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Total phenolic content and antioxidant activity of *Pisum sativum* sprouts extract

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Abstract:
An aqueous extract of dried *Pisum sativum* sprouts (0.1g soluble substances/mL) has been obtained and analyzed. Total phenolic content of the extract was determined using the Folin-Ciocâlteu method and was found to be 138.98±3.16 μg of gallic acid equivalents/mL in the extract, corresponding to 416.9±9.52 mg gallic acid equivalents /100g DM of sprouts. The antioxidant activity of the extract was assayed using the DPPH method. It was expressed as IC_{50} value and was found to be 13.29 mg/mL.

Keywords: *Pisum sativum*, aqueous extract, total phenolic content, antioxidant activity

1. INTRODUCTION

Pisum sativum, (Fabaceae) or green pea is known for its high content of phenolic compounds, including flavonoids, isoflavonoids, phenolic acids, along with other minor phenolics and phytoalexins [1]. Total phenolic content (TPC) and the antioxidant activity (AOx) of *Pisum sativum* are increased by germination [2]. This work describes the following experimental work: (a) preparation of an aqueous extract of pea germs; (b) determination of TPC of the extract using the Folin-Ciocâlteu method; (c) determination of the antioxidant activity of the extract, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method.

2. MATERIALS AND METHODS

2.1. Materials

The pea sprouts were harvested on the fifth day of germination; only the germinated parts were used (the seed coat and cotyledons were not utilised for the experiment). They were dried in an oven at 40 °C for 12 hours, then at 60 °C for 26 hours (until constant mass was reached). The dried pea sprouts were crushed and mortared. 0.5g of dried spouts were extracted with distilled water heated to boiling (10 mL). The mixture was stirred for 10 minutes, then the extract was passed through a cheesecloth. The operation was repeated twice. The combined extracts were centrifuged at 12,000 rpm, at 10 °C, for 20 minutes. The obtained supernatants were filtered, concentrated in a rotary evaporator, and dried in an oven at 60 °C. The obtained concentrate is redissolved in distilled water, to obtain a solution with a concentration of 0.1g dry soluble substances/mL.

2.2. Analysis methods

TPC was determined using the Folin-Ciocâlteu method, with absorbance readings being realized at 765 nm [3-5]. The results were expressed as GAE (gallic acid equivalents), calculated with a calibration curve obtained with gallic acid standard solutions, in the concentration domain 25-400 μg/mL.

AOx activity was determined using the DPPH method [6-8]. Briefly, the 0.1 g/mL extract was diluted in the concentration range 2-20 mg/mL. After incubation with DPPH, A_{sample} were determined at 517 nm and I% was plotted vs. concentration, where I% was calculated as it follows: I%=(Acontrol-A sample)/A control x 100. The results were expressed as IC_{50} value.

Ascorbic acid was also assayed for comparison.

All determinations were realized in triplicate and the results are expressed as mean±standard deviation, calculated using Excel.

2.3. Apparatus

The spectrophotometric measurements were performed with a Varian Cary 50 UV-Vis spectrophotometer using plastic cuvettes, having 1 cm pathlength. A Nuve FNO32 oven was used for heating. A Sigma 2-16K refrigerated centrifuge was used for extract centrifugation. Reagents were weighted using an Adam PW124 Lab Balance.

3. RESULTS AND DISCUSSION

3.1. Results

TPC determination. The equation of the calibration curve for gallic acid was $y = 0.0041x + 0.0415$, with $R^2 = 0.9996$. For the water extract of dried green peas sprout, diluted 10 times, TPC was 138.98±3.16 μg GAE/mL. TPC was 416.9±9.52 mg GAE/100g dried green pea sprouts.

AOx activity determination. For the analysed water extract, by plotting I% versus extract concentration in the domain 2-20 mg/mL, a linear correlation has been obtained, having the equation: $y = 2.9227x + 11.13$, with $R^2 = 0.9888$. IC_{50} value for the water-soluble fraction of green pea sprouts was 13.29 mg/mL. For comparison, AOx was also determined for ascorbic acid and the IC_{50} value was 0.026 mg/ml.

3.2. Discussion

TPC (416.9±9.52 mg GAE/100g DM) of dried green pea sprouts determined in the fifth day of sprouting is similar to other literature data. Borges-Martinez *et al.* found that TPC for green pea sprouts is increasing during germination from 584 (day 0) to 850 (day 10) mg GAE/100g dry matter, with a value of 776.5 mg GAE/100g DM at day 5 [2]. However, the drying method (heat vs. lyophilization), solvent of extraction (water vs. methanol/HCl mixture), temperature of solvent evaporation and drying of the soluble extract (60 °C vs. 30 °C) play a role in the content of total phenols in various extracts, being known that TPC is higher if extraction is performed in alcohols and is less if heat is applied. The same factors play a role in the value of the AOx activity of the water-soluble fraction of the extract, that was very weak (the bigger the IC_{50} value, the smaller the AOx activity).

4. CONCLUSION

The biochemical modifications that appear during *Pisum sativum* germination are attributed to bioactive compounds, plants secondary metabolites, mainly to polyphenols, associated with an increase in AOx activity.

The TPC of dried green pea sprouts, after 5 days of germination, was 416.9±9.52 mg GAE /100g DM, with a weak antioxidant activity of the water-soluble fraction of substances. In future experiments, alcoholic extracts will be obtained and less heat will be applied during the processing of the extracts, in order to achieve higher TPC and AOx values.

REFERENCES

[1] J.R. Fahim, E.Z. Attia and M.S. Kamel, Phytochem Rev 18 (**2019**) 173.
[2] J. Borges-Martinez,T. Gallardo-Velazquez, A. Cardador-Martínez, D.D. Moguel-Concha, G. Osorio-Revilla, J.C. Ruíz-Ruíz and C.J. Martínez, Food Science and Technology, 42 (3) (**2021**) e45920.
[3] H. Noreen, N. Semmar, M.Farman, J. S.O. McCullagh, Asian Pac. J. Trop. Med., 10(8) (**2017**) 792.
[4] S. Kupina, C. Fields, M.C. Roman, S. L. Brunelle J AOAC Int. 101 (5) (**2018**) 1466.
[5] E. Ainsworth, K. Gillespie, Nat Protoc 2, (**2007**) 875.
[6] I.G. Munteanu and C. Apetrei, Int. J. Mol. Sci. 22(7) (**2021**) 3380.
[7] C.S. Tailor, A. Goyal, Am. J. Ethnomed. 1 (4) (**2014**) 244.
[8] R. Wołosiak, B. Drużyńska, D. Derewiaka, M. Piecyk,E. Majewska, M. Ciecierska, E. Worobiej, P. Pakosz, Molecules. 27(1) (**2022**) 50.