Evaluation of bioactive compounds in arugula (Eruca sativa) after lyophilization and tray-drying

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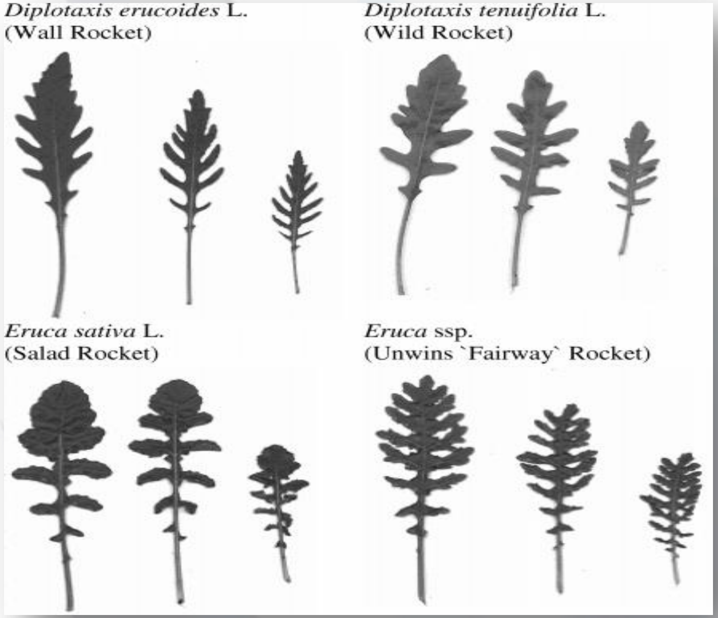
Introduction

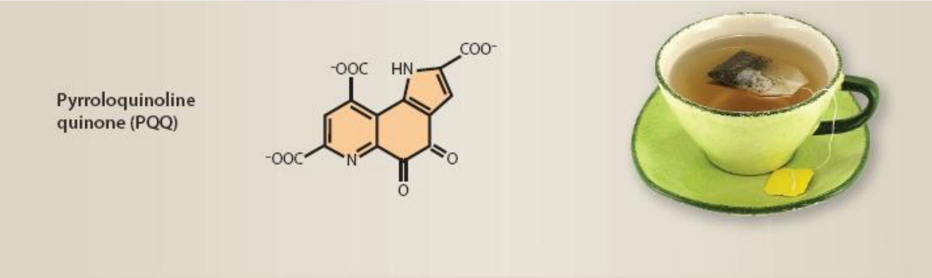
- Family of Brassicaceae
- Origin: Mediterranean region
- Characterized by peppery taste and aroma
- High in vitamins A,C, and K
- Folate, iron, and calcium

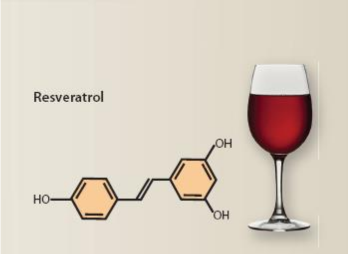


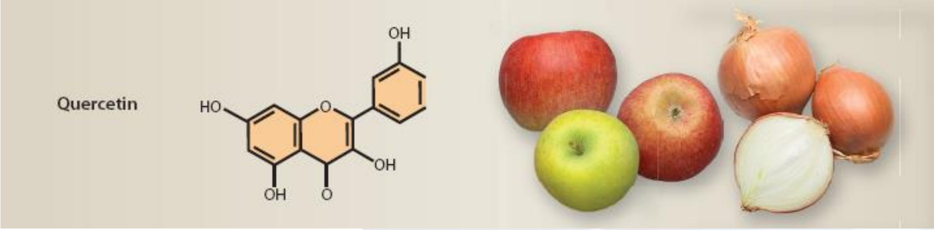
Bennett *et al.*, 2006

Phenolic compounds

- Secondary plant metabolites
- Defense against injury
- Largest subgroup
  - *Flavonoids*







Randhir *et al.*, 2004

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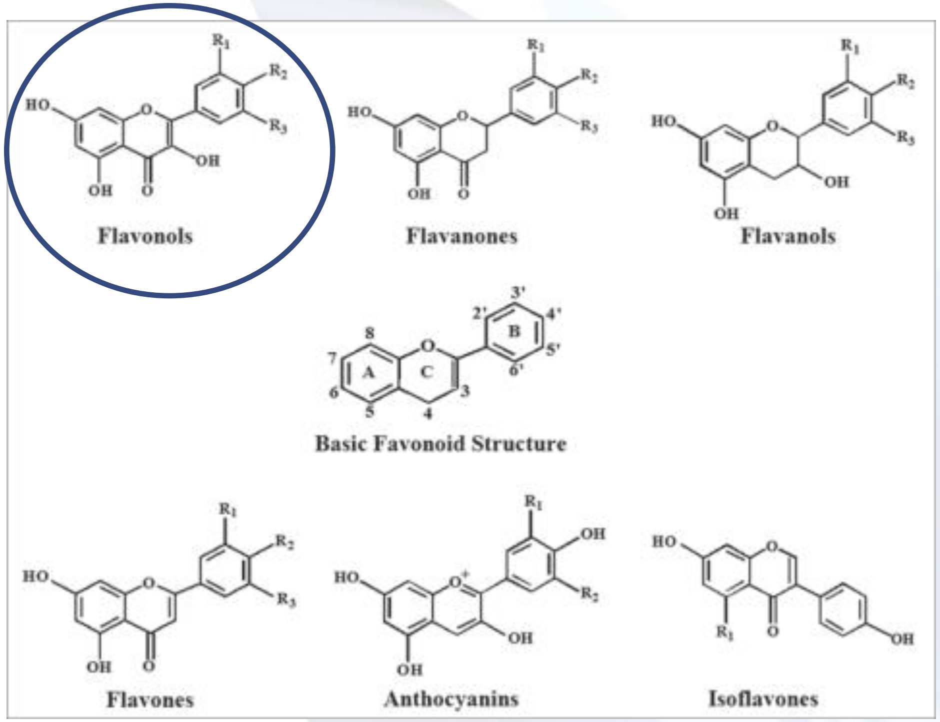
  - *Flavonoids*
    - *Flavonols*



Randhir *et al.*, 2004

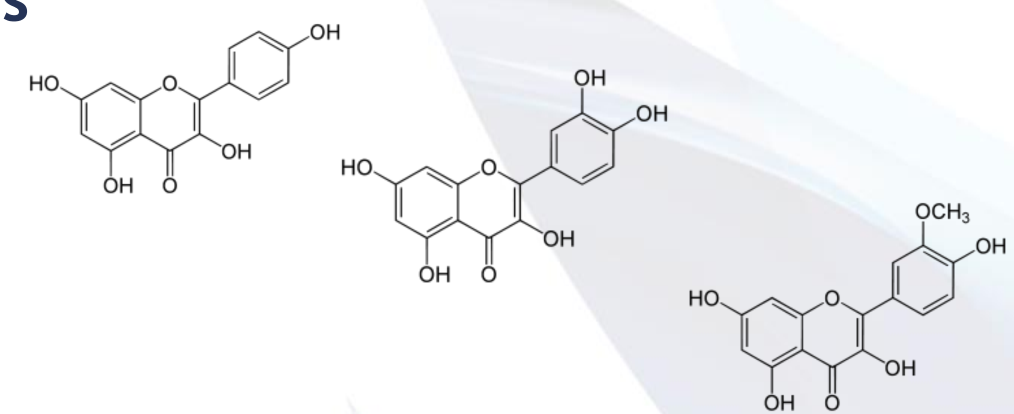
Flavonoids

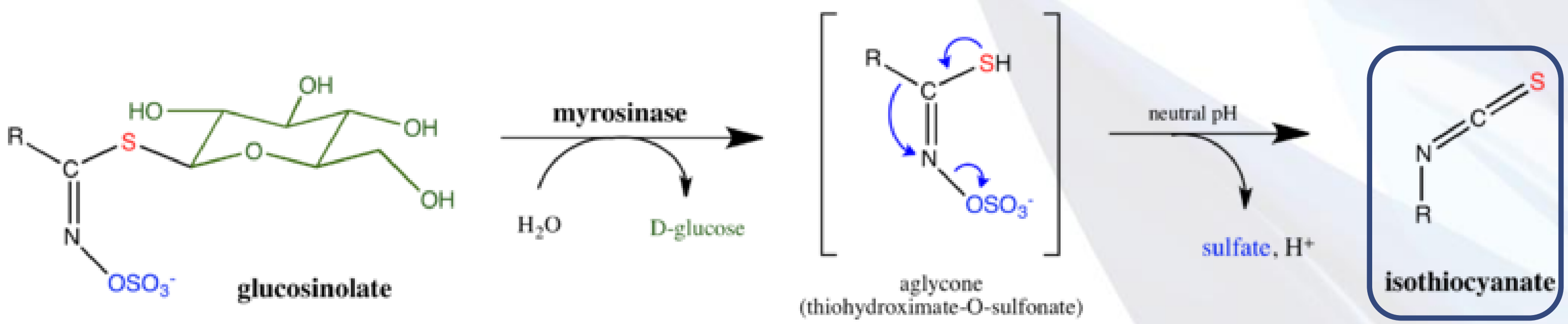
- Organoleptic properties
- Antioxidant properties
- Color differentiation
- Anti-allergenic, anti-inflammatory, anti-microbial

Dai & Mumper, 2010

Bioactive compounds in arugula

- **Glycosylated flavonoids**
  - ***Kaempferol***
  - *Quercetin*
  - *Isorhamnetin*
- **Glucosinolates**
  - *Isothiocyanates*

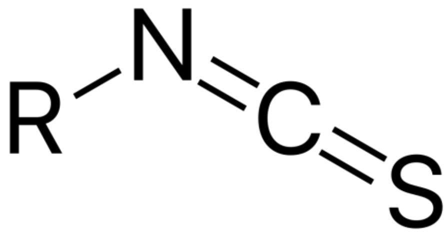




Garg & Sharma, 2014

Isothiocyanates in arugula

- Antibacterial and antifungal properties
- Exert potential anti-carcinogenic benefits
- Interaction of polyphenols, vitamin C and carotenoids lead to strong antigenotoxicity



Bell & Wagstaff, 2014

Rationale

- Arugula is consumed regularly in Kuwait since it is grown in abundant amounts
- Significant quantities are wasted yearly because of the lack of processing and storage facilities
- Raw leafy salad species deliver bioactive compounds

- Investigate effect of pre-processing on the content of bioactive compounds in *arugula leaves*

Overall objective

To compare the bioactive compounds and chemical profiles of lyophilized and tray-dried arugula leaves

Sample preparation: Lyophilization

Arugula cultivated in Kuwait (Wafra farms) harvested after 26 days

Washed, sorted, bunched

Blanching (85°C for 3 min)

Freeze-dried *(GAMMA 2-16 LSC; Martin Christ GmbH, Osterode am Harz, Germany)* for 38 h (-50 °C, 0.7 Pa)

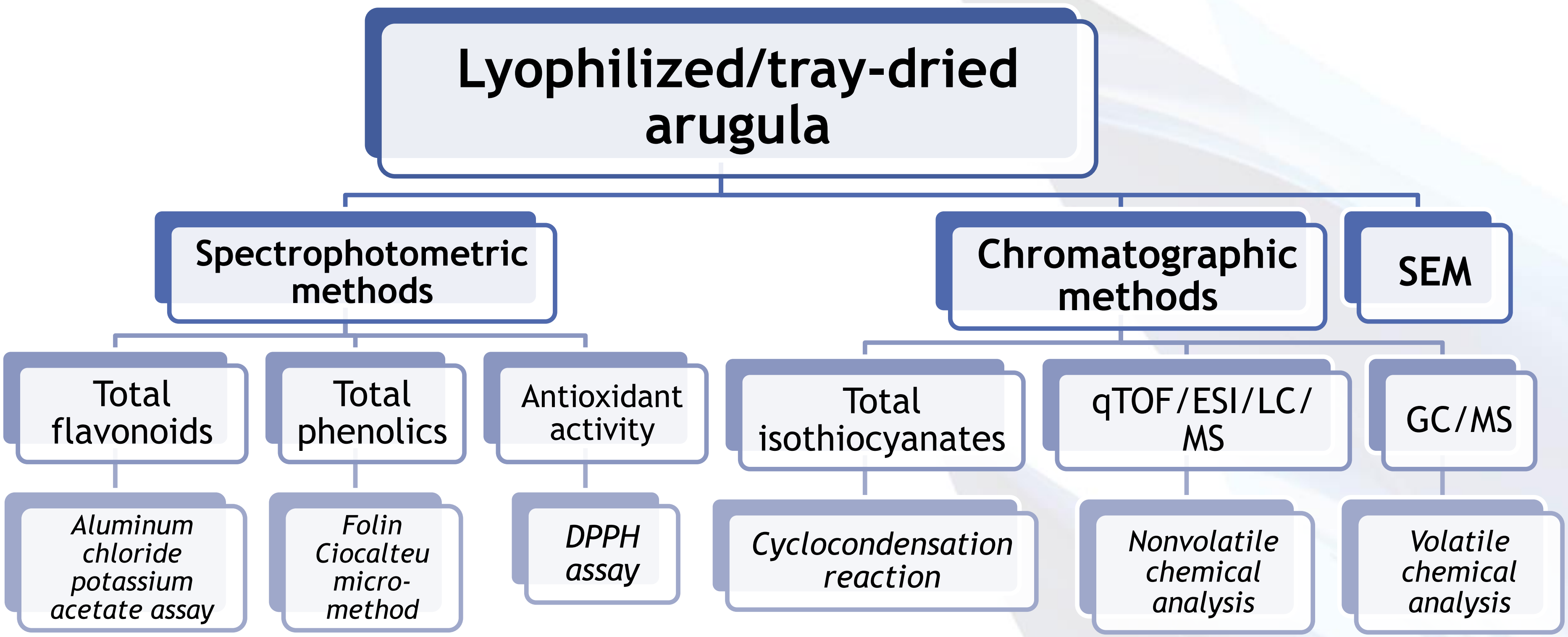
Ground into a powder and stored at 4°C

Sample preparation: Tray drying

Arugula (2 kg) dried uniformly on stainless steel perforated trays in a cabinet dryer at 55°C for 6-7 h.

Ground and stored in air-tight containers at 4°C

Materials and methods



Objectives

To investigate:

- Total flavonoids and phenolic compounds
- Antioxidant activity using DPPH assay
- Total isothiocyanate content
- GC/MS analysis for volatile isothiocyanates
- ESI/qTOF/LC/MS analysis for nonvolatile compounds
- Overall microstructure by Scanning Electron Microscope (SEM)

Results: total flavonoid and phenolic content

Drying method	TFC (g QE/100 g) [a]	Literature (g/100g)	Ref.	TPC (g QE/100g) [a]	Literature (g GAE/100g)	Ref.
Lyophilized arugula	3.29 ± 0.15a	2.35	Pasini et al., (2012)	8.67 ± 0.6a	2.08 (FW)	Heimler et al., (2007)
Tray-dried arugula	2.42 ± 0.22b			8.5 ± 0.8a		

*Values of TFC and TPC of leaves are means ± SD (n=3) measured in dry weight. For each column values followed by the same letter (a-b) are not statistically different at $P < 0.01$.

[a] Values of TFC and TPC are measured as quercetin equivalence.

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Results: antioxidant activity

Drying method	AOA (%DW)[c]	Literature (%FW)	Ref.
Lyophilized arugula	28.01 ± 0.6a	60.8	Heimler et al. (2007)
Tray-dried arugula	27.96 ± 1.0a		Martínez-Sánchez et al. (2005)

*Values of AOA of leaves are means ± SD (n=3) measured in dry weight. For each column values followed by the same letter (a-b) are not statistically different at *P < 0.01.*
[b] Antioxidant activity is measured as average scavenging activity of DPPH.

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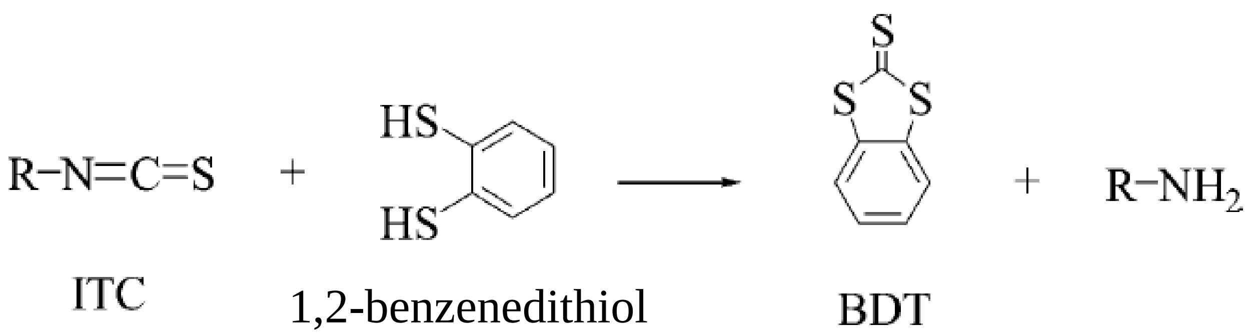
Cyclocondensation reaction



Cyclocondensation reaction of isothiocyanates with 1,2-benzenedithiol

Results: total isothiocyanate content

Drying method	TIC (µg /g)	Literature (µg /g)	Ref.
Lyophilized arugula	3.26 ± 0.59a	8.84 (erucin)	Melchini et al., (2009)
Tray-dried arugula	6.05 ± 0.83a		

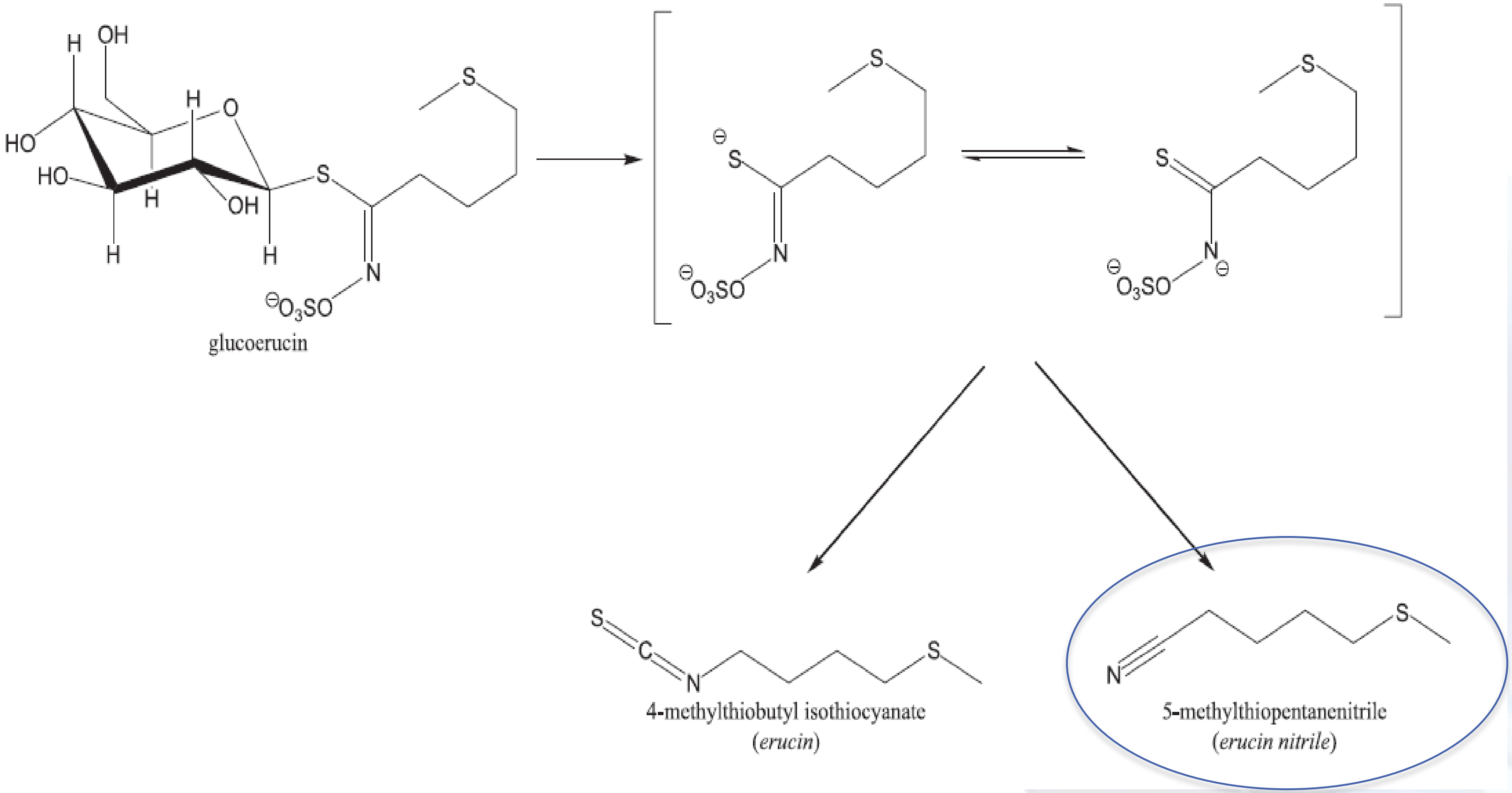
*Values of TIC of leaves are means ± SD (n=3) measured in dry weight. For each column values followed by the same letter (a-b) are not statistically different at *P < 0.01.*

Proposed structure of the volatile isothiocyanate observed in the tray-dried *E.sativa* methanolic extract using GC/MS

RT (min)	Compound	Elemental composition
11.621	5-methylthiopentanenitrile	$C_6H_{11}NS$

- Air-drying of the rocket plant contributes to the degradation of glucoerucin
- No isothiocyanates observed in freeze-dried E.sativa methanolic extract using GC/MS

Volatiles formed by glucoerucin degradation



Blaževic & Mastelic, 2008

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ESI/qTOF/LC/MS – lyophilized vs. tray-dried arugula

Elemental composition	$[M - H]^-$ (m/z)	Chemical name	Error (ppm)
Lyophilized arugula			
$C_{27}H_{29}O_{16}$	609.1382	**Kaempferol 3,4'-diglucoside** ($C_{27}H_{30}O_{16}$)	12.082
$C_{21}H_{19}O_{12}$	463.0817	**Quercetin-3-glucoside** ($C_{21}H_{20}O_{12}$)	12.851
Tray-dried arugula			
$C_{15}H_9O_6$	285.0405	**Kaempferol** ($C_{15}H_{10}O_6$)	2.059
$C_{16}H_{11}O_7$	315.0510	**Isorhamnetin** ($C_{16}H_{12}O_7$)	-2.0
$C_{12}H_{22}NO_9S_3$	420.0460	**Glucoerucin** ($C_{12}H_{23}NO_9S_3$)	0.789

Proposed elemental composition of targeted masses $[M - H]^-$ (m/z) of nonvolatile compounds identified by ESI/qTOF/LC/MS (negative ion mode) of lyophilized and tray-dried arugula.

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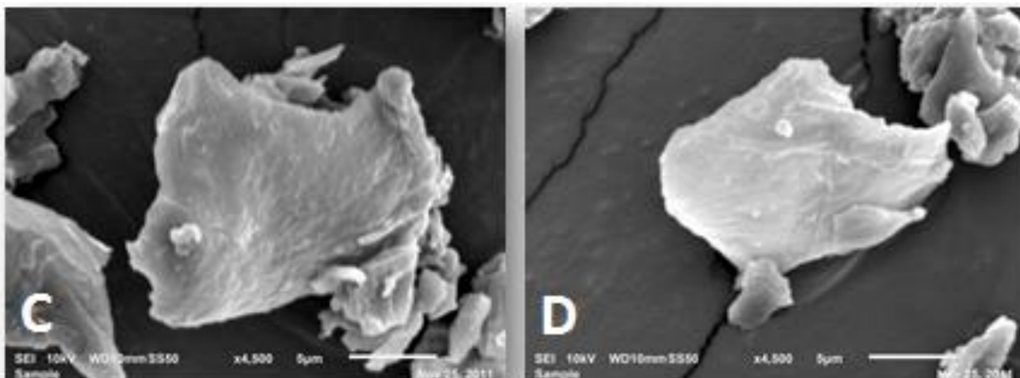
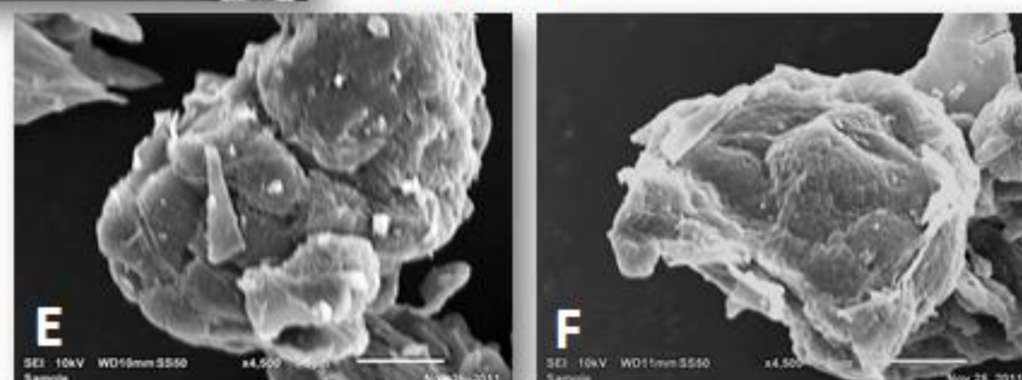
Scanning electron micrographs of lyophilized and tray-dried arugula



(C) and (D) tray-dried rocket at 4,500x 51 to 8 µm

(E) and (F) lyophilized rocket at 4,500x 117 and 10.5 µm



Conclusions

Tray-dried arugula:

- flavonoids mostly present as aglycones
- extract displayed glucosinolate degradation product
- higher isothiocyanate content

Lyophilized arugula:

- flavonoid content as glycosides
- no significant loss of particle integrity
- better retain bioactive compounds in fresh rocket

Further studies

- Further structural identification of compounds using MS/MS
- Comparison between fresh and dried rocket using different drying methods
- Compare isothiocyanate composition of fresh and heat-treated rocket
- Use of dried rocket powder in foods and fate of bioactive compounds

References

Arabbi, P. R., Genovese, M. I. S., & Lajolo, F. M. (2004). Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. *J. Agric. Food. Chem.*, **52**, 1124-1131.

Bell, L., Spadafora, N. D., Müller, C. T., Wagstaff, C., & Rogers, H. J. (2016). Use of TD-GC–TOF-MS to assess volatile composition during post-harvest storage in seven accessions of rocket salad (Eruca sativa). *Food Chem.*, **194**, 626-636.

Bell, L., & Wagstaff, C. (2014). Glucosinolates, myrosinase hydrolysis products, and flavonols found in rocket (Eruca sativa and Diplotaxis tenuifolia). *J. Agric. Food. Chem.*, **62**, 4481-4492.

Bennett, R. N., Carvalho, R., Mellon, F. A., Eagles, J., & Rosa, E. A. S. (2007). 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. *J. Agric. Food. Chem.*, **55**, 67-74.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., & Mérillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC Assays. *J. Agric. Food. Chem.*, **57**, 1768-1774.

Garg, G., & Sharma, V. (2014). Eruca sativa (L.): Botanical description, crop improvement, and medicinal properties. *J. Herbs Spices Med. Plants*, **20**, 171-182.

Heimler, D., Isolani, L., Vignolini, P., Tombelli, S., & Romani, A. (2007). Polyphenol content and antioxidative activity in some species of freshly consumed salads. *J. Agric. Food. Chem.*, **55**, 1724-1729.

Kim, S.-J., & Ishii, G. (2006). Glucosinolate profiles in the seeds, leaves and roots of rocket salad (Eruca sativa Mill.) and anti-oxidative activities of intact plant powder and purified 4-methoxyglucobrassicin. *Soil Sci. & Plant Nutr.*, **52**, 394-400.

Pasini, F., Verardo, V., Caboni, M. F., & D'Antuono, L. F. (2012). Determination of glucosinolates and phenolic compounds in rocket salad by HPLC-DAD–MS: Evaluation of Eruca sativa Mill. and Diplotaxis tenuifolia L. genetic resources. *Food Chem.*, **133**, 1025-1033.

Thank you

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