Trop J Nat Prod Res, August 2025; 9(8): 3729 - 3740 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Diversity of Amino Acids, Sugars, Organic Acids and Phenolic Compounds in Four Cultivars of *Allium cepa* L.

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ARTICLE INFO

Article history:
Received 19 May 2025
Revised 13 July 2025
Accepted 14 July 2025
Published online 01 September 2025

ABSTRACT

The diversity of cultivars of *Allium cepa* L results in varying chemical compositions influenced by genetic and environmental factors. This study aimed to compare the chemical composition of four cultivars of onion (*Allium cepa* L.), namely orange onion (HYR), white onion (HYW), Myanmar red onion (HKM) and Indian red onion (HKI), by analyzing amino acids, sugars, organic acids, phenolic compounds, flavonoids and antioxidant activities using chemical methods combined with multiple statistical analysis (PCA, HCA and VIP score).The results showed that HYR contained the highest total amino acids (5477.39 µg/100g DW) while HYR contained a prominent amount of arginine (2414.45µg/100g DW) and HYW has a high content of umami-related amino acids such as glutamic acid and glutamine. In terms of sugar, HKI had the highest total content (340.26 mg/g DW), while HYR was predominantly glucose and fructose. HKM had the highest fructooligosaccharide (14.53 mg/g DW), while HYW had the highest total organic acids (64.44 mg/g DW), predominantly malic acid and citric acid, while HKI had the highest vanillic acid (305.22 mg/100 g DW), rutin, and myricetin. HKI also showed the highest TPC, TFC, DPPH, and FRAP values (11.56 mg GAE/100g DW, 9.09 mgRE/100g DW, 59.70 mgTE /100gDW, and 72.13 mg/100g DW, respectively), while HYW had the lowest values in all variables. VIP score analysis indicated that essential compounds such as glutamic acid, myricetin, vanillic acid, and glucose are the main variables in classifying the species, displaying the biodiversity that can be used to select suitable cultivars for the development of food products

Keywords: Onion, Antioxidant, Bioactive compounds, Flavonoid compounds, Total phenolic content.

Introduction

The *Allium* genus was an important vegetable plant found worldwide, which includes more than 1100 species that were reported[1]. These are a significant group of plants that are used widely for nutritional, medicinal, and ingredient food[1–3]. Specific species, including shallot (*Allium ascalonicum*), garlic (*A. sativum*), onion (*A. cepa*), and leek (*A. ampeloprasum*), are recognized for their varied compositions of bioactive compounds as well as their unique flavors. In Thailand, various species of onions are commonly utilized in food practices to enhance the flavor, aroma, and complexity of traditional dishes. These include yellow onions (*A. cepa*), regularly referred to locally as hom hua yai (large onions); red onions, known as hom khaek (Indian onions, Myanmar onions or red onions); and shallots (*A. ascalonicum*), traditionally called hom daeng. Each species of onion provides unique sensory attributes, ranging from the mild sweetness of yellow onions to the pungent, aromatic qualities of shallots, which are especially prominent in Thai salads (e.g., laab, yum) and soups (e.g., tom yum).

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Citation: Papayrata C, Saensouk S, Chumroenphat T. The Diversity of Amino Acids, Sugars, Organic Acids and Phenolic Compounds in Four Cultivars of *Allium cepa* L. Trop J Nat Prod Res. 2025; 9(8): 3729 – 3740
https://doi.org/10.26538/tjnpr/v9i8.32

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The extensive utilization of diverse Allium species, characterized by variations in color, size and plant geography, emphasizes the necessity of elucidating their phytochemical compositions, particularly the profiles of amino acids, sugars, organic acids, phenolic compounds, and their associated antioxidant activities[4]. Numerous studies on phytochemical compounds have reported that phenolic compounds, including ferulic acid, were most abundant whit 51 mg/100 g found in onion whit no significant difference with color, followed by chlorogenic, 2,5-dihydroxybenzoic, syringic, protocatechuic and gallic acid[5], respectively. While Korea onion was found to have the caffeic acid highest content in the peel (214 mg/100 g FW), protocatechuic acid and ferulic acid were reported[6]. Additionally, The flavonoid compounds were found as quercetin, epicatechin and rutin individual compounds, as previously reported[6, 7]. Moreover, the predominant amino acids where analysis in onion was found were arginine (12 mg/g DW), glutamic acid (11 mg/g DW) and aspartic acid (6 mg/g DW), respectively[8]. Whist, organic acids in México onions, including malic acid, tartaric acid, oxalic acids and fumaric acid were published[9]. Whereas sugars were one of the phytochemicals found in all species of onions. Several countries were reported to have found sugars, including raffinose, sucrose, glucose, and fructose[10–12] . The phytochemical compounds that are most abundant in cultivars of onion are abundant in many Thai Allium cultivars and have been linked to antioxidant, anti-inflammatory, and antimicrobial activities[13–15]. However, the diversity in the composition and concentration of these metabolites can vary significantly among different Allium species and is influenced by genetic, environmental, and agronomic factors [13, 16, 17]. Moreover, the different cultivars of Allium species significantly influence their chemical composition, including variations in amino

acids, sugars, organic acids, and phenolic contents. Furthermore, the phytochemical diversity among Thai Allium species is essential for investigations with different content in each variety. Consequently, we aimed to provide information comparative studies of amino acids, sugars, organic acids, and phenolic profiles associated with antioxidant activity across different *A. cepa* cultivars in Thailand to expose their potential for information and food applications.

Materials and Methods

Chemicals and reagents

All analytical standards used in this study, including individual amino acids (twenty compounds), individual sugars (eight compounds), individual organic acids (eight compounds), individual phenolic acids (twelve compounds), and individual flavonoid compounds (five compounds), were of HPLC grade purity as ≥99.0% (Sigma–Aldrich Co.; St. Louis, MO, USA). The reagent for antioxidant examines, such as 2,4,6-tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), were also purchased from Sigma–Aldrich. The solvents used for extraction and HPLC analysis were of analytical grade and obtained from RCI Labscan (RCI Labscan Ltd., Bangkok, Thailand).

Plant sample collection and preparation

Four samples (Figure 1) of Allium species (*A. cepa* L.) were purchased from a local market in Kantarawichai district, Maha Sarakham, Thailand (16°15'15.3"N 103°13'50.0"E), in November 2024. The plant species were identified by taxonomist , Assoc. Prof. Dr. Surapon Saensouk, from Walai Rukhavej Botanical Research Institute, Mahasarakham University, and number of voucher specimens as HCT122401-05 was deposited in the herbarium. The samples were dried until moisture content was lower than 7% using a freeze dryer (Scanvac CoolSafe, model 100-9 Pro, LaboGene ApS, Denmark). All authenticated samples for evaluation were deposited in the freezer at -20 °C in the laboratory of Ubon Ratchathani Rajabhat University before analysis.

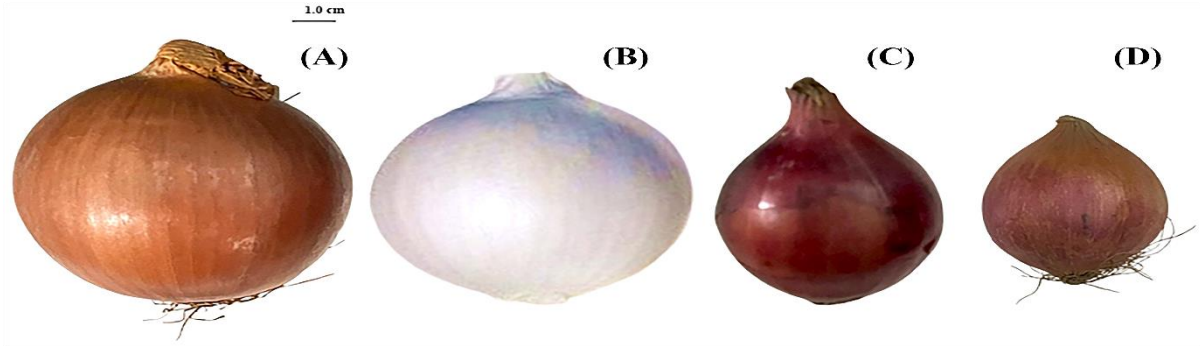

Figure 1: The four Allium species (*Allium* cepa L.) are used in the evaluation. (A): orange onion (HYR); (B): white onion (HYW); (C): Myanmar red onion (HKM); (D): Indian red onion (HKI)

Determination of free amino acids

The twenty individual amino acids were evaluated in this study, including methionine, glycine, histidine, glutamine, proline, lysine, alanine, tyrosine, leucine, cysteine, glutamic acid, tryptophan, asparagine, valine, phenylalanine, serine, arginine, aspartic acid, threonine, and isoleucine. The sample was extracted and analyzed following the previously reported method[13]. demonstration by a triple quadrupole mass spectrometer of Liquid Chromatography-Mass Spectrometer (LC/MS/MS, Shimadzu LCMS-8030, Shimadzu, Kyoto, Japan); electrospray ionization (ESI) mode was operated. Results were reported in micrograms of amino acids in one gram sample on a dry weight (µg/100g DW).

Determination of sugar contents

The analysis of sugars, including monosaccharides, disaccharides, and oligosaccharides, were conducted in accordance with published protocols[18]. The 0.1 g of dried powder sample was extracted with 5 mL of distilled water and stood at 80°C in a hot air oven for 30 minutes. Then, the extracted sample was filled with Whatman no. 4 before filtration through a 0.22 mm filter. The final extracted sample was analyzed by HPLC (Shimadzu 20 series, Japan) with Aminex HPX-87P column (i.d. 7.8 × 300 mm, Bio-Rad, USA) with a guard column operating at a flow rate of 0.5 ml/min. The isocratic conditions, DI water was eluted for 40 min at 90°C as the mobile phase. Refractive index detectors maintained previously monitored calibration curves with displayed individuals of eight external standards (fructooligosaccharide, stachyose, raffinose, sucrose, glucose, mannose, fructose, and xylitol), and the concentrations were calculated.

Determination of organic acids

The dried powder of onion was extracted, and analysis of organic acid followed by previously reported [19]. The sample was 0.5 g extracted with 3% phosphoric acid and mixed by vortex at room temperature for about 2 min and then sonicated with an ultrasonic cleaner (model 6210HP, KUDOS, Japan) for 10 min. The extracted sample was centrifuged (model 2-16 KL, Sigma, Germany) at 25°C at about 12,000 rpm. Supernatant was filtrated by a nylon membrane filter (0.22 µm) before analysis with HPLC (model 20 Series, Shimadzu, Kyoto, Japan). HPLC analysis conditions are described in a previous publication[19]. The results were identified compared with external standards (oxalic acid, citric acid, tartaric acid, malic acid, quinic acid, succinic acid, fumaric acid, and maleic acid), and the calculated concentrations were reported as milligrams per gram dry weight (mg/gDW).

Determination of phenolic acids and flavonoid compounds

The analysis of eleven individual phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, gallic acid, p-coumaric acid, protocatechuic acid, sinapic acid, syringic acid, gentisic acid, cinnamic acid, and vanillic acid) and five individual flavonoid compounds (apigenin, kaempferol, myricetin, quercetin, and rutin) compounds using HPLC(model 20 Series, Shimadzu, Kyoto, Japan) and condition analysis were described in previous studies[18]. The extraction sample was using one gram of sample extracted solvent as 20 mL of 80 % methanol with water/methanol (80:20, v/v) and incubation with shaking for 12 h at 150 rpm (37°C). The extracted sample was filtered with nylon membrane paper through 0.22 µm before using HPLC analysis. The results were displayed as milligrams per gram of 100 dry weight (mg/100g DW) for both phenolic acids and flavonoid compounds.

Determination of total phenolic content (TPC) and total flavonoid content (TFC)

The extraction and analysis of TPC and TFC were followed by previously reported[13]. For TPC, the Folin-Ciocalteu examination was used, and a microplate reader (Varioskan Lux, Thermo Fisher

Scientific, USA) was used for investigation with evaluation of the absorbance at 725 nm. The result was displayed as mg gallic acid equivalents per 100-gram dry weight sample (mg GAE/100gDW). Additionally, TFC was conducted using previous methods and measured by a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA) at absorbance 510 nm immediately after mixing. The results of TFC were demonstrated as mg rutin equivalent per 100-gram dry weight sample (mg RE/100 g DW).

Determination of antioxidant activity

The antioxidant activity was evaluated using two assays, including the DPPH assay (2,2-Diphenyl-1-picrylhydrazyl) and the ferric reducing antioxidant power (FRAP) assay, and followed by previously published methods[20–23]. For the DPPH assay, after the mixed sample with DPPH solution was measured by a microplate reader (Varioskan Lux, Thermo Fisher Scientific, USA) at 517 nm, the calculated value was compared with trolox as reported in mg of trolox equivalent per 100-gram dry weight sample (mgTE/100gDW). While the FRAP assay was performed as previously reported, the final reaction mixture samples were measured using a Varioskan Lux microplate reader (Thermo Fisher Scientific, Waltham, MA, USA) at 593 nm, and FRAP values were expressed as mg $FeSO_4$ per 100-gram dry weight sample (mg $FeSO_4$/100gDW).

Statistical analysis

All data are presented as the mean of three replicates ± standard deviation (SD)To evaluate the results, a one-way ANOVA and the least significant difference (LSD) test were used. $p < 0.05$ indicated significant differences across the samples. The statistical analysis was performed using SPSS software version 17 (Chicago, IL, USA, 2008). In this investigation, MetaboAnalyst 6.0 was used to statistically analyse the observed values[24]. To find important metabolites, principal component analysis (PCA), as the PCA biplot and PCA scores plot for cluster analysis sample. The partial least squares discriminant analysis (PLS-DA) was used; compounds with variable importance in projection (VIP) values higher than 1.5 were deemed relevant. To further group samples according to metabolic profiles, hierarchical clustering analysis (HCA) was performed; a dendrogram was produced to visualise clustering patterns and evaluate group similarities.

Results and Discussion

The diversity of amino acids, organic acids, sugars, phenolic acids and flavonoid compounds, along with different cultivars of Allium species, was investigated using various phytochemical compounds with other variations.

Amino acids content with different cultivars of A. cepa.

Amino acids were evaluated in all cultivars, with their concentrations showing significant differences ($p < 0.05$) across the species. The total amino acid contents of four cultivars ranged from 2771.89 to 5761.36 µg/100g DW and were found in HYW and HKM, respectively. In addition, the highest individual amino acid content was arginine detected in HYW (2414.45 µg/100 g DW) and the lowest was glycine detected in HKM, while cysteine was not detected in all samples as shown in Table 1.

Table 1: The content of amino acids with different cultivars of *Allium cepa*.

Sample name	Amino acids content (µg/100g DW)			
	HYR	HYW	HKM	HKI
Alanine	34.47 ± 1.44^{p}	18.34 ± 0.54^{r}	20.54 ± 0.44^{o}	33.32 ± 0.40^{n}
Arginine	2414.45 ± 43.13^{a}	1856.08 ± 5.93^{a}	1303.41 ± 5.72^{a}	1256.09 ± 1.13^{a}
Asparagine	88.91 ± 0.30^{l}	378.22 ± 7.01^{d}	128.38 ± 1.78^{f}	85.16 ± 4.16^{h}
Aspartic Acid	45.25 ± 1.40^{o}	55.92 ± 0.10^{n}	20.18 ± 0.20^{p}	35.75 ± 0.64^{l}
Cysteine	ND	ND	ND	ND
Glutamine	518.21 ± 6.33^{c}	798.25 ± 15.42^{b}	221.69 ± 4.54^{b}	266.97 ± 1.55^{d}
Glutamic Acid	91.15 ± 1.14^{k}	151.79 ± 4.72^{i}	30.82 ± 0.62^{n}	70.59 ± 1.46^{j}
Glycine	2.22 ± 0.09^{s}	2.00 ± 0.06^{s}	1.41 ± 0.07^{s}	5.73 ± 0.11^{r}
Histidine	134.10 ± 3.57^{h}	108.14 ± 1.51^{k}	69.08 ± 0.32^{i}	71.13 ± 0.19^{i}
Isoleucine	119.82 ± 1.83^{i}	113.21 ± 0.19^{j}	67.57 ± 0.71^{j}	34.84 ± 0.59^{m}
Leucine	106.08 ± 1.44^{j}	98.90 ± 0.41^{l}	60.25 ± 0.44^{k}	33.32 ± 0.49^{n}
Lysine	495.19 ± 7.82^{d}	753.19 ± 4.13^{c}	211.03 ± 0.75^{c}	253.12 ± 1.46^{c}
Methionine	233.91 ± 5.71^{f}	340.88 ± 2.24^{g}	133.06 ± 0.23^{e}	112.19 ± 0.95^{g}
Phenylalanine	176.58 ± 3.11^{g}	352.57 ± 3.17^{f}	99.73 ± 2.03^{h}	157.50 ± 1.08^{f}
Proline	30.43 ± 0.41^{q}	50.89 ± 1.53^{o}	16.72 ± 0.20^{q}	32.63 ± 0.84^{o}
Serine	12.63 ± 0.03^{r}	34.55 ± 2.02^{p}	32.16 ± 0.57^{m}	38.69 ± 0.69^{k}
Threonine	55.11 ± 6.95^{m}	10.24 ± 0.10^{q}	4.74 ± 0.60^{r}	19.50 ± 1.26^{p}
Tryptophan	278.52 ± 0.32^{e}	210.09 ± 1.13^{h}	104.25 ± 2.04^{g}	163.12 ± 0.25^{e}
Tyrosine	593.88 ± 18.32^{b}	362.36 ± 1.24^{e}	209.01 ± 6.06^{d}	299.32 ± 6.36^{b}
Valine	46.48 ± 0.13^{n}	65.74 ± 1.49^{m}	37.87 ± 0.29^{l}	33.01 ± 0.38^{n}
Total	5477.39 ± 5.45^{B}	5761.36 ± 52.94^{A}	2771.89 ± 27.61^{D}	3001.98 ± 22.99^{C}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). ND: Not detected; Means with different lowercase superscripts (a, b, c,…) are significantly different at $p < 0.05$ within the same column. Means with different uppercase superscripts (A, B, C…) are significantly different at $p < 0.05$ within the same row. HYR: orange onion; HYW: white onion; HKM: Myanmar red onion; HKI: Indian red onion

The results of this study are supported by the prior publication, which reported that the most abundant amino acids were arginine and glutamine, found in *A. cepa* L[8, 25] at 12.213 mg/g DW, which is higher than HYW. On the other hand, similarly with the earlier reported that arginine, glutamic acid, phenylalanine, leucines, tyrosine, lysine, and methionine sulfoxide were the amino acids that were more predominant in onion (*A. cepa* L.)[25]. Additionally, the different types and amounts of amino acids found in different cultivars of onion species support previous research that indicated that the amount of amino acids varies depending on the cultivar and cultivated region[16]. Changes in the metabolite compositions of onions, particularly in amino acid profiles, contribute to variations in taste characteristics, notably those responsible for the 'umami' flavor[26, 27]. Consequently, the selection of onion cultivars as processing ingredients differs according to the specific chemical attributes of each tested type[28].

The relationship of amino acids in four different cultivars of onion (*A. cepa*) is shown in Figure 2. The principal component analysis (PCA), as shown in the PCA biplot (Figure 2A) for four cultivars of attributes of the cultivar, namely HKI, HKM, HYR, and HYW, found that PC1 and PC2 explained only 83.42% of the combination (PC1 = 55.17%, PC2 = 28.25%). The result finding presentation that HYR (light blue) was highest in asparagine. HYW (dark blue) was the highest in threonine. HKM (green) was found to have different groups according to the umami taste requirements, including glutamic acid, glutamine, lysine, methionine, isoleucine, and phenylalanine[26]. While HKI (pink) can be found to be high in glycine and tyrosine, which may contribute to the sweet taste[26]. Additionally, Figure 2B showed the PCA scores plot the distribution of samples in each group shows clear differences between the cultivar, particular HKI, HKM, HYR and HYW, which are clustered separately on the PC1 and PC2 axes. Figure 2C. Dendrogram (Hierarchical Clustering Analysis:HCA) as the results indicated a structural relationship between the cultivar, with HYR and HKM formed a closely related cluster, reflecting comparable metabolite profiles between the two cultivars, while HKI and HYW clearly separated into separate groups. HCA clustering analysis indicates that HYR and HYW samples are slightly similar, while HKI and HKM are clearly clustered separately. Heatmap of amino acids showing (Figure 2D) as shown differential accumulation patterns among the cultivars. The result shows that HYR and HYW cultivars were more similar in amino acid composition than the other cultivars, while HKI was clearly separated, associated with the PCA and dendrogram results showing the amino acids differences among the cultivars. The VIP score analysis revealed that each amino acid performed a different role in observing the four groups of onion (*A. cepa*) cultivars (HKI, HKM, HYR, and HYW). Glutamic acid was the variable with the highest VIP value, followed by glutamine, lysine, phenylalanine, and methionine. This indicated that these amino acids contributed a identify in characteristic between cultivars, especially glutamic acid and glutamine, which are related to umami taste and have properties that enhance nutritional value. Comparison of the expression patterns of amino acids in each cultivar revealed that HKM and HYR cultivars had significantly high levels of amino acids with VIP values, which was consistent with the results of the previous PCA and Heatmap analyses. Therefore, these data confirmed that the amino acids that taste and biological function are significantly important for used as important information for the selection of raw materials in food products focusing on flavor and nutraceutical properties.

Sugar content with different cultivars of A. cepa.

The results of sugar content analysis in onion cultivars HYR, HYW, HKM, and HKI showed significant differences ($p < 0.05$) in total sugar content and types of sugar detected in each variety as shown in Table 2. HKI had the highest total sugar content (340.26 mg/gDW), followed by HKM (246.33 mg/gDW), HYW (220.65 mg/gDW), and HYR (166.26 mg/gDW), respectively. Moreover, the individual sugar shown in glucose and fructose were found to the highest content in all cultivars. In particular, HYR cultivar had high glucose levels of 138.64 mg/gDW and fructose of 127.72 mg/gDW, while HKI had glucose and fructose levels of 89.29 mg/gDW and 85.82 mg/gDW, respectively.

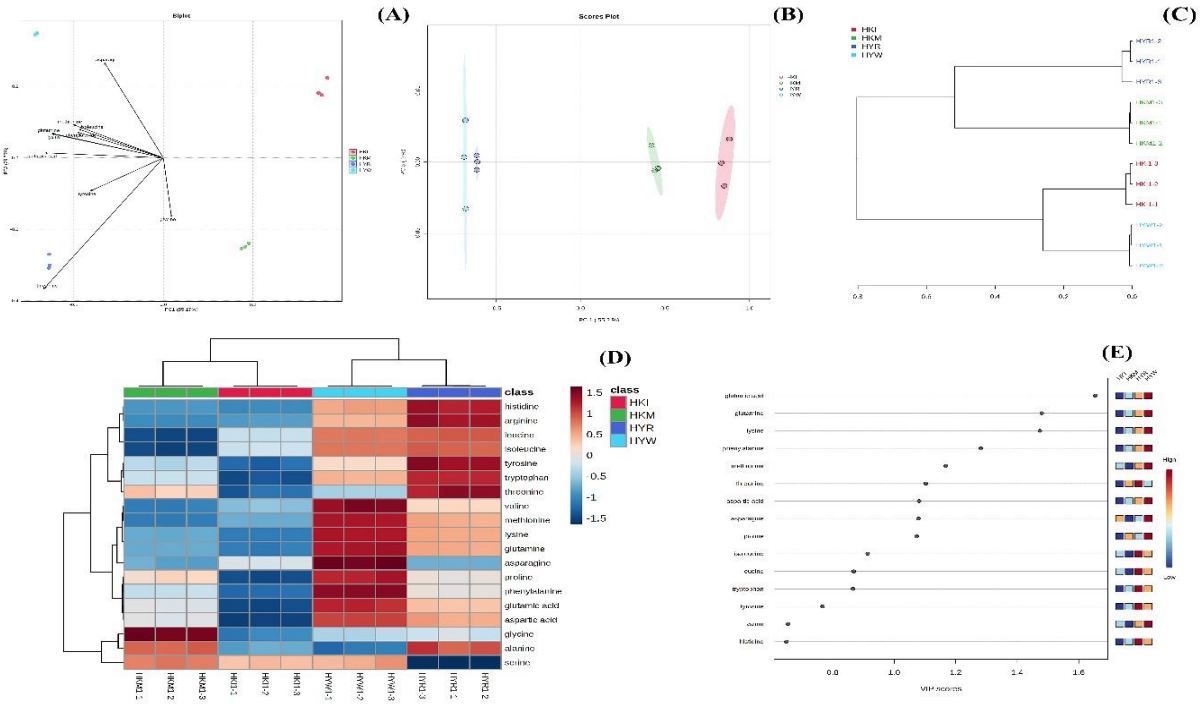

Figure 2: The relationship of free amino acids in different cultivars of *A. cepa* species. (A): PCA biplot; (B): PCA scores plot showing group separation based on amino acids; (C): HCA dendrogram clustering samples by amino acids relationship patterns with cultivars of *A. capa*; (D): Heatmap illustrating amino acids intensity relationships across samples; (E): VIP scores identifying key amino acids contributing to group differences.

Table 2: The content of sugar with different cultivars of *Allium cepa*

parameter	sugar content (mg/gDW)			
	HYR	HYW	HKM	HKI
Fructooligosacharide	4.26±0.05^{d}	6.17±0.04^{e}	14.53±0.43^{d}	9.09±0.07^{d}
Stachyose	ND	ND	ND	ND
Raffinose	ND	11.86±0.26^{d}	8.85±0.20^{e}	7.34±0.21^{e}
Sucrose	31.64±0.32^{c}	31.01±0.25^{c}	32.43±0.28^{c}	29.11±0.21^{c}
Glucose	138.64±1.86^{a}	94.85±0.36^{b}	35.62±0.12^{b}	89.29±0.18^{a}
Mannose	ND	ND	ND	ND
Fructose	127.72±0.38^{b}	102.44±0.82^{a}	74.83±0.28^{a}	85.82±0.37^{b}
Xylitol	ND	ND	ND	ND
Total	166.26±1.31^{D}	220.65±1.04^{C}	246.33±1.73^{B}	340.26±2.61^{A}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). ND: Not detected; Means with different lowercase superscripts (a, b, c,…) are significantly different at $p < 0.05$ within the same column. Means with different uppercase superscripts (A, B, C…) are significantly different at $p < 0.05$ within the same row. HYR: orange onion; HYW: white onion; HKM: Myanmar red onion; HKI: Indian red onion

In addition, HKM was found to have higher fructooligosaccharide content than other cultivars (14.53 mg/gDW), while HYW and HYR had lower fructooligosaccharide contents. The raffinose detection in HYW, HKM, and HKI cultivars but not found in HYR indicated the diversity in the complex sugar composition among cultivars. Stachyose and mannose were not detected in all samples, and no xylitol was detected in any cultivars. From the results of this study, the HKI, which has the highest total sugar content and high levels of single sugars (glucose, fructose), is likely to have a more pronounced sweet flavor than other strains, which is important for its selection as a raw material in the development of food products requiring natural sweet flavor and nutraceutical potential. The result supported that to prior studies predominant sugar found in onion variety cultivar were glucose and fructose[29], which made to sweet test in for ingredients food.

The relation of sugars content in four cultivars of *A. cepa* was shown in Figure 3. The Figure 3A present biplot diagram from PCA analysis shows the distribution of onion cultivars according to sugar composition, with PC1 and PC2 explaining more than 98% of the total variance. HYR and HYW were found to have high correlation with glucose and fructose, while HKM was correlated with fructooligosaccharide and HKI was distant from the total sugar vector, indicating lower content. The PCA scores plot shows (Figure 2B) the distribution of samples in each cultivar, with no overlap between clusters, clearly reflecting the sugar characteristics of each cultivar. HYR and HYW are clustered at opposite locations from HKM and HKI. Furthermore, the specificity of sugar composition in each strain was demonstrated, as was the HCA dendrogram, which grouped HYR and HYW similar clusters, while HKM and HKI were separated into different provides (Figure 3C). In addition, the heatmap analysis (Figure 3D) confirmed the differences in sugars levels among the cultivars, particular HYR with high glucose and fructose levels, HYW with high raffinose and fructooligosaccharide, and HKM showing balanced but unique sugar levels, while HKI had significantly lower sugar content than the other cultivars. The VIP score analysis revealed that glucose and fructose played the most important roles in classifying between cultivars, followed by fructooligosaccharide and raffinose, which are complex sugars with functional properties (Figure 3E). In this study finding that the diversity of sugar composition in different onion cultivars reflects the genetic and environmental differences that affect sugar synthesis in plants, which can be applied to select suitable cultivars for food processing or develop functional food products that emphasize health value and natural sweet taste.

Organic acid content with different cultivars of A. cepa.

The study revealed that the four cultivars onion (*A. cepa*) cultivars, namely HYR, HYW, HKM, and HKI, had significantly different ($p < 0.05$) organic acid compositions (Table 3), with HYW having the highest total organic acid content (64.44 mg/gDW), followed by HKM, HKI, and HYR, respectively, which was consistent with the trend reported in previous research. The variation of cultivars directly affect the synthesis of organic acids that play roles in both flavor and plant metabolism[30, 31]. Among the detected organic acids, malic acid was the major acid found in high amounts in all cultivars, particular in HYW (41.26 mg/gDW) and HKM (35.83 mg/gDW), which is an important organic acid in the tricarboxylic acid cycle (TCA cycle) and shows the sour taste of fruits and vegetable products[32]. Moreover, HKI had the highest levels of citric acid (13.48 mg/gDW), an acid related to antioxidant and antimicrobial properties[1, 6, 27, 33]. In addition, oxalic acid was detected in HYR, HYW, and HKM cultivars, while in HKI was not detected this result may indicate the differences in the metabolic pathways involved in oxalic acid biosynthesis. Even more, the result of succinic acid was found at the highest level in HKI (5.01 mg/gDW), which is consistent with its role in cellular respiration and enhancing the umami taste in some plants[34]. Other organic acids, such as fumaric acid, were found in lowest amounts, and some organic acids, including tartaric acid, quinic acid, and maleic acid, were not detected in all samples, which may be due to genetic variation or environmental conditions during variation that affect the synthesis of these compounds[16]. As reported by the authors, citric acid and malic acid were most abundant in Turkey onion cultivars[35] and white Italy onion cultivar[33].

The relationships of organic acid in onion cultivars including HKI, HKM, HYR and HYW showed obvious chemical differences between each cultivar (Figure 4). The PCA biplot as shown in Figure 4A explained more than 95% of the total variance, indicating that cultivar HKI was related to tartaric acid, while HYR and HYW were related to malic acid and citric acid, while HKM was clearly related to oxalic acid, indication that the organic acid composition unique to each cultivar. Figure 4B shows the PCA scores plot showing the distribution of each group in the Scores plot, showing that all 4 cultivars were significantly different, with HKM and HYW clustered on the right side of PC1, while HYR and HKI were on the left side, indicating the differences in organic acid composition between the groups.

Table 3: The content of organic acids with different cultivars of *Allium cepa*

parameter	organic acid content (mg/gDW)			
	HYR	HYW	HKM	HKI
Oxalic Acid	0.94±0.02^{d}	1.52±0.02^{d}	1.56±0.05^{d}	ND
Citric Acid	12.15±0.13^{b}	17.31±0.88^{b}	22.20±0.49^{b}	13.48±0.41^{b}
Tartaric Acid	ND	ND	ND	6.67±0.04^{c}
Malic Acid	29.44±0.70^{a}	41.26±20.43^{a}	35.83±1.02^{a}	25.06±0.53^{a}
Quinic Acid	ND	ND	ND	ND
Succinic Acid	2.98±0.11^{c}	2.98±0.16^{c}	2.25±0.09^{c}	5.01±0.30^{d}
Fumaric Acid	0.03±0.00^{d}	0.03±0.00^{e}	0.03±0.00^{e}	0.05±0.00^{e}
Maleic Acid	ND	ND	ND	ND
Total	45.54±0.96^{D}	64.44±1.49^{A}	61.53±1.07^{B}	50.60±1.28^{C}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). ND: Not detected; Means with different lowercase superscripts (a, b, c,…) are significantly different at $p < 0.05$ within the same column. Means with different uppercase superscripts (A, B, C…) are significantly different at $p < 0.05$ within the same row. HYR: orange onion; HYW: white onion; HKM: Myanmar red onion; HKI: Indian red onion.

Furthermore, Figure 4C is the HCA Dendrogram, the result of the hierarchical clustering, showing that HKM and HYW were grouped in the same group, while HYR was near the middle group, and HKI was clearly separated, suggesting their separate chemical characteristics. While the heatmap diagram of Figure 4D shows that the concentration levels of six organic acids, especially malic acid, oxalic acid and citric acid, have individual distributions among HKI, HYR, HKM and HYW groups. HKI has high malic and tartaric acids, HYW has high citric and oxalic acids, HYR has moderate acid levels and HKM is dominant in oxalic acid. Furthermore, Figure 4E shows the VIP Score Plot, presenting the VIP score values, showing that oxalic acid and tartaric acid have the highest significance in varied the cultivars, followed by fumaric acid and malic acid, confirming that these organic acids are important variables affecting the genetic differentiation of onion among cultivars[16].

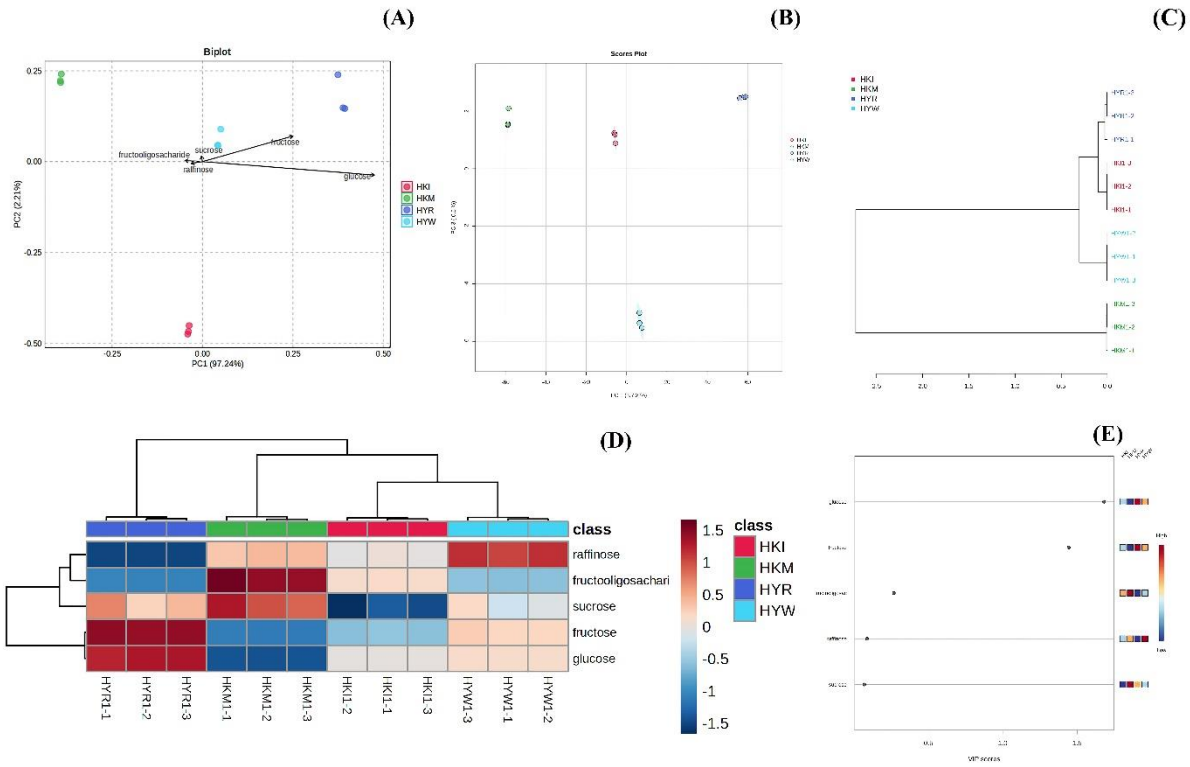

Figure 3: Relationship of sugars in different cultivars of *A. capa*. (A): PCA biplot; (B): PCA scores plot showing group separation based on sugars; (C): HCA dendrogram clustering samples by sugars relationship patterns with cultivars of *A. capa*; (D): Heatmap illustrating sugars intensity relationships across samples; (E): VIP scores identifying key sugars contributing to group differences.

Phenolic acids and flavonoid compounds with different cultivars of A. cepa

The results of phenolic acids and flavonoid compounds in onion (*A. cepa*) cultivars, including HYR, HYW, HKM, and HKI, revealed that each cultivar had significant differences ($p < 0.05$)in both type (Table 4) and amounts of compounds detected, with the HKI cultivar having the highest amount of total phenolic acid and flavonoid compounds (3161.70 mg/100 g), followed by HKM, HYR, and HYW, respectively. In addition, the phenolic acid that was most abundant was vanillic acid, particularly in HYW, which had a high level of 305.22 mg/100gDW, followed by HYR with 78.81 and HKI with 60.78, while HKM had the least amount. Vanillic acid is an important antioxidant in plants and plays a role in protecting against oxidative damage in food[6, 18, 36].

Table 4: The content of phenolic acids and flavonoid compounds with different cultivars of *Allium cepa* cultivars

parameter	content (µg/100gDW)			
	HYR	**HYW**	**HKM**	**HKI**
Phenolic Acids				
Gallic Acid	25.78±0.30^{b}	15.86±0.88^{c}	27.17±2.19^{a}	23.40±0.91^{c}
Protocatechuic Acid	ND	ND	ND	ND
Gentisic Acid	ND	ND	ND	ND
Chlorogenic Acid	ND	ND	ND	ND
Vanillic Acid	78.81±1.03^{a}	305.22±3.45^{a}	29.20±0.71^{a}	60.78±0.42^{a}
Caffeic Acid	ND	ND	ND	ND
Syringic Acid	ND	ND	ND	ND
p-Coumaric Acid	ND	ND	ND	ND
Ferulic Acid	ND	ND	ND	ND
Sinapic Acid	8.47±0.10^{d}	8.4±0.10^{d}	ND	2.33±0.17^{d}
Cinnamic Acid	21.84±0.31^{c}	38.64±0.47^{b}	ND	43.13±0.47^{b}
Total Phenolic Acids	134.89±1.75^{B}	368.20±4.90^{A}	56.37±2.90^{D}	129.64±1.97^{C}
Flavonoid Compounds				
Rutin	623.97±6.96^{b}	24.53±0.65^{c}	556.02±9.03^{b}	696.40±6.99^{b}
Myricetin	1305.94±0.58^{a}	85.00±3.16^{a}	1368.52±8.96^{a}	2252.59±12.62^{a}
Quercetin	39.16±0.10^{c}	38.35±0.02^{b}	36.26±0.02^{c}	83.07±1.20^{c}
Apigenin	ND	ND	ND	ND
Kaempferol	ND	ND	ND	ND
Total flavonoid compounds	2134.26±9.79^{B}	536.19±9.30^{D}	2017.16±20.91^{C}	3032.06±20.81^{A}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). ND: Not detected; Means with different lowercase superscripts (a, b, c…) are significantly different at $p < 0.05$ within the same column.Means with different uppercase superscripts (A, B, C…) are significantly different at $p < 0.05$ within the same row. HYR: orange onion; HYW: white onion; HKM: Myanmar red onion; HKI: Indian red onion

According to the report, vanillic acid content was also found in other onion cultivars. In addition, cinnamic acid was found in three cultivars obtained by HKI (43.13 mg/100gDW), HYR (21.84 mg/100gDW) and HYW (38.64 mg/100gDW), which is related to the antioxidant, antimicrobial, and anti-inflammatory properties[1, 2, 15, 20, 27, 36]. Additionally, the flavonoids such as myricetin were the most prominent compounds in all cultivars, particularly in HKI (2252.59 mg/100gDW), which was the highest compared to other cultivars, followed by HKM and HYR, and lowest in HYW (85.00 mg/100gDW). While rutin was highly enriched in HKI and HYR (696.40 and 623.97 mg/100 g DW, respectively). HKI also had the highest total flavonoid compounds, suggesting its potential as a source of bioactive compounds. This result is in agreement with the finding that onion was used as one of the richest sources of dietary flavonoid compounds, particularly quercetin. The analysis of phenolic acids and flavonoids in onion cultivars HYR, HYW, HKM, and HKI revealed that there were some compounds that could not be detected in all studied cultivars, which may be related to the differences in cultivars and the variation environment. Moreover, protocatechuic acid, gentisic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, apigenin, and kaempferol were not detected in all cultivars of *A. cepa* species.

The relationship of phenolic acids and flavonoid compounds in different onion cultivars (Figure 5) showed that HYW cultivar had significantly different chemical characteristics from other cultivars, especially from the results of PCA biplot (Figure 5A) and PCA score plot (Figure 5B), which explained the total variance was 95.5%, indicating that HYW cultivar was separated from other groups in PC1 axis, with vanillic acid, sinapic acid and cinnamic acid as the main variables affecting the differences. Furthermore, the major compounds such as rutin and myricetin, which were the predominant flavonoid compounds, were highly related to HKI cultivar along the PCA vector pointing in the direction of the HKI group. While, classified by HCA dendrogram (Figure 5C), was found that HKI, HYR and HKM cultivars were similar content of phenolic acid and flavonoid compounds, while HYW was separated.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

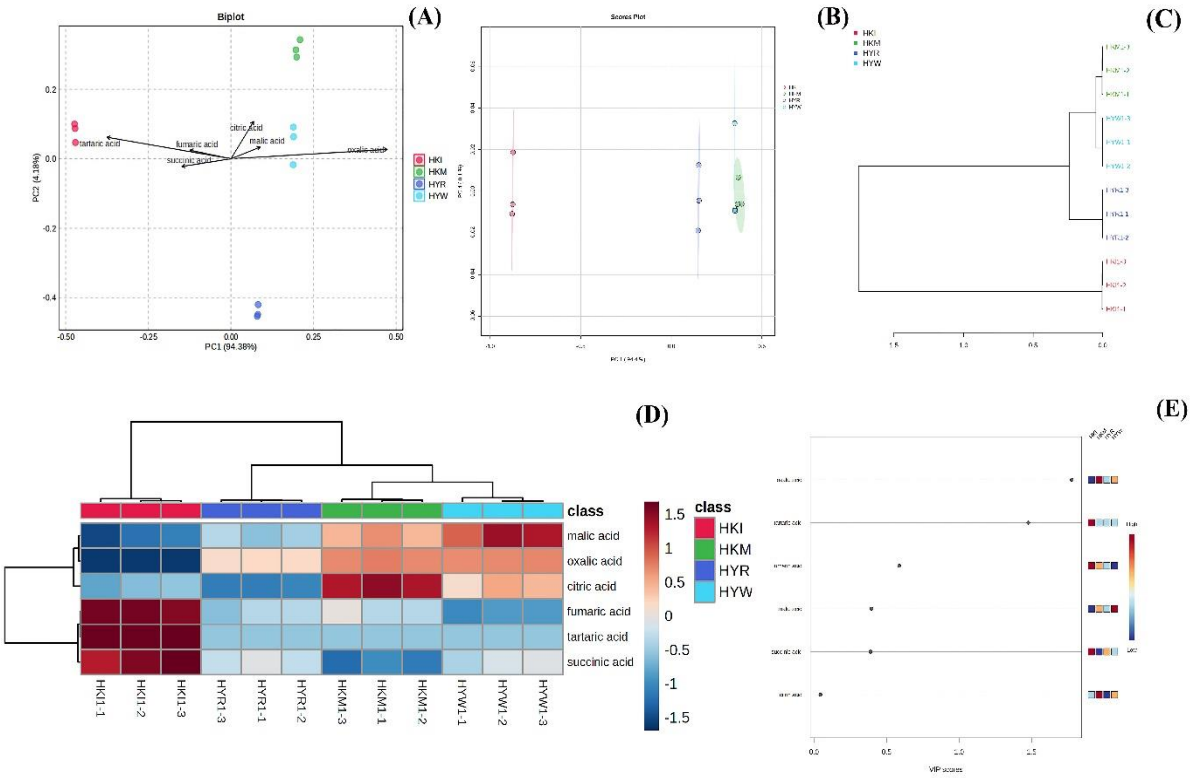

Figure 4: Relationship of organic acids in different cultivars of *A. capa*. (A): PCA biplot; (B): PCA scores plot showing group separation based on organic acids; (C): HCA dendrogram clustering samples by organic acid relationship patterns with cultivars of *A. capa*; (D): Heatmap illustrating organic acids intensity relationships across samples; (E): VIP scores identifying key organic acids contributing to group differences.

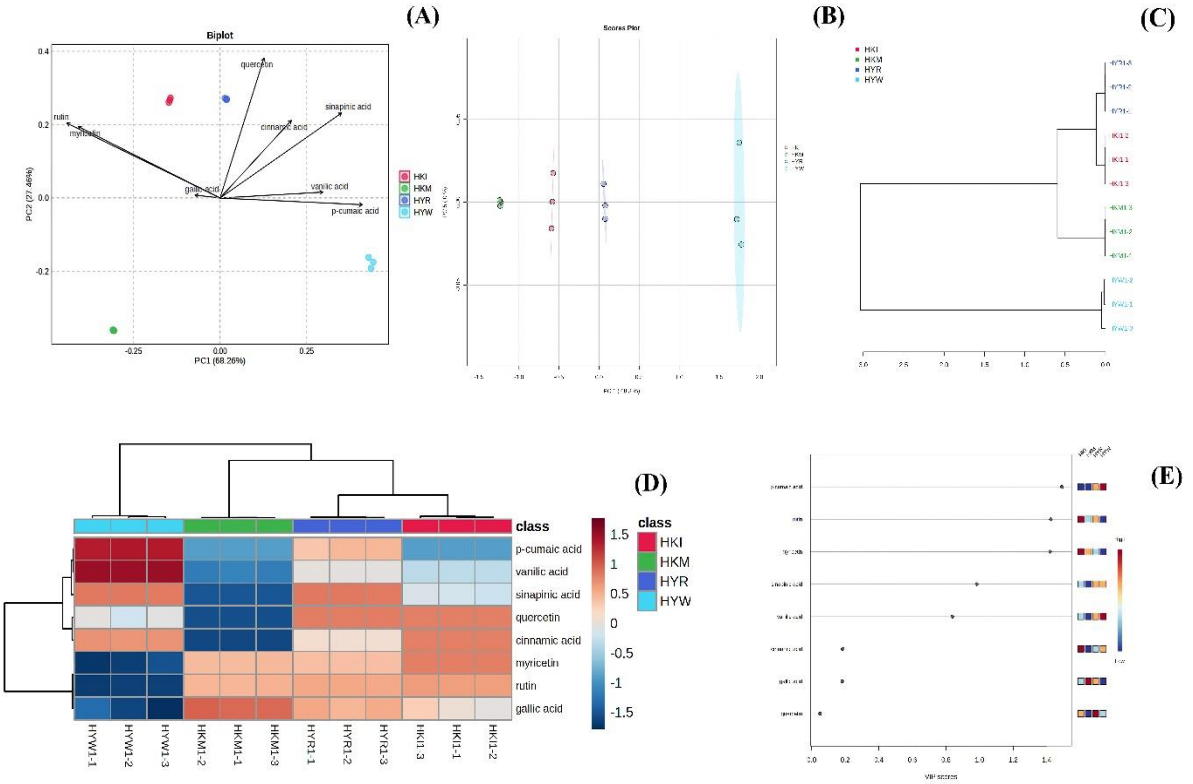

Figure 5: The relationship between phenolic acids and flavonoid compounds in different cultivars of Allium species. (A): PCA biplot; (B): PCA scores plot showing group separation based on phenolic acids and flavonoid compounds; (C): HCA dendrogram clustering samples by phenolic acids and flavonoid compounds relationship patterns with cultivars of *A. capa*; (D): Heatmap illustrating phenolic acids and flavonoid compounds intensity relationships across samples; (E): VIP scores identifying key phenolic acids and flavonoid compounds contributing to group differences.

Supported with the results of PCA biplot and PCA score plot. In addition, the results of heatmap analysis showed the concentration levels of phenolic acids and flavonoids compounds (Figure 5D) using colors to represent the concentration levels (red = high, blue = low). HKI had the highest levels of myricetin, rutin and quercetin, while HYW had significantly higher values of gallic acid, sinapic acid and vanillic acid than HKM and HYR, indicating the diversity of compounds in each cultivar. Myricetin was certainly related to the HKI and HYW groups and had the highest significance value in the VIP score (Figure 5E), indicating that myricetin is a variable that plays a very important role in separating cultivar groups, specifically in relations of antioxidant activity and other biological activities. Rutin, another important flavonoid in onion, was also prominent in the HKI cultivar and had a VIP value higher than 1, indicating its role in separating groups as well. Among the phenolic compounds, vanillic acid and sinapic acid were the main variables in the HYW group, with sinapic acid accumulating the highest in HYW and having a VIP score > 1 (Figure 5E), indicating its importance in species classification. Cinnamic acid was another phenolic acid abundantly found in HYW and HKI, which played an important role in the antioxidant mechanism by acting as a radical scavenger via the hydrogen donation mechanism[37]. In addition, cinnamic acid has been reported to exhibit anti-inflammatory activity[38], contributing to its overall bioactive potential.

Total phenolic content, total flavonoid content and antioxidant activity with different cultivars of A. cepa.

The results showed that different onion cultivars (*A. cepa*) showed significant differences ($p < 0.05$) in total phenolic compounds (TPC), total flavonoids (TFC), and antioxidant activity (DPPH and FRAP assay) shown in Table5.

Table 5: The total phenolic content, total flavonoid content and antioxidant activity with different cultivars of *Allium cepa* cultivars

parameter	HYR	HYW	HKM	HKI
TPC (mg GAE/100g DW)	9.46±0.57^{b}	2.70±0.04^{c}	8.14±0.72^{b}	11.56±1.07^{a}
TFC (mgRE/100g DW)	6.30±0.64^{b}	3.01±0.05^{c}	10.79±0.64^{a}	9.09±0.66^{a}
DPPH (mgTE /100gDW)	57.14±1.33^{a}	1.47±0.15^{b}	60.47±3.23^{a}	59.70±0.35^{a}
FRAP (mg$FeSO_4$ /100gDW)	61.47±2.66^{b}	4.34±0.40^{c}	70.27±1.80^{a}	72.13±0.83^{a}

Note: Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different lowercase superscripts (a, b, c…) are significantly different at $p < 0.05$ within the same row. orange onion. HYR: orange onion; HYW: white onion; HKM: Myanmar red onion; HKI: Indian red onion

The HKI cultivar had the highest total phenolic content (TPC) value (11.56 mg GAE/100g DW), while HYW had the lowest (2.70 mg GAE/100g DW). The highest total flavonoid content (TFC) value was found in HKM (10.79 mg RE/100g DW), followed by HKI (9.00 mg RE/100g DW) and HYR (6.30 mg RE/100g DW), respectively. In more detail, HYW had the lowest TFC value as well as TPC, which, associated with individual phenolic acids and flavonoid compounds analysis by HPLC, showed the lowest total concentration. However, when considering the antioxidant values, the HKI showed the highest activity (no significant difference with HKM) and in both DPPH (59.70 mg TE/100g DW) and FRAP (72.13 mg $FeSO_4$/100g DW), followed by HYR, respectively, while HYW showed the lowest significant antioxidant activity (DPPH: 1.47 mg TE/100g DW and FRAP: 4.34 mg $FeSO_4$/100g DW), indicating that these cultivars may have a lesser amount and type of active compounds that reduce free radicals well under both analysis mechanisms. This result is consistent with previous research that found that red onion has higher phenolic content and antioxidant activity than other cultivars[3, 4, 11], which may be due to the accumulation of various anthocyanins and flavonoids with chemical structures that can donate protons or electrons in the DPPH or FRAP system. In addition, the differences in the content and individual phenolic and flavonoid compounds between onion cultivars may also be influenced by genetic and environmental factors, such as climate, variation methods, and harvesting time[6, 11, 12, 16]. Therefore, the selection of appropriate cultivars is important for the development of food or health supplements that focus on antioxidant activity, especially HKI and HKM cultivars that show high potential in these studies.

The correlation analysis between total phenolic content (TPC), total flavonoid content (TFC), and both DPPH and FRAP antioxidant activities (Figure 6A) showed a statistically significant relationship between the antioxidant activities of the samples, especially between FRAP and DPPH ($r = 0.9943$, $p<0.01$), which reflected the potential for consistent mechanisms of action. In addition, TPC was found to be significantly correlated with both DPPH ($r = 0.8617$, $p<0.01$) and FRAP ($r = 0.8895$, $p<0.01$), while TFC showed a moderate to high correlation with DPPH ($r = 0.9056$, $p<0.01$) but less with FRAP ($r = 0.7085$, $p<0.01$), which is consistent with the observation from the experimental results that some cultivars with high TFC did not show high antioxidant activities.

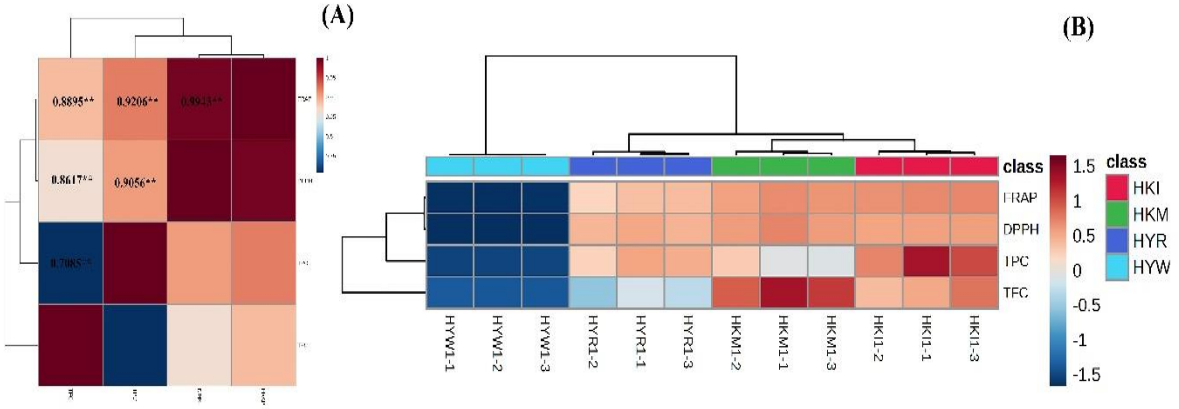

Figure 6: The relationship between total phenolic compounds and total flavonoid compounds associated with antioxidant activity (DPPH and FRAP assay) in different cultivars of *A. cepa*. (A) Correlation analysis of total phenolic compounds and total flavonoid compounds with antioxidant activity (DPPH and FRAP assay) in different cultivars of *A. cepa*. (B) Heatmap illustrates amino acid intensity relationships across samples.

The heatmap (Figure 6B) shows groups of onion cultivars with similar levels of compounds and biological activities, with HKI and HKM cultivars showing deep red color in all four parameters (TPC, TFC, DPPH, FRAP), suggesting their high potential in relationships of bioactive compounds, while HYW cultivars are in deep blue color in almost all parameters, indicating significantly low values. This difference evidently indicates the genetic influence on the phytochemical content of onion, supporting the report by a previous publication[1, 11, 16] that the phenolic composition of onion varies according to the cultivar and the variation environment[11, 16]. Therefore, the heatmap and correlation matrix analysis clearly confirm the role of phenolic compounds in promoting antioxidant activity and support the identification of cultivars with high potential for development as raw materials in health products, especially the HKI and HKM cultivars that show outstanding potential in terms of both quantity and efficacy of active compounds.

Conclusion

This study compared the chemical composition with differences of four cultivars of onion (*A. cepa*) cultivars, namely HKI, HKM, HYR and HYW, by analyzing their amino acids, sugars, organic acids, phenolic compounds, flavonoids and antioxidant activity. It revealed that each cultivar exhibited significant chemical compound diversity, with HKI greating in total amino acids, total sugars and flavonoids (particularly myricetin and rutin), while HYW had the highest levels of some organic acids (e.g. vanillic acid and sinapic acid) despite the lowest total content. HKM had high total flavonoids and fructooligosaccharides, while HYR had high glucose and fructose but relatively low phenolic acids. PCA, Heatmap, Dendrogram and VIP Score analyses confirmed the chemical differences among the cultivars and indicated that some compounds, such as glutamic acid, myricetin, vanillic acid, glucose and fructooligosaccharide, played important roles in cultivar discrimination. The results of this study indicate that each onion variety has its own unique chemical composition, which can be used to select suitable cultivars for the development of food or health products that focus on specific properties such as flavor, natural sweetness, or nutritional value enhancement. Further studies should examine the impact of environmental variables, growing conditions, and processing techniques on the chemical composition of bioactive compounds and their corresponding biological activity.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any responsibility for claims relating to the content of this article shall be borne by them.

Acknowledgement

This research project was financially supported by Mahasarakham University, for which the authors are sincerely grateful. We sincerely appreciate Ubon Ratchathani Rajabhat University, for sharing their invaluable knowledge of phytochemical compounds and laboratory equipment. We are also grateful to the Laboratory Equipment Center and Mahasarakham University for providing the necessary facilities and resources during this study.

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