Phenolic Contents of Different Potato Genotypes Grown in the Central Northern Region in Turkey

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A R T I C L E I N F O

Research Article

Received : 2007/2021
Accepted : 16/08/2021

Keywords:
Solanum tuberosum L.
Phenolic compounds
LC-MS/MS
Quantitative analysis
Spectroscopy

A B S T R A C T

Potato (Solanum tuberosum L.) is one of the most significant vegetable crops for humans along with corn, wheat, and rice. In this study, quantitative analysis of phenolic compounds was carried out for 21 promising potato clones and three commercial cultivars. LC–MS/MS was used for the chemical analyses. The TOGU 3/518 clone had the highest level of 4-hydroxybenzoic acid as 138.51 ± 7.35 µg/kg. TOGU 12/29 and TOGU 2/198 clones, on the other hand, had 126.24 ± 2.29 and 125.29 ± 2.74 µg/kg of 4-hydroxybenzoic acid, respectively. Salicylic acid which is a pharmacologically significant compound was found in TOGU 3/518 clone (125.66 ± 11.51 µg/kg) as a major product. 

Introduction

Potato (Solanum tuberosum L.) is one of the most important vegetable crops produced all over the world and plays a significant role in human nutrition (Ahmed et al., 2018). Potato is the carbohydrate source that has 80% carbohydrate value and protein content. Potatoes are marketed as various forms such as chips, mashed potatoes, frozen, and dehydrated products like granules (Bergthaller et al., 1999). Phenolic compounds in the potatoes contribute to the antioxidant capacity. Especially, caffeic acid, chlorogenic acid and protocatechuic acid increase the corresponding activity of potatoes (Kätkönen et al., 2019). Total phenolic content in potatoes is higher than tomatoes, carrots and onions (Camire et al., 2009). Flavonoids, anthocyanins are found more in the peel than in the inner part of the potatoes (Calliope et al., 2018).

Among the phenolic acids, caffeic acid and chlorogenic acid, which is the ester of quinic acid, are found abundantly in potatoes (Friedman, 1997). Potato peels contain cinnamic acid, ferulic acid, gallic acid, and protocatechuic acid in high percentage. The consumption habit of the potato has been changed recently. While the consumption of fresh potatoes is decreasing, the consumption of chips and frozen potatoes is increasing in developed countries. Potatoes are rich source of dietary energy because of their carbohydrate value and protein content. Potatoes are marketed as various forms such as chips, mashed potatoes, frozen, and dehydrated products like granules (Bergthaller et al., 1999). Phenolic compounds in the potatoes are complex molecules found in all plants and synthesized to protect them against bacteria, fungi, viruses, and insects (Dede et al., 2019; Koysu et al., 2019). According to their chemical structures, they are divided into groups as flavonoids, terpenoids, alkaloids, phenolic acids, tannins, stilbenes, coumarins and lignans (Singh and Saldaña, 2011). The plants synthesize various phenolic compounds with different quantity. After the discovery of spectroscopy, isolation, and identification of secondary metabolites from plants have become the focus of science (Aksit et al., 2014; Demirtas et
al., 2013; Elmastas et al., 2004; Sahin Yaglioglu et al., 2013; Topçu et al., 1999). A great number of secondary metabolites with significant biological activities were isolated from plants (Bayir et al., 2014; Elmastas et al., 2015; Erenler et al., 2015; Erenler et al., 2014).

Environmental factors such as climate, altitude, soil and nutrients, light have considerable effects on the concentration and composition of phenolic compounds. Moreover, these environmental factors are effective on the therapeutic properties of medicinal plants (Burt 2006, Cragg et al., 1997; Kaya et al., 2014). Consequently, determination of the photochemistry of a plant by quantitative analysis is important for medicinal properties. Quantitative analysis means taking a tomographic image of the plant. After biological activity and quantitative analysis, it can be understood which compound cause the activity. Hence, modification of plants by increasing or decreasing the quantity of compounds in the plant by exposure of stress conditions would be effective for plant nutritional values (Harvey, 2010).

In this study, quantitative analysis of phenolic compounds was determined for 21 potato clones and three cultivars grown in Central Northern Region in Turkey.

Materials and Methods

Materials

In the experiment, 21 different promising potato breeding lines and 3 commercially available cultivars were used as plant material. These genotypes were grown in Tokat Artova conditions in 2020. Tokat Artova is located between 40.13° north latitudes and 36.33° east longitudes with an altitude of 1190 m. The pedigrees of potato clones are shown in Table 1.

Table 1. Plant material used in the experiment

| Number | Clones Name   | Pedigree                | Tuber Flesh Color | Tuber Skin Color |
|--------|---------------|-------------------------|-------------------|------------------|
| 1      | TOGU 2/503    |                         | White             | Light Beige      |
| 2      | TOGU 2/198    | A3/110 × A2/11          | White             | Yellow           |
| 3      | TOGU 2/86     | Light Yellow            | Yellow            | White to Yellow  |
| 4      | TOGU 3/640    | Dark Yellow             | Yellow            | White to Yellow  |
| 5      | TOGU 3/518    | Dark Yellow             | Beige             | Light Beige      |
| 6      | TOGU 3/480    | A3/15 × Bafana          | Yellow            | Light Beige      |
| 7      | TOGU 3/110    | Yellow                  | Yellow            | Beige            |
| 8      | TOGU 4/304    | A7/12 × A3/110          | Yellow            | Light Beige      |
| 9      | TOGU 4/103    | A7/12 × A3/110          | Yellow            | Light Beige      |
| 10     | TOGU 6/28     | A4/4 × A6/28            | Yellow            | White to Yellow  |
| 11     | TOGU 6/329    | A4/4 × A6/28            | Yellow            | White to Yellow  |
| 12     | TOGU 6/187    | Light Yellow            | Beige             | Light Beige      |
| 13     | TOGU 7/1      | Yellow                  | Light Beige       | Yellow           |
| 14     | TOGU 7/10     | A7/12 × Melody          | Dark Yellow       | Yellow           |
| 15     | TOGU 7/146    | Light Yellow            | Yellow            | Light Beige      |
| 16     | TOGU 8/150    | A7/12 × Van Gogh        | Light Yellow      | Light Beige      |
| 17     | TOGU 10/676   | White/Cream             | Light Beige       | Yellow           |
| 18     | TOGU 10/403   | Basciftlik Beyazi × A13/1| White turp       | Light Beige      |
| 19     | TOGU 10/487   | White                   | Light Beige       | Yellow           |
| 20     | TOGU 12/29    | Basciftlik Beyazi × Van Gogh| White to Yellow | Light to Yellow  |
| 21     | TOGU 14/353   | Alleddiyan Sarısı × A2/11| Dark Yellow       | Yellow           |
| 22     | Agata          | Light yellow            | White to Yellow   | Light Beige      |
| 23     | Agria          | Dark Yellow             | White to Yellow   | Yellow           |
| 24     | Basciftlik Beyazi |                     | White             | White to Yellow  |
The programme was applied as B (t, min), B%: (0-8, 95), (8.01-12, 95), (12.01-15, 5). The flow rate of solvent was set as 0.4 ml/min, and injection volume was 20 μl. Shimadzu LCMS 8050 model triple quadrupole mass spectrometer equipped with an ESI source operating in positive and negative ionization mode were used for MS detection. The optimum ESI conditions were fixed as interface temperature; 300°C, DL temperature; 250°C, heat block temperature; 400°C, nebulizing gas flow (nitrogen); 10 l min⁻¹ and drying gas flow (nitrogen); 15 l min⁻¹. LabSolutions software (Shimadzu, Kyoto, Japan) was used for data collection.

Validation and Quantitative Analysis

Firstly, optimisation was performed for spectrometric and chromatographic conditions. Various column, mobile phases (acetonitrile-water, methanol-water etc.), stationary phase (ammonium acetate, ammonium formate-formic acid), column temperature and flow rate were optimized for the best chromatographic separation and mass ionization efficiencies.

Linearity

A calibration study was performed showing important phenolic compounds over a wide range of concentrations. Six concentrations were used for the linearity studies of each analysis, and each concentration was performed in triplicate. The method applied indicated to be linear for all compounds for tested concentration while the validation of the method (R² ≥ 0.990) (Table 2).

Accuracy (Recovery) and Precision (Repeatability)

The precision and accuracy were determined by standard addition to the selected potato clone extract. Intra-day and inter-day variability evaluations were executed. For intra-day variability, spiked samples were measured in five replicates in a single day and for inter-day variability, spiked samples were examined in five replicates per day for five consecutive days. Hence, recovery and %RSD values were calculated to present the accuracy and precision (Table 2).

Detection and Quantification Limits (LOD/LOQ)

The analysis mixture was spiked at ten identical samples prepared from selected extract at the lowest concentration by the standards to determine the LOD and LOQ values (Table 2).

Results and Discussion

In addition to showing antioxidant properties, polyphenols have a wide range of biological activities and are the most numerous antioxidants in our diet. The polyphenol compounds in potato make it important in the daily diet (Wierzbićka et al., 2015).

After validation of the method, the phenolic composition of different potato clones methanolic extracts were investigated by the developed and validated LC-MS/MS method. The standard compounds are given in Figure 1. The 2/503 clone which was obtained by hybridisation of A3/110 × A2/11 contained three compounds; the major product was 4-hydroxybenzoic acid with the value of 23.11 ± 1.02 (μg/kg). TOGU 8/150 clone consisted of only two phenolics and 4-hydroxybenzoic acid was detected as the chief compound (5.61 ± 0.30). Another clone, TOGU 14/353 consisted of four phenolic compounds among the tested ones and 4-hydroxybenzoic acid was also detected as the main product (31.31 ± 1.48). 4-hydroxy benzoic acid was found as the chief product in TOGU 4/304 clone (68.17 ± 1.12). The clones TOGU 3/640 and TOGU 3/518 contained the 8 and 9 compounds, respectively. 4-hydroxybenzoic acid was the chief product in both clones. While the 4-benzoic acid concentration was found in the former clone as 82.37 ± 4.15 μg/kg, it was found in later clone as 138.51 ± 7.35 which was the highest concentration among the tested standards. The other clones called TOGU 2/198 and TOGU 7/146 contained the five tested standard compounds. While 4-benzoic acid was the most abundant compound in clone TOGU 2/198, protocatechuic acid was the main compound in 7/146. The clone 7/1 and clone 3/480 also consisted of five compounds. The chief compound, 4-benzoic acid concentration was found in corresponding clones as 36.56 ± 4.15 μg/kg and 59.54 ± 2.33 μg/kg, respectively. TOGU 3/110 and TOGU 12/29 clones included the six and five compounds, respectively. The 4-benzoic acid concentration was the major compounds in clone TOGU 12/29 with the value of 126.24 ± 2.29 μg/kg. The clone TOGU 3/110 included the caffeic acid and the chief product (42.50 ± 3.73 μg/kg). The clones TOGU 10/403, TOGU 6/28 and TOGU 10/676 contained the 4-benzoic acid as the major compound. 4-hydroxybenzoic acid was found as a major product in TOGU 7/F10, TOGU 6/187 and TOGU 10/487 clones as well. The quantitative analysis of phenolic compounds was also investigated in Agata, Basciftlik Beyazi and Agria cultivars. 4-hydroxybenzoic acid was the major compound in Agata. However, Basciftlik Beyazi contained only two compounds. Interestingly, Agria contained gallic acid which was not found in tested clones and cultivars. 4-hydroxybenzoic acid was found as a major compound in all tested materials except three materials (one cultivar and two clones) (Table 3).

Previously reported work indicated that phenolic acids are the most abundant compounds in potatoes. Chlorogenic acid, caffeic acid, vanillic acid, protocatechuic acid, ferulic acid were the main compounds of potatoes in different concentrations (Akyol et al., 2016) and these results were similar to our study. A quantitative study was carried out on four potato cultivars and chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid, trans-cinnamic acid were found as the main products (Im et al., 2008).

The clones include the considerable protocatechuic acid (3,4-dihydroxybenzoic acid) which is a phenolic compound found in many food plants. PCA is the main metabolite of anthocyanins which can reduce the risk of cardiovascular diseases. The beneficial effect of anthocyanins is partly due to the revealing a considerable activity such as anti-inflammatory, antioxidant, peroxidation inhibition effects. PCA was reported to have pharmaceutical properties such as antioxidant, antimicrobial, anti-inflammatory, antihyperglycemia, anti-apoptosis activities (Semaning et al., 2015).

The other secondary metabolite present in clones is 4-hydroxybenzoic acid, a biodegradable bioactive natural product isolated from carrots, oil palm, grapes, peroba, t youth, red sandalwood, southern catalpa, Chinese chest, palm, medlar. 4-hydroxybenzoic acid revealed the antibacterial, antifungal, antialgal, antimutagenic, estrogenic activities. (Khadem and Marles, 2010)
Table 2. Analytical parameters that belong to the LC-MS/MS method

| PC  | RT  | PI   | Product ions | CE   | R²   | LOD | LOQ | Recovery % | RSD % |
|-----|-----|------|--------------|------|------|-----|-----|------------|-------|
| 4HBA| 4.403| 137.20 | 93.10/65.10  | 17.0/ 24.0 | 0.990 | 1.68 | 5.61 | 111.05 | 109.67 |
| SA  | 4.384| 137.20 | 93.10        | 18.0   | 0.993 | 1.18 | 3.93 | 102.05 | 102.35 |
| CA  | 2.776| 179.00 | 134.10/171.10 | 13.0/ 23/0.10 | 0.989 | 0.82 | 2.74 | 93.95  | 91.17  |
| PCA | 2.855| 153.10 | 108.90/108.10 | 20.0/ 11.0 | 0.999 | 3.48 | 11.60 | 98.45  | 105.48 |
| GA  | 2.895| 153.00 | 108.0        | 2.0    | 0.999 | 1.08 | 3.60 | 110.35 | 106.07 |
| QUE | 7.561| 300.90 | 151.10/212.10/107.10 | 15.0/ 24/0.0/20.0 | 0.997 | 1.64 | 5.46 | 96.20  | 96.26  |
| FA  | 3.884| 193.00 | 133.10/105.10 | 24.0/ 20.0 | 0.996 | 2.70 | 9.00 | 91.20  | 89.73  |
| VA  | 2.223| 167.00 | 152.10/108.00 | 28.0/ 20.0 | 0.992 | 4.15 | 13.83 | 98.91  | 92.81  |
| CHA | 3.588| 352.90 | 191.10/127.10 | 21.0/ 25.0 | 0.999 | 1.83 | 6.11 | 96.45  | 98.01  |
| GAA | 1.149| 169.10 | 125.20/79.0   | 24.0/ 30.0 | 0.992 | 1.73 | 5.77 | 100.15 | 96.72  |

PC: Phenolic compound, PI: Precursor ion, 4HBA: 4-hydroxybenzoc acid, SA: salicylic acid, CA: caffeic acid, PCA: protocatechuc acid, GA: gentisic acid, QUE: quercetin, FA: ferulic acid, VA: vanillic acid, CHA: chrolic acid, GAA: gallic acid, RT: Retention time (min), Precursor ion (m/z); Molecular ions of the standard compounds (m/z ratio), CE: Collision energy (eV), R²: Coefficient of determination, RSD: Relative standard deviation, LOD/LOQ (μg/L); Limit of detection/quantification.

Table 3. Quantitative analysis of the potato promising clones and cultivars

| Clone | 4HBA | SA     | CA     | PCA   | GAA   |
|-------|------|--------|--------|-------|-------|
|       | M    | SD     | M      | SD    | M     | SD    | M      | SD    |
| TOGU 2/503 | 23.11 | 1.02  | 20.74  | 1.51  | <LOD  | <LOD  | <LOD   | <LOD  |
| TOGU 8/150  | 5.61  | 0.30  | <LOD   | 4.92  | 0.93  | <LOD  | <LOD   | <LOD  |
| TOGU 14/353  | 31.31 | 1.48  | 28.60  | 1.51  | 5.16  | 0.63  | <LOD   | <LOD  |
| TOGU 4/308   | 68.17 | 1.12  | 62.09  | 0.50  | 6.80  | 0.46  | <LOD   | <LOD  |
| TOGU 3/640   | 82.37 | 4.15  | 73.45  | 5.27  | 21.70 | 1.09  | 20.25  | 1.10  |
| TOGU 3/518   | 138.51| 7.35  | 125.66 | 11.51 | 18.18 | 0.69  | 18.43  | 1.11  |
| TOGU 2/198   | 125.29| 2.74  | 111.27 | 1.31  | 31.26 | 5.62  | 10.66  | 1.76  |
| TOGU 7/146   | 44.73 | 1.09  | 42.59  | 1.24  | 8.52  | 1.04  | 53.98  | 1.47  |
| TOGU 7/81    | 36.56 | 1.94  | 33.41  | 1.19  | 11.64 | 0.37  | 9.62   | 0.71  |
| TOGU 3/480   | 59.54 | 2.33  | 53.88  | 2.66  | 4.53  | 1.03  | 18.91  | 2.73  |
| TOGU 3/110   | 9.20  | 0.79  | 8.42   | 0.14  | 42.50 | 3.73  | 9.75   | 1.48  |
| TOGU 12/29   | 126.24| 2.29  | 111.07 | 3.68  | 6.96  | 1.06  | 13.80  | 2.27  |
| TOGU 10/403  | 9.31  | 0.22  | 8.50   | 0.04  | 5.75  | 0.21  | <LOD   | <LOD  |
| TOGU 6/28    | 7.04  | 0.67  | 6.67   | 0.55  | 4.25  | 0.18  | <LOD   | <LOD  |
| TOGU 10/676  | 13.52 | 0.43  | 12.52  | 0.79  | 5.72  | 1.07  | <LOD   | 0.49  |
| TOGU 7/F10   | 71.03 | 1.15  | 62.57  | 1.93  | <LOD  | 18.96 | 1.68  | 11.84  | 0.18  |
| TOGU 6/187   | 44.04 | 1.04  | 41.07  | 0.44  | <LOD  | 7.38  | 0.18  | <LOD   | <LOD  |
| TOGU 10/487  | 44.45 | 1.63  | 39.16  | 0.71  | <LOD  | 5.72  | 0.54  | <LOD   | <LOD  |
| TOGU 6/329   | 14.88 | 0.70  | 11.97  | 0.94  | 10.67 | 1.61  | <LOD   | <LOD  |
| TOGU 4/103   | 13.95 | 0.18  | 13.28  | 1.07  | 4.00  | 0.10  | <LOD   | <LOD  |
| TOGU 2/86    | <LOD  | <LOD  | 4.53   | 0.49  | <LOD  | <LOD  | <LOD   | <LOD  |
| Agata        | 67.50 | 2.91  | 59.37  | 2.45  | <LOD  | <LOD  | <LOD   | <LOD  |
| Agra         | <LOD  | <LOD  | 6.67   | 1.03  | <LOD  | <LOD  | <LOD   | <LOD  |
| B.Beyazi     | 4.40  | 0.31  | <LOQ   | <LOD  | <LOD  | <LOD  | <LOD   | <LOD  |

Note: <LOD: Limit of detection; <LOQ: Limit of quantification; 4HBA: 4-hydroxybenzoc acid, SA: salicylic acid, CA: caffeic acid, PCA: protocatechuc acid, GA: gentisic acid, QUE: quercetin, FA: ferulic acid, VA: vanillic acid, CHA: chrolic acid, GAA: gallic acid, M: Mean, SD: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification.
Gentisic acid (2,5-dihydroxy benzoic acid) are found in plant kingdom plentifully. Gentisic acid has analgesic, anti-inflammatory, anti-rheumatic, antiarthritic and cytostatic effects, and inhibits low-density lipoprotein oxidation in human plasma (Ashidate et al 2005).

The clones include salicylic acid (2-hydroxy benzoic acid) also found in many aromatic and medicinal plants. Moreover, it is produced by the bacterium *Pseudomonas cepacia*. It has anti-inflammatory, analgesic, antifungal, keratolytic, antipyretic activities (Ashidate et al 2005).

Caffeic acid (3,4-dihydroxy cinnamic acid) is the metabolite occur in plant kingdoms commonly. This compound is present in many foods such as coffee drinks, blueberries, cider, and apples. In addition, it is found in propolis. Caffeic acid was reported to have anticancer, antioxidant, antibacterial properties. Moreover, it can contribute to inhabitation of atherosclerosis and various cardiovascular diseases (Magnani et al 2014).

**Conclusion**

Photochemical properties of 21 potato promising clones and 3 commercial cultivars of *Solanum tuberosum* L. were displayed. Quantitative analysis results of clones and cultivars will be a significant data for potatoes researchers because these kinds of studies will enable us to reveal the best quality of potatoes containing certain quantities of phenolic compounds. Since 3/518 clone included the most phenolic compounds, it could be preferred for production on a large scale.

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