



Article

Analysis of Seed Amino Acids in Vegetable Soybeans Dried by Freeze and Thermal Drying

Guo-Liang Jiang *, William Townsend and Shuxin Ren

Agricultural Research Station, Virginia State University, P.O. Box 9061, Petersburg, VA 23806, USA

* Correspondence: gjiang@vsu.edu or gljiang99@yahoo.com

Abstract: Vegetable soybean (*Glycine max*), known as edamame, has a high nutritional and market value. It is a relatively new crop in North America and Africa. The amino acid profile is important for the nutritional quality of edamame, and a challenge facing its genetic improvement is evaluating its amino acids rapidly. To explore a drying method suitable for the fast evaluation of edamame nutritional profiles, fresh seed samples of 20 soybean genotypes were dried using freeze, low- and high-heat drying methods, and their amino acid contents were analyzed by near-infrared reflectance (NIR) technology. Three-year results indicated that there were significant differences between the years of samplings and among genotypes for all amino acids. Significant differences existed between the drying methods for most amino acids except for leucine and the total amino acid. Low-heat drying at 65 °C and freeze drying showed similar results and were highly comparable to each other for ANOVA and repeatability estimation. The estimates of repeatability under the individual drying methods were 73–94%, except for tryptophan, cysteine and methionine; meanwhile, higher estimates (85–99%) were computed using the combined data of all three drying methods, with few exceptions. Two sulfur-containing amino acids were showed to be more sensitive to high temperature than the others. Six genotypes exhibited higher contents of all the 18 major amino acids and are recommended to be used for the nutritional quality improvement of edamame and other food-grade specialty soybeans. In conclusion, the low-heat drying method can serve as an alternative to freeze drying, and can be used in the large-scale drying of fresh edamame and in the evaluation of seed amino acids in research.



Citation: Jiang, G.-L.; Townsend, W.; Ren, S. Analysis of Seed Amino Acids in Vegetable Soybeans Dried by Freeze and Thermal Drying. *Agronomy* **2023**, *13*, 574. <https://doi.org/10.3390/agronomy13020574>

Academic Editor: Baskaran Stephen Inbaraj

Received: 4 January 2023

Revised: 12 February 2023

Accepted: 15 February 2023

Published: 17 February 2023



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Keywords: edamame; vegetable soybean; seed amino acids; freeze drying; thermal drying

1. Introduction

Vegetable soybean (*Glycine max* (L.) Merr.), originally known as edamame in Japan and as maodou in China, is a consumable food specialty crop. Compared to the commercial grain-type soybean that is harvested after full maturity (R8 growth stage) and is used as a major source of vegetable oil and animal feed, edamame is harvested when the pods and seeds are still green (R6 growth stage) [1]. Vegetable soybean has been traditionally grown and favored in East Asia, and is especially popular in China, Japan and Korea [2,3]. As a diet, edamame can be consumed in different ways, such as boiled or steamed pods or as roasted beans in snacks, or as fried shelled beans in vegetable dishes. Compared with conventional grain-type soybeans, edamame has unique features. These features include a larger seed size, higher contents of sugar and protein, and an appealing taste. Its high nutritional value and health benefits have led to increased attention to and/or interest in edamame in recent decades [4–7]. Edamame also has a relatively higher market value than commercial soybeans and can be grown as a new alternative or niche crop in North America [8,9]. In Africa, edamame is regarded as a superfood crop [7,10]. However, research on edamame is limited due to a relatively small production scale, especially in North America [11–15].

In China and Japan, edamame is highly popular and these countries are the major consuming markets of vegetable soybean worldwide. In addition to its long history of production and consumption for human food, these two countries have also conducted scientific research on edamame for a long time [3,16]. However, accessibility to the scientific reports is limited internationally because of the non-English languages used in the publications [3,17,18]. In Japan, the nutritional composition and quality traits of vegetable soybean have been investigated [19], including its protein and amino acids [20,21], sugars and sweetness [22], and metabolomic profiles and sensory attributes [23]. To increase harvest efficiency and maintain the quality of the products, many harvesters and sorting machines have been used in edamame production in Japan [24]. Mimura et al. [25] investigated the genetic diversity of vegetable soybean cultivars using simple sequence repeat markers. Their results indicated that Japanese cultivars had lower genetic diversity than Chinese cultivars. In China, the objectives of soybean cultivation differ regionally. In the northern regions of China, soybean is produced mainly for the production of soy oil and animal feeds, with a small proportion produced for food uses. However, soybean grown in the southern part of China is usually consumed as food or used in food sources, including tofu, soymilk, soy snacks of boiled or roasted beans, side dishes of fried fresh edamame, douchi, and soy sauce. The studies on vegetable soybean in China are greatly focused on cultivar development and the related basic research [17]. Many new cultivars of vegetable soybean have been released in China during recent years, such as Zhongke Maodou No. 3 and Zhongke Maodou No. 4, developed by the CAS Northeast Institute of Geography and Agroecology [26], Sudou No. 18, developed by the Jiangsu Academy of Agricultural Sciences [27], and Taihu Chunzao, bred by the Zhejiang Academy of Agricultural Sciences [28]. The seed composition and nutritional quality of vegetable soybean, including the proteins, oils, sugars and amino acids, were analyzed using different methods [14,29–31]. Recently, Bu et al. [32] reported a study on the identification of quantitative trait loci (QTL) for seed hardness in vegetable soybean. By QTL mapping, they identified three types of dynamic QTLs associated with seed hardness in a population of 184 recombinant inbred lines derived from the cross Kefeng No. 1 $\times$ Nannong1138-2 [32].

In North America, most of the grown edamame cultivars were developed in Asia and exhibit poor adaptability in general [6,8]. Many of them have undesirable traits, such as a very short plant structure and a low bottom-pod position. Such a trait is unbecoming for mechanical harvesting because it causes increased pod losses and a lower harvest efficiency. On the contrary, excessively tall plants are generally vulnerable to lodging and may also negatively affect harvest efficiency [33]. Shattering is another problem that many edamame cultivars have. It results in seed yield losses and increases production costs, particularly when mechanical harvesting is employed.

Seed composition is important for edamame quality, market acceptability and price. Protein is a major seed component in soybean seeds and its quantity and quality are determined by amino acid profiles. Soybean protein is mainly composed of 18 amino acids, including all the essential amino acids. The amino acid composition is usually determined using wet chemistry techniques and/or high-performance liquid chromatography (HPLC) [34–38]. However, these methods are too slow and expensive for feed formulation and plant breeding applications [39]. They require a trained technician and can be highly labor-intensive, time-consuming and cost-inefficient, in addition to concerns about chemical residues [38,40,41]. The large-scale analysis of seed amino acids is a great challenge for plant breeding practice, where numerous breeding lines need to be evaluated in a timely manner [39,40]. Due to its high efficiency and easy operation, near-infrared reflectance (NIR) technology is considered as an optimal option [42–44]. Moreover, multiple-trait measurements can be achieved simultaneously for a single sample by means of the NIR technology. This method has been extensively used in the evaluation of soybean seed composition, including amino acids [39–41,45,46]. However, the NIR calibrations developed for soybean were based on mature seeds, while fresh edamame contains 60–70% moisture in general [8,47–49], which is far beyond the 4–20% moisture range covered by the calibra-

tions. Clearly, the NIR calibrations cannot be directly used in the analysis of fresh edamame nutrition constituents [8,48]. Therefore, developing a rapid assessment of amino acid composition in fresh seeds is a realistic need for edamame research and breeding [14,50]. There are two ways to approach this goal: developing new calibrations using fresh edamame samples and/or drying the fresh seeds to meet the moisture requirements of established calibrations. Comparatively, the latter is more easily realized and cost-efficient. It could be assumed that the concentration of nutritional compositions, including of amino acids in vegetable soybean or immature soybean seeds, would be still within the ranges covered by the calibrations based on dry seeds [51]. The calibrations should be applicable when the fresh seeds are dried to the range of moisture covered by the calibrations. To use NIR technologies and evaluate nutrient profiles, therefore, a prerequisite is dehydrating fresh edamame samples. There have been reports regarding the drying methods used in plant foods and their potential impacts on nutritional composition [52–56]. However, research on drying methods and their potential effects on the protein quantitation of edamame has been very limited [49,51,57,58].

Jiang and Katuuramu [50] attempted to dry fresh edamame seeds using oven-drying methods at two different temperatures and analyzed the contents of amino acids using NIR spectroscopy. However, the possible influences of heating on seed composition during drying [59,60] were not addressed due to the lack of a reference. Freeze drying can retain the product quality and can have minimum impacts on the nutrient composition [59–61]. Thus, freeze drying is regarded as the optimal method of drying fruits and vegetables [62–66], and it can be used as a reference point for other methods. In this study, the authors used both freeze and thermal drying methods to dehydrate fresh edamame; they explored which heating method was appropriate for fresh edamame drying and could be used in the high-throughput phenotyping of nutritional profiles [47]. To provide useful information for edamame research and breeding, this article addresses seed amino acids in fresh edamame, while the results of seed proteins, sugars, oils and fatty acids have been reported previously [47]. The objectives were (1) to analyze the contents of seed amino acids in edamame, and (2) to compare the differences in amino acid traits between the different drying methods used.

2. Materials and Methods

2.1. Plant Materials and Field Experiments

The plant materials and experimental design were described previously by Jiang et al. [47]. Briefly, 20 soybean genotypes differing in seed size and seed composition traits were planted on the Randolph Farm of Virginia State University, south of Chesterfield County in Virginia. The field soil was a type of sandy soil (series—Bourne, and family—fine, silty mixed thermic). The experiments were conducted in 2018–2020 and four- or two-row plots 3.8 m in length and 0.76 m in row-spacing were employed. In total, 85 seeds were planted per row using a research-plot planter (Amalco, Nevada, IA, USA). No fertilizer was applied, and cultivation was carried out approximately six weeks after planting. Weed control was performed by applying a pre-emergence herbicide treatment and post-emergence treatments with SELECT herbicide at a rate of 0.66–1.17 L per hectare for grass weeds and/or STORM herbicide at 1.75 L per hectare for broadleaf weeds.

2.2. Edamame Sampling and Drying

As described previously [47], fresh edamame sampling was conducted at the R6 stage before yellowing [1]. Sample pods were obtained using a stationary edamame thresher. All genotypes were sampled at once to constitute one batch of samplings. One batch of samplings was made in each year of 2018 and 2019, while two batches were made in 2020. The pods were shelled using a peeling machine to generate fresh seed samples. To conduct three different drying treatments, fresh seed samples from each genotype were randomly divided into three subsamples, and each of them was assigned randomly to one of the three drying treatments. In this way, the homogeneity of samples within the

groups for each batch of samplings and among the three subsamples for each genotype could be maximized.

The subsamples were separately dried using a freeze, low-heat or high-heat method [47]. For freeze drying, the subsamples were pretreated and frozen under -20°C conditions for at least 24 h prior to drying. Then, the frozen seed samples were dried in a freeze dryer for approximately 5 days. In vegetable and fruit drying, different temperatures can be used. It is usually recommended that a temperature of $60\text{--}66^{\circ}\text{C}$ is used for oven drying [65,67–69]. To shorten the drying time, however, higher temperatures could be used [51,68]. According to previous experience and reports [51], in this study, low-heat drying was conducted in an oven at 65°C for approximately 2.5 days, and high-heat drying was performed in an oven at 105°C for approximately 2 days. For further details, refer to the previous report [47].

2.3. Seed Amino Acid Analysis

The amino acid contents in dried samples were evaluated using a DA 7250 NIR analyzer (Perten Instrument AB, Hagersten, Sweden). As described by the manufacturer (Perten Instruments, Inc., Springfield, IL, USA), all individual amino acids were analyzed simultaneously in the machine, but separately based on the different calibrations each of which was established for a specific amino acid. Since the accuracy of the NIR method is dependent on calibrations as well as samples types, three sets of calibrations, referred to as whole seed, ground and combined calibration, were installed in the DA 7250 NIR analyzer to analyze the different types of samples. The combined calibrations are applicable to both the whole seed and the ground samples. In addition, the calibrations have been updated periodically by the manufacturer to improve the prediction accuracy. Considering the differences in the sample shapes and their impact on the analysis [47,70], the dried whole seeds were analyzed first and the process was repeated three times for each subsample in this study. Then, the seeds were ground into flours using an IKA Mill (IKA Works, Inc., Wilmington, NC, USA) and the ground samples were analyzed again. The updated combined calibrations were mainly used because they are generally based on a larger number of samples, including both whole seed and ground flour, and have higher determination coefficients or more precise predictions. If whole and ground calibrations had a higher accuracy for the prediction of a given component, they were also used for different sample types accordingly. Consequently, two sets of data were generated in this way. The seed amino acid contents were presented as mg g^{-1} on a dry weight basis.

2.4. Statistical Analysis

The average of three analyses for each of the subsamples was used as the unit for statistical analysis. As described previously [47], data processing and correlation analysis were performed using Microsoft Excel 2016, and analysis of variance (ANOVA) was conducted using PROC GLM in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The genotypes, drying methods and sample types were treated as fixed effects, and the years or batches of samplings were regarded as replications and random effects. ANOVAs for different considerations were performed separately. For further details of the ANOVAs and estimation of repeatability, refer to the previous article [47].

3. Results and Discussion

3.1. ANOVA and Comparison of Sample Types

A combined ANOVA of all the data across the two types of samples and the three drying methods indicated that the differences between years, as well as among genotypes, were significant at $p = 1\%$ level for all the evaluated amino acids in the fresh edamame seed (Table 1). Significant differences also existed between the drying methods, except for leucine and the total amino acid. No significant difference in the total amino acid between the drying methods was consistent with the crude protein content [47]. The significance of the differences between the sample types varied with the kind of amino acid, consistent with a previous report on the other seed composition traits [47]. Similarly, the interactions

of sample types $\times$ drying methods were significant for some amino acids but insignificant for others. Of the 13 kinds of amino acids that showed a significant difference between years, nine also exhibited a significant sample type $\times$ drying method interaction (Table 1). However, no significant effects of the drying method $\times$ genotype, sample type $\times$ genotype, and sample type $\times$ drying method $\times$ genotype interactions were detected for all the amino acids. In addition, by pooling these three insignificant components of variations into the source of the errors, an adjusted ANOVA (data not shown) exhibited the same or similar results in significance tests for the years, sample types, drying methods, and the interactions of sample type $\times$ drying method as described above.

Table 1. Mean squares from ANOVA of seed amino acid contents in fresh edamame by combining the data of two types of samples and three drying methods.

Trait (mg g ^{−1})	MS _Y ^a	MS _S	MS _M	MS _G	MS _{MG}	MS _{SG}	MS _{SM}	MS _{SMG}
Cysteine	4.91 **	22.86 **	1.75 **	1.18 **	0.09	0.10	4.17 **	0.04
Methionine	0.35 **	0.25 *	0.17 *	0.41 **	0.02	0.03	0.31 **	0.01
Histidine	1.02 **	0.17	3.47 **	3.63 **	0.03	0.04	1.69 **	0.03
Isoleucine	20.33 **	7.73 **	2.90 **	8.51 **	0.10	0.09	1.52 **	0.05
Leucine	41.03 **	3.42 *	0.83	28.24 **	0.20	0.27	0.15	0.14
Lysine	76.84 **	8.13 **	14.44 **	16.07 **	0.22	0.19	1.51 *	0.17
Phenylalanine	56.11 **	2.86 **	6.38 **	13.51 **	0.15	0.28	1.76 **	0.11
Threonine	39.73 **	0.63	4.54 **	7.88 **	0.11	0.16	0.15	0.09
Tryptophan	7.60 **	1.43 **	3.45 **	0.25 **	0.04	0.05	0.36 *	0.02
Valine	66.39 **	0.08	5.34 **	14.16 **	0.13	0.37	3.38 **	0.12
Alanine	54.89 **	2.69 **	5.51 **	6.53 **	0.07	0.26	0.35	0.05
Arginine	245.79 **	5.33	13.76 **	41.04 **	0.62	1.59	1.34	0.25
Aspartic acid	86.44 **	25.11 **	24.57 **	66.51 **	0.57	0.62	15.78 **	0.38
Glutamic acid	153.32 **	57.27 **	172.84 **	226.07 **	1.46	1.58	24.72	1.43
Glycine	56.02 **	0.42	6.96 **	6.12 **	0.13	0.35	0.41	0.09
Proline	65.56 **	3.25	79.65 **	10.80 **	0.48	1.69	1.50	0.25
Serine	246.63 **	10.52 **	66.75 **	10.61 **	0.29	1.44	1.98	0.30
Tyrosine	44.86 **	2.78 *	14.81 **	4.69 **	0.10	0.22	3.71 **	0.04
Other AA ^b	8.08 **	6.04 **	8.44 **	0.80 **	0.10	0.15	2.83 **	0.07
Total AA ^c	13031.00 **	54.56	219.88	4626.93 **	35.31	64.63	35.01	25.25

* and **, Significant at $p = 0.05$ and 0.01 , respectively, based on F tests. ^a MS_Y, MS_S, MS_M, MS_G, MS_{MG}, MS_{SG}, MS_{SM}, and MS_{SMG} represent the mean squares of year, sample type, drying method, genotype, and method $\times$ genotype, sample $\times$ genotype, sample $\times$ method and sample $\times$ method $\times$ genotype interaction effects, respectively. ^b Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine. ^c Sum of all amino acids evaluated.

In the previous report [47], significant differences between the sample types were also detected for seed protein, oil, dietary fiber, stachyose, raffinose, total sugar, palmitic acid, acid detergent fiber, and neutral detergent fiber; meanwhile, no significant differences existed for ash, sucrose, or other fatty acids. These differences between the sample types might be related to factors such as appearance, solidities, structures and unit weight or relative density, which influence sample reflectance and detection by the NIR analyzer [47]. However, the differences between the whole seed and flour samples were very small or ignorable for most amino acids, although they were statistically significant (Table 2). The absolute values of the relative percent of difference (RPD) were 0.10–2.68%, except for cysteine and the sum of the other five minor amino acids. The results indicated that the two types of samples were comparable in the analysis of amino acids in dried edamame seeds.

For the whole seed and flour samples, separate ANOVAs were conducted based on all the data of the three drying methods to more accurately explicate the results. The ANOVAs indicated that the results of the significance tests with the whole seed samples were similar to those of the flour samples in all cases, with few exceptions (Table 3). The same as shown by combined ANOVA, the differences between the years or sets of samplings were significant in all traits for both samples. However, no significant drying method $\times$ genotype interactions were detected in any cases, in agreement with the previous report on other

seed compositions [47]. The differences between the drying methods that used both types of sample were also significant for all traits, except for isoleucine and valine in the ground seeds. Genotypic differences were significant for all amino acids in both the ground and whole seed samples, with an exception only for tryptophan in the flour samples.

Table 2. Means ($\pm$ SD) and differences of seed amino acids for ground and whole seeds of edamame all over three drying methods and three years (2018–2020).

Trait (mg g ⁻¹)	Mean		Difference	
	Ground Samples	Whole Seed Samples	Value	% of Ground Samples
Cysteine	5.6 $\pm$ 0.5	6.1 $\pm$ 0.5	0.45 *	7.99
Methionine	5.0 $\pm$ 0.3	5.1 $\pm$ 0.3	0.04 *	0.85
Histidine	11.5 $\pm$ 0.6	11.4 $\pm$ 0.6	−0.04 NS	−0.33
Isoleucine	20.7 $\pm$ 0.7	21.0 $\pm$ 0.8	0.25 *	1.22
Leucine	33.5 $\pm$ 1.4	33.7 $\pm$ 1.4	0.17 *	0.50
Lysine	27.7 $\pm$ 1.2	27.9 $\pm$ 1.3	0.25 *	0.92
Phenylalanine	22.2 $\pm$ 1.0	22.3 $\pm$ 1.2	0.14 *	0.64
Threonine	16.5 $\pm$ 1.1	16.6 $\pm$ 0.8	0.07 NS	0.43
Tryptophan	3.9 $\pm$ 0.5	4.0 $\pm$ 0.2	0.11 *	2.68
Valine	20.4 $\pm$ 0.9	20.3 $\pm$ 1.4	−0.05 NS	−0.24
Alanine	18.4 $\pm$ 0.7	18.6 $\pm$ 1.1	0.13 *	0.73
Arginine	32.5 $\pm$ 1.8	32.2 $\pm$ 2.5	−0.26 *	−0.81
Aspartic acid	48.5 $\pm$ 2.5	48.0 $\pm$ 2.2	−0.48 *	−0.98
Glutamic acid	76.5 $\pm$ 4.5	75.8 $\pm$ 4.5	−0.69 *	−0.90
Glycine	18.6 $\pm$ 0.7	18.6 $\pm$ 1.2	0.04 NS	0.22
Proline	18.9 $\pm$ 1.4	19.0 $\pm$ 2.3	0.12 NS	0.62
Serine	19.0 $\pm$ 1.2	19.2 $\pm$ 2.4	0.26 *	1.37
Tyrosine	15.3 $\pm$ 0.7	15.5 $\pm$ 1.2	0.14 *	0.91
Other AA ^a	3.8 $\pm$ 0.5	3.6 $\pm$ 0.6	−0.24 *	−6.22
Total AA ^b	418.4 $\pm$ 18.0	418.8 $\pm$ 19.0	0.43 NS	0.10

* and NSs represent significant and not significant differences between the means of ground and whole samples at $p = 0.05$, respectively, based on t tests (LSD) from ANOVA. ^a Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine. ^b Sum of all amino acids evaluated.

Table 3. Mean squares from ANOVA and repeatability estimates (%) of seed amino acid contents in edamame with ground and whole seed samples by the combined data of freeze, low-heat and high-heat drying methods.

Trait (mg g ⁻¹)	Ground Samples					Whole Seed Samples				
	MS _Y ^a	MS _M	MS _G	MS _{MG}	Repeatability	MS _Y	MS _M	MS _G	MS _{MG}	Repeatability
Cysteine	1.83 **	1.84 **	0.81 **	0.06	92.82	3.53 **	4.08 **	0.47 **	0.07	85.05
Methionine	1.40 **	0.12 *	0.23 **	0.02	91.63	0.88 **	0.37 **	0.20 **	0.02	92.26
Histidine	8.74 **	1.16 **	1.73 **	0.03	98.05	5.06 **	4.00 **	1.95 **	0.03	98.48
Isoleucine	10.78 **	0.11	3.96 **	0.08	98.01	12.02 **	4.31 **	4.61 **	0.07	98.41
Leucine	51.76 **	0.49	12.73 **	0.11	99.12	10.08 **	0.50	15.80 **	0.23	98.53
Lysine	32.21 **	5.68 **	7.46 **	0.17	97.69	45.20 **	10.27 **	8.79 **	0.22	97.47
Phenylalanine	24.37 **	2.13 **	5.84 **	0.11	98.11	34.91 **	6.01 **	7.87 **	0.15	98.14
Threonine	52.94 *	3.09 **	3.50 **	0.07	98.11	2.90 **	1.60 **	4.55 **	0.13	97.24
Tryptophan	13.64 **	2.99 **	0.11	0.04	33.36	0.19 **	0.82 **	0.16 **	0.02	86.84
Valine	17.27 **	0.11	6.21 **	0.11	98.09	71.66 **	8.61 **	8.11 **	0.14	98.33
Alanine	9.64 **	1.60 **	2.81 **	0.06	97.95	59.57 **	4.27 **	3.84 **	0.06	98.36
Arginine	43.89 **	4.08 *	20.63 **	0.40	98.05	260.98 **	11.02 **	21.23 **	0.47	97.78
Aspartic acid	228.67 **	11.21 **	31.76 **	0.35	98.91	34.47 **	29.13 **	35.55 **	0.61	98.29
Glutamic acid	595.03 **	47.43 **	108.37 **	0.98	99.09	456.98 **	150.13 **	119.30 **	1.90	98.41
Glycine	8.51 **	2.70 **	2.72 **	0.12	95.77	62.30 **	4.67 **	3.60 **	0.10	97.12
Proline	43.48 **	41.68 **	5.29 **	0.36	93.26	314.57 **	39.48 **	5.97 **	0.37	93.85
Serine	27.39 **	25.35 **	4.28 **	0.19	95.52	305.92 **	43.38 **	7.00 **	0.40	94.34
Tyrosine	4.59 **	7.38 **	2.21 **	0.07	96.70	73.77 **	11.14 **	2.57 **	0.07	97.32
Other AA ^b	2.23 **	8.89 **	0.38 **	0.05	87.05	6.41 **	2.38 **	0.57 **	0.13	77.74
Total AA ^c	7097.16 **	128.52	2130.69 **	24.63	98.84	6255.94 **	126.37	2545.02 **	35.92	98.59

* and **, Significant at $p = 0.05$ and 0.01 , respectively, based on F tests. ^a MS_Y, MS_M, MS_G, and MS_{MG} represent the mean squares of year, drying method, genotype and method $\times$ genotype interaction effects, respectively. ^b Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine. ^c Sum of all amino acids evaluated.

To determine the comparability or consistency of the ground and whole seed samples, the authors further estimated the repeatability of each type of sample, based on the separate

ANOVAs. In most cases, the repeatability estimates based on the genotype means were over 91%, though it was 33.36% for tryptophan with the ground samples (Table 3). The results suggested that the estimates of repeatability were highly comparable between the two types of sample for most amino acids, except for tryptophan and the sum of the other five minor amino acids. The higher estimates of repeatability for amino acids were consistent with the higher repeatability estimates of protein content in edamame with the ground and whole seed samples [47]. In addition, the correlations between the ground and whole seed samples under individual drying methods were also relatively high in most cases, averaging 0.861, 0.864, and 0.829 over all amino acid traits for freeze, low-heat and high-heat drying, respectively (Table 4). Comparatively, the correlations between the two types of samples were lower for tryptophan and the sum of the other five amino acids. These results were consistent with the estimates of repeatability. Together with all the above results, the whole seed and ground samples exhibited highly comparable results to each other. It was suggested that either type of samples could be used in the NIR analysis of amino acids in dried edamame.

Table 4. Correlation coefficients between ground and whole seed samples in seed amino acid traits of edamame dried by different drying methods.

Trait (mg g ⁻¹)	Freeze Drying	Low-Heat Drying	High-Heat Drying
Cysteine	0.810 **	0.799 **	0.725 **
Methionine	0.852 **	0.877 **	0.585 **
Histidine	0.928 **	0.949 **	0.934 **
Isoleucine	0.965 **	0.960 **	0.953 **
Leucine	0.944 **	0.973 **	0.980 **
Lysine	0.952 **	0.948 **	0.925 **
Phenylalanine	0.942 **	0.948 **	0.931 **
Threonine	0.900 **	0.967 **	0.935 **
Tryptophan	0.387	0.555 *	0.621 **
Valine	0.958 **	0.923 **	0.896 **
Alanine	0.946 **	0.938 **	0.829 **
Arginine	0.914 **	0.902 **	0.887 **
Aspartic acid	0.946 **	0.976 **	0.953 **
Glutamic acid	0.928 **	0.979 **	0.972 **
Glycine	0.902 **	0.896 **	0.736 **
Proline	0.719 **	0.674 **	0.656 **
Serine	0.862 **	0.758 **	0.620 **
Tyrosine	0.909 **	0.904 **	0.825 **
Other AA ^a	0.498 *	0.384	0.682 **
Total AA ^b	0.958 **	0.966 **	0.937 **
Average	0.861	0.864	0.829

* and **, Significant at $p = 0.05$ and 0.01 , respectively, based on t tests. ^a Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine. ^b Sum of all amino acids evaluated.

As described above in the materials and methods section, the preciseness of the NIR method depends on calibrations and sample types. Individual calibrations, each of which was formulated specifically for a given compound, were used to separately analyze amino acids, although the analyses of all the amino acids were completed simultaneously on the machine. To be more precise, updated combined calibrations were used for most of the amino acids in this study, because they have a greater coefficient of determination and are based on a larger number of samples, including both whole seeds and ground flours. For the whole seed, the shape of dried vegetable soybean would be somewhat different from the mature soybean seed, especially for the freeze-dried seeds. Powders or ground samples can eliminate or minimize such a difference. Therefore, the whole seed samples were ground into flour after the analysis of the whole seeds, and then the ground samples were analyzed again to avoid the issues or problems related to the shape of the samples. In terms of simplicity and easiness, however, whole seeds have an advantage over ground samples, because the latter type needs additional preparation, i.e., grinding or milling seed into

flour; on the other hand, whole seeds might have less uniformity in their physical features than ground samples [47]. Therefore, subsequent discussions will be mainly focused on the results of whole seed samples unless otherwise specified. However, the data of ground samples will also be presented for further comparisons and discussions.

3.2. Comparison of Edamame Drying Methods

Heating may result in the loss or destruction of amino acids in soybean products [56]. The major affecting factors in the loss of amino acids include the temperature used, the time of heating or treatment, the kind of soybean product and the kind of amino acid [56]. In this study, as described above, significant differences existed between the drying methods for most of the seed amino acids except leucine and the total amino acid (Tables 1 and 3). To better compare the drying methods, the authors also conducted ANOVA separately for each of the three methods used. As shown in Table 5, the results of the significance tests for the years or sets of samplings were similar in all three drying methods in most cases, regardless of the sample type. For whole seed samples, the effects of years or sampling sets and genotypes were significant for all the traits under freeze and low-heat drying, and they were also significant for most of the traits under high-heat drying, but insignificant for methionine (Table 5). The year effects for the sum of the other minor amino acids and the genotype effects for cysteine were not significant under high-heat drying. Furthermore, except for cysteine and methionine, the estimates of repeatability were similar or comparable among the drying methods in most cases (Table 5). The results of the ground samples were highly consistent with those of the whole seeds, with few exceptions; these exceptions included insignificant year effects for tyrosine under freeze drying and insignificant genotype effects and low repeatability for tryptophan, but significant genotype effects for cysteine and methionine under high-heat drying (Table 5). It was indicated that the ANOVA results and repeatability estimates were comparable between the drying methods, especially for freeze drying and low-heat drying at 65 °C. This was also consistent with the results of seed protein content [47]. It was further demonstrated that low-heat drying at 65 °C could serve as an alternative to freeze drying; it could also be suitable for the large-scale drying of fresh edamame and the rapid evaluation of seed composition [47]. During soybean processing, no significant nutrient losses occur when the drying step is performed at 60–70 °C for short periods; most amino acids are stable when heated to a temperature lower than 92 °C [54]. Li et al. [30] used 105 °C for 30 min and then 80 °C for 72 h to dehydrate fresh seed samples in an air oven. Therefore, as was used in this study, low-heat drying at 65 °C for 2.5 days is an appropriate method for edamame drying.

As shown in Table 5, the average estimates of repeatability over all twenty traits with whole seed samples were 81.59% (55.35–91.19%), 84.29% (53.85–93.01%) and 81.08% (15.90–93.49%) for freeze, low-heat and high-heat drying, respectively. For ground samples, the repeatability estimates averaged 84.20% (54.43–95.02%), 84.36% (45.75–94.32%) and 78.88% (26.74–92.30%) for freeze, low-heat and high-heat drying. It seemed that the repeatability estimates under high-heat drying varied more with kind of amino acid than those for freeze and low-heat drying. Among the amino acids, tryptophan exhibited a lower repeatability estimate (<60% mostly), suggesting that it might be less effectively improved compared to most of the other amino acids. Specifically, the repeatability estimates of cysteine and methionine for the whole seed samples under freeze and low-heat drying were 57.63% and 77.99%, and 58.775 and 53.85%, respectively (Table 5). However, these estimates were as low as 15.90% and 34.93% under high-heat drying. It appeared that the higher temperature had more influence on the contents of cysteine and methionine than the other amino acids. In other words, it could be supposed that these two sulfur-containing amino acids were more sensitive to a high temperature than the other amino acids. This result confirmed the unstable features of cysteine and methionine (Mni, 2011). With ground samples, the repeatability estimates of cysteine and methionine were improved under thermal drying, being approximately 10–40% higher than those of whole seed samples;

meanwhile, the whole seed and ground samples exhibited a similar repeatability to these two amino acids under freeze drying (Table 5).

Table 5. Mean squares from ANOVA and estimates of repeatability of amino acid traits for edamame drying methods with whole seed and ground samples.

Whole Seed	Freeze Drying		Low-Heat Drying		High-Heat Drying		Repeatability (%)		
Trait (mg g ⁻¹)	MS _Y ^a	MS _G	MS _Y	MS _G	MS _Y	MS _G	Freeze Drying	Low-Heat Drying	High-Heat Drying
Cysteine	0.39 **	0.19 **	0.92 **	0.24 **	3.25 **	0.19	57.63	58.77	15.90
Methionine	1.00 **	0.09 **	0.29 **	0.09 *	0.08	0.05	77.99	53.85	34.93
Histidine	3.38 **	0.68 **	1.75 **	0.73 **	0.61 **	0.61 **	90.27	91.31	91.36
Isoleucine	4.53 **	1.50 **	7.84 **	1.74 **	1.59 **	1.51 **	86.53	91.72	92.33
Leucine	7.18 **	5.39 **	4.94 **	5.73 **	3.78 **	5.14 **	90.68	93.01	93.49
Lysine	11.66 **	2.94 **	19.40 **	3.09 **	16.43 **	3.20 **	84.59	89.89	93.21
Phenylalanine	5.11 **	2.58 **	21.02 **	2.75 **	12.35 **	2.83 **	86.30	91.03	93.27
Threonine	1.01 **	1.55 **	2.96 **	1.65 **	4.79 **	1.58 **	91.06	91.23	92.16
Tryptophan	0.17 **	0.07 *	0.26 **	0.08 **	0.15 **	0.05 *	55.35	58.07	47.88
Valine	15.46 **	2.70 **	42.60 **	3.16 **	18.02 **	2.52 **	88.75	92.30	90.96
Alanine	11.83 **	1.23 **	26.85 **	1.44 **	22.74 **	1.30 **	88.94	91.87	91.58
Arginine	71.92 **	7.01 **	86.22 **	7.44 **	104.78 **	7.75 **	85.56	91.96	89.79
Aspartic acid	35.58 **	12.27 **	9.07 **	13.92 **	6.35 **	10.56 **	90.30	90.90	90.99
Glutamic acid	306.87 **	40.52 **	113.55 **	44.72 **	88.28 **	37.80 **	91.19	92.55	93.29
Glycine	10.12 **	1.14 **	28.33 **	1.39 **	26.75 **	1.28 **	81.15	87.33	90.82
Proline	143.83 **	2.03 **	107.58 **	2.33 **	70.53 **	2.32 **	73.14	82.56	86.23
Serine	58.16 **	2.03 **	138.84 **	2.66 **	118.97 **	30.5 **	80.36	83.98	89.23
Tyrosine	37.99 **	0.88 **	24.33 **	1.00 **	14.37 **	0.84 **	83.28	88.26	89.07
Other AA ^a	13.02 **	0.14 **	0.38 **	0.18 **	0.09	0.48 **	59.04	72.95	61.90
Total AA ^b	922.66 **	87.56 **	3311.83 **	945.06 **	2502.21 **	825.20 **	89.65	92.17	93.19
Average							81.59	84.29	81.08

Ground Sample	Freeze Drying		Low-Heat Drying		High-Heat Drying		Repeatability (%)		
Trait (mg g ⁻¹)	MS _Y ^a	MS _G	MS _Y	MS _G	MS _Y	MS _G	Freeze Drying	Low-Heat Drying	High-Heat Drying
Cysteine	0.28 **	0.33 **	0.80 **	0.32 **	1.47 **	0.29 **	54.43	68.36	56.62
Methionine	0.85 **	0.08 **	0.37 **	0.10 *	0.89 **	0.09 **	77.92	75.09	59.32
Histidine	5.75 **	0.61 **	2.57 **	0.60 **	1.99 **	0.56 **	91.48	90.62	84.00
Isoleucine	2.55 **	1.26 **	2.17 **	1.60 **	7.04 **	1.27 **	93.93	92.45	90.18
Leucine	22.97 **	4.12 **	14.42 **	4.88 **	17.90 **	3.93 **	93.59	93.21	92.30
Lysine	12.27 **	2.50 **	10.12 **	3.49 **	19.88 **	2.19 **	92.95	88.68	86.81
Phenylalanine	9.93 **	1.75 **	6.18 **	2.30 **	14.86 **	1.97 **	93.15	92.17	90.02
Threonine	20.77 **	1.3 **	9.12 **	1.22 **	25.86 **	1.10 **	89.03	89.14	87.61
Tryptophan	10.69 **	0.04 *	4.19 **	0.06 **	1.23 **	0.07	55.09	65.02	26.74
Valine	5.46 **	2.12 **	4.89 **	2.42 **	10.28 **	1.86 **	95.02	94.32	92.24
Alanine	2.35 **	0.96 **	2.46 **	1.12 **	7.09 **	0.83 **	93.70	91.90	87.80
Arginine	10.66 **	6.13 **	14.25 **	8.28 **	21.20 **	7.00 **	87.90	89.37	82.80
Aspartic acid	79.54 **	10.36 **	71.18 **	11.95 **	83.88 **	10.05 **	91.33	91.10	89.94
Glutamic acid	299.01 **	36.08 **	212.40 **	39.62 **	120.56 **	34.45 **	93.07	92.25	89.70
Glycine	3.22 **	0.83 **	3.73 **	1.28 **	5.99 **	0.79 **	90.11	88.20	78.77
Proline	4.02 **	1.12 **	7.70 **	3.03 **	51.04 **	1.80 **	71.08	80.63	72.21
Serine	5.37 **	1.27 **	5.93 **	2.06 **	27.82 **	1.29 **	80.51	78.26	74.83
Tyrosine	0.15	0.63 **	0.95 **	1.10 **	6.31 **	0.61 **	85.46	88.24	82.45
Other AA ^a	0.41 **	0.12 **	4.07 **	0.19 **	0.03	0.16 **	60.43	45.75	63.45
Total AA ^b	2568.77 **	674.19 **	2251.72 **	840.79 **	3193.47 **	655.89 **	93.87	92.40	89.77
Average							84.20	84.36	78.88

* and **, Significant at $p = 0.05$ and 0.01 , respectively, based on t tests. ^a MS_Y and MS_G represent the mean squares of years/sets of sampling and genotypes, respectively. ^b Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine.

Table 6 presents the means and ranges of amino acid contents in the fresh edamame seeds of twenty genotypes dried by individual drying methods. Overall, the means of the amino acid traits were close or comparable among the drying methods. Dobermann et al. [53] reported that black crickets that were dried at a lower temperature (45 °C) had around a 1% higher protein content than those dried at a higher temperature (120 °C). However, Jiang et al. [47] found that there was no significant difference in the protein content between the fresh edamame dried at 65 and 105 °C. Consistently, this study also indicated that the differences in the total amino acid and most individual amino acids were not significant or were ignorable between the fresh soybean seeds dried at 65 and 105 °C for both of the sample types (Table 6). As discussed previously [47], freeze drying was used as the reference because it minimized the impact of drying on the seed composition. Compared to freeze drying, the RPD averaged 2.50% (0.04–6.51%) and 2.99%

(0.03–8.42%) for low-heat and high-heat drying with whole seeds, respectively (Table 6). Under low-heat drying, only cysteine, proline, serine and tyrosine exhibited an RPD larger than 3%. In addition to these four amino acids, histidine, glutamic acid, tryptophan and the sum of the other five minor amino acids also exhibited an RPD of over 3% under high-heat drying. For ground samples, both low-heat and high-heat drying exhibited an average similar or comparable to that of freeze drying for most amino acids (Table 6), with the exception of the total content of the other five minor amino acids that exhibited an RPD of 13.51% under low-heat drying. It was indicated that the results of low-heat drying with whole seed samples were comparable to those of freeze drying, more so than high-heat drying [47]. This was further confirmed by the correlation analysis. For whole seed samples, the coefficients of correlation between low-heat drying and freeze drying averaged 0.901 (0.679–0.966) over all the 20 amino acid traits investigated; meanwhile, the correlation coefficient between high-heat drying and freeze drying averaged 0.878 (0.483–0.963) (Table 6). For ground samples, the coefficients of correlation between low-heat drying and freeze drying averaged 0.917 (0.660–0.971), but the coefficients of correlation between high-heat drying and freeze drying averaged 0.854 with a low value of 0.091 for tryptophan. In addition, the calculation also indicated that the coefficients of correlation between low-heat drying with whole seed samples and freeze drying with ground samples averaged 0.864 over all 20 amino acids, mostly ranging between 0.742 and 0.970 except for tryptophan (0.382) and the total of the five minor amino acids (0.607).

In soybean seed, the concentrations of amino acid composition varied greatly among individual components (Tables 2 and 6). Some of the components were lower in content, such as the sulfur-containing amino acids cysteine and methionine, which are more important for soybean amino acid profiles and its nutritional value. Tryptophan exhibited the lowest content among all the 18 major amino acids. These three amino acids showed a lower repeatability estimate in most cases (Table 6). Therefore, more attention should be paid to these amino acids in genetic improvement. It was also noticed that some components exhibited larger variation or standard deviation, even for the same genotype. For instance, larger standard deviations were observed for VS15-4018 with whole seed samples and for N6202-8 with ground samples (Tables 7 and S1–S3). Increasing the number of samples and replications could reduce the standard deviation.

3.3. Performance of Twenty Genotypes

The previous reports indicated that there was a large range of variation in the traits of edamame seed proteins, oils and sugars among the genotypes [47,51]. Similarly, the contents of amino acids in the edamame seeds also varied considerably among the cultivars and lines (Table 6). Table 7 presents the averages of 20 cultivars and lines in amino acid contents evaluated with whole seed samples under low-heat drying. Of the 20 genotypes studied, N6202-8, NC Green and VS15-4018 exhibited higher contents of almost all the major amino acids, as well as the total amino acid, followed by VS15-6021, VS15-5148 and VS11-0022. For ground samples under low-heat drying (Supplementary material Table S1) and under freeze drying (Table S2), and whole seed samples under freeze drying (Table S3), these six genotypes were still higher in total amino acid and most of the major amino acids than the others, with a few exceptions. These results were in agreement with the fact that they were among the top genotypes high in protein content [47]. Therefore, the authors would also recommend these genotypes for the improvement of protein and amino acids in edamame and other food-grade specialty soybeans. In addition to NC Green, the new lines VS15-5148, VS15-6021 and VS11-0022 also have larger seed sizes, with an average 100-seed weight of 61.6–62.7 and 20.1–21.7 g for fresh and dried seeds, respectively, compared with the 20-genotype averages of 46.5 g and 15.9 g [47], and 26.3–29.4 g for mature seeds. These new lines exhibited a higher oleic acid content (33.3–35.2%) and lower linoleic acid content (35.1–37.3%) in the dried seeds, compared with the averages of 20 genotypes (29.0 and 42.7% of total oil) [47]. They are also resistant to plant lodging and seed shattering.

Table 6. Means ($\pm$ SD) and ranges of variation of seed amino acid traits in fresh edamame dried by freeze, low-heat and high-heat drying methods, and correlations between heat drying and freeze drying with whole seed and ground samples over three years (2018–2020).

Whole Seed	Freeze Drying		Low-Heat Drying		High-Heat Drying		Relative Difference (%) ^c		Correlation with Freeze Drying	
Trait (mg g ⁻¹)	Mean	Range	Mean	Range	Mean	Range	Low-Heat	High-Heat	Low-Heat	High-Heat
Cysteine	6.3 $\pm$ 0.3	5.4–7.3	6.0 $\pm$ 0.4	5.0–7.0	5.9 $\pm$ 0.5	4.8–7.1	5.30 ^a	7.07 ^a	0.752 **	0.585 **
Methionine	5.2 $\pm$ 0.3	4.4–5.8	5.0 $\pm$ 0.2	4.4–5.5	5.1 $\pm$ 0.2	4.5–5.5	2.62 ^a	1.73 ^a	0.887 **	0.811 **
Histidine	11.7 $\pm$ 0.6	10.2–12.8	11.4 $\pm$ 0.5	9.6–12.4	11.3 $\pm$ 0.5	9.9–12.3	2.83 ^a	3.79 ^b	0.966 **	0.943 **
Isoleucine	20.7 $\pm$ 0.8	18.9–23.3	21.1 $\pm$ 0.9	18.5–23.3	21.2 $\pm$ 0.7	19.1–22.9	1.75 ^a	2.13 ^a	0.945 **	0.942 **
Leucine	33.7 $\pm$ 1.4	30.2–37.7	33.7 $\pm$ 1.4	29.6–37.1	33.6 $\pm$ 1.3	30.0–36.6	0.04	0.45	0.953 **	0.937 **
Lysine	27.5 $\pm$ 1.2	24.6–31.6	28.1 $\pm$ 1.3	24.8–31.3	28.2 $\pm$ 1.3	24.4–31.0	2.05 ^a	2.48 ^a	0.915 **	0.923 **
Phenylalanine	22.0 $\pm$ 1.0	19.7–25.6	22.5 $\pm$ 1.3	19.2–25.5	22.5 $\pm$ 1.2	19.4–25.4	2.20 ^a	2.18 ^a	0.933 **	0.936 **
Threonine	16.6 $\pm$ 0.7	14.7–18.8	16.4 $\pm$ 0.8	14.2–18.3	16.7 $\pm$ 0.8	14.3–19.1	0.99 ^a	0.69	0.930 **	0.885 **
Tryptophan	4.0 $\pm$ 0.2	3.5–4.6	3.9 $\pm$ 0.2	3.5–4.6	4.1 $\pm$ 0.2	3.7–4.6	1.45	3.55 ^a	0.810 **	0.733 **
Valine	20.1 $\pm$ 1.2	17.1–24.0	20.7 $\pm$ 1.6	16.9–24.2	20.1 $\pm$ 1.2	16.8–22.9	2.89 ^a	0.03	0.960 **	0.960 **
Alanine	18.3 $\pm$ 0.9	16.0–20.9	18.7 $\pm$ 1.2	15.9–21.2	18.7 $\pm$ 1.2	16.0–21.0	2.04 ^a	2.35 ^a	0.958 **	0.963 **
Arginine	31.8 $\pm$ 2.3	26.8–37.9	32.5 $\pm$ 2.4	26.8–37.1	32.4 $\pm$ 2.6	26.7–36.7	2.14 ^a	2.06 ^a	0.942 **	0.943 **
Aspartic acid	48.7 $\pm$ 2.3	42.9–53.4	47.6 $\pm$ 2.2	41.9–51.8	47.7 $\pm$ 1.9	42.4–51.5	2.31 ^a	2.08 ^a	0.958 **	0.930 **
Glutamic acid	77.4 $\pm$ 4.9	66.4–86.7	75.4 $\pm$ 4.2	63.3–84.5	74.7 $\pm$ 3.8	63.8–84.2	2.55 ^a	3.54 ^b	0.951 **	0.922 **
Glycine	18.4 $\pm$ 0.9	16.4–21.1	18.9 $\pm$ 1.3	16.4–21.5	18.7 $\pm$ 1.2	16.1–20.9	2.67 ^b	1.67 ^a	0.914 **	0.934 **
Proline	19.0 $\pm$ 2.6	15.1–24.5	19.8 $\pm$ 2.3	15.9–23.6	18.3 $\pm$ 1.9	15.1–22.2	4.21 ^b	3.33 ^a	0.852 **	0.905 **
Serine	18.4 $\pm$ 1.8	14.8–22.8	19.6 $\pm$ 2.6	15.3–24.0	19.7 $\pm$ 2.4	15.4–24.2	6.51 ^a	7.41 ^a	0.842 **	0.924 **
Tyrosine	15.0 $\pm$ 1.4	12.5–18.2	15.7 $\pm$ 1.1	13.4–18.0	15.7 $\pm$ 0.9	13.7–17.9	4.06 ^a	4.71 ^a	0.915 **	0.942 **
Other AA ^d	3.7 $\pm$ 0.8	2.6–5.0	3.6 $\pm$ 0.3	2.9–4.3	3.4 $\pm$ 0.5	2.3–4.7	0.90	8.42 ^a	0.679 **	0.483 *
Total AA ^e	418.3 $\pm$ 17.6	371.9–478.7	420.3 $\pm$ 20.6	363.4–469.1	417.8 $\pm$ 18.7	362.7–464.1	0.47	0.12	0.955 **	0.949 **
Average							2.50	2.99	0.901	0.878

Ground Sample	Freeze Drying		Low-Heat Drying		High-Heat Drying		Relative Difference (%) ^c		Correlation with Freeze Drying	
Trait (mg g ⁻¹)	Mean	Range	Mean	Range	Mean	Range	Low-Heat	High-Heat	Low-Heat	High-Heat
Cysteine	5.6 $\pm$ 0.5	4.8–6.9	5.5 $\pm$ 0.4	4.4–6.5	5.8 $\pm$ 0.5	4.8–6.7	1.84	3.65 ^a	0.920 **	0.734 **
Methionine	5.0 $\pm$ 0.3	4.5–5.7	5.0 $\pm$ 0.2	4.4–5.6	5.1 $\pm$ 0.3	4.5–5.8	0.18	1.52 ^a	0.881 **	0.759 **
Histidine	11.6 $\pm$ 0.7	9.6–12.7	11.3 $\pm$ 0.5	9.5–12.4	11.5 $\pm$ 0.5	9.7–12.5	1.97 ^a	0.22	0.968 **	0.929 **
Isoleucine	20.7 $\pm$ 0.7	19.0–23.5	20.8 $\pm$ 0.8	18.6–22.7	20.8 $\pm$ 0.8	18.5–23.3	0.31	0.34	0.947 **	0.950 **
Leucine	33.6 $\pm$ 1.5	29.2–36.5	33.5 $\pm$ 1.4	28.8–37.0	33.4 $\pm$ 1.4	28.7–36.8	0.35	0.41	0.971 **	0.975 **
Lysine	27.4 $\pm$ 1.1	24.0–29.8	27.9 $\pm$ 1.2	24.5–30.9	27.7 $\pm$ 1.2	23.8–30.8	2.03 ^a	1.30 ^a	0.939 **	0.917 **
Phenylalanine	22.0 $\pm$ 1.0	19.2–23.9	22.4 $\pm$ 1.0	19.2–24.8	22.1 $\pm$ 1.1	18.7–25.2	1.46 ^a	0.34	0.947 **	0.933 **
Threonine	16.5 $\pm$ 1.1	13.5–18.7	16.3 $\pm$ 0.9	13.7–18.1	16.7 $\pm$ 1.2	13.5–18.8	0.98 ^a	1.47 ^b	0.961 **	0.950 **
Tryptophan	3.9 $\pm$ 0.7	3.0–4.9	3.7 $\pm$ 0.4	3.0–4.6	4.1 $\pm$ 0.3	2.8–4.1	4.04 ^b	1.13 ^a	0.736 **	0.091
Valine	20.3 $\pm$ 0.9	17.7–22.2	20.4 $\pm$ 0.9	17.5–22.7	20.1 $\pm$ 1.0	17.4–23.1	0.39	0.13	0.947 **	0.943 **
Alanine	18.3 $\pm$ 0.6	16.3–19.4	18.5 $\pm$ 0.7	16.4–20.1	18.5 $\pm$ 0.7	16.1–20.5	1.40 ^a	1.42 ^a	0.961 **	0.940 **
Arginine	32.2 $\pm$ 1.6	27.1–34.8	32.7 $\pm$ 1.8	26.5–37.4	32.5 $\pm$ 1.9	26.1–36.4	1.47 ^b	0.92 ^{ab}	0.942 **	0.943 **
Aspartic acid	48.5 $\pm$ 2.5	40.3–52.8	48.1 $\pm$ 2.6	39.4–53.1	48.8 $\pm$ 2.6	39.8–53.6	0.96 ^a	0.63	0.971 **	0.965 **
Glutamic acid	77.4 $\pm$ 4.8	62.7–86.3	75.9 $\pm$ 4.5	61.0–84.8	76.3 $\pm$ 4.0	62.7–82.6	1.94 ^a	1.45 ^a	0.974 **	0.974 **
Glycine	18.4 $\pm$ 0.6	16.4–19.4	18.8 $\pm$ 0.8	16.5–20.7	18.5 $\pm$ 0.7	16.1–20.9	2.02 ^a	0.66	0.932 **	0.847 **
Proline	18.6 $\pm$ 0.8	16.9–20.6	19.7 $\pm$ 1.2	17.2–22.7	18.3 $\pm$ 1.7	15.6–22.7	6.10 ^a	1.41	0.873 **	0.810 **
Serine	18.3 $\pm$ 0.8	15.7–19.6	19.4 $\pm$ 1.0	16.5–22.6	19.3 $\pm$ 1.3	15.3–22.2	5.80 ^a	5.22	0.890 **	0.879 **
Tyrosine	15.1 $\pm$ 0.5	14.0–16.4	15.7 $\pm$ 0.73	14.0–17.5	15.3 $\pm$ 0.7	13.4–17.5	4.48 ^a	5.53 ^b	0.945 **	0.896 **
Other AA ^d	3.7 $\pm$ 0.3	3.1–4.4	4.2 $\pm$ 0.5	3.0–5.2	3.5 $\pm$ 0.3	2.8–4.1	13.51 ^b	4.16 ^a	0.660 **	0.680 **
Total AA ^e	416.9 $\pm$ 17.4	360.0–449.8	419.6 $\pm$ 18.6	357.8–461.0	418.6 $\pm$ 18.3	354.3–464.8	0.65	0.41	0.971 **	0.962 **
Average							2.59	1.62	0.917	0.854

* and **, Significant at $p = 0.05$ and 0.01 , respectively, based on t tests. ^a or ^b represents significant difference from freeze drying based on t tests (LSD_{0.05}) from ANOVA. ^c Compared to freeze drying. ^d Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine. ^e Sum of all amino acids evaluated.

Table 7. Average performance of 20 soybean cultivars and lines in seed amino acids (mg g^{−1} on dry weight basis) at edamame or R6 stage with whole samples dried by low-heat drying across three years (2018–2020).

Genotype	Cysteine	Methionine	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Tryptophan	Valine
Asmara	6.0 ± 0.3	4.8 ± 0.1	11.3 ± 0.2	21.0 ± 0.9	33.8 ± 0.8	28.0 ± 1.2	22.4 ± 1.3	16.4 ± 0.7	3.9 ± 0.3	20.8 ± 1.8
Moon Cake	5.7 ± 0.3	5.0 ± 0.3	11.2 ± 0.0	20.8 ± 0.7	33.1 ± 0.6	28.1 ± 1.3	22.2 ± 1.1	16.0 ± 0.5	3.8 ± 0.2	20.4 ± 1.3
N6202-8	6.3 ± 0.5	5.3 ± 0.2	12.0 ± 0.3	21.9 ± 0.7	35.3 ± 0.8	29.2 ± 1.1	23.6 ± 1.2	17.1 ± 0.5	3.9 ± 0.1	21.6 ± 1.4
NC 346	6.1 ± 0.3	5.0 ± 0.3	11.4 ± 0.1	21.4 ± 1.1	34.3 ± 1.1	28.4 ± 1.9	22.9 ± 1.8	16.8 ± 0.9	4.2 ± 0.3	20.9 ± 2.0
NC Green	6.3 ± 0.2	5.2 ± 0.2	11.9 ± 0.3	22.0 ± 1.2	35.5 ± 0.9	29.2 ± 1.7	23.7 ± 1.7	17.4 ± 0.9	4.3 ± 0.4	22.2 ± 2.2
NC Raleigh	5.5 ± 0.4	4.7 ± 0.4	10.1 ± 0.4	19.2 ± 0.5	30.5 ± 0.7	25.6 ± 0.7	20.2 ± 0.8	14.6 ± 0.5	3.9 ± 0.2	18.1 ± 1.0
Randolph	6.1 ± 0.2	5.1 ± 0.2	11.7 ± 0.0	21.3 ± 1.0	34.3 ± 1.0	28.6 ± 1.5	22.9 ± 1.5	16.7 ± 0.6	4.0 ± 0.2	20.9 ± 1.7
VS11-0022	6.1 ± 0.8	5.0 ± 0.4	11.5 ± 0.2	21.5 ± 0.7	34.3 ± 0.6	28.6 ± 0.9	22.9 ± 1.0	16.7 ± 0.8	3.9 ± 0.1	21.3 ± 1.9
VS11-0112	6.0 ± 0.2	5.0 ± 0.2	11.2 ± 0.4	20.8 ± 0.5	33.1 ± 0.6	27.8 ± 0.9	22.1 ± 0.9	16.2 ± 0.4	3.8 ± 0.1	20.2 ± 1.2
VS11-0137	5.8 ± 0.3	5.0 ± 0.1	11.1 ± 0.5	20.4 ± 0.4	32.5 ± 0.8	27.4 ± 0.4	21.6 ± 0.5	15.9 ± 0.1	3.8 ± 0.2	20.0 ± 1.0
VS12-0021	5.8 ± 0.4	5.0 ± 0.2	11.3 ± 0.5	21.0 ± 0.5	33.5 ± 0.6	28.0 ± 0.7	22.3 ± 0.8	16.4 ± 0.5	3.7 ± 0.2	20.1 ± 1.1
VS12-0161	5.9 ± 0.1	5.0 ± 0.3	11.5 ± 0.4	20.8 ± 0.9	33.5 ± 0.6	27.9 ± 1.1	22.3 ± 1.2	16.5 ± 0.5	3.8 ± 0.1	20.7 ± 1.6
VS15-4007	5.8 ± 0.5	4.9 ± 0.2	11.4 ± 0.4	21.3 ± 0.4	34.0 ± 0.1	28.0 ± 1.1	22.6 ± 0.9	16.3 ± 0.2	3.9 ± 0.1	20.8 ± 1.6
VS15-4049	5.8 ± 0.4	5.0 ± 0.2	11.0 ± 0.5	20.6 ± 0.8	32.7 ± 1.3	27.2 ± 1.0	21.7 ± 1.0	15.7 ± 0.5	3.9 ± 0.1	20.0 ± 1.1
VS15-5148	6.3 ± 0.4	5.1 ± 0.0	11.6 ± 0.3	21.7 ± 0.6	34.6 ± 0.4	28.7 ± 1.1	23.1 ± 1.2	16.8 ± 0.6	3.9 ± 0.1	21.2 ± 1.9
VS15-6005	5.8 ± 0.4	4.8 ± 0.2	11.0 ± 0.6	20.7 ± 0.6	32.7 ± 1.0	27.1 ± 0.7	21.8 ± 0.8	15.8 ± 0.4	3.9 ± 0.2	20.0 ± 1.4
VS15-6077	6.1 ± 0.4	5.1 ± 0.2	11.5 ± 0.5	20.9 ± 0.6	33.7 ± 0.7	27.9 ± 1.2	22.4 ± 1.2	16.6 ± 0.2	4.1 ± 0.3	20.6 ± 1.4
VS15-4018	6.2 ± 0.2	5.2 ± 0.1	12.1 ± 0.2	22.4 ± 1.4	36.0 ± 1.5	30.0 ± 1.8	24.2 ± 1.9	17.8 ± 0.7	4.0 ± 0.1	22.6 ± 2.3
VS15-6021	6.5 ± 0.1	5.3 ± 0.1	11.6 ± 0.4	21.4 ± 0.5	34.2 ± 0.2	28.6 ± 0.9	23.0 ± 0.9	16.9 ± 0.3	4.2 ± 0.3	21.5 ± 1.7
VS15-6023	6.1 ± 0.3	5.1 ± 0.2	11.0 ± 0.6	20.8 ± 0.5	33.0 ± 0.3	27.5 ± 0.7	22.2 ± 0.7	16.4 ± 0.2	4.1 ± 0.2	20.6 ± 1.4
LSD _{0.05}	0.45	0.29	0.6	0.55	0.92	0.81	0.72	0.55	0.27	0.72
Genotype	Alanine	Arginine	Aspartic Acid	Glutamic Acid	Glycine	Proline	Serine	Tyrosine	Other AA ^a	Total AA ^b
Asmara	18.7 ± 1.4	32.3 ± 2.3	47.5 ± 0.5	75.0 ± 1.6	19.0 ± 1.4	20.0 ± 2.9	19.9 ± 3.3	15.6 ± 1.3	3.5 ± 0.2	419.8 ± 17.9
Moon Cake	18.6 ± 1.3	32.9 ± 2.9	47.0 ± 0.8	73.8 ± 1.6	18.8 ± 1.3	19.8 ± 2.0	19.4 ± 2.6	15.5 ± 1.0	3.7 ± 0.2	415.7 ± 17.0
N6202-8	19.3 ± 1.2	34.2 ± 2.3	50.0 ± 1.0	80.5 ± 2.8	19.5 ± 1.4	20.6 ± 2.3	20.1 ± 2.6	16.3 ± 1.1	3.3 ± 0.4	439.8 ± 14.8
NC 346	18.8 ± 1.7	32.3 ± 2.8	48.8 ± 1.1	77.1 ± 0.5	19.0 ± 1.9	19.6 ± 3.2	19.8 ± 3.7	15.8 ± 1.6	3.8 ± 0.3	426.3 ± 25.4
NC Green	19.6 ± 1.7	33.9 ± 2.7	50.4 ± 1.0	79.9 ± 1.7	19.8 ± 1.8	20.8 ± 3.0	20.9 ± 3.5	16.4 ± 1.5	3.6 ± 0.4	442.8 ± 23.5
NC Raleigh	16.9 ± 1.0	28.2 ± 1.7	42.6 ± 1.0	66.2 ± 2.7	17.2 ± 0.9	17.9 ± 2.1	17.5 ± 2.4	14.4 ± 0.9	4.0 ± 0.1	377.2 ± 10.7
Randolph	18.9 ± 1.4	33.5 ± 3.1	48.8 ± 1.2	77.6 ± 1.3	19.1 ± 1.5	20.0 ± 2.7	19.7 ± 2.9	15.8 ± 1.4	3.8 ± 0.3	428.7 ± 21.4
VS11-0022	19.1 ± 1.3	33.1 ± 2.2	48.2 ± 1.0	76.8 ± 2.9	19.4 ± 1.3	20.7 ± 2.5	20.5 ± 3.1	16.1 ± 1.3	3.5 ± 0.2	429.1 ± 12.7
VS11-0112	18.4 ± 1.1	32.0 ± 2.1	46.7 ± 1.3	73.8 ± 3.6	18.6 ± 1.2	19.5 ± 2.5	19.2 ± 2.9	15.4 ± 1.0	3.7 ± 0.2	413.5 ± 10.8
VS11-0137	18.2 ± 0.7	31.8 ± 1.4	45.8 ± 2.0	72.3 ± 4.4	18.4 ± 0.6	19.5 ± 1.5	19.1 ± 2.0	15.2 ± 0.7	3.4 ± 0.2	407.2 ± 5.2
VS12-0021	18.4 ± 1.0	31.2 ± 1.5	46.5 ± 1.5	74.1 ± 3.6	18.6 ± 0.9	19.0 ± 2.1	19.4 ± 2.8	15.6 ± 1.2	3.0 ± 0.1	412.6 ± 7.8
VS12-0161	18.7 ± 1.2	33.0 ± 2.3	47.4 ± 0.9	75.4 ± 2.5	18.9 ± 1.2	19.4 ± 2.7	19.5 ± 2.6	15.5 ± 1.3	3.5 ± 0.2	419.1 ± 16.1
VS15-4007	18.7 ± 1.2	32.5 ± 2.5	48.0 ± 0.8	76.1 ± 3.2	18.8 ± 1.4	19.4 ± 2.7	19.1 ± 2.6	15.6 ± 1.2	3.7 ± 0.2	420.8 ± 11.3
VS15-4049	18.0 ± 1.0	31.2 ± 1.4	46.1 ± 2.4	73.2 ± 4.6	18.2 ± 0.9	18.8 ± 1.0	18.2 ± 1.5	15.3 ± 0.7	3.7 ± 0.5	406.2 ± 14.9
VS15-5148	19.1 ± 1.4	33.0 ± 2.5	49.2 ± 0.6	78.2 ± 2.7	19.2 ± 1.5	20.4 ± 3.1	19.9 ± 3.2	15.9 ± 1.4	3.9 ± 0.1	431.6 ± 15.1
VS15-6005	18.1 ± 0.9	31.3 ± 1.7	46.0 ± 2.1	73.0 ± 4.5	18.2 ± 1.0	18.7 ± 2.0	18.3 ± 1.9	15.1 ± 0.9	3.9 ± 0.3	405.9 ± 10.1
VS15-6077	18.6 ± 1.2	32.4 ± 2.4	47.7 ± 1.0	76.0 ± 3.1	18.8 ± 1.2	19.3 ± 2.7	19.5 ± 2.8	15.5 ± 1.2	3.7 ± 0.3	420.3 ± 14.3
VS15-4018	20.0 ± 1.7	35.3 ± 2.7	50.8 ± 1.1	80.9 ± 0.9	20.1 ± 1.8	21.6 ± 2.8	21.4 ± 3.0	16.9 ± 1.7	3.6 ± 0.2	451.1 ± 25.0
VS15-6021	19.3 ± 1.2	33.8 ± 2.2	48.5 ± 1.0	76.9 ± 3.2	19.4 ± 1.2	21.0 ± 2.4	20.7 ± 2.9	15.8 ± 1.2	3.7 ± 0.0	432.1 ± 11.0
VS15-6023	18.7 ± 1.1	32.2 ± 1.3	46.5 ± 1.5	73.1 ± 3.8	18.7 ± 1.0	20.0 ± 2.2	19.9 ± 2.4	15.3 ± 1.1	3.8 ± 0.2	414.9 ± 8.1
LSD _{0.05}	0.50	1.12	1.63	2.65	0.61	0.93	0.95	0.50	0.32	12.50

^a Sum of hydroxylysine, hydroxyproline, lanthionine, ornithine and taurine. ^b Sum of all amino acids analyzed.

In addition, the authors also noticed that of the six genotypes mentioned above, NC Green and VS15-6021 had higher or had the highest content of tryptophan in whole seed samples under both low-heat and freeze drying, while others were in the moderate range (Tables 7 and S3). However, the highest total content of the other five minor amino acids was not detected in these genotypes. Surprisingly, NC Raleigh, a commercial soybean cultivar with a higher oil content [71], had the highest total content of the five minor amino acids of all these twenty cultivars and lines; this was in all cases, in both sample types and for both thermal and freeze drying (Tables 7 and S1–S3), although it exhibited the lowest contents of most of the amino acids, total amino acid and crude protein [47]. It seems that these minor amino acids might not be associated with major amino acids, but further investigation is needed to elucidate the phenomenon.

4. Conclusions

To dehydrate fresh edamame seeds, freeze drying, low-heat drying at 65 °C, and high-heat drying at 105 °C were used in this study. The contents of amino acids in the dried seed samples of 20 soybean genotypes were evaluated using NIR technology in three consecutive years, and the effects of the drying methods on the estimates were compared. There were significant differences between the years or batches of samplings for all amino acids. The differences among the genotypes were also significant for all the traits of seed amino acids, except for cysteine and methionine with whole seed samples and for tryptophan with ground samples under high-heat drying. There were significant differences between the drying methods for most of the amino acid traits, except for leucine and the total amino acid in both the whole seed and ground samples, as well as in isoleucine with the ground samples; meanwhile, no significant effects of the genotype × drying method interaction were found. However, the ANOVA results and estimates of repeatability were similar and/or comparable between low-heat drying at 65 °C and freeze drying, and the coefficients of correlation between these two methods were considerably high, averaging 0.901 (0.679–0.966) and 0.917 (0.660–0.971) for individual amino acids with whole seed and ground samples, respectively. Thus, the low-heat drying method can serve as an alternative of freeze drying, and it is suitable for the large-scale drying of fresh edamame and the rapid evaluation of seed amino acids.

The estimates of repeatability under individual drying methods were as high as 71–95%, except for tryptophan, cysteine, methionine, and the total of the other five minor amino acids. Using the combined data of all three drying methods, the estimates of repeatability were as high as 85–99%, with the exception of tryptophan with the ground samples and the sum of the five minor amino acids with the whole seed samples. Two sulfur-containing amino acids, cysteine and methionine, were more sensitive to high temperatures than the others were. Of the 20 genotypes, six cultivars and lines exhibited higher contents of all the 18 major amino acids and can be used for the nutritional quality improvement of edamame and other food-grade specialty soybeans.

Supplementary Materials: The following supporting information Tables S1–S3 can be accessed/downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13020574/s1>.

Author Contributions: Conceptualization and funding acquisition: G.-L.J. Data collection and analysis: G.-L.J. and W.T. Result interpretation: G.-L.J. and S.R. Manuscript preparation: G.-L.J. Manuscript review and approval: G.-L.J., W.T. and S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported in part by the USDA-NIFA Capacity Building Grant (2017-38821-26413), and the National Science Foundation HBCU Excellence in Research Grant (2200575).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: This study was supported in part by the USDA-NIFA Evans-Allen Research Program, the USDA-NIFA Capacity Building Grant (award number: 2017-38821-26413), and the National Science Foundation HBCU Excellence in Research Grant (award number: 2200575). Dennis Katuuramu, Yuet Hah Cheung and Sadal Hwang provided assistance in the field trials and sampling. Edward Sismour assisted in seed sample drying. David Lipston, Haley Berry and Kyle Epps, the undergraduate students of Virginia State University, participated in the project. This article is a contribution of the Virginia State University, Agricultural Research Station (Journal Series No. 388).

Conflicts of Interest: The authors declare no conflict or competing of interests.

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