

Characterization of red raspberry (*Rubus idaeus* L.) for their physicochemical and morphological properties

I. Augšpole^{1*}, F. Dimiņš¹, I. Romanova² and A. Liniņa²

¹Latvia University of Life Sciences and Technologies, Faculty of Food Technology, Rīgas iela 22, LV-3001 Jelgava

²Latvia Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Lielā iela 2, LV-3001 Jelgava, Latvia

*Correspondence: ingrida.augspole@llu.lv

Received: January 30th, 2021; Accepted: May 9th, 2021; Published: May 13th, 2021

Abstract. Different raspberry cultivars are grown in Latvia suitable both for fresh market and for processing. Fresh local red raspberry is available for consumers from July to October. Information about the physical, chemical, and morphology properties of raspberry fruit is essential for understanding the behavior of the product during the postharvest operations such as harvesting, transporting, sorting, grading, packaging and storage. Knowledge of the physicochemical properties of red raspberries is essential because variations in the levels of these properties may exist between cultivars. New varieties of plants were used to describe and compare the fruit quality of red raspberry cultivars: ‘Daiga’, ‘Shahrazada’, ‘Norna’ and ‘Polana’ grown in Zemgales region of Latvia. The samples were collected from farm ‘Plūģi’ the full stage of ripening. The current research aimed to investigate and determine the chemical composition (total phenolic content (TPC), total flavonoid content (TFC), titratable acidity (TAc), total soluble solids content of raspberry fruits, the physical parameter pH, and color. Presented morphological parameters of fruit included their weight, receptacle length (Rl), receptacle width (Rw), fruit length (Fl), fruit width (Fw), weight of fruit (M), number of seeds. Research results showed significant differences in all chemical and physical characteristics as well as in morphology properties ($P < 0.05$) between cultivars.

Key words: raspberry, *Rubus idaeus* L, physicochemical, physical and morphological properties.

INTRODUCTION

Raspberries (*Rubus idaeus* L.) are a member of the *Rosaceae* family, grown as a perennial crop. Red raspberry contains numerous phenolic compounds with potential health benefits (Ilhami et al., 2011). Phenolic compounds are ubiquitous in plants which collectively synthesize several thousand different chemical structures characterized by hydroxylated aromatic ring(s). These compounds play several important functions in plants. They represent a striking example of metabolic plasticity enabling plants to adapt to changing biotic and abiotic environments and provide to plant products color, taste, technological properties and putative health promoting benefits (Augšpole et al., 2018b).

Fresh raspberries have a very short shelf life and are generally only readily available around summer. Most of the produced raspberries worldwide are processed, i.e., frozen and sold within different frozen fraction blocks or in jams and sauces. However, there has been an increasing demand for fresh raspberries out-of-season lately, and so many producers appear to be interested in growing primocane fruiting raspberry cultivars (Beekwilder et al., 2005; Atkinson et al., 2006). Today, raspberry fruit is being used in the production of bakery goods, jams, jellies, beverages, dairy products like ice cream and yogurt, fruit syrups, and many other specialty products such as fruited honey. Red raspberry juice is used in blended fruit drinks and other food products (Riaz & Bushway, 1996). The most significant health benefits of raspberry fruits are attributed to the phenolic compounds, such as flavonoids, phenolic acids, and tannins (Paredes-Lopez et al., 2010). It is believed that raspberry has a higher antioxidant capacity than most other fruits and vegetables (Alibabić et al., 2018). Polyphenols are a group of compounds synthesized exclusively by plants, especially for the protection against UV-radiation and the activity of pathogens. About 8,000 plant polyphenol compounds have been identified so far, whereas only some hundred occur in edible plants (Manach et al., 2004; Linina et al., 2020). Phenolic compounds of plants can be divided into two broad categories: 1) phenolic acids (oxometallic) and their derivatives, and 2) flavonoids (polyphenols). Phenolic acids and their derivatives, mainly esters, have more basic structures (Dimiņš & Augšpole, 2019). The flavonoid class of compounds has a more complex molecular structure, which is usually heterocyclic with an attached phenolic ring(s).

Flavonoids include anthocyanidins (water-soluble pigments, which are oxidized flavonoles), catechins, isoflavones, and proanthocyanidins (David et al., 2008). In addition, flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclooxygenase and lipoxygenase enzyme activities, flavonoids act as antioxidants, free radical scavengers or chelators of divalent cation (Augšpole et al., 2018b).

Organization (WHO) emphasized the importance of the antioxidant activity of phenolic components, especially from small colorful fruits, for prevention of the most important health problems namely cardiovascular diseases, diabetes, cancer, and obesity (The World Health Report, 2002; Stapleton et al., 2008). Raspberry is also an excellent source of vitamin C, a very powerful antioxidant with anticancer and immunomodulatory properties and known to prevent colds (Alibabić et al., 2018).

Therefore, the goal of raspberry production is to grow a cultivar with good productivity, large fruit, and excellent quality. This study aimed to explore the four most common cultivars of raspberry 'Daiga', 'Shahrazada', 'Norna' and 'Polana', which are grown in Zemgales region farm 'Plūģi' of Latvia. The study aimed to compare the chemical and morphological characteristics of different raspberry cultivars.

MATERIALS AND METHODS

Investigations were carried out at the Latvia University of Life Sciences and Technologies, Institute of Soil and Plant Sciences. In the experiment, the following red raspberry cultivars: 'Daiga', 'Shahrazada', 'Norna' and 'Polana' grown in the Zemgales region (GPS-coordinates: N56° 33' 29.5302", E23° 46' 26.04") of Latvia. The samples were collected from the farm 'Plūģi' at fully stage of ripening.

Titration acidity (TAc) was determined titrimetrically (Duma et al., 2019) with a solution of sodium hydroxide 2 ± 0.0001 g of raspberry was quantitatively transferred in 100 mL tubes, added 40 mL of distilled water ($0.055 \mu\text{S cm}^{-1}$) and mixed. After 30 minutes, the solutions were centrifuged for 10 min at 5,000 rpm. For determination, 10 mL of the supernatant was titrated with 0.1 M NaOH in the presence of indicator phenolphthalein, and results expressed as g of citric acid 100 g^{-1} raspberry sample fresh matter (FM).

The total soluble solids content (expressed as BRIX degree) was measured with a digital refractometer (A.KRÜSS Optronic Digital Handheld Refractometer DR301-95), calibrated at $+20 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$ with distilled water (deviation of the measuring instrument face value $\pm 0.1\%$) by standard method ISO 2173:2003.

The total phenolics content (TPC) of raspberries was analysed spectrometrically according to the Folin-Ciocalteu method (Dewanto et al., 2002; Kaškonienė et al., 2009). Each sample of raspberries was diluted with distilled water and filtered. This solution was then mixed with 0.2 N Folin - Ciocalteu reagent for 5 min and then a solution of sodium carbonate was added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank. Gallic acid was used as a standard to produce the calibration curve. The total phenolic content was expressed in mg of gallic acid equivalents (GAE) and 100 g^{-1} of raspberry fresh matter (FM).

The total flavonoid content (TFC) was expressed as quercetin equivalent mg QE 100 g^{-1} was determined using the Dowd method. 2% AlCl_3 solution in methanol was mixed with raspberries. Absorbance readings at 415 nm were taken after 10 min against a blank sample consisting of 5 mL coffee solution with 5 mL methanol without AlCl_3 . The total flavonoid content was determined using a standard curve with quercetin as a standard. The total flavonoid content was expressed in mg of quercetin equivalents (QE) 100 g^{-1} of raspberry fresh matter (FM), described by (Meda et al., 2005; Xu & Chang, 2007; Augšpole et al., 2018a) with some modifications.

The colors of the raspberry samples were evaluated by measuring CIE L^* , a^* , b^* parameters using “ColorTec-PCM/PSM” (ColorTec Associates, Clinton, USA). L^* , a^* , and b^* indicate whiteness/darkness, redness/greenness, and blueness/yellowness values, respectively. The maximum color value for L^* is 100, which would be a perfect reflecting diffuser. The minimum color for L^* would be zero, which would be black. The value colors of the a^* and b^* axes have no specific numerical limits. Positive a^* is red and negative a^* is green. Positive b^* is yellow and negative b^* is blue (Augšpole & Rakcejeva, 2013).

pH value was measured using Jenway 3510 pH meter, by the standard method LVS ISO 5542:2010.

Statistical analysis. The data of the research was analysed by the statistical and mathematical methods (standard deviation, mean). Data compared by the analysis of variance (ANOVA) and significance was defined at $P < 0.05$. For the data analysis the Microsoft Excel software of the version 2019 was used.

RESULTS AND DISCUSSION

The mean of total phenolic content (TPC), total flavonoid content (TFC), titration acidity (TAc), total soluble solids (TSS) content, and pH in the raspberry cultivars

selected for the study are shown in Table 1. TPC significantly ranged between raspberry cultivars ($P < 0.05$). The highest TPC was detected for the ‘Shahrazada’ raspberry cultivars (148.87 ± 3.43 mg GAE 100 g^{-1} FM). The lowest TPC for the ‘Polana’ raspberry cultivars (111.96 ± 3.11 mg GAE 100 g^{-1} FM). In turn, Alibabić et al. (2018) from Bosnia and Herzegovina University of Bihać reported significantly higher and similar values - total phenols of different raspberry cultivars were 102.0–521.4 mg GAE 100 g^{-1} , (FM). Latvian researchers Ozola et al. (2019) reported that the total phenol content depends on the extracting solvent. In turn, Turkmen et al. (2006) mentioned that it is higher in ethanol extract than aqueous extract, but Shirin & Jamuna (2010) found the highest total polyphenol content in water extracts.

Flavonoids are phenolic compounds that are common in different plants. These compounds have a wide range of biological functions - they protect plants from biotic and abiotic stresses, and they actively participate in the interaction between plants and the environment (Amalesh et al., 2011). Flavonoid content is one of the important influencing plant nutrition quality. Flavonoids content affects the color, flavor, and fragrance of plants (Sergejeva et al., 2018). The total flavonoid content in analyzed raspberry cultivars ranged from 411.36 ± 2.99 to 475.00 ± 2.04 mg QE 100 g^{-1} , FM (Table 1). It can be explained by the fact that the total phenolic properties of the raspberry samples are determined not only by phenolic compounds, but also trace elements, vitamins, individual amino acids and enzymes, etc. (Dimiņš & Augšpole, 2019).

However, the relationship between total soluble solids (TSS) (°Brix) and pH may be observed (Table 1). That is, the higher the content of TSS, the greater the pH content. The content of soluble solids (°Brix) depended on raspberry cultivar. Slightly higher content was observed in cultivars ‘Daiga’ 10.58 ± 0.75 °Brix and ‘Norna’ 10.28 ± 0.32 °Brix. Raspberry cultivars ‘Norna’ and ‘Daiga’ distinguish themselves with the significantly lowest amount of TAc (1.81 ± 0.32 and $2.06 \pm 1.81 \pm 0.32$ g 100 g^{-1} , FM). In turn researcher Sergejeva et al. (2018) reported that the soluble sugars are main product of photosynthesis and effects plant nutrition.

Table 1. Raspberry cultivars average total phenolics, total flavonoids, titrable acidity, and total soluble solids, and pH

Raspberry cultivars	TPC, mg GAE 100 g^{-1} , (FM)	TFC, mg QE 100 g^{-1} , (FM)	TAc, g 100 g^{-1} , (FM)	TSS (°Brix)	pH
‘Daiga’	141.76 ± 2.53	475.00 ± 2.04	2.06 ± 0.21	10.58 ± 0.75	3.23 ± 0.12
‘Shahrazada’	148.87 ± 3.43	456.06 ± 3.44	2.42 ± 0.11	9.78 ± 0.11	3.03 ± 0.32
‘Norna’	142.35 ± 1.05	465.91 ± 2.11	1.81 ± 0.32	10.28 ± 0.32	3.21 ± 0.12
‘Polana’	111.96 ± 3.11	411.36 ± 2.99	2.42 ± 0.11	8.33 ± 0.21	2.91 ± 0.41

TPC – total phenolic; TFC – total flavonoid; TAc – titrable acidity; TSS – total soluble solids.

It was found that the titratable acidity in fresh raspberry cultivar samples at harvest was in the range of 1.81 ± 0.32 g 100 g^{-1} (FM) till 2.42 ± 0.11 g 100 g^{-1} . Our findings are lower than the results noted by Riaz & Bushway (1996) who determined the titratable acidity was from 2.35 g 100 g^{-1} (FM) till 2.64 g 100 g^{-1} (FM) in fresh raspberry cultivar samples. Researchers Vinha et al. (2013) reported that high levels of acidity are responsible for the stability of vitamin C during storage of fruits and vegetables.

The results of pH level performed on the fresh raspberry cultivar samples formulation revealed that the pH ranged from 2.91 to 3.23 (Table 1), which indicates an acidic environment. The difference between the lowest and highest pH was not significant - 0.32 units ($P > 0.05$). The highest pH level was found in fresh raspberry cultivar samples in descending order: raspberry cultivar 'Daiga' 3.23 ± 0.12 , 'Norna' 3.21 ± 0.12 , 'Shahrazada' 3.03 ± 0.32 and 'Polana' 2.91 ± 0.41 . The lowest pH value results were gained in Moore (2006) where the pH value in raspberries ranged from 2.41 to 2.76. The obtained results indicated that the selected red raspberry cultivar fruits can be estimated as a rich source of biological active compounds. This study demonstrated that different red raspberry cultivar fruits have high potential value for fruit growers as well as for food manufacturers because of their high polyphenolic contents.

The results of fruit dimensions (fruit length and width, seed number, receptacle length, and width) as well as fruit weight are shown in Table 2. Significant differences ($P < 0.05$) can be observed between the cultivar 'Shahrazada' and the other three examined cultivars. All morphological parameters were higher ($P < 0.05$) than the dimensions of the 'Daiga', 'Norna' and 'Polana' raspberry cultivars. In turn, the total seed number of 'Shahrazada' was significantly lower compared to the other four raspberry cultivars. The morphological characteristics of the fruit, including chemical and sensory characteristics, vary among cultivars and depend on many factors, such as environmental factors (temperature, rainfall, soil type), irrigation, yield efficiency, ripeness of harvested fruits, and agrotechniques (Alibabić et al., 2018).

Table 2. Raspberry fruit morphological characteristics

Raspberry cultivars	M, g	Lf, mm	Wf, mm	TSN	Rl, mm	Rw, mm
'Daiga'	2.78 ± 0.33	6.0 ± 1.3	49.83 ± 3.5	73 ± 9	49.83 ± 3.40	4.00 ± 0.50
'Shahrazada'	4.40 ± 0.81	11.0 ± 2.3	72.55 ± 4.2	66 ± 4	72.55 ± 4.10	4.75 ± 1.50
'Norna'	3.68 ± 0.21	7.25 ± 2.0	62.55 ± 2.1	57 ± 5	62.55 ± 2.63	4.13 ± 1.00
'Polana'	2.74 ± 0.41	5.25 ± 1.7	54.77 ± 3.0	49 ± 7	54.83 ± 3.11	4.50 ± 1.00

M – fruit weight; Lf – fruit length; Wf – fruit width; TSN – total seed number of fruit; Rl – receptacle length; Rw – receptacle width.

It is assumed that the greater intensity of the color of plants indicates its higher nutritive value (Shibghatallah & Suhandono, 2013). Significant differences were found between the values of the color components L^* , a^* , and b^* of 'Norna', 'Daiga', 'Shahrazada', and 'Polana' raspberry cultivars (Table 2). The highest L^* values, related to the lightness, were found for fresh 'Daiga', 'Shahrazada' and 'Polana' raspberry cultivars, respectively (29.13 ± 1.43), (27.09 ± 1.74) and (27.18 ± 1.84) (Table 3). The lowest value of this color parameter was determined for 'Norna' raspberry cultivar (23.69 ± 2.42) (showing a darker color intensity). For fresh raspberry cultivar samples 'Daiga' (17.32 ± 1.42) and 'Shahrazada' (16.29 ± 1.32) the

Table 3. Raspberry fruit color L^* , a^* , b^*

Raspberry cultivars	L^*	a^*	b^*
'Daiga'	29.13 ± 1.43	17.32 ± 1.42	7.77 ± 2.11
'Shahrazada'	27.09 ± 1.74	16.29 ± 1.32	13.56 ± 1.42
'Norna'	23.69 ± 2.42	13.56 ± 1.22	6.03 ± 0.94
'Polana'	27.18 ± 1.84	15.81 ± 2.03	6.26 ± 1.05

highest b^* value was determined. This color component is related to the yellowness. In turn, the highest values of parameter a^* were obtained for raspberry cultivar samples (13.56 ± 1.42). This color component is related to the redness. Our results are also a little lower than the data of Patrick P. Moore (2006) who analysed the raspberry fruit colors L^* , a^* , b^* . That can be explained with the fact that there are many factors - raspberry cultivar, as well as environmental growth factors such as temperature, light, and soil properties, that affect raspberry fruit color. As well as the region and conditions for growing, the studied plants are essential.

CONCLUSIONS

1. This study generated valuable information on the biologically active compounds of different red raspberry cultivar species grown in Zemgales region farm 'Plūģi' and it highlights the crucial influence of cultivar on the physiochemical properties of raspberry fruits.

2. Raspberry represents a diverse source of potentially healthy antioxidants and as such can provide a useful component to our daily diet. The raspberry cultivar fruits are rich source of total phenolics, total flavonoids, titrable acidity, and total soluble sugar, demonstrating its potential use as a food additive.

3. Significant differences were determined between the color components $L^*a^*b^*$ of fresh raspberry cultivar samples.

4. Knowledge of raspberry composition will give the food processor the option to select the proper cultivar for a particular use. Information on raspberry composition at harvest will also help for the fresh market.

REFERENCES

- Alibabić, V., Skender, A., Bajramović, M., Šertović, E. & Bajrić, E. 2018. Evaluation of morphological, chemical, and sensory characteristics of raspberry cultivars grown in Bosnia and Herzegovina. *Turkish Journal of Agriculture and Forestry* **42**, 67–74.
- Amallesh, S., Gouranga, D. & Sanjoy, K.D. 2011. Roles of flavonoids in plants. *International Journal of Pharmacy and Pharmaceutical Sciences* **6**(1), 12–35.
- Atkinson, C.J., Doddis, P.A.A., Ford, Y.Y., J.Le, Taylor, J.M. & Blake, P.S. 2006. Effects of cultivar, fruit number and reflected photosynthetically active radiation on *Fragaria x ananasa* productivity and fruit ellagic and ascorbic acid concentrations. *Annals of Botany*, **97**, 429–441.
- Augšpole, I., Dūma, M. & Ozola, B. 2018a. Bioactive compounds in herbal infusions. *Agronomy Research* **16**(2), 1322–1330.
- Augšpole, I., Dūma, M., Cinkmanis, I. & Ozola B. 2018b. Herbal teas as a rich source of phenolic compounds. *Chemija*. **29**(4), 257–262.
- Augšpole, I. & Rakcejeva, T. 2013. Effect of hydrogen peroxide on the quality parameters of shredded carrots. **In: Proceedings of Annual 19th International Scientific Conference Research for Rural Development**, Jelgava, Latvia, 91–97.
- Beekwilder, J., David Hall, R. & De Vos, R. 2005. Identification and dietary relevance of antioxidants in raspberry. *BioFactors* **23**(4), 197–205.
- David, W., Reische, Dorris, A., Lillard, Ronald, R. & Eiten, M. 2008. Antioxidants, in: Casimir C.Akoh and David B.Min (Eds.), *Food Lipids. Chemistry, Nutrition, and Biotechnology*, CRC Press (Taylor & Francis Group), New York, 409–430.
- Dewanto, V., Wu, X., Adom, K.K. & Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* **50**(10), 3010–3014.

- Dimiņš, F. & Augšpole, I. 2019. Total phenolic, antioxidant activities and flavonoid contents of herbal syrups. *Key Engineering Materials. Materials Science and Applied Chemistry II* **800**, 60–64.
- Duma, M., Alsina, I., Dubova, L., Augšpole, I. & Erdberga, I. 2019. Suggestions for consumers about suitability of differently coloured tomatoes in nutrition. **In: FoodBalt 20019: 13th Baltic conference on food science and technology “Food. Nutrition. Well-Being”:** conference proceedings. Jelgava: LLU, 261–264.
- Ilhami, G., Fevzi, T., Ramazan, C., mine, B., Ahmet, C.G. & Ummugulsum, E. 2011. Pomological Features, Nutritional Quality, Polyphenol Content Analysis, and Antioxidant Properties of Domesticated and 3 Wild Ecotype Forms of Raspberries (*Rubus idaeus* L.). *Journal of Food Science* **76**(4), 585–593.
- Kaškoniene, V., Maruška, A., Kornyšova, O., Charczun, N., Ligor, M. & Buszewski, B. 2009. Quantitative and qualitative determination of phenolic compounds in honey, *Chemine Technologija* **3**(52), 74–80.
- Linina, A., Augšpole, I., Romanova, I. & Kuzel, S. 2020. Winter rye (*Secale cereale* L.), antioxidant capacity, total phenolic content and quality indices. *Agronomy Research* **18**(S3), 1751–1759.
- Manach, C., Scalbert, A., Morand, Ch., Remesy, Ch. & Jimenez, L. 2004. Polyphenols: Food source and bioavailability. *The American Journal of Clinical Nutrition* **5**, 727–747.
- Meda, A., Lamien, C.E., Romito, M., Millogo, J. & Nacouluma, O.G. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity, *Food Chemistry* **91**, 571–577.
- Moore, P.P. 2006. ‘Cascade Dawn’ Red Raspberry. *HortScience* **41**(3), 857–859.
- Ozola, B., Augšpole, I., Duma, M. & Kreicbergs, V. 2019. Bioactive compounds in fresh and dried ginger root (*Zingiber officinale*). **In: FoodBalt 2019: 13th Baltic conference on food science and technology „Food. Nutrition. Well-Being“:** conference proceedings, Jelgava: LLU, 265–268.
- Paredes-Lopez, O., Cervantes-Ceja, M.L., Vigna-Perez, M. & Hernandez-Perez, T. 2010. Berries: Improving Human Health and Healthy Aging, and Promoting Quality of Life-A Review. *Plant Foods for Human Nutrition* **65**(3), 299–308.
- Riaz, M.N. & Bushway, A.A. 1996. Compositional analysis of red raspberry cultivars grown in Maine. *Journal of Food Quality* **19**, 457–465.
- Sergejeva, D., Alsina, I., Duma, M., Dubova, L., Augšpole, I., Erdberga, I. & Berzina, K. 2018. Evaluation of different lighting sources on the growth and chemical composition of lettuce. *Agronomy Research* **16**(3), 892–899.
- Shibghatallah, K. & Suhandono, V.K. 2013. Measuring leaf chlorophyll concentration from its color: A way of monitoring environment change in plantations. *Biological Physics*. **In: AIP Conference Proceedings** **1554**(1), 210–213.
- Shirin, A.P.R. & Jamuna, P. 2010. Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *Journal of Medicinal Plants Research* **4**(24), 2674–2679.
- Stapleton, A.P., James, E.M., Goodwill, G.A. & Frisbee, J.C. 2008. Obesity and vascular dysfunction. *Pathophysiology* **15**, 79–89.
- The World Health Report 2002. Reducing risks and promoting healthy life. Geneva, *World Health Organization*, 2002.
- Turkmen, N., Sari, F. & Velioglu, Y. 2006. Effects of extraction solvents on the concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chemistry* **99**(4), 835–841.
- Vinha, A.F., Barreira, S.V.P., Castro, A., Costa, A. & Oliveira, M.B.P.P. 2013. Influence of the Storage Conditions on the Physicochemical Properties, Antioxidant Activity, and Microbial Flora of Different Tomato (*Lycopersicon esculentum* L.) Cultivars. *Journal of Agricultural Science* **5**(2), 118–128.
- Xu, B.J. & Chang, S.K.C. 2007. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. *Journal of Food Science* **72**(2), 159–166.