

Antioxidant activity characterisation of rose petal agro-waste feedstuff

Francesco Vizzarri^{1*}, Aneta Kišová², Lubomir Ondruška¹, Ivana Speváková¹, Nikolay Kolev³, and Mihaela Ivanova⁴

¹National Agricultural and Food Centre, Department of Nutrition and Small farm animals, Hlohovecká 2, Lužianky, Slovakia

²Faculty of Agrobiolgy and Food Resources, Institute of Animal Husbandry, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, Nitra, Slovakia

³Department of Meat and Fish Technology, University of Food Technologies – Plovdiv, Plovdiv, Bulgaria

⁴Department of Milk Technology, University of Food Technologies – Plovdiv, Plovdiv, Bulgaria

Abstract. Agro-industrial residues are by-products rich in organic compounds and hold great potential for transformation into valuable resources. Rose petal agro-waste refers to the discarded floral material produced during commercial rose cultivation and its processing in the cosmetic, food, and herbal industries. The objective of this study was to evaluate the antioxidant activities of the rose petal dried waste for potential consideration as livestock feedstuff. Rose petal waste was obtained from a Bulgarian producer of aromatic natural products located in the Sofia-Oblast Region. All samples were dried, milled, and further processed for the characterization of the antioxidant activity. The spent *Rosa damascena* petal dried waste showed a considerable amount of total phenol (341.17 ± 7.20 mg GAE/g) and a remarkable antioxidant activity, in terms of FRAP (3911.55 ± 86.43 mM TE/g) and ORAC (4015 ± 77.34 mg TE/g) values. The in vitro activity also resulted very promising. All these findings together confirm that the rose petal dried waste can be considered as a potential candidate to produce natural functional feedstuff for livestock application.

1 Introduction

Agro-industrial residues, often left unmanaged, can pose significant risks to the environment, as well as to human and animal health. However, these by-products are rich in organic compounds and hold great potential for transformation into valuable resources. For example, agro-food waste materials contain varying levels of lignin, ash, moisture, carbon, nitrogen, cellulose, and hemicellulose components that make them suitable for applications such as biogas production, biofuels, animal feed, and other value-added products [1]. The increasing resistance of pathogens to antibiotics, coupled with concerns over antibiotic residues in animal products, has prompted researchers to seek alternative solutions that promote animal health, support growth, enhance production performance, and reduce emissions and environmental impact [2]. Rose petal agrowaste refers to the discarded floral material produced during commercial rose cultivation and its processing in the cosmetic, food, and herbal industries. Rather than being used solely as compost or fertilizer, this plant biomass can be repurposed as a feed additive [3], thanks to its rich content of antioxidants and other health-promoting phytochemicals. Phytochemical analysis of *Rosa damascena* Mill. identified phenolic acids and flavonoids as the predominant bioactive compounds, present in the highest concentrations. These were

followed by moderate levels of alkaloids and glycosides, while carbohydrates were detected in the lowest amounts among the identified phytochemical groups [4]. The objective of this study was to evaluate the antioxidant activities of the rose petal dried waste for potential consideration as livestock feedstuff.

2 Material and Method

Rose petal waste was obtained from a Bulgarian producer of aromatic natural products located in the Sofia-Oblast Region. All samples were dried in oven (Memmert UN Universal Oven, Germany) at 55°C till constant weight. Later, rose petal waste were milled using a 1.5 mm sieve and a water extract (100 mg/mL) was prepared and further processed for the characterization of the antioxidant activity.

2.1 Spectrophotometer determinations

Total polyphenols content (TPC) were determined according to the method of Singleton and Rossi [5] using the Folin-Ciocalteu's reagent. Gallic acid was employed as a calibration standard, and the results were expressed in mg Gallic acid equivalents (GAE) per g. The ferric (Fe³⁺) reduction ability of plasma -FRAP assay was performed following the method of Benzie

* Corresponding author: francesco.vizzarri@nppe.sk

and Strain [6]. Oxygen radical absorbance capacity (ORAC) activity was measured on a microplate reader FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany) with excitation at $\lambda = 485$ nm and emission at $\lambda = 520$ nm, according to the method of Ou et al. [7]. Trolox was used for building the standard curve and results were expressed in micromole Trolox equivalents ($\mu\text{mol TE}$) per gram.

2.2 *In vitro* antioxidant determinations

Blood samples were obtained from 6 healthy adult rabbits at the experimental rabbit unit of the Department of Nutrition and Small Farm Animals in Luzianky (Slovak Republic; Permission code: SK CH 17 021). Blood was collected from ear vein in ethylenediaminetetraacetic acid (EDTA)-treated tubes and centrifuged ($2300\times g$ at 4°C for 10 min). Plasma and buffy coat were removed, and erythrocytes were washed twice with PBS pH 7.4. Later, the antioxidant activity in Red Blood Cells (CAA-RBC) and Hemolysis Test were performed. The antioxidant activity of water extract of rose petal waste (100 mg/mL) was spectrophotometrically evaluated in an *in vitro* system with a modified assay in red blood cells as described by Frassinetti and colleagues [8]. Each value was expressed as CAA units, as follows:

$$\text{CAA unit} = 100 - \left(\frac{\int\text{SA}}{\int\text{CA}}\right) \times 100 \quad (2)$$

where $\int\text{SA}$ is the integrated area of the sample curve and $\int\text{CA}$ is the integrated area of the control curve.

Hemolysis of rose petal waste extract (100 mg/mL) was analyzed according to the protocol described by Frassinetti and colleagues [8] using AAPH, a generator of peroxy radicals, to cause the red blood cell lysis. The reported values are the percentage of hemolysis compared with the control. All analyses were performed in triplicate.

2.3 Statistical analysis

Descriptive statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) software (IMB, version 29.0, 2022) and analyzed through a one-way ANOVA and Tukey's test for post-hoc with significance at $p \leq 0.05$. Values are reported as means \pm standard deviation.

3 Result and Discussion

Results on the antioxidant characterization of the rose petal dried waste are reported in Table 1. The concentration of total phenolic content is similar to what reported in literature [9], indicating that the studied agro-waste contains high value of active compounds. Analysing the results regarding the FRAP and ORAC activities, the rose petal dried waste exhibited a strong iron-reducing potential and a very significant oxygen radical absorbance capacity, respectively. These results are in line with previous research studies evaluating the present agro-waste [9-10].

Table 1. Antioxidant profile of the rose petal waste

Item*	TPC (mg GAE/g DM)	FRAP (mM TE/g DM)	ORAC (mg TE/g DM)
Rose petal dried waste	341.17 \pm 7.20	3911.55 \pm 86.43	4015 \pm 77.34

*TPC = total phenol content; FRAP = ferric (Fe³⁺) reduction ability of plasma; ORAC = Oxygen radical absorbance capacity; GAE = gallic acid equivalent; TE = Trolox equivalent. All analysis were performed in triplicate.

In vitro antioxidant activity of pretreated erythrocytes with the water extract of rose petal dried waste (100mg/mL), showed a significantly higher cellular antioxidant activity (CAA unit = 58.49 \pm 1.26) compared with untreated cells (CAA = 0; $p \leq 0.001$), comparable to 100 μM Trolox (CAA unit = 15.92 \pm 1.02; $p \leq 0.001$) and 500 μM Trolox (CAA unit = 41.17 \pm 1.58; $p \leq 0.001$). Taking these results into consideration, the EC₅₀ of rose petal dried waste extract for antioxidant activity in red blood cells was 50 mg/mL. The antihemolytic activity of the studied extract was screened in erythrocytes exposed to high doses of AAPH, causing a strong oxidative hemolysis. Rose petal waste extract exerted a significant inhibition of AAPH-induced hemolysis compared with the control erythrocytes (AAPH-treated). Rose petal waste extract (100 mg/mL) pretreated cells demonstrated a marked antihemolytic effect (87% hemolysis inhibition) compared with AAPH-treated cells ($p \leq 0.001$), with a reduction of the hemolysis similar to that of the highest concentration of the reference standard (500 μM Trolox). To the best of our knowledge, no previous studies determined these parameters (CAA and hemolysis test) in rose petal dried waste, then further investigation is needed to confirm these results.

4 Conclusion

The spent *Rosa damascena* petal dried waste showed a considerable amount of total phenol and a remarkable antioxidant activity, in terms of FRAP and ORAC values. The *in vitro* activity also resulted very promising. All these findings together confirm that the rose petal dried waste can be considered as a potential candidate for the production of natural functional feedstuff for livestock application.

Acknowledgement

This research was funded by the Bulgarian National Science Fund for bilateral projects, grant number KP-06-Slovakia/7 from 13 August 2024 and APVV-SK-BG-23-0002 (grant 27A4-A-64039).

References

1. J. Madureira, L. Barros, S. Cabo Verde, F. Margaca, C. Santos Buelga, I.C.F.R. Ferreira, Ionizing radiation technologies to increase the extraction of bioactive compounds from agro-industrial residues: a

- review. *J. Agric. Food Chem.*, **68(40)**, 11054-11067 (2020)
2. S. Jiménez, N. Jiménez-Moreno, A. Luquin, M. Laguna, M.J. Rodríguez-Yoldi, C. Ancín-Azpilicueta, Chemical composition of rosehips from different *Rosa* species: An alternative source of antioxidants for the food industry. *Food Addit. Contam. Part A*, **34(7)**, 1121–1130 (2017)
3. F. Vizzarri, M. Polacikova, M. Ivanova, N. Kolev, L. Ondruska, I. Spevakova, A. Kisova, Valorization of phytonutrients in rose petal agro-waste feedstuff. In proceedings: XXVth Jubilee International Conference 10th – 12th September 2025 Siedliska, Poland
4. M. Szumacher-Strabel, A. Cieślak, P. Zmora, E. Pers-Kamczyc, P. Bielak, E. Stanisławska, The potential of the wild dogrose (*Rosa canina*) to mitigate *in vitro* rumen methane production. *J. Anim. Feed Sci.* **20(3)**, 285-299 (2011)
5. V. Singleton, J. Rossi, Colorimetry of total phenolic with phosphomolibdophosphotungstic acid reagents. *Am. J. Enol. Vitic.*, **16**, 144–158 (1965)
6. I.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem.*, **239(1)**, 70–76 (1996)
7. B. Ou, M. Hampsch-Woodill, R.L. Prior, Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescence probe. *J. Agric. Food Chem.*, **49**, 4619–4626 (2001)
8. S. Frassinetti, M. Gabriele, L. Caltavuturo, V. Longo, L. Pucci, Antimutagenic and antioxidant activity of a selected lectin-free common bean (*Phaseolus vulgaris* L.) in two cell-based models. *Plant Foods Hum. Nutr.*, **70**, 35–41 (2015)
9. N. Kolev, M. Ivanona, D. Vlahova-Vangelova, A. Balabanov, Y. Tumbariski, M. Dimitrova-Dicheva, F. Vizzarri, L. Ondruska, Bioactive potential of spent *Rosa damascena* Mill. petals extract: Antioxidant and antimicrobial properties, *Sci. Papers Ser. A*, **68(1)**, 792-799 (2025)
10. A. Slavov, P. Denev, I. Panchev, V. Shikov, N. Nenov, N. Yantcheva, I. Vasileva, Combined recovery of polysaccharides and polyphenols from *Rosa damascena* wastes. *Ind. Crops Prod.*, **100**, 85–94 (2017)