ORIGINAL PAPER

VITAMIN C CONTENT OF NEW ECOTYPES OF CORNELIAN CHERRY (*Cornus mas* L.) DETERMINED BY VARIOUS ANALYTICAL METHODS

Małgorzata Kostecka✉, Iwona Szot, Tomasz Czernecki, Paweł Szot

University of Life Sciences in Lublin, Poland

ABSTRACT

Food can be a rich source of nutrients that are required for optimal health. Vitamin C is an antioxidant that protects cells and bodily fluids against oxidative stress. Cornelian cherry (*Cornus mas* L.) is widely recognized for its health benefits, taste and qualitative attributes. It is a source of biologically active compounds, including vitamin C. The determination of the vitamin C content of new ecotypes of cornelian cherry supported the identification of the most cost-efficient and accurate analytical method. The results of this study revealed that new ecotypes of cornelian cherry differed significantly with respect to their vitamin C content. Ecotypes 5, 10, 12 and 14 had the highest concentrations of vitamin C, which were determined at $201.61–210.75$ mg·100 g^{-1} by the titration (Tillmans) method and $70.90–82.30$ mg·100 g^{-1} by the spectrophotometric method. Ecotype 6 had the lowest vitamin C content which ranged from 177.19 mg·100 g^{-1} (titration method) to 54.68 mg·100 g^{-1} (spectrophotometric method). The vitamin C content of cornelian cherry fruit, measured by HPLC, reached 63.1 mg·100 g^{-1} and it was 4-fold higher than in other analyzed fruits.

Key words: biologically active compounds, vitamin C, instrumental methods, HPLC, dry matter content, individual fruit mass

INTRODUCTION

Fresh fruits and vegetables are the main sources of vitamin C in the human diet [Melo et al. 2006]. Fruits are abundant in antioxidants, including vitamin C, bioflavonoids and polyphenols [Benvenuti et al. 2004, Rinaldo et al. 2014]. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables (including potatoes). Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of L-ascorbic acid. Ascorbic acid is the principal biologically active form but L-dehydroascorbic acid, an oxidation product, also exhibits biological activity. Vitamin C plays an important role in human health by protecting cells and bodily fluids against oxidative stress. It scavenges free radicals that are produced during food preparation and metabolic processes in the body. Vitamin C prevents initial stages of oxidation and disrupts this process by protecting LDL cholesterol against oxidation. Recent research has demonstrated that both ascorbate and dehydroascorbate effectively protect LDL particles against adverse changes [Duthie et al. 2005].

Vitamin C can prevent certain types of cancer, it boosts resistance against bacterial and viral diseases, participates in collagen synthesis and facilitates iron absorption [Zuo et al. 2002, Edefonti at al. 2015]. Vitamin C present in food is sensitive to heating above the temperature of 60°C [Njoku et al. 2011], in particular in the presence of oxygen and metal ions, mainly iron and copper. The breakdown of L-ascorbic acid is also accelerated by an alkaline or neutral environment, drying, long-term storage or exposure to antiseptic agents [Lee and Kader 2000].

✉ malgorzata.kostecka@up.lublin.pl

The fruit of cornelian cherry is widely recognized for its taste, quality attributes and health benefits. It is characterized by high dry matter content. Before World War II, cornelian cherry was widely planted in Polish manors and highly valued for its fruit, leaves and wood. Its popularity has been recently revived, which spurred research into the collection and selection of cornelian cherry varieties [Klimenko 2004]. The physicochemical properties and concentrations of active substances in cornelian cherry fruit differ across varieties, and they are also determined by the local habitat and weather conditions (temperature and precipitation) [Demir and Kalyoncu 2003]. Cornelian cherry is rich in vitamin C whose content ranges from 50 to 100 mg·100 g^{-1} [Pantelidis et al. 2007].

The fruit of bog cranberry and American cranberry is also abundant in vitamin C, but it should be noted that ascorbic acid is not the main determinant of cranberries' antioxidant capacity. According to research, vitamin C content is influenced by numerous factors, including variety, climate, storage conditions and – in processed fruit – by the parameters of the production process [Gumul et al. 2005, Witkowska and Zujko 2009].

Apples are not a major source of vitamin C, but they are the most popular fruit in Poland. The average vitamin C content of apples is estimated at 9–11 mg per 100 g of fresh weight [Rad et al. 2014]. Due to the high popularity and wide availability of apples, they may constitute the main source of vitamin C in certain populations.

The aim of this study was to evaluate the vitamin C content of new ecotypes of cornelian cherry and to identify the most rapid and cost-effective method of determining vitamin C concentrations. The effectiveness of the proposed method was verified by analyzing the vitamin C content of other fruits with health-promoting properties.

MATERIALS AND METHODS

The fruits of 14 ecotypes of cornelian cherry (*Cornus mas* L.) were analyzed in the study. The berries were harvested at the turn of August and September 2014 in Ronald Choina's plantation in Dąbrowica near Lublin (latitude 51°48'51"N; longitude 22°57'83"E).

All ecotypes were developed by the owner in 1998. Only fully ripened, healthy and undamaged fruits were harvested for the experiment. Samples of 1 kg each were collected twice during harvest.

Bog cranberries (*Vaccinium oxycoccos* L.) from a peatland on Lake Czarne near Sosnowica (Lublin Region), American cranberries (*Vaccinium macrocarpon* Aiton) and apples (*Malus domestica* Borkh.) cv. 'Braeburn Mariri Red' from an orchard in Stryjno near Piaski were used in comparative analyses.

Determination of vitamin C content

Vitamin C concentrations were determined with the use of the methods described in the literature for analyzing the vitamin C content of fruit and food products.

Titration method (Tillmans method) L-ascorbic acid determination. Fruit samples of 5 g each, accurate to 0.001 g, were used in analysis. Vitamin C levels were determined in three replicates for each cornelian cherry ecotype, bog cranberry, American cranberry and apples. Fruits were crushed in a homogenizer for 5 minutes. The homogenate was filtered into a suction flask and 45 cm^3 of 3% metaphosphoric acid was added to produce the extract with a final volume of 50 cm^3. Samples of 5 cm^3 were collected from the resulting solution, and they were titrated with freshly prepared 2,6-dichlorophenolindophenol with a concentration of 0.0009 M·dm^{-3} [ISO 6557-2:1984; PN-A-04019:1998].

Iodometric titration. Iodine and potassium iodide in amount of 1 g each were ground in a mortar and dissolved in distilled water in a 1 dm^3 flask. L-ascorbic acid in the amount of 0.25 ±0.0001 g was dissolved in a 250 cm^3 flask.

The concentration of iodine in the potassium iodide solution was determined with a solution of L-ascorbic acid using starch as the indicator. Titration was continued until the solution began to turn dark blue [Dasiewicz and Dobrosz-Teperek 2008].

Fruit samples of 10 g each were ground in a mortar with 10% oxalic acid. The mixture was transferred to a conical flask, combined with 20 cm^3 of water and 1 cm^3 of starch solution, and titrated with I$_2$/KI until it turned dark blue.

Spectrophotometric method. Extracts of cornelian cherry, cranberry and apples in 3% metaphosphoric acid were sampled for analysis in the amount of 1 cm^3 each. They were combined with 9 cm^3 oxalic acid in EDTA, 2 cm^3 of 50% sulfuric (VI) acid and 4 cm^3 of 5% ammonium molybdate and mixed thoroughly. Absorbance was measured at λ = 705 nm at room temperature for 3 minutes at Spectrophotometer Shimadzu UV-160A [Bajaj and Kaur 1981]. Assay of vitamin C was performed using the standard curve.

Fluorimetric method. Samples of the fruit extract and ascorbic acid standard solution of 2 cm^3 each were transferred to 100 cm^3 flasks, combined with 0.005 M potassium iodide solution, shaken for 1 minute and combined with 0.1 M sodium thiosulfate. Their pH was adjusted to 6 by adding 1 M of sodium hydroxide and derivatization was carried out with 0.3 cm^3 o-phenylenediamine solution [Wu et al. 2008]. Flasks were centrifuged for 30 minutes. Fluorescence was measured at λ = 365 nm and λ = 425 nm wavelength with a fluorimeter (Vrian Cary 50 Bio Cry Eclipse).

Enzymatic method. The quantity of L-ascorbic acid was measured an enzymatic assay kit (Boehringer Mannheim, Cat. No. 409677) used in the food industry. Measurements were performed in 10 g samples of fruit extract, in 3 replications.

High-performance liquid chromatography (HPLC). Fruit samples were ground with the addition of 3% metaphosphoric acid and filtered into a suction flask to produce the extract. Dehydro-L-(+)-ascorbic acid was transformed to L-(+)-ascorbic acid with the use of a reducing solution. The total content of L-(+)-ascorbic acid was determined in 3 replications by RP-HPLC on a C18 column with UV detection at 265 nm with the use of an external standard (PN-EN 14130: 2004). The limit of detection was 1.5 µg·cm^{-3}, and the limit of quantification was 5 µg·cm^{-3}. Column temperature: 22°C, composition of the eluent: 95/5 $CH_3COOH : CH_3OH$, flow rate 0.7 cm^3·min^{-1}.

Determination of dry matter content. Dry matter content was determined by the using of weighing vessels lined with filter paper. Vessels containing approximately 3 g of dried cornelian cherry fruit were dried at 105°C for 30 minutes. Dried samples were cooled in an exicator and weighed within an accuracy of 0.00002 g. Fruit samples were dried until the difference between two weight measurements did not exceed 0.0002 g. The samples were dried for approximately 2 hours.

Determining of individual fruit mass. Individual fruit mass was determined by an electronic balance with the accuracy of 0.01 g. The fruit mass was measured on 100 randomly selected samples for every cornelian cherry ecotype.

Statistical analysis. Data were processed in the Statistica 10.0 program (StatSoft, Poland) with the use of routine statistical techniques, including calculation of the means, standard deviation, analysis of variance, multiple regression analysis and correlation analysis. The Pearson's correlation coefficient (r) was applied to measure the strength of the relationship between different methods of determining the vitamin C content, dry matter and individual fruit mass. PCC is a usual measure of linear association which varies from −1 to +1 with 0 indicating no relationship. A value +1 indicates perfect relationship and −1 perfect negative relationship. The level of statistical significance for all analyses was at $p < 0.05$.

RESULTS AND DISCUSSION

The ascorbic acid content of food products can be determined with the use of various methods that differ in complexity, speed, cost and reagents. Titration method for the determination of vitamin C are very popular and often use in research [Tareen et al. 2015]. The results of the experiment indicate that vitamin C concentrations differed significantly between all the analyzed ecotypes and the applied analytical methods.

In the Tillmans method, which is recommended by the Internal Standardization Organization (ISO) and the Association of Official Analytical Chemists (AOAC) [Danielczuk et al. 2004], vitamin C concentrations are determined by titration with 2,6-dichlorophenolidophenol solution until the analyzed sample changes color. This method is simple, easy to use and rapid, but it is characterized by low selectivity, which implies that the presence of reducing substances can influence the result. Sample prep-

aration (cleaning) methods will significantly affect analyses of specimens containing large amounts of reducing substances. The analyzed solutions were bright pink, which did not influence the quality of the read-out, and darker solutions were obtained only for ecotypes 6 and 7. Those solutions were titrated by a modified Tillmans method, and they were titrated with 2,6-dichlorophenolindophenol in the presence of chloroform as an organic solvent of choice. The results of Tillman's method (tab. 1) indicated that vitamin C concentrations were highest in ecotypes 5, 14 and 10 and lowest in ecotypes 11, 9, 13, 2 and 6.

Table 1. Content of vitamin C and dry matter content in fruit of cornelian cherry ecotypes determined by different methods

Ecotype	Content of vitamin C (mg·100 g^{-1} FW)				Dry matter (%)	Individual fruit mass (g)
	Tillmans method	iodometric method	spectrophoto-metric method	fluorimetric method		
1	190.75 ±0.92 bc*	210.14 ±0.81 i	62.74 ±0.42 c*	68.45 ±0.24 e	24.88 ±0.001 m*	2.36 g
2	175.46 ±1.0 a	186.34 ±0.74 e	61.70 ±0.45 b	78.60 ±0.12 g	22.15 ±0.02 g	1.57b–d
3	183.53 ±1.55b	196.04 ±1.01 g	68.38 ±0.14 e	75.30 ±0.1 f	22.36 ±0.034 i	1.98 ef
4	190.90 ±1.43 bc	202.54 ±0.86 h	66.45 ±0.18 d	85.5 ±0.15 j l	22.19 ±0.025 h	2.13 fg
5	210.75 ±1.26 d	225.40 ±0.91 l	70.90 ±0.3 f	82.53 ±0.02 i	25.07 ±0.004 n	1.39 a–c
6	177.19 ±1.32 a	153.14 ±1.12 a	55.27 ±0.12 a	54.68 ±0.14 a	21.06 ±0.04 a	1.38 ab
7	181.26 ±0.88 b	160.50 ±0.83 b	61.60 ±0.43 b	65.65 ±0.14 d	22.57 ±0.003 k	1.19 a
8	189.35 ±1.11 bc	186.12 ±1.23 e	55.30 ±0.1 a	62.27 ±0.2 c	21.45 ±0.024 d	1.96 ef
9	173.95 ±1.79 a	192.94 ±0.64 f	71.05 ±0.24 f	74.85 ±1.05 f	21.34 ±0.007 b	1.96 ef
10	208.54 ±1.05 d	222.36 ±1.29 k	82.50 ±0.2 h	85.50 ±0.15 j	21.85 ±0.01 e	2.13 fg
11	169.65 ±1.2 a	183.06 ±1.43 d	54.90 ±1.07 a	59.90 ±0.3 b	22.41 ±0.0025 j	1.70 de
12	201.61 ±1.63 cd	237.16 ±1.23 m	82.30 ±0.24 h	85.50 ±0.15 j	21.36 ±0.0061 c	1.59 b–d
13	175.06 ±0.71 a	176.30 ±1.11 c	70.65 ±0.3 f	75.45 ±0.1 f	22.07 ±0.01 f	1.68 c–e
14	209.50 ±0.89 d	219.56 ±1.19 j	75.97 ±0.26 g	80.55 ±0.18 h	23.58 ±0.02 l	2.23 fg

* Means within the table followed by the same letter do not differ significantly at p < 0.05

Table 2. Pearson's correlation coefficient *r* between the analyzed parameters

		Vitamin C content			Dry matter content	Individual fruit mass
		spectrophotometric method	fluorimetric method	iodometric method		
Vitamin C content	spectrophoto-metric method	–	0.83*	0.74*	–0.10	0.06
	fluorimetric method	0.83*	–	0.78*	0.08	0.07
	iodometric method	0.74*	0.78*	–	0.17	0.11
Dry matter content		–0.10	0.08	0.17	–	0.06
Individual fruit mass		0.06	0.07	0.11	0.06	–

Coefficients marked with asterisks (*) are statistically significant at p < 0.05

Table 3. Vitamin C content of healthy fruit determined by various methods

Fruits	Tillmans method	Iodometric method	Spectrophoto-metric method	Fluorimetric method	HPLC	Enzymatic method
	(mg·100 g^{-1} fresh matter)					
Bog cranberry	52.96 ±0.12 b	24.26 ±0.07 c	16.44 ±0.14 c	19.54 ±0.2 b	16.40 ±0.1 b	22.10 ±0.3 c
American cranberry	77.51 ±0.7 c	20.93 ±0.2 b	15.58 ±0.21 b	18.74 ±0.15 b	15.20 ±0.22 b	18.00 ±0.21 b
Apple	41.79 ±0.12 a	3.40 ±0.5 a	9.19 ±0.10 a	8.67 ±0.11 a	13.70 ±0.17 a	11.46 ±0.17 a
Cornelian cherry	189.92 ±0.14 d	196.31 ±0.23 d	67.47 ±0.14 d	73.97 ±0.14 c	63.1 ±0.11 c	55.10 ±0.13 d
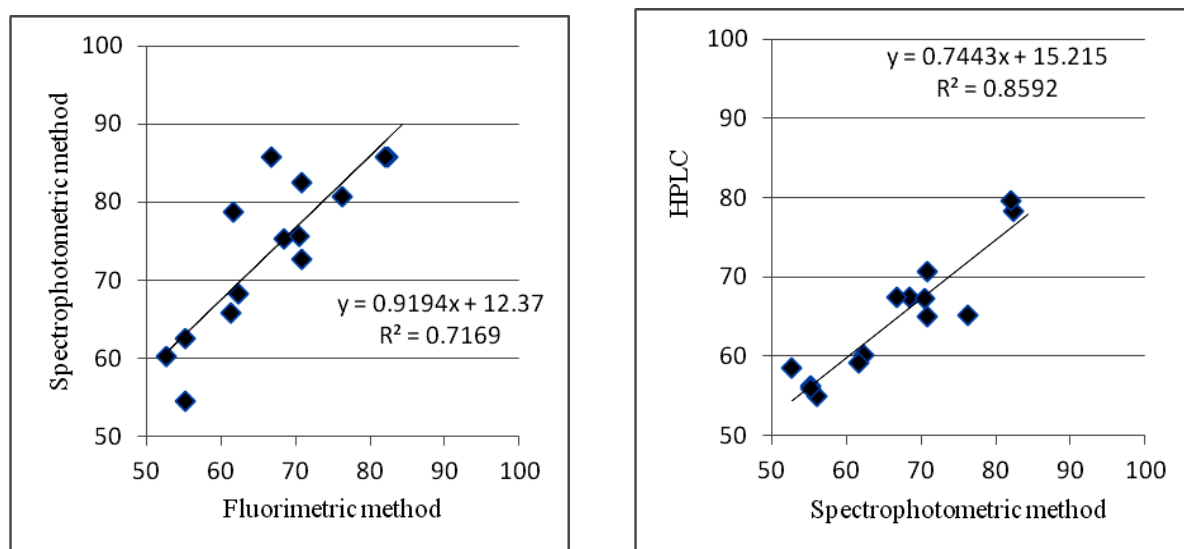
Fig. 1. Comparison of methods for determining vitamin C concentrations

Iodometric titration is also a simple method of determining vitamin C content, and similarly to the Tillmans method, it is characterized by low selectivity because iodine reacts with a number of reducing compounds in plant material, which can lead to the overestimation of results [Dasiewicz and Dobrosz-Teperek 2008]. The method is suitable for use with vitamin C tablets, fresh or packaged fruit juices and solid fruits and vegetables [Determination of vitamin C… 2012]. Vitamin C concentrations determined by iodometric titration were 10–15% higher on average than the results obtained by the Tillmans method. The highest vitamin C content was also noted in ecotype 12 and the lowest – in ecotype 6 (tab. 1).

The spectrophotometric method is an instrumental technique that eliminates the risk of error when observing changes in the color of the examined solution. Spectrophotometric analyses produce rapid results, and sample preparation does not require specialist reagents, which reduces analytical costs. This method is also characterized by a wide linear range of the L-ascorbic acid standard curve. Despite the above, spectrophotometric equipment is quite expensive [Danielczuk et al. 2004, Rutkowski and Grzegorczyk 2007]. One of the greatest disadvantages of spectrophotometric analysis is that it has to be carried out in an alkaline environment, which could destabilize ascorbic acid present in the sample [Kapur et al.

2012]. In this study, the spectrophotometric method was modified, and analyses were performed in an acidic environment by adding sulfuric (VI) acid which stabilizes vitamin C. The resulting content of vitamin C was significantly lower than that determined by titration. The difference in vitamin C concentrations exceeded 60% in some ecotypes, and it was determined at 54 ±19% on average.

The fluorimetric method is more time-consuming, and the linear range of the L-ascorbic acid standard curve is very narrow. The analyzed samples have to be diluted to produce a range that corresponds to the standard curve, which could obfuscate the results. In the fluorimetric method, the pH of the environment in which vitamin C is analyzed has to be controlled to produce reliable results [Arya et al. 2000].

High-performance liquid chromatography has been recommended by the International Federation of Fruit Juice Producers as the reference method for determining vitamin C concentrations [IFFJP 1985]. It is suitable for all types of fruit, regardless of their color or pectin content, and it delivers rapid and reliable results. Despite those advantages, HPLC is an expensive technique that requires specialist equipment. Due to the reaction environment, it carries a high risk of damage to column packing, which shortens column life and increases costs. Kucharska [2012] comparing the vitamin C content determined by HPLC for some Polish cultivars, in two subsequent vegetative seasons, stated that values of mentioned feature were significant dependent on cultivar and the year of harvest. In this study, vitamin C concentrations measured by HPLC were similar to the results noted in spectrophotometric analysis. The average vitamin C content of all cornelian cherry ecotypes was determined at 63.1 ±5.6 mg·100 g^{-1} of fresh weight (tab. 3).

In the last analytical method, vitamin C concentrations were determined with the use of enzymatic test kits applied in the food industry. This method is characterized by high specificity, short preparation time, availability of reagents and short time of analysis. It has been recommended by the JIAN Code of Practice for determining the vitamin C content of fruit juices and nectars in the European Union [Danielczuk et al. 2004]. The average vitamin C content of all cornelian cherry ecotypes was determined at 55.1 ±1.1 mg·100 g^{-1} of fresh weight (tab. 3). The main drawback of the enzymatic method is its high price.

The results noted in parallel samples were compared by regression analysis to identify the optimal analytical method with the highest repeatability. In fluorimetric and spectrophotometric methods, the distribution of points in parallel samples diverged from linearity. The highest correlation coefficient (fig. 1) was noted in HPLC and spectrophotometric analysis ($r^2 = 0.8592$). Those methods can be used interchangeably, and the choice of spectrophotometric detection is dictated by the simplicity and ease of identification, no additional sample processing, ease of interpretation and maximum elimination of readout error. The spectrophotometric method is also recommended by other authors [Kamlesh et al. 2005, Rahman et al. 2006, Kapur et al. 2012].

Dry matter content (tab. 1) varied significantly between the analyzed ecotypes of cornelian cherry. This parameter was highest in ecotype 5 (25.07%) and lowest in ecotype 6 (21.06%). In a study of Lachman et al. [1995], dry matter content was correlated with cultivation method, and it ranged from 15.7% in wild fruit to 36% in commercially produced fruit. Similar results were reported by other authors. The dry matter content of cornelian cherry fruit was determined at 20–24% in Ukrainian varieties [Klimenko 2004] and 16–28% in fruit harvested in Turkey [Tural and Koca 2008].

Individual fresh fruit mass varied between fruits of studied cornelian ecotypes. The biggest fruit characterized the ecotype 1 (2.36 g) and the smallest – ecotype 7 (1.19 g). The fruit mass above 2 g characterized the ecotype 14, 10 and 4. The obtained results are higher than for native Turkish cornelian cherry fruits [Tural and Koca 2008], but smaller than most ecotypes from Serbia [Bijelić et al. 2011] were the range of fruit mass was 2.26–6.37 g.

In this study, statistically significant correlations between dry matter content as well as individual fruit mass and vitamin C content of the analyzed ecotypes were not determined (tab. 2).

The repeatability of results in the compared methods was validated by measuring the vitamin C con-

tent of bog cranberries, American cranberries and apples (tab. 3). The results noted for the analyzed ecotypes of cornelian cherry (the average for the studied ecotypes) are consistent with the findings of Pantelidis et al. [2007] in whose study. Kucharska demonstrated that the Cornelian cherry is a rich source of vitamin C. The studied ecotypes were characterized by higher vitamin C content than those analyzed by Kucharska, with the exception of Szafer which was more abundant in vitamin C [Kucharska et al. 2011].

Cornelian cherry fruit from natural mountainous localities in Vermio, Greece, were characterized by a higher content of vitamin C than Polish fruits that are generally regarded as a rich source of ascorbic acid: raspberry, blackcurrant, redcurrant, blackberry and gooseberry. Vitamin C concentrations in the examined ecotypes of cornelian cherry were significant higher than in bog cranberry and American cranberry (tab. 3). Other authors also demonstrated that the examined species is more abundant in vitamin C than strawberries (46 mg·100 g^{-1} of fresh weight), oranges (31 mg·100 g^{-1} of fresh weight) [Roberts and Gordon 2003] and kiwi fruit (29–80 mg·100 g^{-1} of fresh weight) [Nishiyama et al. 2004]. In the present study, the applied analytical methods produced different concentrations of vitamin C in cornelian cherry. Vitamin C concentrations determined by the Tillmans method were 3- to 4-times higher than those noted in the remaining analytical techniques, due to the color of the samples and the presence of other reducing compounds. The average content of vitamin C in bog and American cranberries, determined by spectrophotometry and HPLC, was similar. Those results are consistent with published data which indicate that bog cranberry can be a more abundant source of vitamin C than American cranberry (19.28 ±1.03 mg·100 g^{-1} of fresh weight vs. 17.51 ±0.36 mg·100 g^{-1} of fresh weight), subject to variety [Mazur et al. 2009]. Spectrophotometric and enzymatic analyses produced similar values in apples, which is consistent with the published data (9.79 mg of ascorbic acid per 100 mg of fresh weight on average). Vitamin C concentrations determined by HPLC were 30% higher on average [Wojdyło et al. 2010].

CONCLUSIONS

1. Cornelian cherry fruit can be a rich source of vitamin C in the human diet. The new ecotypes of cornelian cherry analyzed in this study differed significantly in their vitamin C content, also subject to the applied analytical methods. Ecotypes 5, 10, 12 and 14 were most abundant in ascorbic acid.

2. No statistically significant correlations were found between vitamin C content and dry matter content as well as an individual fresh fruit mass in different ecotypes of cornelian cherry.

3. The enzymatic assay was the most selective but also the most expensive analytical technique. Despite their simplicity, methods characterized by low selectivity, such as titration analysis, are not recommended for analyzing the vitamin C content of cornelian cherry. Due to the complexity of the examined matrix, those methods are not cost-effective, and they may produce inaccurate results.

4. The spectrophotometric method with appropriate sample preparation is the most cost-effective and accurate method of determining the vitamin C content of cornelian cherry fruit. It can be a method of choice, used interchangeably with enzymatic analysis and HPLC.

REFERENCES

Arya, S.P., Mahajan, M., Jain, P. (2000). Non-spectrophotometric methods for the determination of Vitamin C. Anal. Chim. Acta, 417, 1–14.

Bajaj, K.J., Kaur, G. (1981). Spectrophotometric determination of L-ascorbic acid in vegetables and fruits. Analyst, 106, 117–120.

Bijelić, S.M., Gološin, B.R., Ninić Todorovič, J.I., Cerović, S. B., Popović, M.M. (2011). Physicochemical fruit characteristics of Cornelian Cherry (*Cornus mas* L.) genotypes from Serbia. HortScience, 46(6), 849–853.

Benvenuti, S., Pellati, F., Melegari, M., Bertelli, D. (2004). Polyphenols, anthocyanins, ascorbic acid and radicalscavenginy activity of Rubus, Ribes and Aronia. J. Food Sci., 69, 164–169.

Danielczuk, J., Pietrzykowski, R., Zieliński, W. (2004). Comparative study of the enzymatic method for deter-

mination of vitamin C with routine methods according to ISO. Pol. J. Food Nutr. Sci., 13(54), 41–46.

Dasiewicz, B., Dobrosz-Teperek, K. (2008). Oznaczanie witaminy C w owocach roślin dziko rosnących – propozycja ćwiczenia studenckiego. Chem. Dydakt. Ekol. Metrol., 13(1–2), 51–53.

Demir, F., Kalyoncu, I.H. (2003). Some nutritional, pomological and physical properties of cornelian cherry (*Cornus mas* L.). J. Food Eng., 60, 335–341.

Determination of Vitamin C concentration by titration. Redox titration using iodine solution. University of Canterbury, Christchurch, 2012.

Duthie, G.G., Kyle, J.A., Jenkinson, A.M. (2005). Increased salicylate concentrations in urine of human volunteers after consumption of cranberry juice. J. Agric. Food. Chem., 53, 2897–2900.

Edefonti, V., Hashibe, M., Parpinel, M., Turati, F., Serraino, D., Matsuo, K., Olshan, A.F., Zevallos, J.P., Winn, D.M., Moysich, K., Zhang, Z.F., Morgenstern, H., Levi, F., Kelsey, K., McClean, M., Bosetti, C., Galeone, C., Schantz, S., Yu, G.P., Boffetta, P., Amy Lee, Y.C., Chuang, S.C., La Vecchia, C., Decarli, A. (2015). Natural vitamin C intake and the risk of head and neck cancer: a pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Int. J. Cancer., 137(2), 448–462.

Gumul, D., Korus, J., Achremowicz, B. (2005). Wpływ procesów przetwórczych na aktywność przeciwutleniającą surowców pochodzenia roślinnego. Żywn. Nauka Technol. Jakość, 4(45), 41–48.

IFFJP (1985). Analyses. Determination of vitamin C. HPLC method, 55, 1077–1080.

ISO 6557-2:1984. Fruits, vegetables and derived products – Determination of ascorbic acid content – Part 2: Routine methods. Geneva, International Organization for Standardization.

Kamlesh, S., Kavita, A., Devendra, K.P.A. (2005). Spectrophotometric determination of ascorbic acid. J. Chin. Chem. Soc., 52(3), 503–506.

Kapur, A., Hasković, A., Čopra-Janićijević, A., Klepo, L.A, Topčagić, A., Tahirović, I., Sofić, E. (2012). Spectrophotometric analysis of total ascorbic acid content in various fruits and vegetables. Bull. Chem. Technol. Bosnia Herzegov., 38, 39–42.

Klimenko, S. (2004). The cornelian cherry (*Cornus mas* L.) collection preservation and utilization of genetic resources. J. Fruit Ornam. Plant Res. 12, 93–98.

Kucharska, A.Z., Sokół-Łętowska, A., Piórecki, N. (2011). Morfologiczna, fizykochemiczna i przeciwutleniająca charakterystyka owoców polskich odmian derenia właściwego (*Cornus mas* L). Żywn. Nauka Technol. Jakość, 3(76), 78–89.

Kucharska, A.Z. (2012). Active compounds of cornelian cherry fruit (*Cornus mas* L.). Wyd. UP, Wrocław.

Lachman, J., Pivec, V., Surbutova, D. (1995). Anthocyanins in the fruits if Wild dogwood (*Cornus mas* L.) of central Bohemia origin. Sci. Agric. Biochem., 4(26), 259–268.

Lee, S.K., Kader, A.A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20, 207–220.

Mazur, B., Borowska, E.J., Polak, M. (2009). Zawartość witaminy C i pojemność przeciwutleniająca owoców i przecierów z żurawiny błotnej i wielkoowocowej. Żywn. Nauka Technol. Jakość, 2(63), 130–137.

Melo, E. de A., Lima, V.L.A.G., Maciel, M.I.S., Caetano, A.C. da S., Leal F.L.L. (2006). Polyphenol, ascorbic acid and total carotenoid contents in common fruits and vegetables. Braz. J. Food Technol., 9, 89–94.

Nishiyama, I., Yamashita, Y., Yamanaka, M., Shimohashi, A., Fukuda, T., Oota, T. (2004). Varietal difference in vitamin C content in the fruit kiwifruit and other *Actinidia* species. J. Agric. Food Chem., 52, 5472–5475.

Njoku, P.C., Ayuk, A.A., Okoye, C.V. (2012). Temperature effects on vitamin C content in citrus fruits. Pak. J. Nutr., 10(12), 1168–1169.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., Diamantidis, G.R. (2007). Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, red currants, gooseberries and cornelian cherries. Food Chem., 102, 777–783.

PN-A-04019:1998. Produkty spożywcze – Oznaczanie zawartości witaminy C. Polski Komitet Normalizacyjny.

Rad, A.H., Falahi, E., Ebrahimzadeh, F. (2014). Recent patents on physical, mineral & organic acid composition of Golden Delicious and Red Delicious apples (*Malus × domestica* Borkh) grown at West of Iran. Recent Pat. Food Nutr. Agric., 6(2), 93–99.

Rahman Khan, M.M., Rahman, M.M., Islam, M.S., Begum, S.A. (2006). A simple UV-spectrophotometric method for the determination of vitamin C content in various fruits and vegetables at Sylhet Area in Bangladesh. J. Biol. Sci., 6, 388–392.

Rinaldo, D., Fils-Lycaon, B. Mbéguié-A-Mbéguié, D. (2014). Antioxidant activity of tropical fruits as related to their polyphenol, vitamin C and carotenoid contents: a review. Acta Hortic., 1040, 261–268.

Roberts, W.G., Gordon, M.H. (2003). Determination of the total antioxidant activity of fruit and vegetables by liposome assay. J. Agric. Food Chem., 51, 1486–1493.

Rutkowski, M., Grzegorczyk, K. (2007). Modification of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practise. Acta Sci. Pol. Technol. Aliment., 6(3), 17–28.

Tareen, H., Mengal, F., Masood, Z., Mengal, Z., Ahmed, S., Bibi, S., Shoaib, S., Sami, U., Mandokhail, F., Riaz, M., Farman. N., Nawaz, Z. (2015). Determination of vitamin C content in citrus fruits and in non-citrus fruits by titrimetric method, with special reference to their nutritional importance in human diet. Biol. Forum Internat. J., 7(2), 367–369.

Tural, S., Koca, I. (2008). Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grow in Turkey. Sci. Hortic., 116(4), 362–366.

Witkowska, A., Zujko, M.E. (2009). Antioxidant activity of wild berries. Bromat. Chem. Toksykol., 42(3), 900–903.

Wojdyło, A., Oszmiański, J., Bielecki, P. (2010). Chemical composition, phenolic compounds and antioxidant activity of three varieties of apple from organic and conventional farming. J. Res. Appl. Agric. Eng., 55(4), 173–177.

Wu, X., Diao, Y., Sun, Ch., Yang, J., Wang, Y., Sun, S. (2008). Fluorimetric determination of ascorbic acid with o-phenylenediamine. Talanta, 59(1), 95–99.

Zuo, Y., Wang, C., Zhan, J. (2002). Separation, characterization and quantification of benzoic and phenolic antioxidants in American Cranberry fruit by GC-MS. J. Agric. Food Chem., 50, 3789–3794.