



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

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ARTICLE INFO

Research Article

Received : 20/07/2021

Accepted : 16/08/2021

Keywords:

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

ABSTRACT

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 $138.51 \pm 7.35 \mu\text{g/kg}$. TOGU 12/29 and TOGU 2/198 clones, on the other hand, had 126.24 ± 2.29 and $125.29 \pm 2.74 \mu\text{g/kg}$ of 4-hydroxybenzoic acid, respectively. Salicylic acid which is a pharmaceutically significant compound was found in TOGU 3/518 clone ($125.66 \pm 11.51 \mu\text{g/kg}$) as a major product. This compound was also found in TOGU 2/198 ($111.27 \pm 1.31 \mu\text{g/kg}$) and TOGU 12/29 clones ($111.07 \pm 3.68 \mu\text{g/kg}$) as the third and fourth most abundant. In terms of caffeic acid, TOGU 3/110 clone contained the highest level ($42.50 \pm 3.73 \mu\text{g/kg}$). While TOGU 7/146 clone included the most protocatechuic acid ($53.98 \pm 1.47 \mu\text{g/kg}$), TOGU 3/480 clone consisted of most gentisic acid ($30.79 \pm 0.51 \mu\text{g/kg}$). Quercetin, an important flavonoid found many aromatic and medicinal plants, was highest in TOGU 12/29 clone ($6.27 \pm 0.15 \mu\text{g/kg}$).

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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% water and 20% dry matter in the harvested fresh state. Potatoes, which are especially rich in vitamin C, contain vitamins B1, B3, B6, E and minerals such as calcium, potassium, phosphorus, and magnesium (Ezekiel et al., 2013). Total phenolic content in potatoes is higher than other commonly used fruits and vegetables such as 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 contribute to the antioxidant capacity. Especially, caffeic acid, chlorogenic acid and protocatechuic acid increase the corresponding activity of potatoes (Kähkönen et al., 1999).

Phenolic compounds, known as secondary metabolites, 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; Topcu 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
4	TOGU 3/640		Dark Yellow	Yellow
5	TOGU 3/518		Dark Yellow	Beige
6	TOGU 3/480	A3/15 × Bafana	Dark Yellow	Yellow
7	TOGU 3/110		Yellow	Yellow
8	TOGU 4/304	A7/12 × A3/110	Cream	Yellow
9	TOGU 4/103		White	White to Yellow
10	TOGU 6/28		Yellow	Yellow
11	TOGU 6/329	A4/4 × A6/28	Dark yellow	Yellow
12	TOGU 6/187		Light Yellow	Beige
13	TOGU 7/1		Yellow	Light Beige
14	TOGU 7/10	A2/11 × Melody	Dark Yellow	Yellow
15	TOGU 7/146		Light Yellow	Yellow
16	TOGU 8/150	A7/12 × Van Gogh	Light Yellow	Light Beige
17	TOGU 10/676		White/Cream	Light Beige
18	TOGU 10/403	Basciftlik Beyazi × A13/1	White turp	Light Beige
19	TOGU 10/487		White	White to Yellow
20	TOGU 12/29	Basciftlik Beyazi × Van Gogh	Dark Yellow	Yellow
21	TOGU 14/353	Alleddiyan Sarısı × A2/11	Dark Yellow	Yellow
22	Agata		Light yellow	White to Yellow
23	Agria		Dark Yellow	White to Yellow
24	Basciftlik Beyazi		White	White to Yellow

Chemicals

Gentisic acid, caffeic acid, quercetin, protocatechuic acid, 4-hydroxybenzoic acid, salicylic acid, ferulic acid, and vanillic acid were purchased from Sigma Aldrich Company. Methanol and other solvents with HPLC grade were bought from Merck.

Extraction Protocol

Potato powder of each clone (1.0 g) was extracted with methanol (50%) and acetic acid (0.5%) for three hours at room temperature (RT). Later, vortex process was carried out for 20 seconds at five times. The centrifugation continued at 10000 rpm for 20 min, then, the supernatant was taken to a new tube. The process was repeated four times and supernatants were combined. A last centrifugation was performed to remove the remaining tissue suspension and stored at deep fridge (-80°C) for further analysis (Nusret et al., 2020).

Chromatographic Conditions and instrumentation

Tubers were harvested in the mid of October in Artova. After harvest, tubers were stored at +4°C for 8 weeks. Quantitative analysis of phenolic compounds was carried out using Shimadzu LCMS-8050 instrument. LC-30AD binary pumps, DGU-20A3r degasser, CTO-10ASvp column oven and SIL-30AC autosampler were used for liquid chromatography. C18 Inertsil ODS-4 (3µm; 2.1×150 mm) analytical column was employed for separation. The column temperature was adjusted at 40°C. The elution gradient contained mobile phase A (water, 5mM ammonium formate) and B (methanol, 5 mM ammonium formate).

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 μ l. 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 ($R^2 \geq 0.990$) (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 (Wierzbicka 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 \times A2/11 contained three compounds; the major product was 4-hydroxybenzoic acid with the value of 23.11 \pm 1.02 (μ g/kg). TOGU 8/150 clone consisted of only two phenolics and 4-hydroxybenzoic acid was detected as the chief

compound (5.61 \pm 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 \pm 1.48). 4-hydroxy benzoic acid was found as the chief product in TOGU 4/304 clone (68.17 \pm 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 \pm 4.15 μ g/kg, it was found in later clone as 138.51 \pm 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 \pm 4.15 μ g/kg and 59.54 \pm 2.33 μ g/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 \pm 2.29 μ g/kg. The clone TOGU 3/110 included the caffeic acid and the chief product (42.50 \pm 3.73 μ g/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 inhabitation effects. PCA was reported to have pharmaceutical properties such as antioxidant, antimicrobial, anti-inflammatory, antihyperglycemia, anti-apoptosis activities (Semaming 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, taheebo, 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	R ²	LOD	LOQ	Recovery %		RSD %	
								Inter-day	Intra-day	Inter-day	Intra-day
4HBA	4.403	137.20	93.10/65.10	17.0/ 24.0	0.990	1.68	5.61	111.05	109.67	3.34	3.05
SA	4.384	137.20	93.10	18.0	0.993	1.18	3.93	102.05	102.35	6.30	3.33
CA	2.776	179.00	134.10/117.10/109.00	13.0/ 23.0/ 10.0	0.999	0.82	2.74	93.95	91.17	14.42	9.08
PCA	2.855	153.10	108.90/108.10	20.0/ 11.0	0.999	3.48	11.60	98.45	105.48	11.24	7.50
GA	2.895	153.00	108.,0	2.0	0.999	1.08	3.60	110.35	106.07	6.02	7.45
QUE	7.561	300.90	151.10/212.10/107.10	15.0/ 24.0/ 20.0	0.997	1.64	5.46	96.20	96.26	8.06	8.52
FA	3.884	193.00	133.10/105.10	24.0/ 20.0	0.996	2.70	9.00	91.20	89.73	10.37	13.21
VA	2.223	167.00	152.10/108.00	28.0/ 20.0	0.992	4.15	13.83	99.50	92.81	9.57	19.58
CHA	3.658	352.90	191.10/127.10	20.0/ 25.0	0.999	1.83	6.11	96.45	98.01	7.88	7.77
GAA	1.149	169.10	125.20/79.0	24.0/ 30.0	0.992	1.73	5.77	100.15	96.72	5.09	4.48

PC: Phenolic compound, PI: Precursor ion, 4HBA: 4-hydroxybenzoic acid, SA: salicylic acid, CA: caffeic acid, PCA: protocatechuic acid, GA: gentisic acid, QUE: quercetin, FA: ferulic acid, VA: vanillic acid, CHA: chlorgenic 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		GA	
	M	SD	M	SD	M	SD	M	SD	M	SD
TOGU 2/503	23.11	1.02	20.74	1.51	<LOD		<LOD		<LOD	
TOGU 8/150	5.61	0.30	<LOD		4.92	0.93	<LOD		<LOD	
TOGU 14/353	31.31	1.48	28.60	1.51	5.16	0.63	<LOD		<LOD	
TOGU 4/304	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	30.08	1.27
TOGU 3/518	138.51	7.35	125.66	11.51	18.18	0.69	18.43	1.11	23.75	0.99
TOGU 2/198	125.29	2.74	111.27	1.31	31.26	5.62	10.66	1.76	9.10	0.56
TOGU 7/146	44.73	1.09	42.59	1.24	8.52	1.04	53.98	1.47	25.62	0.94
TOGU 7/1	36.56	1.94	33.41	1.19	11.64	0.37	9.62	0.71	9.82	1.14
TOGU 3/480	59.54	2.33	53.88	2.66	4.53	1.03	18.91	2.73	30.79	0.51
TOGU 3/110	9.20	0.79	8.42	0.14	42.50	3.73	9.75	1.48	<LOD	
TOGU 12/29	126.24	2.29	111.07	3.68	6.96	1.06	13.80	2.27	<LOD	
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		5.99	0.49
TOGU 7/F10	71.03	1.15	62.57	1.93	<LOD		19.86	1.68	11.84	0.18
TOGU 6/187	44.04	1.04	41.07	0.44	<LOD		7.38	0.18	<LOD	
TOGU 10/487	44.45	1.63	39.16	0.71	<LOD		5.72	0.54	<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	
Agata	67.50	2.91	59.37	2.45	<LOD		<LOD		23.28	1.72
Agria	<LOD		<LOD		6.67	1.03	<LOD		<LOD	
B.Beyazi	4.40	0.31	<LOQ		<LOD		<LOD		<LOD	
Clone	QUE		FA		VA		CHA		GAA	
	M	SD	M	SD	M	SD	M	SD	M	SD
TOGU 2/503	<LOD		5.70	1.16	<LOD		<LOD		<LOD	
TOGU 8/150	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 14/353	5.00	0.55	<LOD		<LOD		<LOD		<LOD	
TOGU 4/304	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 3/640	<LOD		30.65	2.47	28.84	2.79	11.05	1.27	<LOD	
TOGU 3/518	5.45	0.19	6.11	0.22	11.60	0.75	6.71	0.54	<LOD	
TOGU 2/198	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 7/146	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 7/1	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 3/480	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 3/110	<LOD		<LOD		<LOD		6.71	0.31	<LOD	
TOGU 12/29	6.27	0.15	<LOD		<LOD		<LOD		<LOD	
TOGU 10/403	5.57	0.57	<LOD		<LOD		<LOD		<LOD	
TOGU 6/28	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 10/676	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 7/F10	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 6/187	4.46	0.18	<LOD		<LOD		<LOD		<LOD	
TOGU 10/487	5.47	0.35	<LOD		<LOD		<LOD		<LOD	
TOGU 6/329	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 4/103	<LOD		<LOD		<LOD		<LOD		<LOD	
TOGU 2/86	<LOD		<LOD		<LOD		<LOD		<LOD	
Agata	<LOD		<LOD		<LOD		<LOD		<LOD	
Agria	<LOD		<LOD		<LOD		<LOD		7.32	0.16
B.Beyazi	<LOD		<LOD		<LOD		<LOD		<LOD	

4HBA: 4-hydroxybenzoic acid, SA: salicylic acid, CA: caffeic acid, PCA: protocatechuic acid, GA: gentisic acid, QUE: quercetin, FA: ferulic acid, VA: vanillic acid, CHA: chlorgenic acid, GAA: gallic acid, M: Mean, SD: Standard deviation, LOD: Limit of detection, LOQ: Limit of quantification.

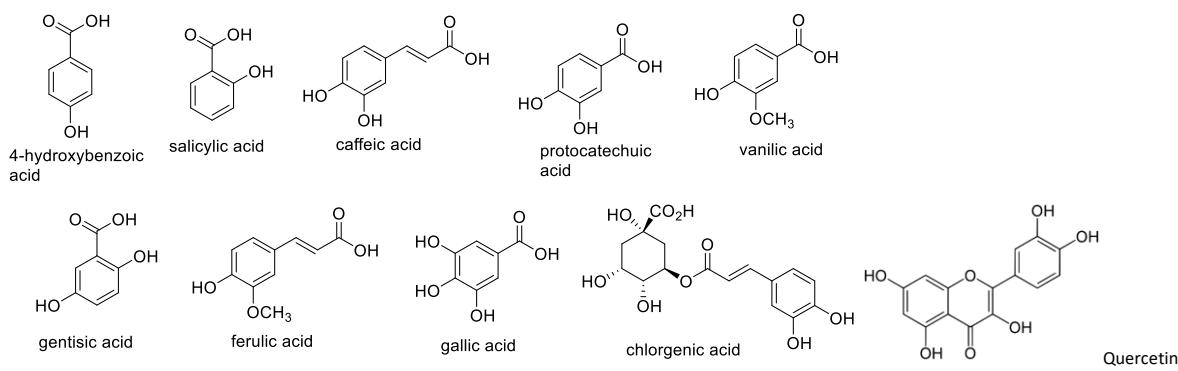


Figure 1. The standard phenolic compounds for quantitative analysis

Gentisic acid (2,5-dihydroxy benzoic acid) are found in plant kingdom plentifully. Gentisic acid has analgesic, anti-inflammatory, antirheumatic, 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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