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ANTIOXIDANT CAPACITY, TOTAL PHENOLIC AND FLAVONOID CONTENT OF EDIBLE ROSE PETALS FROM ORGANIC PRODUCTION

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Abstract

In 2015, in the Experimental Field of Faculty of Horticulture within USAMV Bucharest an organic edible rose plantation with three varieties from David Austin collection: 'Crown Princess Margareta', 'Falstaff' and 'Brother Cadfael' was established. Before and after planting, three ameliorative species were sown in seven variants for increasing the soil biological activity. Two types of mulches were applied on the rose rows with special results on the agrochemical parameters. Organic plant protection and fertiliser schemes were used. The petal antioxidant capacity, total phenol and flavonoid content were determined from fresh petals. Part of them were dried at 45°C during four hours and analysed. Three variants of jam were made from each variety, using different ingredients as lemon, sea buckthorn and ginger and analysed. The extracts were subjected to analysis using spectrophotometric methods. Antioxidant capacity was similar in all the three variants. Total phenolic and flavonoid content varied according to the experimental variants, 'Falstaff' registering the highest amount. Dried petals registered similar biochemical parameters or higher than the fresh or processed ones, drying being recommended as an efficient preserving method.

Key words*: Rosa sp., varieties, drying, jam, spectrophotometry, free radicals.*

INTRODUCTION

Rose is a complex plant. Considered the Queen of plants, the symbol of love, purity, victory, beauty, friendship and respect among men (Milică et al., 2010), nobility and refinement (Bojor & Dumitru, 2007), the edible rose has special nutraceutic properties.

This is due to its content in carotenoids, anthocyanins, flavon detivatives, tannins, vitamins, ethereal oils etc (Milică et al., 2010) with anticancerous, slightly laxative, aphrodisiac, decongestant, disinfectant, antiseptic, hemostatic properties (Bojor & Răducanu, 2010), antimicrobial (Park et al., 2016; Sengul et al., 2017).

The rose has been used since ancient times as a medicinal plant (Lambraki, 2001; Hessayon, 2005; Milică et al., 2010; Lia et al., 2014; Dong et al., 2017; Fernandes et al., 2017). Milică et al. (2010) mentioned the role of rose jam in the treatment of lung and oral cavities.

The nutritional and biochemical parameters of rose petals can become a source of bioactive compounds along with other edible flowers (Pires et al., 2017; Santos et al., 2018). Riffault-Valois recalls the role of polyphenols in plant defense mechanisms, attracting pollinators and color shades. Ouerghemmi et al. (2016) analyzed the antioxidant activity and phenolic profile of the organic extract from the leaves of several varieties of roses, also mentioning the important role of polyphenols both as a source of antioxidants in the human diet but also as an important factor of the plant's immune system, indicating the adaptability of the plant of adaptation to the environment, also mentioned by Hashidoko (1996); Coruh and Ercisli (2010).

The antioxidant activity of three species of roses *Rosa damascena* Mill., *Rosa bourboniana* Desp. and *Rosa Brownies* Lindl., from fresh petal extracts was determined by Kumar et al. (2009). *Rosa rugosa* Thunb. at Lia et al. (2014), who studied phenolic content and antioxidant activity in 51 species of edible flowers, ranks first in terms of antioxidant capacity.

Ge and Ma (2013), studying the content of anthocyanins and polyphenols in edible roses, suggested that the varieties studied should be used to replace artificial colors in food.

MATERIALS AND METHODS

In 2015, in the Experimental Field of Faculty of Horticulture within USAMV Bucharest an organic edible rose plantation with three varieties from David Austin collection: 'Crown Princess Margareta', 'Falstaff' and 'Brother Cadfael' was established. Before and after planting, three ameliorative species were sown in seven variants for increasing the soil biological activity (V1-V8). Two types of mulches (wool and wood chips) were applied on the rose rows with special results on the agrochemical parameters (Vn.1. wood chips and Vn.2. wool, while the control Vn.3., was represented by unmulched soil) (Butcaru et al, 2017). Organic plant protection and fertiliser schemes were used. The antioxidant capacity, total phenol and flavonoid content were determined from fresh and dry petals and from jam rose petal. The extracts were subjected to analysis using spectrophotometric methods.

Total content in polyphenols.
The total content in polyphenols was determined spectrophotometrically with the Folin-Ciocalteu method after Skupień (2006), Khanizadeh (2008), Delian (2011), Mureşan (2014), Bezdadea Catuneanu et al. (2017), with some modifications. 25 µl sample (hydro-alcoholic extract) + 1.975 H_20 + 125 µl Folin-Ciocalteu + 375 µl Na_2CO_3 (20%) were homogenized, followed by incubation for 2 hours at ambient and dark temperature. Then the samples were read on the spectrophotometer to the blank at wavelength λ = 750 nm. In the case of hydroalcoholic extracts obtained from rose petals, it was necessary to make 1:10 dilutions in order to fit the results on the calibration curve. The total polyphenol content was expressed in M / ml, and the calculation was performed using the calibration curve: y = 0.0008x + 0.0003.

Total content in flavonoids.
The total flavonoid content was determined spectrophotometrically after a method adapted after Zilić (2011), Shen (2016), Bezdadea Cătuneanu et al. (2017). 250 µl of sample (hydro-alcoholic extract) + 1.25 ml H_2O + 75 µl $NaNO_2$ (5%) were homogenized, after which incubation was carried out for 5 minutes at ambient temperature. 75 µl of $AlCl_3$ (10%) was added and incubated for 6 minutes, after which 500 µl of NaOH (1M) was added. Samples were read on the spectrophotometer relative to the blank at wavelength λ = 510 nm. Dilutions of 1: 2 were used to align the results on the calibration curve. The total flavonoid content was expressed in M/ml, and the calculation was performed using the calibration curve:

$$y = 0.000627455 * x + 0.0068.$$

Antioxidant capacity.
The evaluation of antioxidant activity was performed according to the spectrophotometric method after Khanizadeh (2008), Mureşan (2014), Drogoudi (2016), Bezdadea Catuneanu et al. (2017).
500 µl sample + 1 ml DPPH (0.1 mM) was homogenized and incubated for 30 minutes at ambient temperature and dark. Samples were read on the spectrophotometer relative to the blank at the wavelength λ = 515 nm. The results were calculated according to the formula:
AADPPH (%) = (Acontrol-Asample)/(A control) x 100, where Acontrol is the absorbance of the control sample (not containing the hydroalcoholic extract) and Asample is the sample absorbance. Dilutions of 1: 10 were used. Expression of results was made in percentages.

Rose jam variants.
Three variants of jam were made from each variety, using different ingredients as lemon, sea buckthorn and ginger and analysed (Table 1).

Table 1. Rose petal jam variants

Variant	Description
D1	'Crown Princess Margareta' + lemon
D2	'Falstaff' + lemon
D3	'Brother Cadfael' + lemon
D4	'Crown Princess Margareta' + lemon
D5	'Falstaff' + ginger
D6	'Brother Cadfael' + ginger
D7	'Crown Princess Margareta' + sea buckthorn juice
D8	'Falstaff' + sea buckthorn juice
D9	'Brother Cadfael' + sea buckthorn juice

Dry rose petals
Part of the petals were dried at 45°C during four hours and analysed.

RESULTS AND DISCUSSIONS

Total polyphenols and flavonoid content, antioxidant capacity of fresh petals

Total content in polyphenols.

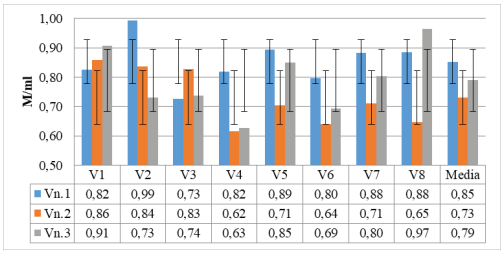
In 'Crown Princess Margareta' variety there are significant differences between the wool mulched variants and the others.

In the wood chips mulched variants, values between variants do not differ significantly being from 0.73 M/ml (V3.1) to 0.99 M/ml (V2.1).

In the wool mulched variants, there are significant differences between the variants, the lowest values being present in variants V4.2 (0.62 M/ml), V6.2 (0.64 M/ml) and V8.2 (0.65 M/ml) and the highest values being V1.2 (0.86 M/ml), V2.2 (0.84 M/ml) and V3.2 (0.83 M/ml).

There are also significant differences in the unmulched rows, ranging from 0.63 (V4.3 M/ml) to 0.97 (V8.3 M/ml) and 0.91 (V1.3 M/ml) (Figure 1).

Comparing experimental variants V1-V8, there are significant differences, the lowest values being found in V4, V6, V3 variants and the highest in variants V1, V2, V5 and V8.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,82	0,99	0,73	0,82	0,89	0,80	0,88	0,88	0,85
Vn.2	0,86	0,84	0,83	0,62	0,71	0,64	0,71	0,65	0,73
Vn.3	0,91	0,73	0,74	0,63	0,85	0,69	0,80	0,97	0,79

Figure 1. Total polyphenols content in petals at 'Crown Princess Margareta' variey

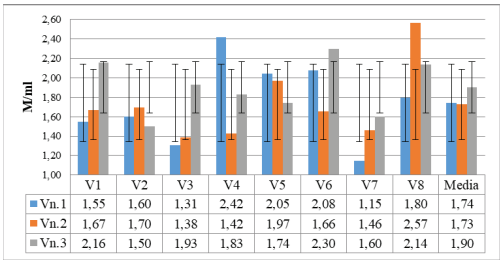
At the 'Falstaff' variety there are significant differences between variants.

In the wood chips mulched variants, the values range from 1.15 M/ml (V7.1) to 2.42 M/ml (V4.1).

In the wool mulched variants, the lowest values are present in variants V3.2 (1.38 M/ml), V4.2 (1.42 M/ml) and V7.2 (1.46 M/ml) and the highest values at V8.2 (2.57 M/ml).

In the non-mulched raws, the lowest values are in variants V2.3 (1.50 M/ml), V5.3 (1.74 M/ml) and V7.3 (1.60 M/ml) values in V6.3 (2.30 M/ml), V1.3 (2.16 M/ml) and V8.3 (2.14) (Figure 2).

Comparing the variants, there are significant differences, the smallest values being found in V7 and the highest in the V8.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	1,55	1,60	1,31	2,42	2,05	2,08	1,15	1,80	1,74
Vn.2	1,67	1,70	1,38	1,42	1,97	1,66	1,46	2,57	1,73
Vn.3	2,16	1,50	1,93	1,83	1,74	2,30	1,60	2,14	1,90

Figure 2. Total polifenols content in petals at 'Falstaff' variety

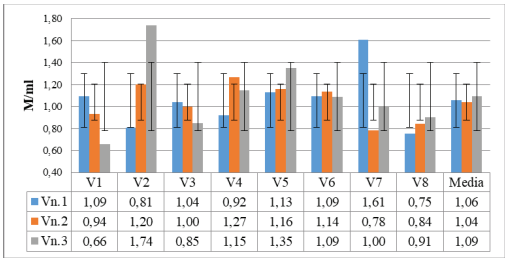
At the 'Brother Cadfael' variety there are also significant differences between variants.

In the wood chips mulched variants, the values range from 0.75 M/ml (V8.1) and 0.81 M/ml (V2.1) to 1.61 M/ml (V7.1), respectively.

In the wool mulched variants, the smallest values are present in variants V7.2 (0.78 M/ml) and V8.2 (0.84 M/ml) and the highest values at V4.2 (1.27 M/ml), V2.2 (1.20 M/ml), V5.2 (1.16 M/ml) and V6.2 (1.14 M/ml).

In the unmulched rows, the lowest value is present in variant V1.3 (0.66 M/ml) and the highest value at V2.3 (1.74 M/ml) (Figure 3).

By comparing the variants, the smallest values are found in the V8 variant and the highest in the variants V2 and V5.

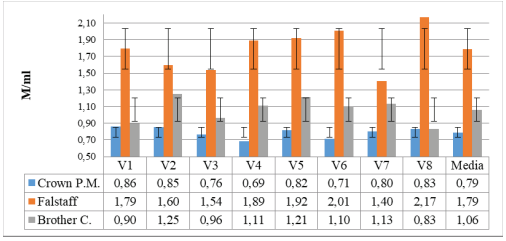


	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	1,09	0,81	1,04	0,92	1,13	1,09	1,61	0,75	1,06
Vn.2	0,94	1,20	1,00	1,27	1,16	1,14	0,78	0,84	1,04
Vn.3	0,66	1,74	0,85	1,15	1,35	1,09	1,00	0,91	1,09

Figure 3. Total polyphenols content in petals at 'Brother Cadfael' variety

There are significant differences between the total content of polyphenols in the three varieties of roses (Figure 4). 'Falstaff' variety has almost double values (1.79 M / ml) than 'Crown Princess Margareta' (0.79 M / ml) and

significantly higher than 'Brother Cadfael' (1.06 M / ml).



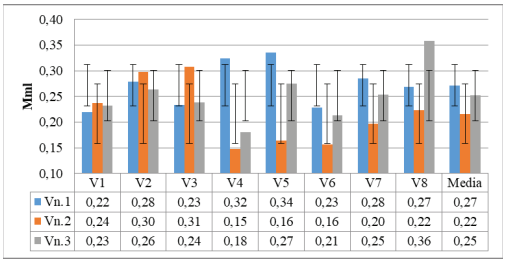
	V1	V2	V3	V4	V5	V6	V7	V8	Media
Crown P.M.	0,86	0,85	0,76	0,69	0,82	0,71	0,80	0,83	0,79
Falstaff	1,79	1,60	1,54	1,89	1,92	2,01	1,40	2,17	1,79
Brother C.	0,90	1,25	0,96	1,11	1,21	1,10	1,13	0,83	1,06

Figure 4. Total polyphenols content in petals at the three rose varieties

Total content in flavonoids.

In the 'Crown Princess Margareta' variety, in the wood chips mulched rows, the smallest values are V1.1 (0.22 M/ml), V3.1 (0.23 M/ml) and V6.1 (0.23 M/ml) M / ml) and the highest values are at V5.1 (0.34 M/ml) and V4.1 (0.32 M/ml). In the wool mulched rows, the lowest values are V4.2 (0.15 M/ml), V6.2 (0.16 M/ml) and V5.2 (0.16 M/ml), and the higher values at V3.2 (0.31 M/ml) and V2.2 (0.30 M/ml). There are also significant differences in the unmulched variants, ranging from 0.18 M/ml (V4.3) and 0.21 M/ml (V6.3) to 0.36 M/ml (V8.3).

By comparing the variants, the smallest values are in V6 and V4 variants and the highest in V8 and V2 variants (figure 5).



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,22	0,28	0,23	0,32	0,34	0,23	0,28	0,27	0,27
Vn.2	0,24	0,30	0,31	0,15	0,16	0,16	0,20	0,22	0,22
Vn.3	0,23	0,26	0,24	0,18	0,27	0,21	0,25	0,36	0,25

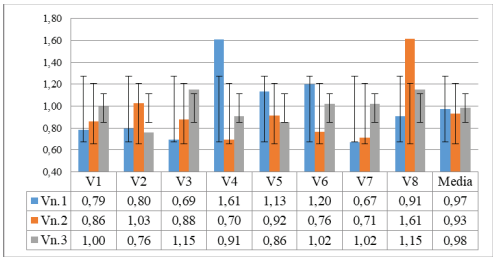
Figure 5. Total content of flavonoid in petals at 'Crown Princess Margareta' variety

At the 'Falstaff' variety there are significant differences between variants. In the wood chips mulched rows, the values range from 0.67 M/ml (V7.1) and 0.69 M/ml (V3.1) to 1.61 M/ml (V4.1), respectively.

On the wool mulched rows, the smallest values are present in variants V4.2 (0.70 M/ml) and V7.2 (0.71 M/ml) and the highest values at V8.2 (1.61 M/ml).

In the unmulched variants, the lowest value is present in variant V2.3 (0.76 M/ml) and the highest values are at V8.3 (1.15 M/ml) and V3.3 (1.15 M/ml) (Figure 6).

By comparing the variants, there are significant differences, the smallest values being found in the V7 and V1 variants, and the largest in the V8 variant.

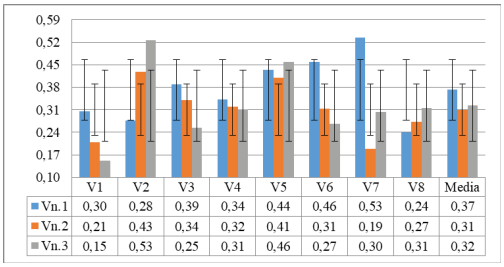


	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,79	0,80	0,69	1,61	1,13	1,20	0,67	0,91	0,97
Vn.2	0,86	1,03	0,88	0,70	0,92	0,76	0,71	1,61	0,93
Vn.3	1,00	0,76	1,15	0,91	0,86	1,02	1,02	1,15	0,98

Figure 6. Total content of flavonoid in petals at 'Falstaff' variey

There are also significant variations at the 'Brother Cadfael' variety. In the wood chips variants, the values range from 0.24 M/ml (V8.1) to 0.53 M/ml (V7.1). On the wool mulched rows, the smallest value is present at V7.2 (0.19 M/ml) and highest at V2.2 (0.43 M/ml). In the unmulched rows, the lowest value is present in variant V1.3 (0.15 M/ml) and the highest value at V2.3 (0.53 M/ml). (Figure 7)

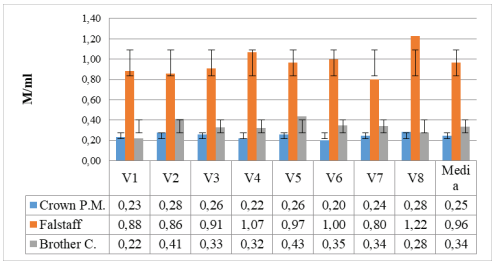
By comparing the variants, the smallest values are in V1 and the highest in V5.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	0,30	0,28	0,39	0,34	0,44	0,46	0,53	0,24	0,37
Vn.2	0,21	0,43	0,34	0,32	0,41	0,31	0,19	0,27	0,31
Vn.3	0,15	0,53	0,25	0,31	0,46	0,27	0,30	0,31	0,32

Figure 7. Total content of flavonoid in petals at 'Brother Cadfael' variety

By comparing the flavonoid content to the three varieties, there are significant differences between them (Figure 8). 'Falstaff' variety has values four times bigger (0.96 M/ml) than 'Crown Princess Margareta' (0.25 M/ml) and almost three times bigger than 'Brother Cadfael' (0.34 M/ml).



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Crown P.M.	0,23	0,28	0,26	0,22	0,26	0,20	0,24	0,28	0,25
Falstaff	0,88	0,86	0,91	1,07	0,97	1,00	0,80	1,22	0,96
Brother C.	0,22	0,41	0,33	0,32	0,43	0,35	0,34	0,28	0,34

Figure 8. Total content of flavonoid in petals at the three rose varieties

In general, there are significant positive correlations between the content of polyphenols and flavonoids in petals in all varieties of roses, in the experimental variants (Figures 9 and 10).

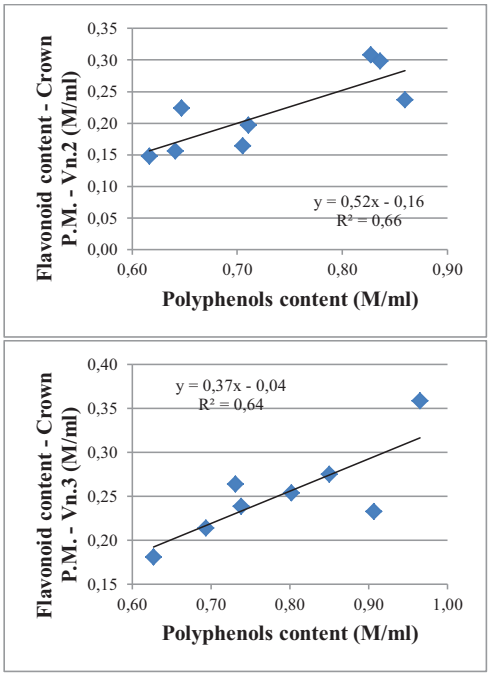


Figure 9. Correlations between flavonoid and polifenols content in fresh petals (Vn.2 and Vn.3)

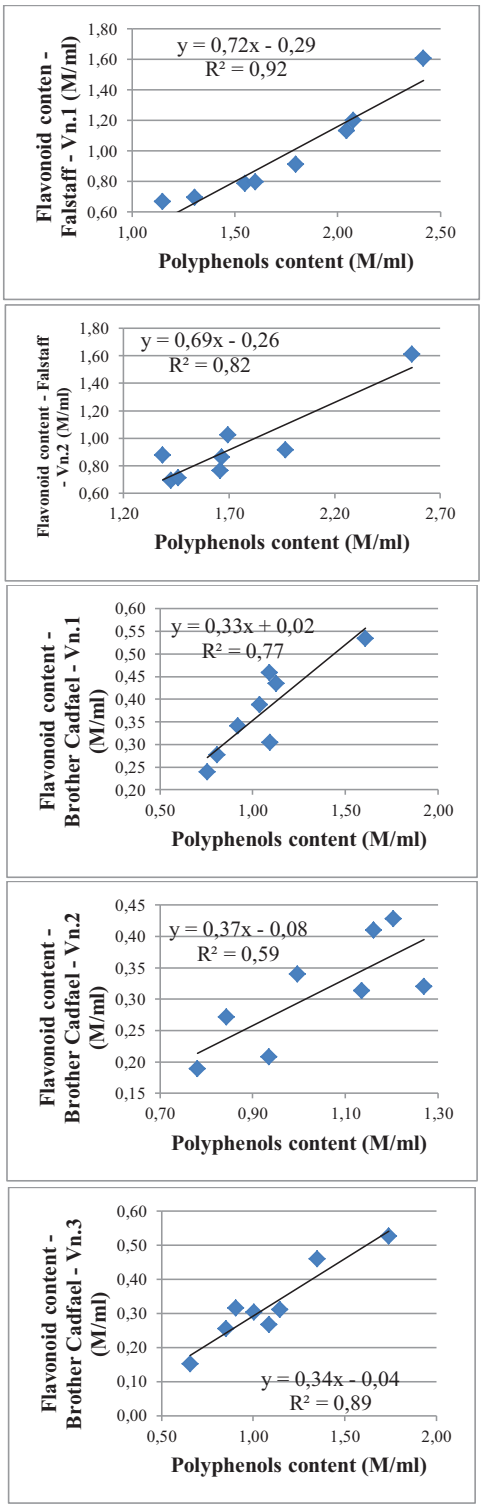


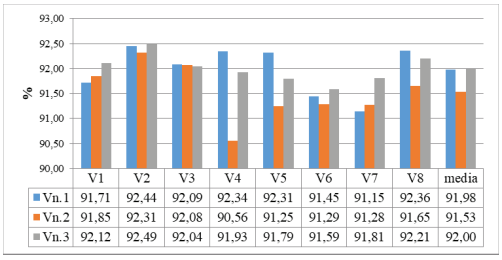
Figure 10. Correlation between flavonoid and polyphenols content în petale

Antioxidant capacity.

In 'Crown Princess Margareta' variety, the lowest values are present in variant V7.1 (91.15%) and the highest values are at V2.1 (92.44%). On the wool mulched rows, the lowest values are in V4.2 (90.56%) and the highest values are in V2.2 (92.31%) and V3.2 (92.08%). On the unmulched raws, the values range from 91.59% (V6.3) to 92.49% (V2.3) (Figure 11).

Comparing variants V1-V8, there are significant differences, with the lowest values being found in V7 variants and the highest in V2.

The values obtained are comparable to those determined by Sengul M. et al. (2017), who determined at *Rosa damascena* Mill. a high value of antioxidant activity, highlighting the importance of rose and its use alone or in combination with other herbal teas.

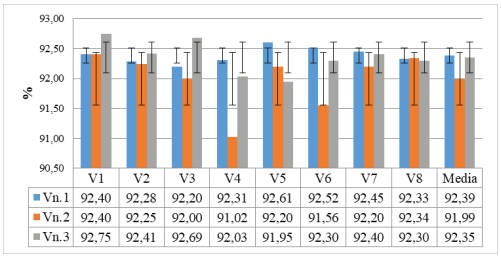


	V1	V2	V3	V4	V5	V6	V7	V8	media
Vn.1	91,71	92,44	92,09	92,34	92,31	91,45	91,15	92,36	91,98
Vn.2	91,85	92,31	92,08	90,56	91,25	91,29	91,28	91,65	91,53
Vn.3	92,12	92,49	92,04	91,93	91,79	91,59	91,81	92,21	92,00

Figure 11. Antioxidant capacity of fresh petals at 'Crown Princess Margareta'

At the 'Falstaff' variety there are significant differences between variants. On wood chips mulched rows, the values range from 92.20% (V3.1) to 92.61% (V5.1). In the wool mulched raws, the lowest values are present in variant V4.2 (91.02%) and the highest values are at V1.2 (92.40%). In the unmulched raws, the lowest value is present in variant V5.3 (91.95%) and the highest values at V1.3 (92.75%) (Figure 12).

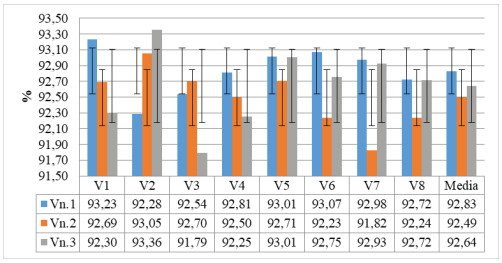
By comparing the variants, the smallest values are found in V4 and the highest in V1.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	92,40	92,28	92,20	92,31	92,61	92,52	92,45	92,33	92,39
Vn.2	92,40	92,25	92,00	91,02	92,20	91,56	92,20	92,34	91,99
Vn.3	92,75	92,41	92,69	92,03	91,95	92,30	92,40	92,30	92,35

Figure 12. Antioxidant capacity of fresh petals at 'Falstaff' variety

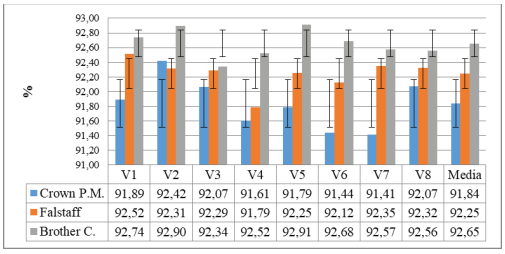
For the 'Brother Cadfael' variety, the values from the wood chips mulch raws ranged from 92.28 (V2.1) to 93.23% (V1.1). In the wool mulched variants, the smallest values are present in variant V7.2 (91.82%) and the highest values in V2.2 (93.05%). In the unmulched variants, the lowest value is present in variant V3.3 (91.79%) and the highest values are at V2.3 (93.36%) (Figure 13). By comparing the variants, the smallest values are in V3 and the highest in V5.



	V1	V2	V3	V4	V5	V6	V7	V8	Media
Vn.1	93,23	92,28	92,54	92,81	93,01	93,07	92,98	92,72	92,83
Vn.2	92,69	93,05	92,70	92,50	92,71	92,23	91,82	92,24	92,49
Vn.3	92,30	93,36	91,79	92,25	93,01	92,75	92,93	92,72	92,64

Figure 13. Antioxidant capacity of fresh petals at 'Brother Cadfael' variety

By comparing the antioxidant capacity of the three varieties of roses, there are significant differences between them (Figure 14).

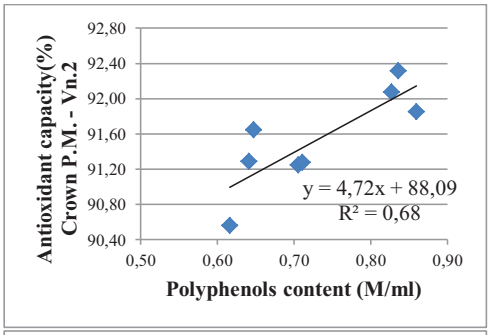


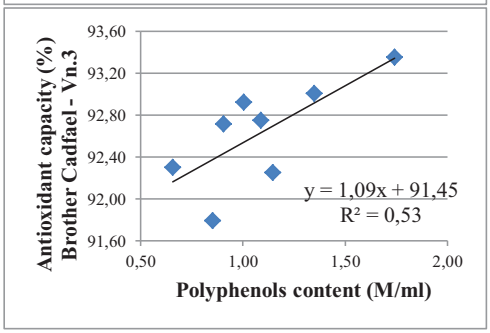
	V1	V2	V3	V4	V5	V6	V7	V8	Media
Crown P.M.	91,89	92,42	92,07	91,61	91,79	91,44	91,41	92,07	91,84
Falstaff	92,52	92,31	92,29	91,79	92,25	92,12	92,35	92,32	92,25
Brother C.	92,74	92,90	92,34	92,52	92,91	92,68	92,57	92,56	92,65

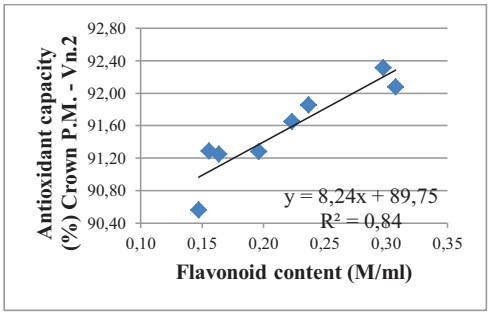
Figure 14. Antioxidant capacity of fresh petals to the three rose varieties

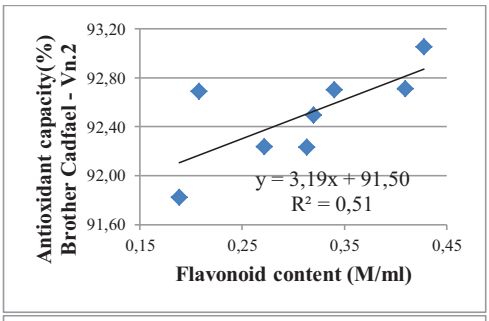
Significant positive correlations are noted between the antioxidant capability of petals and

the polyphenol content at the 'Crown Princess Margareta' variety in wool mulched variants and at the 'Brother Cadfael' variety in the unmulched variants. Also, significant positive correlations between the antioxidant capability of petals and the flavonoid content of the 'Crown Princess Margareta' and 'Brother Cadfael' varieties in wool mulched variants and the 'Brother Cadfael' variety in the unmulched variants (Figure 15).









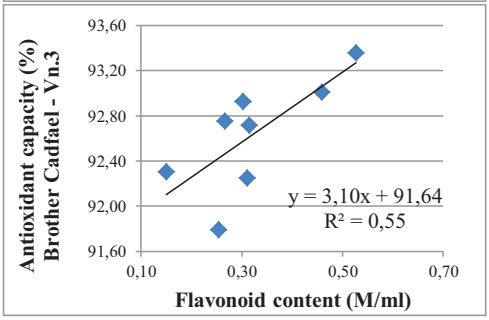


Figure 15. Correlation between antioxidant capacity and total polyphenols and flavonoids in fresh petals

Total polyphenols and flavonoid content, antioxidant capacity of rose petal jam

In Table 2 are presented the total content in polyphenols, flavonoids and the antioxidant capacity in the 9 rose petal jam variants.

Table 2. Total polyphenol, flavonoids and antioxidant capacity in rose petal jam

Variant	Polyphenol content (M/ml)	Flavonoid content (M/ml)	Antioxidant capacity (%)
D1	0.08±0.01	0	88.58±0.00
D2	0.17±0.01	0.25±0.01	93.15±0.00
D3	0.10±0.02	0	92.36±0.00
D4	0.07±0.00	0	87.82±0.00
D5	0.13±0.00	0.23±0.02	92.95±0.00
D6	0.08±0.00	0.02±0.00	92.64±0.00
D7	0.07±0.00	0.02±0.01	88.31±0.00
D8	0.15±0.00	0.29±0.01	92.91±0.00
D9	0.08±0.00	0.04±0.01	92.76±0.00

The values of total polyphenol content differ significantly between variants, those with 'Falstaff' petals (D2, D5 and D8, respectively) having almost double values compared to variants in the other two varieties. For all three varieties, the polyphenol content of rose petal jam is much lower than in fresh petals.

Variants of the 'Falstaff' variety have similar flavonoid values, significantly higher than those of the other variants, where the values are low or 0.

The values for antioxidant capacity are high in all variants. 'Crown Princess Margareta' jam has an average value of 88.24%, less than in fresh petals. The 'Falstaff' variety averages 93.00% similar to those of fresh petals and the 'Brother Cadfael' variety averages 92.59%, similar to those of fresh petals.

Total polyphenols and flavonoid content, antioxidant capacity of dry petals

In Table 3 are presented total content in polyphenols, flavonoids and antioxidant capacity in the dehydrated petals of the three varieties.

Table 3. Polyphenol, flavonoids and antioxidant capacity in the dehydrated petals

Variant	Polyphenol content (M/ml)	Flavonoid content (M/ml)	Antioxidant capacity (%)
'Crown Princess Margareta'	7.81±0.15	2.09±0.09	90.30
'Falstaff'	15.74±0.90	6.88±0.11	86.79
'Brother Cadfael'	8.91±0.07	2.41±0.11	90.20

Polyphenol content values differ significantly between variants, those of the 'Falstaff' variety having almost double values than those of the other two varieties. For all three varieties, the polyphenol content in petals is much higher than in fresh petals. Values of flavonoids in dehydrated petals also exceed the values found in fresh petals, the 'Falstaff' variety having more than double values than the other two varieties.

Antioxidant capacity values are high in all variants, for 'Crown Princess Margareta' and 'Brother Cadfael', similar to those in fresh petals. The 'Falstaff' variety has a smaller value than in fresh and jam.

CONCLUSIONS

Antioxidant capacity was similar in all the three variants. Total phenolic and flavonoid content varied according to the experimental variants, 'Falstaff' registering the highest amount. Dried petals registered similar biochemical parameters or higher than the fresh or processed ones, drying being recommended as an efficient preserving method.

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