.056	-	0.173	0.146	0.432	1.522	0.437	0.032	0.399	0.259	0.425	0.716	9.008	42.08	7.29	67.17
DH20	1.166	1.319	1.912	0.561	0.091	-	0.244	0.185	0.327	1.165	0.622	0.056	0.453	0.508	0.356	0.722	9.687	40.07	7.70	65.55
DH21	0.337	0.652	1.018	0.260	0.057	-	0.152	0.106	0.247	1.186	0.628	0.046	0.351	0.390	0.168	0.778	6.203	55.80	5.73	58.62
DH22	0.815	0.938	2.015	0.499	0.132	-	0.154	0.152	0.351	1.910	0.983	0.050	0.364	0.368	0.156	0.523	9.407	46.30	6.93	67.22
DH23	1.750	1.586	0.626	0.639	0.057	0.028	0.226	0.216	0.583	2.234	1.131	0.140	0.498	0.464	0.327	0.689	11.165	48.85	7.66	62.32
DH24	1.583	1.339	0.780	0.541	0.059	-	0.153	0.146	0.294	0.989	0.262	0.043	0.337	0.267	0.170	0.404	7.366	33.56	9.33	71.13
DH25	1.822	0.931	0.592	0.517	0.070	-	0.125	0.149	0.406	1.423	0.379	0.090	0.356	0.382	0.196	0.467	7.905	41.66	8.43	67.28
DH26	1.802	1.661	0.793	0.683	0.062	0.037	0.191	0.165	0.702	2.558	0.564	0.097	0.378	0.472	0.205	0.475	10.826	43.89	7.65	68.79
DH27	1.940	2.843	0.867	0.714	0.079	0.028	0.180	0.214	0.631	2.288	0.869	0.070	0.317	0.432	0.190	0.541	12.214	38.54	7.60	69.79
DH28	0.643	0.997	1.359	0.866	0.097	-	0.211	0.170	0.326	4.311	2.147	0.090	0.521	0.317	0.281	0.482	12.819	63.58	8.08	63.50
DH29	1.802	1.880	0.679	0.908	0.080	0.089	0.264	0.278	0.454	1.938	0.660	0.098	0.499	0.342	0.303	0.720	10.985	41.43	10.80	65.02
DH30	0.472	1.272	2.019	0.973	0.069	0.042	0.142	0.134	0.352	1.326	0.512	0.076	0.468	0.498	0.258	0.540	9.144	40.23	12.10	62.96

(Contd.)

DH31	1.511	1.843	1.460	0.940	0.057	-	0.165	0.132	0.565	1.966	0.436	0.071	0.355	0.297	0.235	0.506	10.539	36.69	10.18	69.80
DH32	0.948	1.230	0.975	0.670	0.061	0.106	0.460	0.252	0.459	3.330	1.671	0.145	0.573	0.407	0.291	0.615	12.195	57.66	7.56	62.15
DH33	0.251	1.263	1.048	0.697	0.059	0.037	0.226	0.298	0.756	2.768	1.044	0.148	0.525	0.517	0.365	1.317	11.318	59.07	8.79	54.24
DH34	0.684	1.663	0.530	0.604	0.061	-	0.179	0.219	0.600	2.167	0.599	0.117	0.495	0.404	0.283	0.573	9.180	50.53	8.97	62.97
DH35	2.379	1.788	0.818	0.737	0.077	-	0.317	0.198	1.080	2.878	0.487	0.117	0.500	0.340	0.286	0.677	12.678	41.68	7.38	69.06
DH36	1.033	1.997	3.489	1.609	0.108	-	0.182	0.103	0.154	1.718	0.280	0.087	0.417	0.354	0.226	0.546	12.302	29.49	13.91	72.70
DH37	2.029	1.668	0.906	0.997	0.071	-	0.373	0.194	0.351	3.530	0.364	0.133	0.529	0.537	0.305	0.578	12.565	47.57	9.48	72.47
DH38	0.397	1.194	1.642	0.897	0.078	-	0.229	0.162	0.079	1.069	0.776	0.070	0.446	0.415	0.172	0.495	8.114	42.43	13.05	62.23
DH39	1.027	1.693	1.459	0.806	-	-	0.293	0.225	0.200	1.488	1.003	0.091	0.465	0.585	0.205	0.513	10.055	43.27	10.26	63.91
DH40	0.409	1.405	1.113	0.693	0.041	-	0.229	0.165	0.095	2.947	0.685	0.091	0.574	0.402	0.330	0.747	9.925	58.20	8.64	67.68
DH41	1.338	1.766	1.038	0.695	0.038	0.336	0.475	0.204	0.166	2.666	0.927	0.158	0.467	0.452	0.309	0.687	11.722	48.33	7.67	66.44
DH42	0.795	1.335	1.700	1.016	0.045	-	0.238	-	0.122	2.924	1.005	0.085	0.345	0.424	0.261	0.541	10.835	51.55	9.37	68.12
DH43	2.094	2.038	0.602	0.613	0.068	-	0.522	-	0.231	4.029	0.761	0.144	0.575	0.524	0.341	0.675	13.217	53.34	4.64	75.11
DH44	1.536	1.473	2.453	0.505	0.149	-	0.574	-	0.263	3.202	0.975	0.166	0.816	0.591	0.486	0.863	14.052	50.52	3.59	72.61
DH45	0.634	1.794	1.034	0.620	0.084	-	0.340	-	0.127	1.934	0.682	0.152	0.745	0.593	0.311	0.819	9.861	53.01	6.29	66.58
DH46	1.175	1.253	0.468	0.555	0.094	-	0.162	0.154	0.128	2.229	0.553	0.100	0.399	0.423	0.259	0.758	8.710	54.21	8.14	66.35
DH47	1.171	1.916	0.821	0.663	0.084	-	0.150	0.169	0.092	1.202	0.400	0.075	0.477	0.355	0.303	0.586	8.444	40.27	9.85	68.69
DH48	1.408	2.331	2.480	1.360	0.101	-	0.350	0.377	0.145	3.488	0.786	0.112	0.443	0.405	0.358	0.748	14.893	42.58	11.67	71.19
DH49	1.287	1.275	0.516	0.723	0.088	-	0.213	0.306	0.129	3.474	0.890	0.147	0.575	0.428	0.324	0.804	11.178	59.43	9.20	66.44
DH50	0.470	0.404	1.575	0.643	0.065	-	0.316	-	0.113	6.428	2.451	0.104	0.630	0.367	0.302	0.785	14.653	75.52	4.39	67.48
DH51	1.875	2.404	3.775	1.414	0.127	-	0.326	-	0.144	3.911	0.018	0.147	0.526	0.427	0.370	0.839	16.284	38.18	8.68	79.37
DH52	2.778	2.940	0.514	0.913	0.081	-	0.489	0.281	0.162	4.420	1.087	0.169	0.650	0.467	0.339	0.967	16.256	49.82	7.35	73.03
DH53	1.712	2.439	1.129	0.686	0.070	-	0.427	-	0.088	2.718	0.692	0.114	0.562	0.490	0.280	0.840	12.247	46.52	5.60	73.95
DH54	1.327	2.249	1.108	1.151	0.168	-	0.533	-	0.156	5.249	1.978	0.260	0.720	0.580	0.488	1.477	17.443	61.64	6.60	65.09
DH55	1.441	2.322	1.377	1.079	0.206	-	0.857	-	0.311	6.173	1.279	0.317	0.743	0.719	0.612	1.419	18.956	59.94	5.69	69.73
DH56	0.975	1.809	3.606	0.000	0.074	-	0.404	-	0.227	4.344	1.058	0.146	0.662	0.601	0.370	1.631	15.906	55.40	0.00	74.64
DH57	4.620	4.987	3.282	0.644	0.154	0.178	0.183	-	0.219	6.702	0.717	0.277	0.807	0.729	0.326	1.340	25.185	43.31	2.46	82.40
DH58	1.234	0.529	0.020	0.800	0.168	0.616	0.586	-	0.472	3.162	2.890	0.102	1.418	0.881	0.354	2.728	15.961	72.27	5.01	44.60
DH59	0.976	0.792	0.576	0.792	0.094	-	2.295	0.917	0.248	8.727	0.420	0.328	0.650	1.088	0.418	2.336	20.658	67.61	8.37	68.28
DH60	3.381	1.494	1.498	0.256	0.080	0.072	0.132	0.148	0.203	1.834	0.308	—	0.236	0.230	0.063	0.359	10.295	29.43	3.94	84.29
Average	1.367	1.884	1.428	0.794	0.096	0.146	0.335	0.218	0.287	2.669	0.866	0.131	0.525	0.484	0.362	0.824	12.226	46.64	8.45	67.58

(1) ▲ Essential amino acid (E); △ Non Essential amino acid; ★ Child Essential amino acid(CE); ■ Flavor amino acid; ◆ Sweet amino acid; ● Aromatic amino acid; (2) TAA: Total amino acid; (3)-: Not detected and (4) Delicious amino acid（DAA）=■+◆+●.

Sixteen kinds of free amino acids were comprehensively analysed in 60 waxy maize DH lines. Each amino acid content and total amount of all kinds of free amino acids were highest in DH57, DH59 and DH55 lines (Table 2). This result was consistent with the overall score result, indicating that the nutritional and eating quality of the three inbred lines were optimal. The result that DH21 had the lowest score was consistent with the fact that its free amino acids content was the lowest, indicating that amino acids quality in DH21 was poor. Currently, the ear taste identification for waxy maize DH breeding process was usually carried out after the hybrids were planted and seldom carried out in inbred lines. The artificial taste identification was not only affected by the food quality, but also by the people's taste preferences. Those results were largely influenced by human error (Liu *et al.* 2009), resulting in unclear direction of the genetic improvement of waxy maize. By measuring and analysis of flavour amino acids in DH lines, combining the eating quality with artificial tasting of DH lines can significantly improve the efficiency of waxy maize DH breeding.

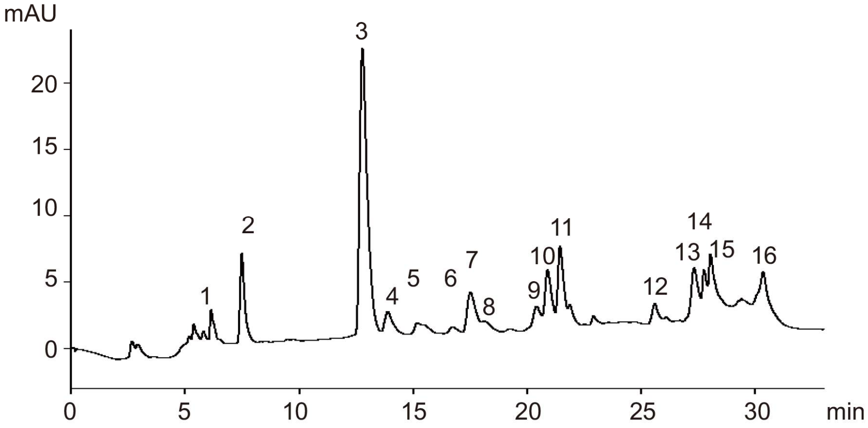

Fig. 2. A HPLC chromatogram of free amino acids in the sample. 1. Asp 2, Glu 3, Ser 4, His 5, Gly 6, Thr 7, Ala 8, Arg 9, Cys 10, Tyr 11, Val 12, Met 13, Phe 14, Ile 15, Leu 16. Lys.

Detected 16 kinds of free amino acids were chosen and SPSS20 software was used to analyse principal component of their main contents, and obtained 16 principal components which represent 100% of all the original information.

The first principal component was PC1 (51.68%), the second principal component was PC2 (20.28%) and the third principal component was PC3 (12.49%), the above three principal components contained 84.22% of all the original information, which were usually considered to represent the majority of original information (Massart *et al.* 1997). Thus, the present investigators decided to extract the main ingredients from these three principal components. The first principal component included eight indicators: PC1= 0.472 Gly + 0.597 Ala + 0.989 Tyr + 0.769 Met + 0.498 Phe + 0.574 Ile + 0.466 Leu + 0.612 Lys; the second principal component included two indicators PC2 = 0.768Asp + 0.848Glu, the third principal component included one indicator PC3 = 0.882Ser. When using the principal component to make a comprehensive evaluation, main ingredients can be selected, namely $Y_{1,}$ $Y_{2\,...}$ Y_m, with variance contribution rate of each principal component as weights to construct comprehensive evaluation index. In this paper, according to the three extracted principal components, investigators calculated the comprehensive evaluation indexes of different waxy maize DH lines (Table 4). Usually the larger the index, the better the comprehensive quality (Wang and Yang 2010). The order of the comprehensive evaluation indexes of three inbred lines was DH57 > DH59 > DH55, they were greater than 1. This result showed that the comprehensive evaluations index of three waxy maize DH lines were the best; the comprehensive evaluation index of DH21 was the lowest.

Table 3. Variance of amino acid content of different waxy maize inbred lines (mg/g).

	Min.	Max.	Extreme	Average	Sd	CV/%
Asp	0.248	4.624	4.368	1.378	0.854	62.028
Glu	0.403	4.878	4.584	1.876	0.768	41.946
Ser	0.022	3.757	3.756	1.448	0.816	56.746
His	0.000	1.612	1.612	0.796	0.296	37.060
Gly	0.042	0.208	0.164	0.084	0.045	40.698
Thr	0.000	0.616	0.626	0.048	0.098	255.262
Ala	0.132	2.294	2.172	0.335	0.336	102.482
Arg	0.000	1.046	1.065	0.183	0.178	97.822
Cys	0.062	1.080	1.026	0.287	0.212	72.543
Tyr	0.414	8.727	8.325	2.698	1.602	59.284
Val	0.028	2.892	2.874	0.886	0.556	62.538
Met	0.000	0.338	0.338	0.129	0.068	53.782
Phe	0.242	1.422	1.182	0.545	0.182	33.842
Ile	0.230	1.088	0.868	0.474	0.156	32.708
Leu	0.060	0.612	0.552	0.312	0.114	33.964
Lys	0.362	2.734	2.379	0.820	0.436	51.952
Total	6.405	25.175	18.762	12.246	3.522	28.714

Through analyzing types and contents of free amino acid in grains of 60 waxy maize DH lines, the following conclusions can be made: (1) Sixteen kinds of free amino acids were detected in waxy maize DH lines including seven kinds of essential amino acids and nine kinds of non-essential amino acids. At least 14 kinds of amino acids were detected, and the variation coefficients of ten kinds of amino acid were greater than 50%. The total content of free amino acids was between 6.203 and 25.185 mg/g, showing a significant genetic diversity in each DH line. (2) Of the total free amino acids, umami amino acids content accounted for 6.09 - 47.48%, sweet amino acids content accounted for 4.97 - 30.84% and aromatic amino acids content accounted for 8.71 - 48.28%. Those results indicated that the nutritional quality and eating quality were different in each inbred line. (3) The method of principal component analysis was used to comprehensively evaluate 60 DH lines and construct comprehensive evaluation index, 23 DH lines were higher than the average score and the rest of DH lines were close to the average score, in which scores of DH57, DH59 and DH55 lines were outstanding. (4) Based on the analysis of the total content of free amino acids, essential amino acids content, flavor amino acids content and the main component of comprehensive evaluation score, it was concluded that DH57, DH55 and DH59 had the most optimal quality, while DH21 had the worst quality.

Table 4. Comprehensive value index of different samples of waxy maize DH lines

No.	Comprehensive value index	No.	Comprehensive value index	No.	Comprehensive value index	No.	Comprehensive value index	No.	Comprehensive value index	No.	Comprehensive value index
DH1	–0.1682	DH11	0.7878	DH21	–0.8102	DH31	–0.2044	DH41	–0.1255	DH51	0.9835
DH2	–0.4081	DH12	0.4332	DH22	–0.3171	DH32	–0.0904	DH42	–0.0313	DH52	0.5747
DH3	–0.6388	DH13	–0.7203	DH23	–0.3306	DH33	–0.2702	DH43	0.2745	DH53	0.0525
DH4	0.2201	DH14	–0.6146	DH24	–0.6898	DH34	–0.4668	DH44	0.3127	DH54	0.6991
DH5	0.2994	DH15	–0.4621	DH25	–0.6427	DH35	–0.0113	DH45	–0.3854	DH55	1.0698
DH6	0.2664	DH16	–0.0533	DH26	–0.1884	DH36	0.1634	DH46	–0.4575	DH56	0.8618
DH7	–0.3011	DH17	–0.2221	DH27	–0.0666	DH37	0.1517	DH47	–0.5746	DH57	2.2788
DH8	–0.0383	DH18	0.0237	DH28	0.1875	DH38	–0.6163	DH48	0.5147	DH58	–0.328
DH9	–0.1341	DH19	–0.3951	DH29	–0.3333	DH39	0.0002	DH49	–0.0869	DH59	1.3571
DH10	0.2202	DH20	–0.4305	DH30	–0.4421	DH40	–0.1783	DH50	0.7172	DH60	–0.1085

Acknowledgements

The authors gratefully appreciate the helpful comments and suggestions from Dr. Rengui Zhao and other anonymous reviewers. Thanks for PhD Research Foundation 2018[5002] and the Scientific Research Project Fund of Jilin Provincial Education Department (JJKH20190979KJ).

References

Beauregard M and Hefford MA 2006. Enhancement of essential amino acid contents in crops by genetic engineering and protein design. Plant Biotechnol. J. **4**(5): 561-574.

Caňas RA, Quillerė I, Chirst A and Hirel B 2009. Nitrogen metabolism in the developing ear of maize (*Zea mays*): analysis of two lines contrasting in their mode of nitrogen management. New Phytol. **184**(2): 340-352.

Dong ZS, Lu H, Chai YC and Cai Z 2015. Concept and practices of maize commercial breeding in China. J. Maize Sci. **23**: 1-9.

Galili G and Amir R 2013. Fortifying plants with the essential amino acids lysine and methionine to improve nutritional quality. Plant Biotech. J. **11**(2):211-222.

Gong KJ, Liu ZX and Chen LR 2012. Discussion on the fresh waxy corn served as staple food. Chinese Agric Sci Bulletin, **28**(36): 312-316.

Hao XQ, Wu ZK and Zhao G 2008. Studies on the amino acid content of sweet-waxy maize kernels for fresh food. J. Maize Sci. **16**(6): 62-67.

Huang YB and Rong TZ 1998. The genetic diversity and evolutionary origins of waxy maize germplasm resources in China. Crops (supplement), pp. 77-80.

Jiang L, Ci JB, Cui XY and Zhang Y 2014. Study on induction rate and doubling rate of maize haploid under different ecological conditions. J Jilin Agric Univ **36**(2): 139-143.

Ke YP, Shi HC and Niu YZ 2006. Genetic and combining ability analysis of nutritional quality traits in maize. J Sichuan Univ: Natural Sci. **43**(5): 1146-1153.

Li RX, Yin JF and Shen SD 2011.Analysis of free amino acid composition in *Porphyra yezoensis*. Amino Acids & Biotic Resources **33**(1): 4-9.

Lindroth P and Mopper K 1979. High Performance Liquid Chromatographic Determination of Subpicomole Amounts of Amino Acids by Precolumn Fluorescence Derivatization with *o*-Phthaldialdehyde. Analytical Chemistry **51**(11): 1667-1674.

Liu P, Lu WP and Lu DL 2009. Quality differences and physicochemical index screening for quality evaluation in waxy corn. J. Yangzhou Univ. (Agric and Life Sci. edn.) **30**(3):16-21.

Liu RS, Yang HQ and Huang YW 2007. Extraction, purification and analysis methods of free amino acid in the plant. J of Henan Univ of Sci and Tech: Natural Sci. **28**(3): 76-79.

Massart DL, Vandeginste BGM, Buydens LMC, de Jong S, Lewi PJ, Smeyers-Verbeke J and Mann CK 1998. Handbook of Chemometrics and Qualimetrics. Applied Spectroscopy **52**(8): 302A.

Qi X, Zhao YJ and Liang J 2001. Analysis of heterosis on amino-acids of maize. J. Jilin. Agric. Univ. **23** (2): 17-20.

Seebauer JR, Moose SP and Fabbri BJ 2004. Amino acid metabolism in maize earshoots. Implications for assimilate preconditioning and nitrogen signaling. Plant Physiol. **136**(4): 4326-4334.

Shi ZS, Li FH and Wang ZB 2002. Study on current situation and problem of fresh eating research and development. J. Maize Sci. (supplement) **10**: 93-96.

Sun LJ, Guo YR and Li JJ 2012. Analysis of regional characteristics of free amino acids in pulp of “Nagafu2” apples from different habitats. Food Sci. **33**(5): 539-557.

Wang M, Zhang JS and Zhao YL 2010. Determination of free amino acids content in corn syrup by HPLC. China Brewing **5**(218): 156-159.

Wang QD and Yang J 2010. Food experimental design and statistical analysis. pp. 343-366. Beijing, China.

Wenefrida I, Utomo HS, Blanche SB and Linscombe SD 2009. Enhancing essential amino acids and health benefit components in grain crops for improved nutritional values. Recent Patents on DNA & Gene Seq. **3**(3): 219-225.

Yang BM, Yao LX and Guo OB 2011. Analysis of free amino acids in litchi fruits from different cultivars. Food Sci. **32**(16): 249-252.

Zhang SH, Yi HH and Cai ZR 2008. Advances in research on waxy maize germplasm. J. Maize Sci. **16**(3): 44-46.

Zhao T, Shi HZ and Ji XM 2011. Difference in composition and content of free amino acids among various tobacco types. Acta Tabacaria Sinica **17**(2): 13-17.

(Manuscript received on 16 July, 2018; revised on 26 March, 2019)