UNIVERSITÀ DEGLI STUDI DI SALERNO

Department of Industrial Engineering

Master's Degree in Food Engineering

Development and optimization of extraction conditions of Erucin from Eruca sativa

Thesis in

Transport Phenomena

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Abstract

Cruciferous vegetables, such as rocket salad, are rich of secondary metabolites like glucosinolates. Upon tissue damage resulting from plant injury or chewing, the endogenous plant enzyme myrosinase is released and catalyzes the hydrolysis of glucosinolates into isothiocyanates. Among these isothiocyanates, erucin has emerged as a natural compound of considerable interest in both nutraceutical and pharmaceutical industries due to its promising health-promoting properties, including its potential as a chemo-preventive agent and its demonstrated antioxidant and anti-inflammatory effects. Nutritional and health studies require gram quantities of isothiocyanates, and the utilization of these compounds is often limited because either they are not commercially available, or their cost is very high. Transforming surplus rocket salad leaves ant their by-products into nutraceuticals through enzymatic erucin extraction offers a sustainable and economically viable solution to both reduce waste and create health-promoting products, thereby enhancing the circularity of the rocket salad production chain. The present study develops a viable method for the extraction and quantification of erucin from rocket salad leaves, ensuring attainable concentration for subsequent analysis. Furthermore, the same extraction and quantification method is applied to rocket salad seeds. High-Performance Liquid Chromatography is employed as the analytical technique to detect and estimate the amount of erucin in the extracted samples. However, this analysis presents several challenges due to the low concentration of erucin in the plant material as well as the presence of other compounds that may interfere during the analysis. Overcoming these challenges is crucial to obtain accurate and reliable results. Eruca sativa seeds exhibited the highest erucin content at 272.2 micrograms per gram of dry weight, while the leaves contained erucin at levels of 18.1 and 19.1 micrograms per gram of dry weight (18.59 ± 0.71).

Chapter One

Introduction

The objective of this chapter is to present an overview of rocket salad and its active compounds, focusing the attention on glucosinolates from which erucin is obtained. The glucosinolate-myrosinase system, the extraction techniques, and the health-promoting activity of erucin are discussed. Finally, the aims of the thesis work are reported.

References

1. Possenti, M., et al., *Glucosinolates in food.* Glucosinolates. Ref. Ser. Phytochem, 2017: p. 87-132.
2. Hall, M., J. Jobling, and G. Rogers, *Some perspectives on rocket as a vegetable crop: A review.* Journal of Fruit and Ornamental Plant Research, 2012. **76**(1): p. 21-41.
3. Barazani, O. and J. Ziffer-Berger, *Eruca sativa, a tasty salad herb with health-promoting properties.* Medicinal and Aromatic Plants of the Middle-East, 2014: p. 269-279.
4. Esiyok, D., S. Padulosi, and D. Pignone, *Rocket: A Mediterranean Crop for the World.* 1997.
5. Alruwaih, N., *Chemical Profiling and Comparative Evaluation of Bioactive Compounds in Lyophilized and Tray-dried Rocket (Eruca sativa)*. 2016: McGill University (Canada).
6. Pagnotta, E., et al., *Bioactive Compounds from Eruca sativa Seeds.* Encyclopedia, 2022. **2**(4): p. 1866-1879.
7. *Eruca sativa Mill. | Plants of the World Online*. Available from: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:1110374-2#source-KBD.
8. Cavaiuolo, M. and A. Ferrante, *Nitrates and glucosinolates as strong determinants of the nutritional quality in rocket leafy salads.* Nutrients, 2014. **6**(4): p. 1519-1538.
9. Arora, R., S. Bhushan, and S. Arora, *Changing Trends in the Methodologies of Extraction and Analysis of Hydrolytic Products of Glucosinolates: A Review.* Glucosinolates, 2017: p. 383-405.
10. De Nicola, G.R., *Isolation and Modification of Plant Glucosinolates and their Role in the Prevention of Pathologies of the Central Nervous System.* 2018.
11. Falk, K.L., et al., *Glucosinolate biosynthesis: demonstration and characterization of the condensing enzyme of the chain*

elongation cycle in Eruca sativa. Phytochemistry, 2004. **65**(8): p. 1073-1084.

12. Marchioni, I., et al., *Small functional foods: Comparative phytochemical and nutritional analyses of five microgreens of the Brassicaceae family.* Foods, 2021. **10**(2): p. 427.
13. Lee, M.C., *The Development, Sensory Evaluation and Interconversion of Bioactive Isothiocyanates in a Tomato-Soy-Arugula Seed Beverage*. 2015, The Ohio State University.
14. You, Y., et al., *Screening of Chinese brassica species for anti-cancer sulforaphane and erucin.* African Journal of Biotechnology, 2008. **7**(2).
15. Omirou, M., et al., *Microwave-assisted extraction of glucosinolates from Eruca sativa seeds and soil: comparison with existing methods.* Phytochemical analysis, 2009. **20**(3): p. 214-220.
16. ELSadek, M., *Chemical constituents of Eruca sativa and treatment activity against paracetamol inducing hepatic injury in experimental rats.* Egyptian Journal of Nutrition and Health, 2014. **9**(1): p. 1-12.
17. Singh, S., et al., *Development and optimization of nanoparticles loaded with erucin, a dietary isothiocyanate isolated from Eruca sativa: Antioxidant and antiproliferative activities in ehrlich-ascites carcinoma cell line.* Frontiers in Pharmacology, 2023. **13**: p. 1080977.
18. Franco, P., et al., *Development of a liquid chromatography–electrospray ionization–tandem mass spectrometry method for the simultaneous analysis of intact glucosinolates and isothiocyanates in Brassicaceae seeds and functional foods.* Journal of Chromatography a, 2016. **1428**: p. 154-161.
19. Fahey, J.W., A.T. Zalcmann, and P. Talalay, *The chemical diversity and distribution of glucosinolates and isothiocyanates among plants.* Phytochemistry, 2001. **56**(1): p. 5-51.
20. Abdel-Massih, R.M., et al., *Glucosinolates, a natural chemical arsenal: More to tell than the myrosinase story.* Frontiers in Microbiology, 2023. **14**: p. 1130208.
21. Burmeister, W.P., et al., *The crystal structures of Sinapis alba myrosinase and a covalent glycosyl–enzyme intermediate provide insights into the substrate recognition and active-site machinery of an S-glycosidase.* Structure, 1997. **5**(5): p. 663-676.

22. Nugrahedi, P.Y., M. Dekker, and R. Verkerk, *Processing and Preparation of Brassica Vegetables and the Fate of Glucosinolates 14.* Glucosinolates, 2017. **4**: p. 407.
23. Yehuda, H., et al., *Isothiocyanates inhibit psoriasis-related proinflammatory factors in human skin.* Inflammation Research, 2012. **61**: p. 735-742.
24. Bell, L. and C. Wagstaff, *Glucosinolates, myrosinase hydrolysis products, and flavonols found in rocket (Eruca sativa and Diplotaxis tenuifolia).* Journal of agricultural and food chemistry, 2014. **62**(20): p. 4481-4492.
25. Jasper, J., C. Wagstaff, and L. Bell, *Growth temperature influences postharvest glucosinolate concentrations and hydrolysis product formation in first and second cuts of rocket salad.* Postharvest Biology and Technology, 2020. **163**: p. 111157.
26. Melchini, A. and M.H. Traka, *Biological profile of erucin: a new promising anticancer agent from cruciferous vegetables.* Toxins, 2010. **2**(4): p. 593-612.
27. YADAV, K. and J. DHANKHAR, *Isothiocyanates-A Review of their Health Benefits and Potential Food Applications.* Current Research in Nutrition & Food Science, 2022. **10**(2).
28. Martelli, A., et al., *Erucin exhibits vasorelaxing effects and antihypertensive activity by H2S-releasing properties.* British Journal of Pharmacology, 2020. **177**(4): p. 824-835.
29. Melchini, A., et al., *Erucin, a new promising cancer chemopreventive agent from rocket salads, shows anti-proliferative activity on human lung carcinoma A549 cells.* Food and Chemical Toxicology, 2009. **47**(7): p. 1430-1436.
30. Citi, V., et al., *Anticancer properties of erucin, an H2S-releasing isothiocyanate, on human pancreatic adenocarcinoma cells (AsPC-1).* Phytotherapy Research, 2019. **33**(3): p. 845-855.
31. Wang, Q. and Y. Bao, *Nanodelivery of natural isothiocyanates as a cancer therapeutic.* Free Radical Biology and Medicine, 2021. **167**: p. 125-140.
32. Lamy, E., et al., *Antigenotoxic properties of Eruca sativa (rocket plant), erucin and erysolin in human hepatoma (HepG2) cells towards benzo (a) pyrene and their mode of action.* Food and chemical toxicology, 2008. **46**(7): p. 2415-2421.

33. Kaur, P., et al., *Neuromodulatory effect of 4-(methylthio) butyl isothiocyanate against 3-nitropropionic acid induced oxidative impairments in human dopaminergic SH-SY5Y cells via BDNF/CREB/TrkB pathway.* Scientific Reports, 2023. **13**(1): p. 4461.
34. Martelli, A., et al., *The H2S-donor erucin exhibits protective effects against vascular inflammation in human endothelial and smooth muscle cells.* Antioxidants, 2021. **10**(6): p. 961.
35. Karanikolopoulou, S., et al., *Current Methods for the Extraction and Analysis of Isothiocyanates and Indoles in Cruciferous Vegetables.* Analytica, 2021. **2**(4): p. 93-120.
36. Pilipczuk, T., et al., *Simultaneous determination of individual isothiocyanates in plant samples by HPLC-DAD-MS following SPE and derivatization with N-acetyl-l-cysteine.* Food chemistry, 2017. **214**: p. 587-596.
37. Coskun, O., *Separation techniques: chromatography.* Northern clinics of Istanbul, 2016. **3**(2): p. 156.
38. Ziegler, W., *Extraction of grape pomace polyphenols and validation by means of antioxidant content and activity*. 2013, Graz.
39. *Basic Guide on How to Use the HPLC*. Available from: https://www.youtube.com/watch?v=9ZqL4MOWAi8.
40. Sahu, P.K., et al., *An overview of experimental designs in HPLC method development and validation.* Journal of pharmaceutical and biomedical analysis, 2018. **147**: p. 590-611.
41. Arora, R., et al., *Evaluating extraction conditions of glucosinolate hydrolytic products from seeds of Eruca sativa (Mill.) Thell. using GC-MS.* Journal of food science, 2014. **79**(10): p. C1964-C1969.
42. Bennett, R.N., et al., *Identification and quantification of glucosinolates in sprouts derived from seeds of wild Eruca sativa L.(salad rocket) and Diplotaxis tenuifolia L.(wild rocket) from diverse geographical locations.* Journal of agricultural and food chemistry, 2007. **55**(1): p. 67-74.
43. Ku, K.-M., et al., *Profiles of glucosinolates, their hydrolysis products, and quinone reductase inducing activity from 39 arugula (Eruca Sativa Mill.) accessions.* Journal of agricultural and food chemistry, 2016. **64**(34): p. 6524-6532.
44. Crescenzi, M.A., et al., *Metabolite Profiling for Typization of “Rucola Della Piana del Sele”(PGI), Eruca sativa, through*

UHPLC-Q-Exactive-Orbitrap-MS/MS Analysis. Foods, 2023. **12**(18): p. 3384.

45. Villatoro-Pulido, M., et al., *An approach to the phytochemical profiling of rocket [Eruca sativa (Mill.) Thell].* Journal of the Science of Food and Agriculture, 2013. **93**(15): p. 3809-3819.
46. Bell, L., et al., *Changes in rocket salad phytochemicals within the commercial supply chain: Glucosinolates, isothiocyanates, amino acids and bacterial load increase significantly after processing.* Food chemistry, 2017. **221**: p. 521-534.
47. Lv, C., et al., *Simultaneous hydrolysis and extraction increased erucin yield from broccoli seeds.* ACS omega, 2021. **6**(9): p. 6385-6392.
48. Fusari, C.M., et al., *Phytochemical profile and functionality of Brassicaceae species.* Food Bioscience, 2020. **36**: p. 100606.
49. Cuong, D.M., et al., *Evaluation of Phytochemical Content and the Antioxidant and Antiproliferative Potentials of Leaf Layers of Cabbage Subjected to Hot Air and Freeze-Drying.* Journal of Food Quality, 2022.
50. Khoobchandani, M., et al., *Antimicrobial properties and analytical profile of traditional Eruca sativa seed oil: Comparison with various aerial and root plant extracts.* Food Chemistry, 2010. **120**(1): p. 217-224.
51. Fusari, C.M., D.A. Ramirez, and A.B. Camargo, *Simplified analytical methodology for glucosinolate hydrolysis products: a miniaturized extraction technique and multivariate optimization.* Analytical Methods, 2019. **11**(3): p. 309-316.
52. Vaughn, S.F., et al., *Herbicidal activity of glucosinolate-containing seedmeals.* Weed Science, 2006. **54**(4): p. 743-748.