Variation of Phytochemicals Content in Pulp and Skin of Seven Romanian Apple Cultivars

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The amounts of anthocyanins, flavonoids, total polyphenol and ascorbic acid in seven apple cultivars from Romania were investigated. The amounts of polyphenol and flavonoids in pulp and skin of apple cultivars ranged between 17.18–7.52 mg GAE/g DW and 20.10–11.06 mg CE/g DW as well as 3.64–1.18 mg GAE/g DW and 10.31–5.57 mg CE/g DW, respectively. The highest and smallest values of ascorbic acid both in the skin and the pulp were observed in the cultivars Mutsu and Starkrimson. The anthocyanin content is positively correlated with the colour intensity of the apple epicarp, being about three-fold higher in Prima than Golden Delicious cultivar. The better antioxidant activity provided by the content of polyphenol, ascorbic acid and flavonoids was in apple fruits of Mutsu cultivar and for this reason it should be regarded as a valuable source of antioxidants.

Keywords: Total polyphenols; Flavonoids; Anthocyanins; Ascorbic acid; Apple cultivars

Since early time, fruits and vegetables have been used as medicinal agents. Nowadays the scientific community recognizes the importance value of fruits and vegetables, besides their nutritive role; that is the role in preventing vitamin deficiencies [1]. Apples are commonly eaten in Middle Europe during the whole growth period and are large contributors of secondary plant metabolites in human diets [2]. The chemical composition of apples is very complex. Apples, one of the most frequently consumed fruits, represents a source of sugar, acids and various biologically active compounds, such as phenolic compounds that are responsible for most of the antioxidant activities of the fruit [3, 4]. The benefits of fruits and vegetables are often attributed to their high antioxidant content. Our human body possesses many self protection means but sometimes we need to supply it with antioxidants substances that have the power of preventing or inhibiting the oxidation process. Apple fruits are an important source of secondary plant metabolites in human nutrition and one of the major phenol sources being consumed during the whole year [5, 6]. Antioxidant compounds of apples may play an important role in physiological functions related to human health [7]. Antioxidants are mainly localized in the apple peel, but cultivars exhibit a wide variation in the distribution pattern [8,9]. Epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes. In addition, there are many other healthy effects, such as *scavengers of free radicals*, they inhibit oxidation of low-density lipoproteins, lower cholesterol levels, decrease fragility of blood vessels and increase their permeability, decrease heart coronary risk, etc [5].

Ascorbic acid is an important quality characteristic of apple fruit, specially desired for its antioxidant properties [9]. Flavonoids are one of the better-known groups of polyphenols and they are most common in edible plant products, particularly fruits and vegetables [10]. Apples are a very significant source of flavonoids in people's diet in USA and in Europe [11]. Apples are also excellent sources of several polyphenols with high antioxidant capacities [12]. The concentration of phenolic compounds, particularly anthocyanins, is strongly dependent on the apple cultivar as well as the maturity of the apples and is closely associated with their nutritional and sensory qualities, such as taste and colour [4]. In the apple fruit anthocyanin pigments are found in the epicarp and represent the colour of the fruits. Interest in anthocyanin pigments in the consumer market has increased recently due to their potential health beneûts as dietary properties with one of the most important benefits beeing their antioxidant activity. Consumption of fresh fruits and vegetables is associated with lower risk of chronic diseases due to their antioxidant content.

For these reason the aim of this study was to compare the total polyphenols anthocyanins, flavonoids, ascorbic acid both in the pulp and skin of seven apple cultivars as well as to evaluate the cultivar influence on these investigated parameters.

Experimental part

Sample collection

The biological material was represented by seven cultivars of apple trees (Starkrimson, Idared, Golden Delicious, Jonathan, Mutsu, Prima and Wagner), some of them with significant predominance in cultures in Romania and the most consumed. Samples of apple (*Malus domestica* Borkh L) fruits were harvested from the "Marul de aur" Orchard in Vaslui county, at commercial maturity stage, during the autumn of 2012. All apple cultivars fruits were harvested from the part extern of crown tree. The unhealthy, diseased and bruised fruits were discarded while only fruits of uniform size were selected for the study. All apple fruit cultivars were screened for their total polyphenol, flavonoid and ascorbic acid levels being analyzed both in pulp (mesocarp) and skin (epicarp) or only in the skins for anthocyanin,. After harvesting the pulp and skins of these apple cultivars were dried and powdered to obtain uniform particles. These dry weights (DW) of pulp and skin were used only for the extraction of flavonoids and polyphenols content while for ascorbic acid and anthocyanin content fresh weight (FW) was used

Assay of total anthocyanis content

Anthocyanins were extracted from apples by homogenizing 4g of apple skin from each cultivar with

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acidified 70% ethanol solution using the Fuleki & Francis (1968) method and the absorbance beeing measured at 515 nm [13].

Assay of ascorbic acid content

For the extraction of ascorbic acid from the skin and pulp of apple cultivars was used the method with potassium ferricyanide 1% and the absorbance of the final solution was measured at 680 nm [14].

Assay of total polyphenols content

The total polyphenols content was determined by using a modified Folin-Ciocalteu method [15]. The amount of the total phenolic content was expressed as mg galic acid equivalents per g of dry weight (R^2=0.99).

Assay of total flavonoids content

The flavonoids content was measured following a spectrophotometric method [16]. Absorbance of resulting pink-coloured solution was read at 510 nm against the blank (distilled water). Flavonoid content was expressed as mg catechin equivalents per g of dry weight (R^2 =0.98).

Statistical analysis

All experiments were carried out with three independent repetitions and the results were expressed as the mean values ± standard error (SE). All the data were submitted to the analysis of variance using F-Test Two sample for variance.

Results and discussions

The anthocyanin composition is an important quality parameter of apples due to the importance of these compounds in terms of the color of the fruits and of the respective products [3]. The anthocyanin pigments, important for the colour of many fruits content vary in the apple cultivars studied between 10.21 mg/100 FW (Golden Delicious) and 32.30 mg100 FW (Prima) (fig. 1). This pigment amount was approximately three times higher in red skin (Prima) than in green skin (Golden Delicious). The anthocyanin pigment content is positively correlated with the colour intensity of the epicarp. The skin of Prima, Idared, Wagner, Jonathan and Starkrimson apple cultivars is red, also, the pigment content values are very close and range between 22.69 (Idared) and 15.30 mg100 FW (Starkrimson).

As Golden Delicious cultivar is green, in this case the pigment content is less. Bae Ro-Na et al., (2006) find that the different profiles of total anthocyanin accumulation of apple fruit depends on the skin color [17]. Thereby the pigment content was approximately six to seven times higher in red skin than in green skin. In agreement with these results were those of other studies [18]. On the other hand the biosynthesis of anthocyanin in many plants is affected by environmental conditions. In apple concentrations of fruit anthocyanins are lower under hot climatic conditions [19]. In apple fruits numerous varieties have been produced in which levels of anthocyanin pigment vary widely and change in response to environmental and developmental stimuli. With exposure to light, anthocyanin is observed to accumulate during ripening in epidermal and hypodermal cell of apple fruit skin when examined by light microscopy [17]. The apple fruit cortex is usually colourless, although germplasm does exist where the cortex is highly pigmented due to the accumulation of either anthocyanins or carotenoids [20]. In addition, Leja et al. (2001) detected a decrease in anthocyanins level with simultaneously increasing phenol content which is in agreement with our Mutsu cultivar [21].

Ascorbic acid is an important quality characteristic of apple fruit, especially desired for its antioxidant properties [9]. The differences of ascorbic acid content between the two anatomic parts (pulp and skins) of apples fruits are shown in figure 2. In apple fruit skin, the maximum ascorbic acid content was recorded in Mutsu cultivar while the least was observed in Starkrimson cultivar. In fact, the level of the ascorbic acid in apple fruits pulp ranged between 7.19 mg/100 FW and 2.98 mg/100 FW in Mutsu and Starkrimson cultivars, respectively. It is also interesting to note that both values of ascorbic acid, maximum and minimum, were obtained at the same cultivars. The amount of vitamin C was between 4.5 and 10.5 times higher in the skin than in the pulp of the apple fruit cultivars studied (Starkrimson, Idared, Golden Delicious, Jonathan, Mutsu, Prima and Wagner). Our results confirm the data of Boyer & Liu (2004) [22] where the skin of apple fruit is a richer source of vitamin C than the pulp but correlated with each other. According to Patykowski et al. (2007), apple peel contains about six fold more ascorbate than pulp [23].

In agreement with our present data Campeanu et al. (2009) reported that Mutsu and Jonathan cultivars registered the highest value for vitamin C. Overall, apple fruits contain 2-30 mg ascorbic acid per 100 g, depending on the cultivar and this concentration decreases progressively from the peel to the core of the fruit [24]. The storage conditions were important for the content of ascorbic acid in apple fruits and after one week of domestic storage. The study was found a decrease of this parameter by 75% [25]. The ascorbic acid content of apple fruits may also depends on the stage at which the fruits are harvested.

Generally, the fruit harvested at early maturity had lower ascorbic acid content than later stages of harvest, indicating that the fruit may have still been synthesizing ascorbic acid when harvested at early mature stages [26]. Likewise, Davey et al., (2007) found that the apple cultivar harvested later tend to have higher concentrations in

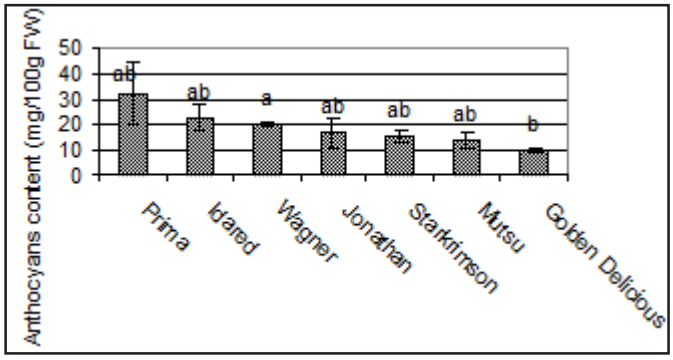

Fig. 1. The anthocyanin content of the skin in apple cultivars (mean ± SE, n = 3) (Mean values indicated by the same letter are not significant different, p<0.05)

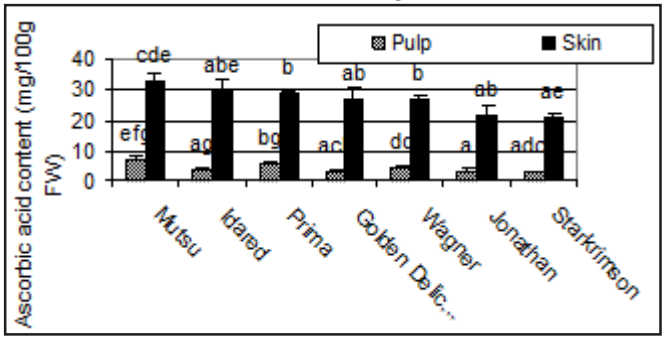

Fig. 2. Ascorbic acid content of the pulp and skin in apple cultivars (mean ± SE, n = 3) (Mean values indicated by the same letter are not significant different, p<0.05)

ascorbic acid than the early harvested cultivar [27]. However, Blazek et al. (2003) established that only about half of the original content of vitamin C remained in fruits after 5 months in the air light storage [28].

In addition, the location of the fruits on the same tree makes a difference because apples exposed to sunlight contain more ascorbic acid than the apples on the shaded side of the same tree [29]. According to several previous reports on ascorbic acid content in apples, a large variation can be found among different cultivars in different geographical locations. For example the cultivar *Jonathan* presents different ascorbic acid content in the USA (17 mg/100g), Canada (15.4 mg/100g) or France (10.8 mg/100g) [29]. The variation may be explained in part by a difference in the maturity of the apples of a given cultivar when analyzed. In our experiment in the Jonathan cultivar the content of ascorbic acid in pulp was 3.51 mg/100g and in skin 21.33 mg/100g. In some Romanian apple cultivars studied, Nour et al. (2010) found that the ascorbic acid contents were low (6.18 mg/100 g) with the exception of Red Boskoop and Idared [30].

Total polyphenol content (TPC) in seven apple cultivars were compared in skin and pulp (fig. 3). Thus, TPC level was higher in the skin within almost all three apple cultivars than pulp values. In Jonathan and Golden Delicious cultivars the values of polyphenol content were very similar in both parts of the fruit. The skin of Mutsu cultivar had the highest TPC whereas Wagner had the lowest. In pulp, the TPC ranged from 7.52 to 17.18 mg GAE/g DW, with the highest amount found in Mutsu and the lowest amount measured in Starkrimson. Mutsu apple cultivar had the highest TPC both in skin and pulp. The distribution and composition of phenolic phytochemicals are affected by a number of factors: cultivar, fruit part, growing season, environmental conditions, horticultural practices, geographic origin, postharvest storage conditions and processing procedures [12].

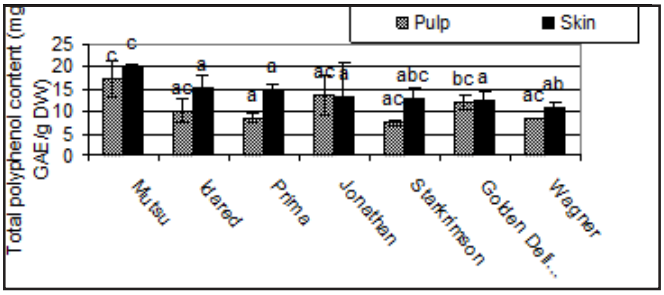

Fig. 3. Total polyphenol content of the pulp and skin in apple cultivars (mean ± SE, n = 3) (Mean values indicated by the same letter are not significant different, $p<0.05$)

Phenolic compounds are important secondary plant metabolites and contribute to any health promoting effects of fruits and vegetables. Apples contain many types of phenolic derivates and flavonoids (flavan-3-ols, flavonols, procyanidins, chalcones, and anthocyanins) [31-33]. The most common phenolic compounds in apples were epicatechin, chlorogenic acid, quercetin galactoside, quercetin glucoside, quercetin xyloside, quercetin arabinoside, quercetin rhamnoside, phloretin glucoside, phloretin xylogalactoside, and procyanidin B2. Several studies indicate that skin of apple fruits contains higher concentrations of polyphenols [34-36]. On the other hand, Schmitz Eiberger et al. 2003 found that the polyphenol content was cultivar dependent in those 31 apple cultivars studied [2]. These phenolic compounds are mainly located in the skin and their concentration decreased sharply during the early stage of development and then remained relatively constant during maturation and storage. More

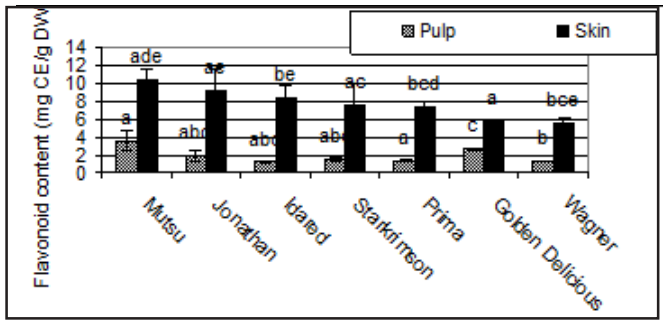

Fig. 4. Flavonoids content of the pulp and skin in apple cultivars (mean ± SE, n = 3) (Mean values indicated by the same letter are not significant different, $p<0.05$)

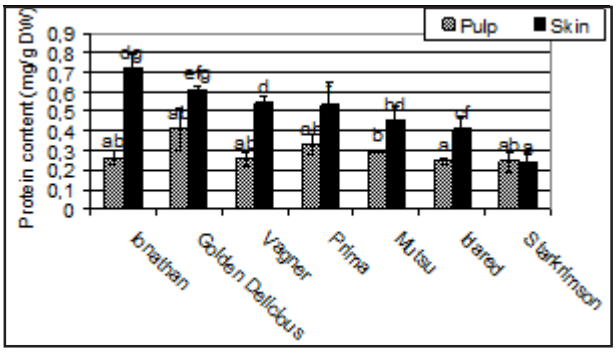

Fig. 5. Protein content of the pulp and skin in apple cultivars (mean ± SE, n = 3) (Mean values indicated by the same letter are not significant different, $p<0.05$)

recent discoveries show that apples are also an excellent source of bioactive phenolic phyto-chemicals that may provide special nutraceutical benefits compared to other common fruits [29].

The amount of flavonoids content, expressed as catechin equivalent (CE), in the pulp of fruits of seven apple cultivars ranged from 3.64 to 1.18 mg CE/g DW. As it can be seen in figure 4 the pulp of Mutsu apple cultivar exhibited the highest flavonoid content followed by Golden Delicious. For skin the amounts of total flavonoids varied from 10.31 mg CE/g DW (Mutsu) to 5.57 mg/100g (Wagner). The skin of all seven cultivars had significantly higher concentrations of flavonoid content than those determined for pulp. In agreement with these data, the research groups have also noted that apple skin extract has higher amount of phenolics and flavonoids than pulp extracts [37, 38, 3]. Significant differences on total polyphenol content between skin and pulp were observed in Idared, Prima, Starkrimson cultivars being 1.5 or 1.71 and 1.72 times greater in skin than pulp, respectively. In another study, Drogoudi et al. (2003) have found similar results where apples peel was found to be 1.2 to 3.3 times greater in total phenolic content compared with flesh [39].

Conclusions

The content of polyphenol compounds, flavonoids, anthocyanins and ascorbic acid of pulp and skin from seven cultivars of apple fruits grown in Romania were compared. The results revealed that there was no considerable variation in the amount of flavonoids and total phenolic content among the apple cultivars studied, however these levels differed significantly between the two parts (skin and pulp) tested. As expected, the skin of all the cultivars analyzed had significantly higher concentrations of flavonoids than those determined for the pulp. With the exception of the Jonathan cultivar in almost all apple cultivars tested the total polyphenol content was higher in skin than pulp. The maximum anthocyanin content was

recorded in Prima apple cultivar while the least was observed in Golden Delicious cultivar, the difference being approximately three fold higher in red skin apple than in green skin. As regards the ascorbic acid content there was considerable variation between the pulp and skin, the values being high in skin. Both values the maximum and minimum of ascorbic acid were obtained in the same cultivar Mutsu and Starkrimson, respectively. Between all the varieties tested the better antioxidant activity provided by the content of polyphenol, ascorbic acid and flavonoids was in apple fruits of Mutsu cultivar and for this it should be regarded as a valuable source of antioxidants.

References

1. LAMPE, J., Am. J. Clin. Nutr. **70**, no 3, 1999, p. 475.
2. SCHMITZ-EIBERGER, M., WEBER, V., TREUTTER, D., BAAB, G., LORENZ, J., J. Appl. Bot. – Angewandte Botanik, **77**, 2003, p. 167.
3. VIEIRA, F.G.K., BORGES, G.D.S.C., COPETTI, C., AMBONI, R.D.D.M.C., DENARDI, F., FETT, R., Sci. Hort., **122**, no 3, 2009, p. 421.
4. WU, J., CAO, H. ZHAO, L., LIAO, X., CHEN, F., WANG, Y., HU, X., Food Chemistry, **103**, 2007, p. 88.
5. LACHMAN, J., ORSAK, M., PIVEC, V., KUCERA, J., Czech J. Food Sci., **18**, 2000, p. 71.
6. MATTHES, A., SCHMITZ-EIBERGER, M., J. Appl. Bot. Food Qual. **82**, 2009, p. 152.
7. TSAO, R., YANG, R., YOUNG, J.C., ZHU, H., J. Agri. Food Chem., **51**, no 21, 2003, p. 6347.
8. EBERHARDT, M.V., LEE, C.Y., LIU, R.H., Nature. **405**, 2000, p. 903.
9. £ATA, B., J. Agri. Food Chem, **55,** 2007, p. 663.
10. BRAVO, L., Nutr. Rev. **11,** 1998, p. 317.
11. SOLER, C., SORIANO, J.M., MAÑES, J., Nat. Product Comm., **4**, no 5, 2009, p. 659.
12. KHANIZADEH, S., LI, DING, RONG, T., DJAMILA, R., YANG, R., MARIE-THERESE, VIGNEAULT, C., VASANTHA, RUPASINGHE, H.P., NOVA, S., J. Agri. Food Chem. Sci., **1**, no 2, 2007, p. 1.
13. FULEKI, T., FRANCIS, F.J., J. Food Sci., **33**, 1968, p. 78.
14. ARTENIE, V., TANASE, E., Ed.Universitatii "Al. I Cuza" Publishing House, 1981, p. 291.
15. SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTOS, R.M., Methods Enzymol., **299,** 1999, p. 152.
16. DEWANTO, V., WU, X., ADOM, K.K., LIU, R.H., J. Agri. Food Chem., **50**, 2002, p. 3010.
17. BAE, RO-NA, KI-WOO K., TAE-CHOON K., SEUNG-KOO L. Hort. Sci., **41**, no 3, 2006, p. 733.
18. KIM, J.B., PhD thesis. Dept. Hort., Seoul Natl. Univ., Suwon, Korea, 1990, p. 79.
19. LIN-WANG, K., MICHELETTI, D., PALMER, J, VOLZ, R., LOZANO, L., ESPLEY, R., HELLENS, RP., CHAGNE, DD., ROWAN, DD., TROGGIO, M., IGLESIAS, I., ALLAN, A.C., Plant Cell Environ. **34**, no 7, 2011, p. 1176.
20. ESPLEY, R., HELLENS, R., PUTTERILL, JO, STEVENSON, D., KUTTY-AMMA, S., ALLAN, A., Plant J. 49, no 3, 2007, p. 414.
21. LEJA, M., MAREZEK, A., BEN, J., Food Chem., 80, 2001, p. 303.
22. BOYER, J., RUI, HAI L., Nutr. J., 12, 2004, p. 3.
23. PATYKOWSKI, J., MAJCZAK, A., BERGIER, K., SKLODOWSKA, M., J. Fruit Ornam. Plant Res, 15, 2007, p. 21.
24. CAMPEANU, G., NEATA, G., DARJANSCHI, G., Not. Bot. Horti. Agrobot., 37, no 2, 2009, p. 161.
25. KEVERS, C., PINCEMAIL, J., TABART, J., DEFRAIGNE, J.O., DOMMES, J., J. Agri. Food Chem., s**59**, no 11, 2011, p. 6165.
26. JAN, I., RAB, A., SAJID, M., J. An. Plant Sci., **22**, no 2, 2012, p. 438.
27. DAVEY, M.W., AUWERKERKEN, A., KEULEMANS, J., J. Sci. Food Agri., **87,** 2007, p. 802.
28. BLAŽEK, J., HLUSIEKOVA, I., VARGA, A., Hort. Sci., **30**, no 3, 2003, p. 81.
29. CHANG, Y.L., New York Fruit Quarterly, **20**, no 3, 2012, p. 3.
30. NOUR, V., TRANDAFIR, I., IONICA, M.E., Not. Bot. Horti. Agrobot., **38**, no 3, 2010, p. 228.
31. MANGAS, J.J., RODRÍGUEZ, R., SUAREZ, B., PICINELLI, A., DAPENA, E., J. Agri. Food Chem., **47**, no 10, 1999, p. 4046.
32. PODSEDEK, A., WILSKA-JESKA, J., ANDERS, B., MARKOWSKI, J., Eur. Food Res. Technol., **10,** no 4, 2000, p. 268.
33. SHOJI, T., MUTSUGA, M., NAKAMURA, T., KANDA, T., AKIYAMA, H., GODA, Y., J. Agri. Food Chem., **51**, no 13, 2003, p. 3806.
34. LATTANZIO, V., DI, VENERE, LINSALATA, V., BERTOLINI, P., IPPOLITO, A., SALERNO, M., J. Agri. Food Chem, **49,** 2001, p. 5817.
35. WOLFE, K., XIANZHONG, W., RUI HAI, L., J. Agri. Food Chem., **51,** 2003, p. 609.
36. £ATA, B., TRAMPCZYNSKA, A., PACZESNA, J., Sci.Hort., **121**, no 2, 2009, p. 176.
37. MALEEHA, MANZOOR, FAROOQ, A., NAZAMID, S., ASHRAF, M., Molecules, **17**, 2012, p. 390.
38. ABROSCA, B.D., PACIFICO, S., CEFARELLI, G., MASTELLONE, C., FIORENTINO, A. Food Chem., **104,** 2007, p. 1333.
39. DROGOUDI, P.D., MICHAILIDIS, Z., PANTELIDIS, G., Sci. Hort., **115,** 2008, p. 149.

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