

INVESTIGATION OF CHANGES IN THE BIOACTIVE PROPERTIES AND PHENOLIC COMPOSITIONS OF DIFFERENT LAYERS OF THE LEEK PLANT

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ABSTRACT

Background. Leek is one of the most widely cultivated winter vegetables in Turkey and Western Europe, with large-scale production in Turkey, France, Belgium and Poland.

Material and methods. The bioactive properties and phenolic compounds of leek layers, numbered from the outermost to the innermost, were determined using spectrophotometric and chromatographic methods, respectively.

Results. Total phenol and flavonoid contents were highest in the outermost (1st layer) and innermost (9th layer) parts of the leek, while intermediate layers (3rd–4th layers) showed comparatively lower levels. Total phenol content ranged from 90.92 mg GAE/100 g (4th layer) to 177.13 mg GAE/100 g (9th layer), and total flavonoid content ranged from 171.84 mg/100 g (4th layer) to 340.58 mg/100 g (1st layer). The predominant phenolic compounds were quercetin, kaempferol, cinnamic acid, gallic acid and catechin. Quercetin content ranged from 49.09 mg/100 g (1st layer) to 207.56 mg/100 g (7th layer), and kaempferol from 35.52 mg/100 g (1st layer) to 291.81 mg/100 g (8th layer). Cinnamic acid content ranged from 5.22 mg/100 g (1st layer) to 184.40 mg/100 g (9th layer). Gallic acid and 3,4-dihydroxybenzoic acid levels ranged from 39.39 mg/100 g (5th layer) to 75.67 mg/100 g (3rd layer) and from 2.22 mg/100 g (8th layer) to 47.57mg/100g (9th layer), respectively.

Conclusion. Both the outermost and innermost layers exhibited the highest total phenol and flavonoid contents. The higher concentration in the outermost layer compared to the innermost layer may be attributed to a relative decrease in water content, which concentrates bioactive compounds.

Keywords: leek, layers, bioactive properties, phenolic compounds, PCA, HPLC

INTRODUCTION

The leaves and stems of leek (*Allium porrum*), a member of the garlic family (Amaryllis), are widely used in soups, stews, and as fillings in bakery products. Due

to its medicinal properties, leek has also been traditionally used for insect bites, tuberculosis, and even bleeding (Kratchanova et al., 2010). The height of the

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leek's inflorescence shoot has been reported to range from 30 cm to 200 cm (Van Der Meer and Hanelt, 1990). Leek is one of the most widely cultivated winter vegetables in Turkey and Western Europe, with large-scale production in Turkey, France, Belgium and Poland (Bernaert et al., 2012). Turkey has recently become a significant producer, with an annual production exceeding 200,000 tons (TÜİK, 2021). Leek is produced and consumed in all regions of the country, forming an important part of winter vegetable intake, particularly in regions with a continental climate (Vural et al., 2000). Rich in fiber, leek plays a valuable role in human nutrition. After harvest, leeks require a period of storage before reaching the market, during which significant internal and external quality losses can occur, as with other horticultural products. Packaging and marketing, whether of whole leeks or cut forms, can greatly influence the extent of these losses (Gülal et al., 2023). The edible parts of the *Allium* species, mostly perennial and bulbous plants, exhibit great morphological diversity and have the potential to grow in a wide range of environments (Stearn, 1992). Leeks contain various biologically active compounds, including fibers, sugars, polyphenols, and S-alkenyl-L-cysteine sulfoxides (Proteggente et al., 2002; Golubkina et al., 2018). Phenolic compounds, including flavonoids and phenolic acids, constitute the secondary metabolites in vegetable leaves, with the main phenolic compounds identified as chlorogenic acid, quercetin, ferulic acid, kaempferol and caffeic acid (Barba et al., 2014; Yalcin and Çapar, 2017). Growing consumer awareness of the health and nutraceutical benefits of fruits, vegetables and medicinal plants has increased interest in the phytochemical content of these products, which are considered functional foods (Chandrashekar et al., 2011; Lee et al., 2012). Vegetables of the *Allium* genus have been reported to protect against cardiovascular diseases due to their antiplatelet and antioxidant activities (Beretta et al., 2017). Organosulfur and phenolic compounds contribute to the antiplatelet activity of *Allium* plants, and in vitro studies have shown that four thiosulfonates inhibit platelet aggregation. Moreover, a relationship has been reported between the phenolic content of *Allium* vegetables (onion, shallot, garlic, and leek) and their antioxidant activities (Queiroz et al., 2009; Lu et al., 2011; Bernaert et al., 2012). *Allium* vegetables,

including leek, are produced for both fresh consumption and industrial processing in winter and summer. Among these, leek is widely grown in Turkey. It is primarily consumed fresh in winter, providing economic importance as production increases. Fresh onion, garlic, and leek are staple salad ingredients and remain in demand all year-round. Leek is particularly sought after in winter markets. The popularity of *Allium* vegetables is attributed to their flavor and antioxidant capacity, which is linked to their content of flavonoids, especially flavonols, flavanones (e.g., naringenin) and flavones (Singh et al., 2018). The aim of this study was to investigate differences in total phenol and flavonoid contents, antioxidant activity, and specific phenolic compound levels among different layers of the bulb portion of the leek plant.

MATERIAL AND METHODS

Material

Leek (*Allium porrum*) samples were purchased from a local market in Konya, Turkey. The layers were numbered from the outermost to the innermost part, with the outermost layer coded as 1, and the innermost as 9 (Fig. 1).



Fig. 1. The layers of a leek

Methods

Moisture content

The moisture content of the leek layers was determined using a KERN & SOHN GmbH infrared moisture analyzer.

Extraction procedure

Leek extracts were prepared using a partially modified method proposed by Strati et al. (2018). Briefly, 15 ml of methanol:water (80:20, v/v) was added to 2 g of crushed sample. The mixture was stirred in an ultrasonic water bath for 30 min and then centrifuged at 6000 rpm for 10 min. The supernatants were collected for analysis.

Total phenolic content

Total phenolic content of the leek layer extracts was determined using the Folin-Ciocalteu method as described by Yoo et al. (2004). After preprocessing, absorbance was measured at 750 nm. Results are expressed as mg GAE/100 g dw.

Total flavonoid content

Total flavonoid content of the leek extracts was determined according to the method described by Hogan et al. (2009), using 0.3 ml of NaNO₂, 0.3 ml of AlCl₃ and 2 ml of NaOH per 1 ml of extract. Results are expressed as mg QE/100 g dw.

DPPH free radical scavenging activity

The antioxidant activity of the extracts was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) according to Lee et al. (1998). Results are expressed as mmol trolox (TE)/kg dw.

Determination of phenolic compounds

Phenolic compounds in the leek layer extracts were separated using HPLC (Shimadzu) equipped with a PDA detector and an Inertsil ODS-3 column (5 µm; 4.6 × 250 mm). The total running time per sample was 60 min. Results are expressed as mg/100 g dw.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 22. Data are presented as mean ±SD (*n* = 3). Significant differences among means were determined using Duncan's Multiple Comparison Test at *p* < 0.05

with the MSTAT program. PCA and correlation analyses were performed using the JMP Pro statistical program.

RESULTS AND DISCUSSION

Moisture amounts and bioactive properties of the leek layers

The moisture content, total phenolic content, total flavonoid content, and antioxidant capacity of different leek layers are presented in Table 1. Total phenolic and flavonoid contents were highest in the outermost (1st layer) and innermost (9th layer) leek layers, whereas intermediate layers (3rd–4th layers) showed comparatively lower levels. Moisture content did not differ significantly among layers, ranging from 84.83% (2nd layer) to 87.05% (9th layer). The slightly lower moisture levels observed in the 1st and 2nd layers may be due to increased evaporation from greater exposure to air. Total phenolic content ranged from 90.92 mg GAE/100 g dw (4th layer) to 177.13 mg GAE/100 g dw (9th layer), while total flavonoid content ranged from 171.84 mg QE/100 g dw (4th layer) to 340.58 mg QE/100 g dw (1st layer). Although both phenolic and flavonoid levels were elevated in the outermost and innermost layers, the higher values in the 1st layer compared to the 9th may be attributed to concentration effects caused by lower water content. The 5th and 6th layers showed similar phenolic contents (106.81 and 100.95 mg GAE/100 g dw, respectively), whereas the lowest total phenolic contents were detected in the 4th and 7th layers, which were comparable to each other. Antioxidant activity ranged from 2.16 mmol/kg dw (1st layer) to 6.41 mmol/kg dw (8th layer). The 1st and 2nd layers exhibited lower antioxidant activities than the other layers. Although antioxidant activity partially reflected total phenolic and flavonoid contents, some discrepancies suggest that differences in phenolic compound distribution among layers influence antioxidant potential. The highest antioxidant activities were observed in the 6th, 7th, and 8th layers.

Factors affecting phytochemical composition in *Allium* species

Factors such as geographical origin, genotype, physiological stage, growing conditions and analytical methods were found to affect the phytochemical content

Table 1. Bioactive properties of leek layer samples

Part of sample	Moisture content %	Total phenolic content mg/100 g	Total flavonoid content mg/100 g	Antioxidant activity mmol/kg
1	85.48 ±0.77*f	173.75 ±15.43a	340.58 ±8.52a	2.16 ±0.10i
2	84.83 ±0.07g**	120.27 ±3.64c	236.60 ±18.69b	3.86 ±0.30h
3	86.82 ±0.97b	99.29 ±12.08g	190.96 ±8.13g	5.75 ±0.08f
4	86.39 ±0.87bcd	90.92 ±6.19hi	171.84 ±37.19h	5.83 ±0.08d
5	86.74 ±0.11b	106.81 ±6.36e	203.35 ±4.64f	5.89 ±0.09de
6	86.02 ±0.74e	100.95 ±1.90ef	215.87 ±15.95de	5.70 ±0.26bc
7	86.65 ±0.10bc	96.54 ±3.03h	255.48 ±4.63c	6.10 ±0.09b
8	86.73 ±0.97b	115.99 ±1.33d	203.25 ±23.31f	6.41 ±0.09a
9	87.05 ±0.33a	177.13 ±4.15b	230.28 ±29.06d	5.72 ±0.44fg

*Values are reported as mean ±SD. Values with the same letters in a column are not significantly different ($p < 0.05$).

and composition in papaya (Duma et al., 2014; Blasi et al., 2016), which may explain the variability observed among different leek layers. Research on other *Allium* species has also documented differences in phenolic content and antioxidant activity. For example, chives (*Allium schoenoprasum*) contain anthocyanins, carotenoids, flavonoids, phenols and tannins, with total phenolic contents of 52.65 mg/100 g (leaves) and 53.52 mg/100 g (roots), and antioxidant capacities of 10.43% and 13.71% for stems and leaves, respectively (Štajner et al., 2011). Gorinstein et al. (2009) determined the antiradical activities of various *Allium* species—garlic, and red, white, and yellow onions as 7 µM TE/g dw, 22 µM TE/g dw, 21 and 20 µM TE/g DW, respectively. In *Allium ampeloprasum*, total phenolic and flavonoid contents in bulbs from 10 wild accessions ranged from 16.78–21.51 mg GAE/g dm to 4.10–4.40 mg CE/g dm, respectively (Arfa et al., 2015). For leek, native to the temperate regions of Europe and Western Asia, Pellegrini and Ponce (2020) reported antioxidant activity (DPPH inhibition) of 48.6% and total phenolic content of 262.7 mg GAE/g dw in ethanolic extracts of dried leaves.

Phenolic compounds and quantitative values of leek layers

Phenolic compounds in leek layers identified by HPLC are presented in Table 2. The amounts of these

compounds varied among the layers. The predominant phenolic compounds were quercetin, kaempferol, cinnamic acid, gallic acid and catechin (Fig. 2). Quercetin and kaempferol contents ranged from 49.09 mg/100 g (1st layer) to 207.56 mg/100 g (7th layer) and from 35.52 mg/100 g (1st layer) to 291.81 mg/100 g (8th layer), respectively. Cinnamic acid levels varied between 5.22 mg/100 g (1st layer) and 184.40 mg/100 g (9th layer). Gallic acid and 3,4-dihydroxybenzoic acid ranged from 39.39 mg/100 g (5th layer) to 75.67 mg/100 g (3rd layer) and from 2.22 mg/100 g (8th layer) to 47.57mg/100 g (9th layer), respectively. Rutin contents ranged from 6.12 mg/100 g (6th layer) to 35.96 mg/100 g (1st layer), while catechin varied between 4.59 mg/100 g (8th layer) and 165.65 mg/100 g (4th layer). Caffeic acid contents ranged from 2.94 mg/100 g (1st layer) to 10.24 mg/100g (3rd layer), and syringic acid ranged from 1.04 mg/100 g (3rd layer) to 10.86 mg/100 g (9th layer). The highest resveratrol concentration (10.00 mg/100g) was detected in the 4th layer. Overall, 3,4-dihydroxybenzoic acid, catechin, rutin, and p-coumaric acid decreased from the outer to inner layers, whereas quercetin, cinnamic acid and kaempferol increased from the inner to outer layers. The highest kaempferol levels were observed in the 4th, 7th, and 8th layers. The lowest phenolic compounds detected were syringic acid, p-coumaric acid, ferulic acid and resveratrol. Interestingly, the

Table 2. Phenolic compounds of leek layer samples (mg/100 g)

	Leek layers								
	1	2	3	4	5	6	7	8	9
Gallic acid	52.82 ±13.76*cd	41.66 ±7.85f	75.67 ±8.43a	54.90 ±4.94de	39.39 ±6.64gh	55.09 ±1.46c	42.02 ±8.07g	42.04 ±5.14i	69.31 ±7.96b
3,4-dihydroxybenzoic acid	3.34 ±0.39f**	19.13 ±1.65c	10.87 ±3.54d	16.93 ±2.08b	3.40 ±0.17fg	2.79 ±0.20h	6.34 ±2.70e	2.22 ±0.45i	47.57 ±3.80a
Catechin	13.25 ±0.65f	26.47 ±2.25c	22.40 ±0.130d	165.65 ±11.04a	16.77 ±2.04e	15.52 ±5.36e	10.43 ±2.45g	4.59 ±1.60h	84.24 ±5.84b
Caffeic acid	2.94 ±0.10i	10.02 ±3.69a	10.24 ±3.66	9.72 ±0.70b	8.44 ±3.89c	6.87 ±2.51ef	3.84 ±1.79g	3.73 ±0.40gh	7.84 ±4.33d
Syringic acid	7.70 ±3.87b	6.56 ±1.12d	1.04 ±0.39g	7.07 ±2.34cd	7.31 ±4.12c	6.97 ±2.60c	3.12 ±1.13f	4.75 ±2.75e	10.86 ±1.59a
Rutin	35.96 ±4.23a	8.07 ±4.36d	12.19 ±2.85c	7.46 ±2.40e	7.75 ±2.74e	6.12 ±1.06f	6.29 ±1.10fg	6.33 ±1.04fg	33.14 ±2.25b
<i>p</i> -coumaric acid	6.11 ±2.54a	3.55 ±0.12d	4.75 ±0.80c	3.71 ±0.33de	4.79 ±1.24c	2.99 ±0.23fg	2.30 ±0.51h	3.28 ±0.24f	5.11 ±0.99b
Ferulic acid	1.47 ±0.31c	1.35 ±0.48c	1.76 ±0.62b	1.41 ±0.63e	3.45 ±1.53a	0.75 ±0.05fg	1.53 ±0.14cd	0.81 ±0.15fg	0.89 ±0.25f
Resveratrol	3.20 ±0.76h	5.61 ±1.68de	4.00 ±1.74fg	10.00 ±0.87a	7.30 ±1.38c	7.32 ±2.58b	6.44 ±2.21d	4.09 ±2.19f	1.03 ±0.24i
Quercetin	49.09 ±1.13g	171.14 ±20.53b	117.81 ±3.58ee	156.30 ±10.64c	115.41 ±7.54	180.43 ±8.78b	207.56 ±6.74a	57.17 ±1.42f	161.03 ±9.00d
Cinnamic acid	5.22 ±0.22i	65.67 ±0.43e	69.67 ±6.28ef	22.81 ±1.29h	62.27 ±5.03g	92.94 ±11.48c	82.34 ±2.44d	104.08 ±14.13b	184.40 ±5.64a
Kaempferol	35.52 ±2.11i	175.90 ±31.78d	189.64 ±1.34e	289.22 ±5.14a	107.09 ±3.60h	163.35 ±10.98f	268.56 ±21.63c	291.81 ±21.83b	135.45 ±5.48g

*Values are reported as mean±SD. Values with the same letters in a row are not significantly different ($p < 0.05$).

highest rutin and syringic acid contents were found in the 1st and 9th layers, with very low rutin detected in the intermediate layers. Beretta et al. (2017) reported 0.8 mg/100g quercetin, 0.3 mg/100g kaempferol, 0.24 mg/100g catechin, 0.32 mg/100g myricetin, 0.29 mg/100g coumaric acid, 1.6 mg/100g ferulic acid, and 0.09 mg/100g caffeic acid in leek. Additionally, Beretta et al. (2017) found 8.7 mg/100 g rutin, 2.0 mg/100 g kaempferol, 0.80 mg/100 g ferulic acid, 0.44 mg/100 g catechin, 0.31 mg/100 g chlorogenic acid, 0.20 mg/100 g coumaric acid, and 0.19 mg/100 g quercetin (fw) in leek leaves. While the phenolic compounds identified in leek in this study were similar

to the literature data, their quantities differed. These differences are likely due to factors such as growing conditions, harvest time, and genetic variation in leek.

Principal component analysis (PCA)

Principal component analysis (PCA) was performed to evaluate the effect of leek layers on total phenolic content, total flavonoid content, antioxidant activity, and individual phenolic compounds. PCA modeling exhibited about 38.743% of explained variance for PC1 and 20.546% of variance for PC2, as shown in Table 3. PC1 was highly positively correlated with rutin (0.955), coumaric acid (0.882), and total phenolic

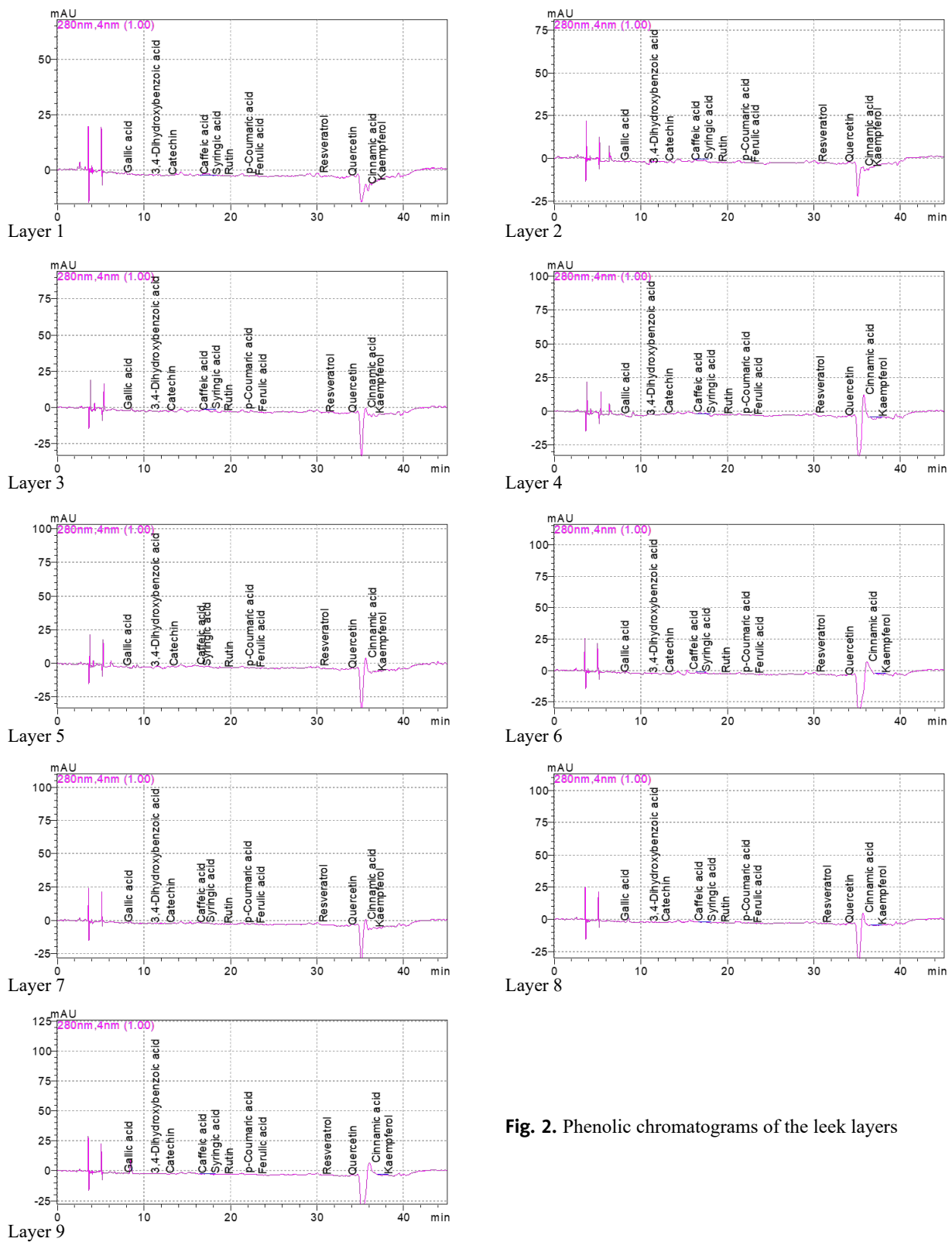


Fig. 2. Phenolic chromatograms of the leek layers

Table 3. PCA results in relation to bioactive properties of leek

	PC1	PC2
Eigenvalue	5.812	3.082
Variability, %	38.743	20.546
Cumulative, %	38.743	59.290
Correlation		
Gallic	0.338	0.499
3,4-dihydroxybenzoic acid	0.243	0.926
Catechin	-0.159	0.652
Caffeic	-0.278	0.560
Syringic	0.518	0.496
Rutin	0.955	0.188
Coumaric	0.882	-0.027
Ferulic	-0.007	-0.349
Resveratrol	-0.652	-0.147
Quercetin	-0.483	0.498
Cinnamic	-0.072	0.602
Kaempferol	-0.839	0.082
TPC	0.944	0.169
TFC	0.765	-0.339
AA	-0.856	0.225

content (0.944), while it showed a strong negative correlation with antioxidant activity (-0.856) and kaempferol (-0.839). PC2 was identified with 3,4-dihydroxybenzoic acid (0.926). The highest total phenolic content, total flavonoid content, and rutin levels were detected in sample P1 (outermost layer), which was located in the positive region of PC1. Sample P9 (innermost layer) was located in the positive region of both PC1 and PC2, and was characterized by higher amounts of 3,4-dihydroxybenzoic acid. Sample P4 (approximately the middle layer) was located in the negative region of PC1 and the positive region of PC2, and exhibited the highest catechin content (Fig. 3).

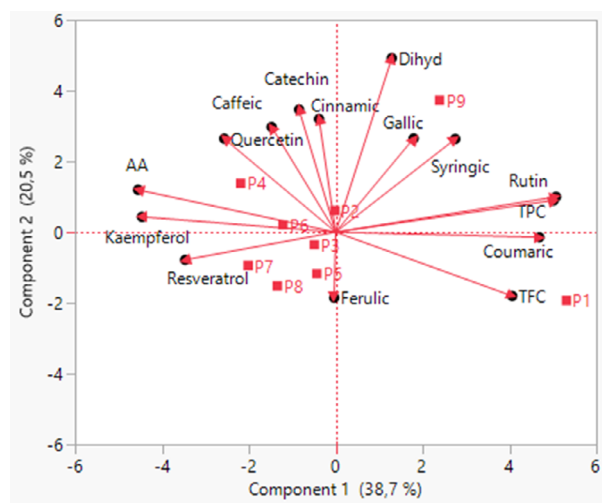


Fig. 3. Biplot graph drawn from PCA results (P1–P9 – part of leek; P1 – outer part, P9 – inner part; TPC – total phenolic content; TFC – total flavonoid content; AA – Antioxidant activity; Dihyd – 3,4-dihydroxybenzoic acid)

Correlation analysis between individual phenolic compounds and antioxidant activity

The correlation between individual phenolic compounds and antioxidant activity is presented in Table 4. Kaempferol (0.6816) showed the strongest positive correlation with antioxidant activity, followed by cinnamic acid (0.5110). The contributions of other phenolic compounds – such as 3,4-dihydroxybenzoic acid, catechin, caffeic acid, ferulic acid, resveratrol and quercetin – to antioxidant activity were relatively low. A moderate negative correlation was observed for both rutin (-0.5970) and coumaric acid (-0.5634) with antioxidant activity.

CONCLUSION

Although the highest total phenol and flavonoid quantities were observed in both the outermost and innermost leek layers, the outermost layer exhibited higher levels than the innermost layer, which may have been due to the increased concentration of bioactive components as water decreased. No significant differences in moisture content were observed across leek layers. Antioxidant activity varied partially with total phenol and flavonoid contents, which may reflect the uneven distribution of phenolic compounds contributing to antioxidant activity in the different layers. The highest

Table 4. Correlation analysis between individual phenolic compounds and antioxidant activity

	Galic	Dihyd	Catechin	Caffeic	Syringic	Rutin	Coumaric	Ferulic	Resveratrol	Quercetin	Cinnamic	Kaempferol	AA
Galic	1.0000	0.4708	0.3476	0.4957	0.1003	0.4193	0.4151	0.0276	-0.1341	0.3467	0.0461	-0.3896	-0.1678
Dihyd	0.4708	1.0000	0.5348	0.3992	0.5438	0.4755	0.2469	-0.2787	-0.4423	0.3377	0.6334	-0.0658	0.0625
Catechin	0.3476	0.5348	1.0000	0.4663	0.3823	0.0766	0.0618	-0.1264	0.3459	0.2558	-0.0472	0.3003	0.1635
Caffeic	0.4957	0.3992	0.4663	1.0000	0.0085	-0.2632	0.0576	0.2560	0.2801	0.3742	0.0403	0.0567	0.1994
Syringic	0.1003	0.5438	0.3823	0.0085	1.0000	0.5326	0.3822	-0.1007	-0.1652	0.0123	0.2735	-0.4573	-0.2650
Rutin	0.4193	0.4755	0.0766	-0.2632	0.5326	1.0000	0.8043	-0.1609	-0.7243	-0.3644	0.1283	-0.6798	-0.5970
Coumaric	0.4151	0.2469	0.0618	0.0576	0.3822	0.8043	1.0000	0.2933	-0.5160	-0.6026	-0.1481	-0.7778	-0.5634
Ferulic	0.0276	-0.2787	-0.1264	0.2560	-0.1007	-0.1609	0.2933	1.0000	0.2941	-0.1165	-0.3527	-0.3153	0.0257
Resveratrol	-0.1341	-0.4423	0.3459	0.2801	-0.1652	-0.7243	-0.5160	0.2941	1.0000	0.3699	-0.5288	0.4156	0.2686
Quercetin	0.3467	0.3377	0.2558	0.3742	0.0123	-0.3644	-0.6026	-0.1165	0.3699	1.0000	0.3041	0.3062	0.3521
Cinnamic	0.0461	0.6334	-0.0472	0.0403	0.2735	0.1283	-0.1481	-0.3527	-0.5288	0.3041	1.0000	0.1110	0.5110
Kaempferol	-0.3896	-0.0658	0.3003	0.0567	-0.4573	-0.6798	-0.7778	-0.3153	0.4156	0.3062	0.1110	1.0000	0.6816
AA	-0.1678	0.0625	0.1635	0.1994	-0.2650	-0.5970	-0.5634	0.0257	0.2686	0.3521	0.5110	0.6816	1.0000

antioxidant activities were detected in the 6th, 7th and 8th layers. In general, 3,4-dihydroxybenzoic acid, catechin, rutin, and *p*-coumaric acid decreased from the outer layers to the inner layers, whereas quercetin, cinnamic acid, and kaempferol increased from the inner to the outer layers. The highest kaempferol levels were found in the 4th, 7th and 8th layers. The lowest amounts of phenolic compounds were syringic acid, *p*-coumaric acid, ferulic acid and resveratrol. Notably, the highest rutin and syringic acid levels were detected in the 1st and 9th layers. For practical applications, the selection of leek layers during cooking should consider the distribution of phytoconstituents and bioactive components to maximize health benefits.

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DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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