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# *Quality characteristics of red fruits fresh and after lyophilization*

**Keywords:** fruit, lyophilization, freeze drying, Total phenolic content (TPC), flavonoid, vitamin C

## 1. SUMMARY

**In our work, the quality characteristics and nutritional parameters of red fruits were examined in their fresh state and after lyophilization. The purpose of the comparison was to assess the effect of freeze drying on these fruits. The elemental and dry matter content of fresh fruits was determined, and the changes in their total phenolic compound and flavonoid content, as well as their vitamin C and acid content. A slight increase in the total phenolic compound and flavonoid content and a smaller decrease in the vitamin C content were observed, while the quantity of total acids was reduced in the sample to almost one-third after lyophilization.**

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## 2. Introduction

Over the past few decades, there has been a steady increase in interest in research into the antioxidant effects of fruits, especially red fruits, as they support the proper functioning of the human body through their prominent role in nutritional physiology **[1, 2]**. Fruits are extremely rich in phenolic compounds, such as tannins, anthocyanins and flavonoids, and are considered a very good source of vitamins. They are high in sugar, contain dietary fiber and organic acids (oxalic acid, malic acid, citric acid, fumaric acid), while low in calories and fat **[11]**. These plant substances are present in higher concentrations in small fruits (blueberries, blackberries, strawberries, sour cherries and raspberries) **[13]**, thus having a positive effect on the health and performance of the human body, and may provide protection against, for example, digestive, cardiovascular and other chronic diseases **[3, 4, 5, 6, 7, 8]**.

Phenolic compounds present in fruits form a very large group of plant metabolites and exert their defense mechanisms over a very wide range **[9, 14]**. These compounds also affect the organoleptic properties and quality of the fruits **[11, 12]**. Flavonoids are secondary plant metabolites that have a protective function in fruits, against dehydration, infections, mechanical damage, etc. **[15]**. Vitamin C is a water-soluble vitamin that is essential for the human body, as it plays an important role in the defense against scurvy, as well as in maintaining healthy skin, gums and blood vessels, among other things **[16]**. Not only bioactive compounds, but also minerals may be responsible for the antioxidant effect. Exogenous antioxidants, such as vitamins C and E, flavonoids, carotenoids and elements with antioxidant effects, such as selenium, zinc, manganese, etc., are also key to the functioning of the human body's defense mechanism. Red fruits contain higher amounts of the elements that are essential for the healthy functioning of the human body. For example, several studies have reported high potassium, calcium and magnesium contents in such fruits, as well as their low sodium content **[17, 18, 19, 20]**.

In their fresh state, the fruits spoil in a short time, and their shelf life can be extended by reducing their moisture content, i.e., by drying. The production of such fruits is a major challenge for the food industry, as some drying processes can damage the antioxidant effects of the plants **[10]**. Therefore, it may be interesting to assess how freeze drying (as a gentle method) affects the bioactive content and antioxidant effects of the fruits.

## 3. Materials and methods

The fruits examined by us were strawberries (*Fragaria x ananassa)*, raspberries (*Rubus idaeus)*, sour cherries (*Prunus cerasus*), blackberries (*Rubus*) and blueberries (*Cyanococcus)*. Fresh fruits were obtained from the same commercial unit, their place of cultivation was the north-eastern region of Hungary. The tests were started by examining the total polyphenol, flavonoid, acid and vitamin C contents of the fresh fruits. Following this, fresh fruits were lyophilized using a Heto Powerdry PL 9000 lyophilizer at -45 °C for 24 to 48 hours, and then the above tests were again carried out on the freeze-dried samples. Element content was tested only in the case of fresh samples, as neither drying ovens nor lyophilization has not an effect on the element content of the plants.

### *3.1. Determination of dry matter content*

In the case of fresh fruits, the dry matter content was determined using a drying oven (Memmert UF 75 Universal Oven, Memmert GmbH+Co. KG, Schwabach, Germany). Samples were dried at 55 °C to constant weight for 12 hours, and then the moisture and dry matter content of the samples was determined using a formula. As lyophilization is a freeze-drying method, no further drying was performed on the lyophilized samples.

### *3.2. Total phenolic content (TPC)*

The total phenolic content was determined using Folin-Ciocalteu reagent according to the method described by Singleton et al. **[21]**. After homogenization, the samples were soaked in an 80:20 mixture of methanol (Scharlab S. L., Spain) and distilled water, then they were filtered through fluted filter paper (Sartorius Stedim Biotech S.A., Gottingen, Germany). 0.5 ml of the samples was pipetted into a test tube, followed by the addition of 2.5 ml of Folin-Ciocalteu reagent (VWR International S.A.S., France) and 2 ml of 75 g/l sodium carbonate (Scharlab S. L., Spain) solution. For the formation of the colored compound, the samples were allowed to stand for 2 hours at room temperature in a light-protected area, and then the absorbance of the samples was measured in a 1 cm cuvette at 760 nm using a spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England). The calibration solution used in the determination of the total phenolic content was prepared from a stock solution of gallic acid (Alfa Aesar GmbH&Co. KG, Karlsruhe, Germany), so the results were obtained in mg GAE/100 g (Gallic Acid Equivalent).

### *3.3. Determination of flavonoid content*

A spectrophotometric method was used to determine the total flavonoid content. Samples were once again soaked in an 80:20 mixture of methanol (Scharlab S. L., Spain) and distilled water, then they were filtered through fluted filter paper (Sartorius Stedim Biotech S.A., Gottingen, Germany). 1 ml of the filtered samples was pipetted into test tubes containing 4 ml of a 20:80 methanol:distilled water mixture and 0.3 ml 5% of sodium nitrite (Scharlau Chemie S.A., Spain), then 5 minutes were allowed to pass. At the end of the waiting time, 0.3 ml 10%of aluminum chloride (Scharlab S.L., Spain) and 2 ml of 1 M sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany) solution were pipetted to the samples, and the volume was filled to 10 ml using a methanol: distilled water mixture. Finally, the absorbance of the samples was measured in a 1 cm cuvette using a spectrophotometer (Evolution 300 LC, Thermo Electron Corporation, England) at 510 nm. A stock solution of catechin (Cayman Chemical Company, USA) was used for the calibration solutions, and the results were obtained in mg CE/100 g (Catechin Equivalent) **[22]**.

### *3.4. Determination of vitamin C content*

The vitamin C content of the samples was determined using a metaphosphoric acid solution [**23**]. To 5 g of the samples was added 100 ml of a 3% metaphosphoric acid (Thermo Fischer GmbH, Germany) solution, then it was mixed. It was then washed into a 250 ml volumetric flask and an additional 50 ml of metaphosphoric acid was added. The mixture was filtered through fluted filter paper (Sartorius Stedim Biotech S.A., Gottingen, Germany). 50 ml of the filtrate was pipetted into a titration flask, then 30 ml of distilled water, 5 ml of 2% hydrochloric acid (VWR International S.A.S, France), 5 ml of 1% potassium iodide (Sigma-Aldrich Chemie GmbH, Germany) and 1 ml of starch indicator (VWR International S.A.S., France) were added. The resulting solution was finally titrated with a 0.004 M potassium iodate (Sigma-Aldrich Chemie GmbH, Germany) solution. Results are given in mg/100 g.

### *3.5. Determination of total acid content*

Acid content was determined according to the method described by Czipa (2014) **[23]**. Fresh samples were homogenized, lyophilized samples were pulverized, and then 20 g was weighed into an Erlenmeyer flask and 150 ml of distilled water was added. After thorough stirring, it was heated on a water bath at 85-95 °C for 30 minutes, and then it was allowed to cool to room temperature. The mixture was filtered through cotton wool and then made up to the mark with distilled water in a 250 ml volumetric flask. 25 ml of the resulting filtrate was pipetted out and made up to 100 ml with distilled water ml. Titration was carried out in the presence of a few drops of phenolphthalein indicator with 0.1 M sodium hydroxide (Sigma-Aldrich Chemie GmbH, Germany). Results are given in %.

### *3.6. Determination of element content*

Sample preparation was performed according to the method of Kovács et al. **[24]**. During the test, 3 g of the sample was weighed into a 100 ml digestion tube. Concentrated nitric acid (10 ml) was added to the samples, they were allowed to stand overnight, and then were heated at 60 °C for 30 minutes. Following this, hydrogen peroxide (3 ml) was added to the samples and they were heated again at 120 °C for 90 minutes. At the end of this time, the samples were made up to 50 ml with high purity distilled water (Milli-Q water purification system; Millipore SAS, Molsheim, France), and filtered through 388 filter-paper (Sartorius Stedim Biotech S.A., Gottingen, Germany). Element content was measured with an ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) instrument (Thermo Scientific iCAP 6300, Cambridge, UK). The elements were measured at the following wavelengths: Ca (317.9 nm), K (766.4 nm), Mg (279.5 nm), Na (589.5 nm), P (185.9 nm), S (180.7 nm), Mn (259.3 nm), Zn (213.8 nm). The Rf power of the ICP instrument was set to 1200 W.

### *3.7. Statistics*

Analytical testing of the samples was performed in triplicate in each case. SPSS software (version 13; SPSS Inc. Chicago, Illinois, USA) was used for the evaluation of the results. Using the program, the mean and standard deviation were determined, and then Tukey and Dunnett's T3 test (one-way analysis of variance) was used to determine statistically significant differences between the results.

## 4. Results

### *4.1. Dry matter content*

Among the fruits we examined, strawberries and sour cherries had the lowest dry matter content (12.6%), while the highest results were obtained for blackberries (16.8%). In order to make the results of the different tests comparable, the values are given on a dry matter basis in each case.

### *4.2 Total phenolic compounds (TPC)*

The total phenolic content of the fresh and lyophilized fruits is summarized in ***Figure 1***. Very high values (945-1363 mg GAE/100 g) were obtained for all samples. Higher values were measured for the lyophilized samples than for the fresh fruits. In our opinion, this may be due to the fact that lyophilization for these compounds is a more gentle drying method than using an oven.

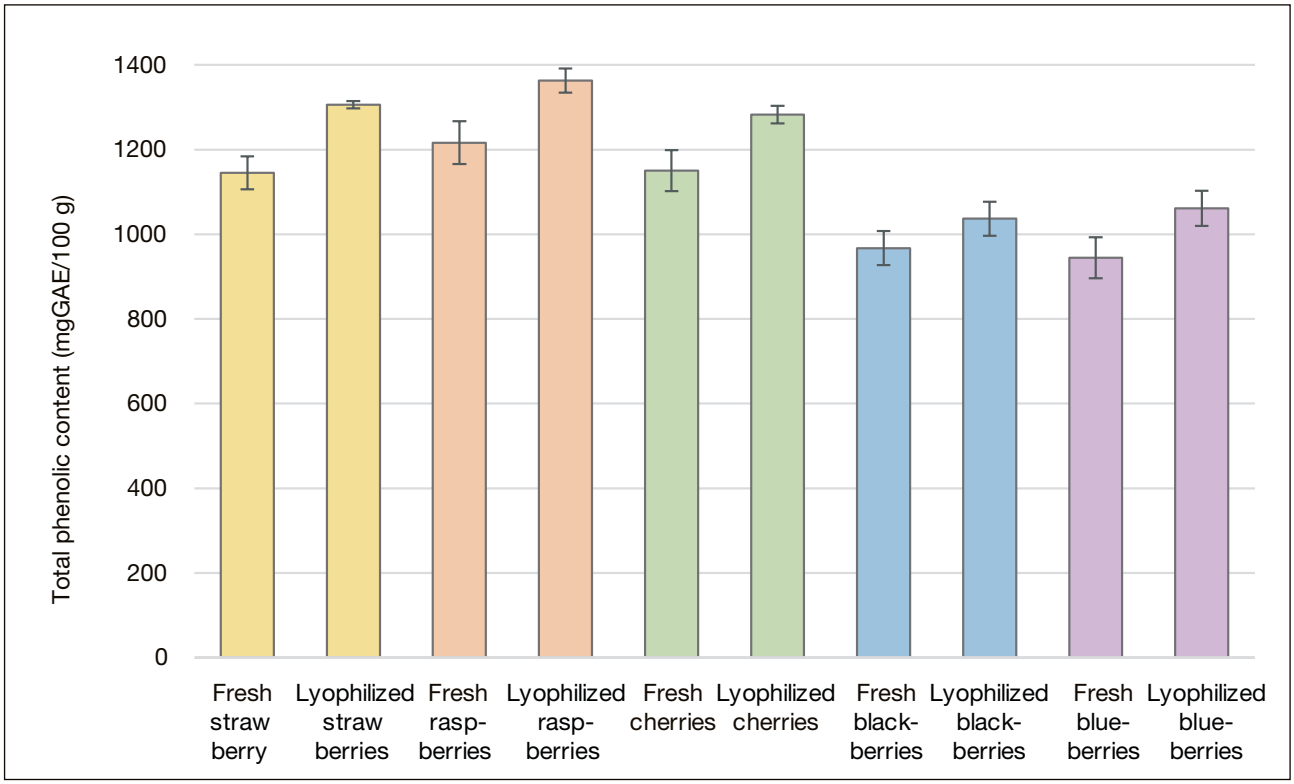


*Figure 1. Total phenolic content of fresh and lyophilized fruits*

As shown in the figure, there is no significant difference between fresh blackberries (967 mg GAE/100 g) and fresh blueberries (945 mg GAE/100 g). In the case of these fruits, the phenolic content is not significantly higher even after lyophilization (1037-1061 mg GAE/100 g). In contrast, for strawberries, raspberries and sour cherries, values between 1,145 and 1,363 mg GAE/100 g were obtained. No significantly different values were obtained for fresh strawberries and fresh sour cherries (1,145 and 1,150 mg GAE/100 g), and for lyophilized strawberries and lyophilized sour cherries (1,306 and 1,283 mg GAE/100 g). These differences could not be verified statistically.

### *4.3. Flavonoid content*

The flavonoid content of the tested samples is shown in ***Figure 2***. It is clear that there is no significant difference between the fresh and lyophilized samples, i.e., lyophilization does not significantly affect the presence of these compounds in the samples. In contrast to the phenolic compounds content, raspberries had the lowest flavonoid content (309-340 mg CE/100 g), while blueberries had the highest values (647-707 mg CE/100 g). In the case of strawberries and blackberries, almost the same results were obtained, and the differences were statistically verifiable in each case.

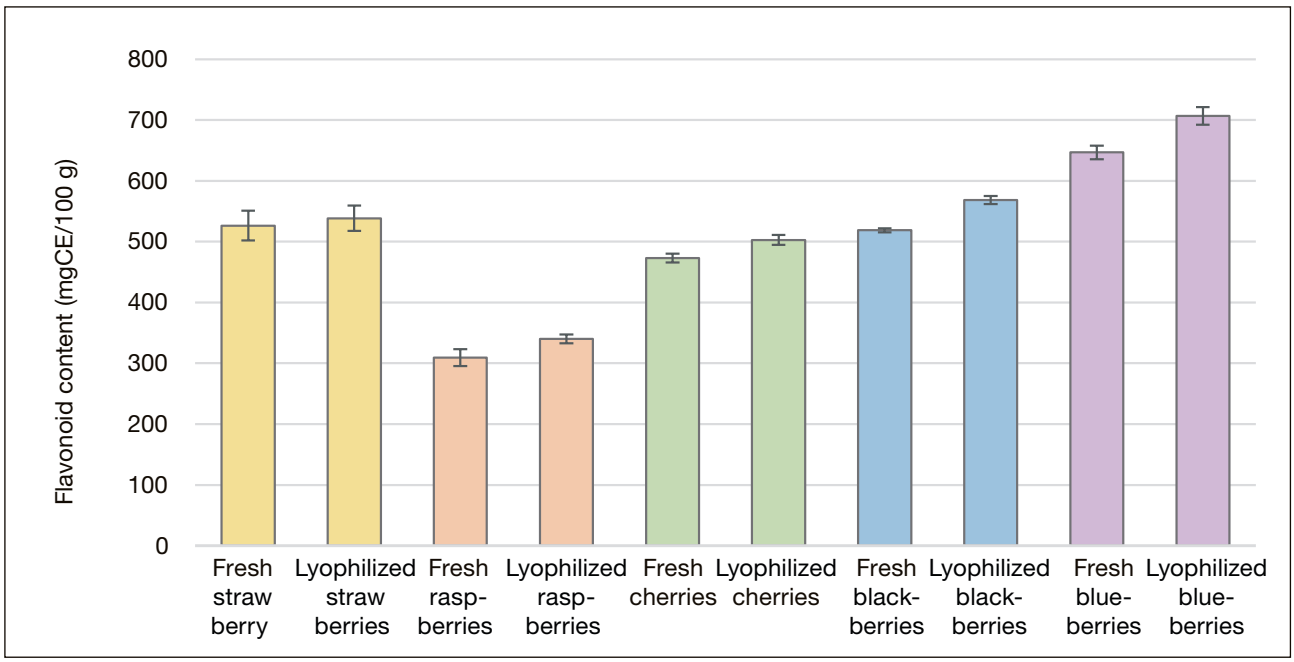


*Figure 2. Total flavonoid content of fresh and lyophilized fruits*

### *4.4. Total acid content*

The total acid content is shown in ***Figure 3***. It can be clearly seen that lyophilization did not have a beneficial effect on these compounds, as significantly lower results were obtained for all samples compared to the fresh fruits. Very high acid contents were measured for fresh raspberries and fresh sour cherries (54.4-54.5%). As a result of lyophilization, these values decreased by two-thirds (16.6-16.7%). In the case of the other fruits, the acid content did not even reach 30%. Statistically verifiable differences were obtained in all cases, except for lyophilized sour cherries and lyophilized raspberries (P=0.167).

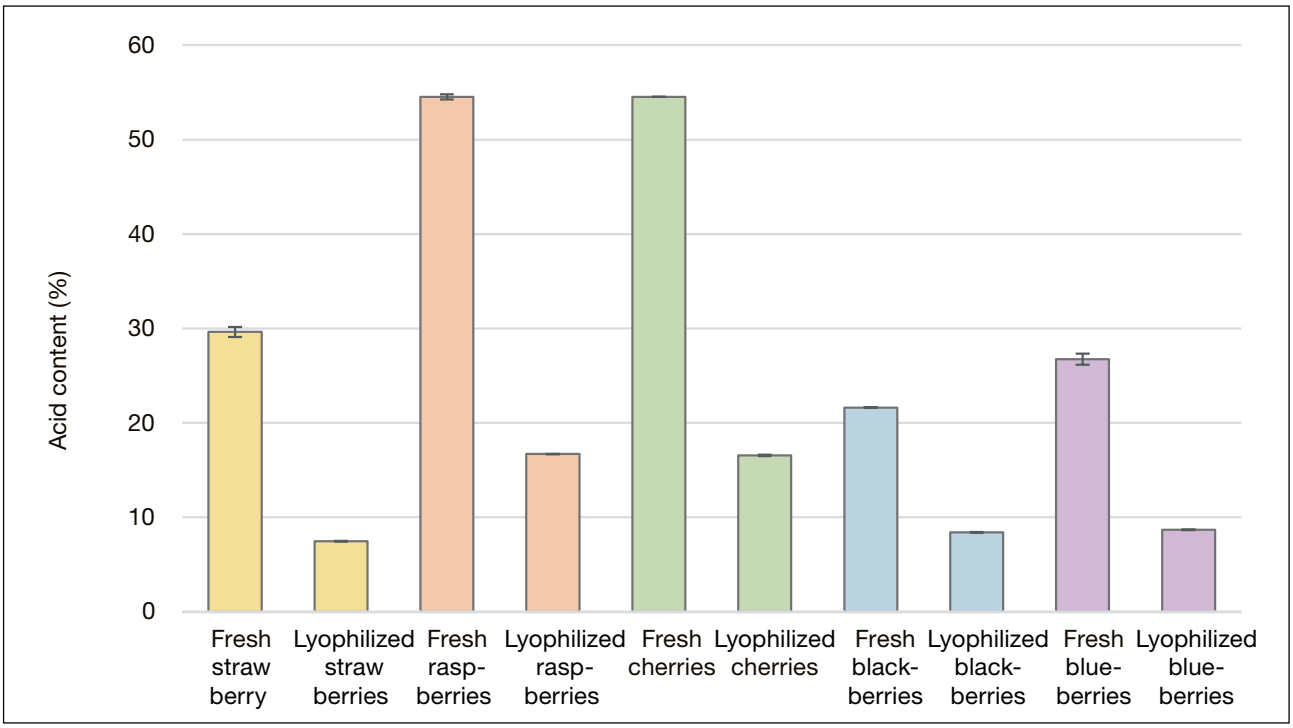


*Figure 3. Total acid content of fresh and lyophilized fruits*

### *4.5. Vitamin C content*

The vitamin C content of the fruits is shown in ***Figure 4***. In this case, lyophilization did not have a large effect on the vitamin C content, since, as shown in the figure, the results after lyophilization were lower for all samples. The highest values (236 and 242 mg/100 g) were obtained for strawberries. Similar results were obtained for raspberries and blackberries (172-199 mg/100 g), and for sour cherries and blueberries (102-128 mg/100 g). Significant results were obtained in almost all cases, except for fresh strawberries with fresh raspberries, fresh raspberries with fresh blackberries, and for fresh sour cherries with fresh blueberries.

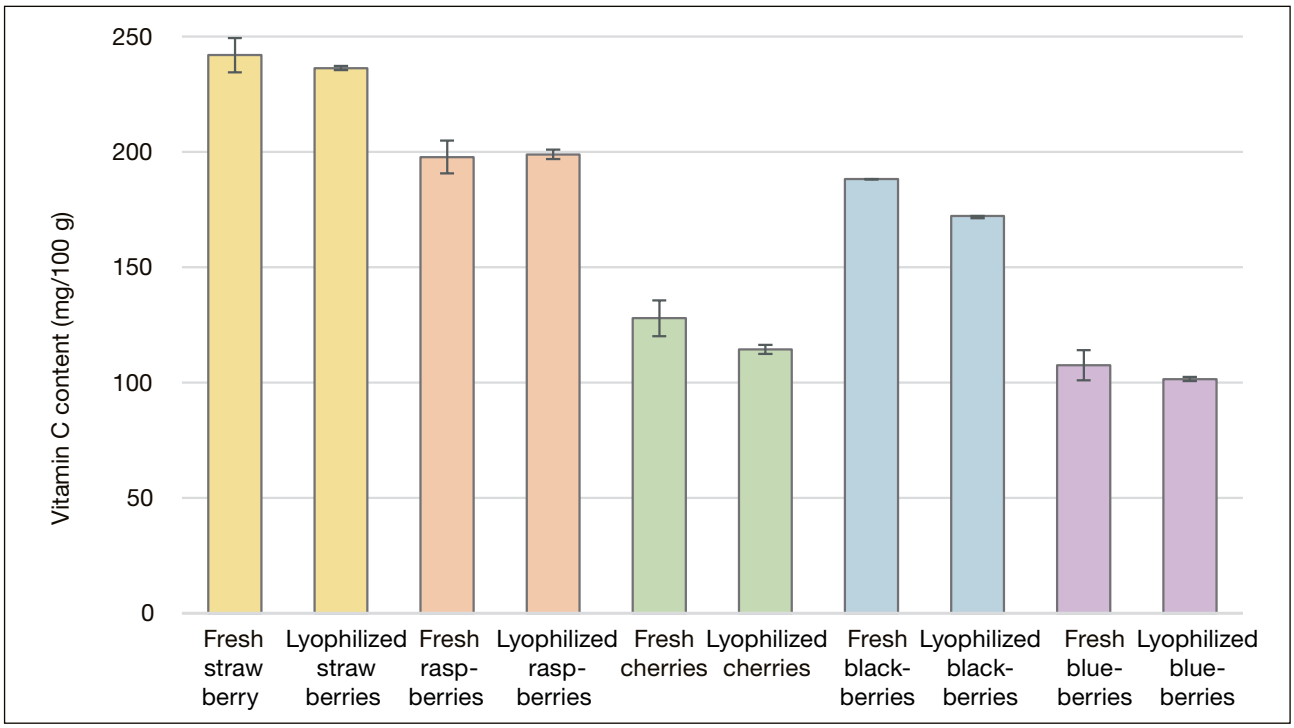


*Figure 4. Vitamin C content of fresh and lyophilized fruits*

### *4.6. Element content*

The element contents of the samples are shown in ***Table 1***. Although several elements were measured, only the most important results are highlighted. The calcium content of the fruits was between 240 and 2302 mg/kg. Among the results, the calcium content of blueberries was extremely low compared to the other samples (240 mg/kg). There was no statistically verifiable difference between strawberries and blackberries (P=0.096).

*Table 1. Element results of fresh and lyophilized fruits*

| Sample | Ca (mg/kg) | K (mg/kg) | Mg (mg/kg) | Na (mg/kg) | P (mg/kg) | S (mg/kg) |
|---|---|---|---|---|---|---|
| Strawberries | 2,302±2 | 12,693±9 | 1,383±7 | 22.9±0.5 | 2,012±2 | 541±1 |
| Raspberries | 1,286±1 | 6,983±4 | 1,143±4 | 4.47±0.01 | 2,024±3 | 500±1 |
| Sour cherries | 1,458±2 | 9,521±13 | 945±1 | 4.24±0.01 | 1,677±3 | 533±0 |
| Blackberries | 1,956±8 | 6,582±22 | 1,225±8 | 23.1±0.2 | 1,806±5 | 695±2 |
| Blueberries | 240±1 | 3,765±7 | 195±2 | 5.15±0.14 | 863±2 | 445±1 |

The potassium content was highest in the case of strawberries (12,693 mg/kg). In contrast, blueberries have an extremely low potassium content of 3,765 mg/kg. Values between 6,582 and 9,521 mg/kg were obtained for the other samples. The differences were statistically verifiable in all cases. The magnesium content of the fruits was between 195 and 1,383 mg/kg. Once again, blueberries exhibited an extremely low value of 195 mg/kg. At the same time, the magnesium content of strawberries was 1,383 mg/kg. Significant differences were obtained in almost all cases, with the exception of strawberries-raspberries and sour cherries-raspberries. The sodium content values of the fruits was between 5.15 and 23.1 mg/kg. Compared to the other samples, the sodium content of strawberries and blackberries was very high (22.9 and 23.1 mg/kg). Phosphorus content results were between 863 and 2,024 mg/kg. Similarly to calcium, potassium and magnesium, blueberries had the lowest result for phosphorus (863 mg/kg) as well. Significant results were obtained for all samples. In the case of sulfur, values between 445 and 695 mg/kg were measured.

The lowest result was obtained for blueberries, while the highest was obtained for blackberries. Significant results were obtained in all cases except for blueberries-strawberries-sour cherries **[25]**.

### *4.7. Conclusions*

The nutritional parameters of different red fruits were examined. Our aim was to compare the examined parameters (total phenolic content, flavonoid, acid and vitamin C content) in the fresh state of the fruits and after lyophilization. In addition, the major element contents (calcium, potassium, magnesium, sodium, phosphorus, sulfur) of the fresh samples were also determined. In terms of the total phenolic content and the flavonoid content, higher results were obtained for all fruits after lyophilization. The reason for this may be that lyophilization does not have as adverse an effect on these compounds as the use of a drying oven. Positive results were also obtained for vitamin C. The presence of this vitamin was slightly reduced in these samples by lyophilization. In contrast, much lower acid content results were obtained after lyophilization. In terms of their element content, blueberries had the lowest values, while the highest values were obtained in the case of strawberries. Based on the results obtained, it can be stated that in the case of the parameters examined (except for the acid content), freeze drying, also known as lyophilization, is a much more gentle drying method than the use of an oven.

## 5. References


**[1]** Fu L., Xu B.-T., Xu X.-R., Gan R.-Y., Zhang Y., Xi E.-Q., & Li H.-B. (2011): Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry* **129** (2) 345–350. pp. https://doi.org/10.1016/j.foodchem.2011.04.079

**[2]** Imeh U., Khokhar S. (2002): Distribution of Conjugated and Free Phenols in Fruits: Antioxidant Activity and Cultivar Variations. *Journal of Agricultural and Food Chemistry* **50** (22) 6301–6306. pp. https://doi.org/10.1021/jf020342j

**[3]** de Souza V. R., Pereira P. A. P., da Silva, T. L. T., de Oliveira Lima L. C., Pio R., Queiroz F. (2014): Determination of the bioactive compounds, antioxidant activity and chemical composition of Brazilian blackberry, red raspberry, strawberry, blueberry and sweet cherry fruits. *Food Chemistry* **156** 362–368. pp.

**[4]** Denardin C. C., Hirsch G. E., da Rocha R. F., Vizzotto M., Henriques A. T., Moreira J. C. F., Emanuelli T. (2015): Antioxidant capacity and bioactive compounds of four Brazilian native fruits. *Journal of Food and Drug Analysis* **23** (3) 387–398. pp. https://doi.org/10.1016/j.jfda.2015.01.006

**[5]** Moo-Huchin V. M., Moo-Huchin M. I., Estrada-León R. J., Cuevas-Glory L., Estrada-Mota I. A., Ortiz-Vázquez E., Sauri-Duch E. (2015): Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. *Food Chemistry* **166** 17–22. pp. https://doi.org/10.1016/j.foodchem.2014.05.127

**[6]** Yildiz H., Ercisli S., Hegedus A., Akbulut M., Topdas E. F., Aliman J. (2014): Bioactive content and antioxidant characteristics of wild (Fragaria vesca L.) and cultivated strawberry (Fragaria × ananassa Duch.) fruits from Turkey. *Journal of Applied Botany and Food Quality* **87** 274–278. pp. https://doi.org/10.5073/JABFQ.2014.087.038

**[7]** Slatnar A., Jakopic J., Stampar F., Veberic R., Jamnik P., (2012): The Effect of Bioactive Compounds on In Vitro and In Vivo Antioxidant Activity of Different Berry Juices. *PLoS ONE* **7** (10) 1-8. pp.

**[8]** Namiesnik J., Vearasilp K., Nemirovski A., Leontowicz H., Leontowicz M., Pasko P., Martinez-Ayala A.L., González-Aguilar G.A., Suhaj M., Gorinstein S. (2014): In vitro studies on the relationship between the antioxidant activities of some berry extracts and their binding properties to serum albumin. *Applied Biochemistry and Biotechnology* **172** 2849–2865. pp.

**[9]** Baiano A. (2014): Influence of genotype, pedoclimatic conditions, viticultural practices and ripening on the phenolic composition of grapes. A review. In J. S. Cámara (Ed.), Grapes: Production, phenolic composition and potential biomedical effects. *Food and Beverage Consumption and Health Series. New York, NY: Nova Science* 1–26. pp. ISBN: 978-1-63321-410-1

**[10]** Lutz M., Hernández J., Henríquez C. (2015): Phenolic content and antioxidant capacity in fresh and dry fruits and vegetables grown in Chile. *CyTA - Journal of Food. Taylor & Francis.* 1–7. pp.

**[11]** Aly A., Maraei R., El-Leel O. A. (2019): Comparative study of some bioactive compounds and their antioxidant activity of some berry types. Slovak Journal of Food Sciences. *Potravinarstvo Slovak Journal of Food Sciences* **13** (1) 515–523. pp. https://doi.org/10.5219/1132

**[12]** Lachowicz S., Kolniak-Ostek J., Oszmianski J., Wisniewski R. (2017): Comparison of phenolic content and antioxidant capacity of bear garlic (Allium ursinum L.) in different maturity stages. *Journal Food Processing and Preservation* **41** (1) 1–10. pp. https://doi.org/10.1111/jfpp.12921

**[13]** Toledo-Martín E., García-García M., Font R., Moreno-Rojas J., Salinas-Navarro M., Gómez P., del Río-Celestino M. (2018): Quantification of Total Phenolic and Carotenoid Content in Blackberries (Rubus Fructicosus L.) Using Near Infrared Spectroscopy (NIRS) and Multivariate Analysis. *Molecules* **23** (12) 3191. p. https://doi.org/10.3390/molecules23123191

**[14]** Zapata, P. J., Martínez-Esplá, A., Gironés-Vilaplana, A., Santos-Lax, D., Noguera-Artiaga, L., Carbonell-Barrachina, Á. A. (2019): Phenolic, volatile, and sensory profiles of beer enriched by macerating quince fruits. *LWT – Food Science and Technology* **103** 139–146. pp. https://doi.org/10.1016/j.lwt.2019.01.002

**[15]** Oliveira K. G., Queiroz V. A. V., Carlos L. A., Cardoso L. M., Pinheiro-Sant'Ana H. M., Anunciacao P. C., Menezes C. B., Silva E. C., Barros F. (2017): Effect of the storage time and temperature on phenolic compounds from sorghum grain and flour. *Food Chemistry* **216** 390–398. pp. https://doi.org/ 10.1016/j.foodchem.2016.08.047

**[16]** Rekha C., Poornima G., Manasa M., Abhipsa V., Pavithra Devi J., Vijay Kumar H. T., Prashith Kekuda T. R. (2012): Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chem Sci Trans*. **1** (2) 303–310. pp. https://doi.org/10.7598/cst2012.182

**[17]** Nour V., Trandafir I., Ionica M. E. (2011): Ascorbic acid, anthocyanins, organic acids and mineral content of some black and red currant cultivars. *Fruits* **66** 353–362. pp. https://doi.org/ 10.1051/fruits/2011049

**[18]** Plessi M., Bertelli D., Albasini A., (2007): Distribution of metals and phenolic compounds as a criterion to evaluate variety of berries and related jams, *Food Chemistry* **100** 419–427. pp.

**[19]** Nile, S. H., & Park, S. W. (2014). Edible berries: Bioactive components and their effect on human health. *Nutrition* **30** (2) 134–144. pp. https://doi.org/ 10.1016/j.nut.2013.04.007

**[20]** Rodler I. 2008. Élelmezés- és táplálkozás-egészségtan. *Medicina Könyvkiadó*, Budapest.

**[21]** Singleton, V. L., Orthofer, R., Lamuela Raventos, R. M. (1999): Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin Ciocalteu reagent. *Methods in Enzymology* **299** 265–275. pp.

**[22]** Kim, D.O, Jeong, S.W., Lee, C.Y. (2003): Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* **81** 321–326. pp.

**[23]** Czipa, N. (2014): Élelmiszeranalitika gyakorlati jegyzet élelmiszermérnök BSc III. évfolyam részére. *Debreceni Egyetem*, Debrecen.

**[24]** Kovács, B., Győri, Z., Csapó, J., Loch, J., Dániel, P. (1996): A study of plant sample preparation and inductively coupled plasma emission spectrometry parameters. *Communication in Soil Science and Plant Analysis* **27** (5-8) 1177–1198. pp.

**[25]** Słupski, J., Lisiewska, Z., Kmiecik, W. (2005): Contents of macro and microelements in fresh and frozen dill (Anethum graveolens L.). *Food Chemistry* **91** (4) 737–743. pp. https://doi.org/10.1016/j.foodchem.2004.06.046