



9th International Food data Conference, Norwich, UK, September 14-16, 2011

Variability of glucosinolates and phenolics in local kale populations from Turkey, Italy and Portugal

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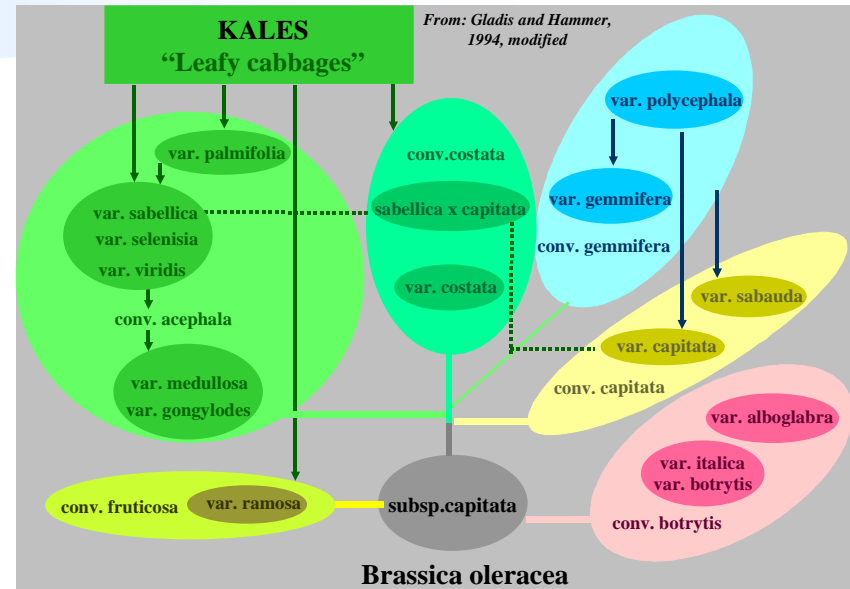
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Kales, collards

- Non-heading forms of *Brassica oleracea* L.
- Plants not forming any head: nor vegetative, like cabbage or reproductive, like cauliflower or broccoli.
- Taxonomy: still under review
- They are considered primitive types of *B. oleracea*, the closest to wild types.
- The leaves are used, at different developmental stages.
- In Europe, substantially neglected by breeding: no modern varieties do exist.



Local types, Turkey



Old and young crops, Portugal



Commercial crop, Italy



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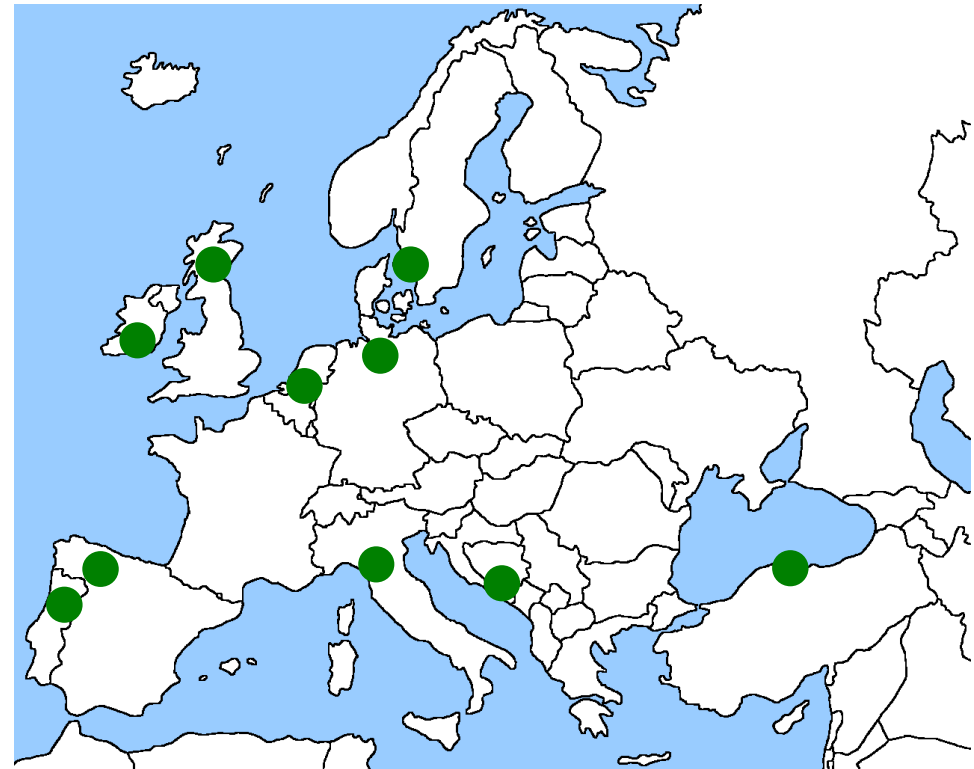
Local kale populations are still cultivated in several European areas

- Also popular in southern United States, India, East Africa
- A cool season vegetable, among the few able to grow during winter



Local types, under snow, Portugal

Local types, in winter, Italy



- Also used as a famine or war time crop (Dig for Victory campaign)

Sean Poulter

World War Two vegetable comes back as 'superfood'

Mail on line, 3 October 2007



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Kales represent basic ingredients of local traditional dishes



rumbledethumps



boerenkool stamppot



grøn lankgål



kailkenny



gruenkhol und pinkel



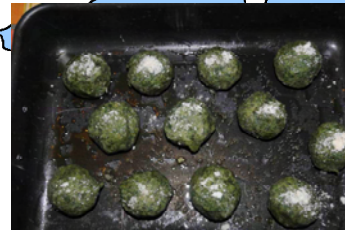
colcannon



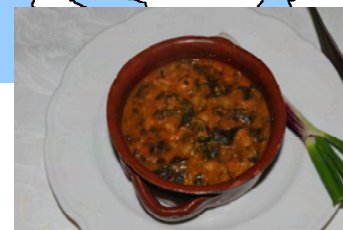
kara lahana chorbasi



caldo verde



prebugiun



ribollita toscana



kara lahana dolma





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Putative health-promoting compounds of kales

Glucosinolates

- Kales represent the more relevant potential dietary source of the glucobrassicin / indole-3- carbinol system

glucobrassicin (% total glucosinolates) of kale populations

north Spain ¹	Turkey ²	Italy ³
19.3-36.9	78.9-84.1	47.7-90.7

- Indole-3-carbinol is being investigated as a protective agent against cancer development and other immune and hormone-related diseases ⁴

1. Velasco P., Cartea M.E., Gonzales C., Vilar M., Ordas A., 2007. Factors affecting the glucosinolate content of kale (*Brassica oleracea acephala* group). *J. Agric. Food Chem.*, 55, 955-962

2. Sarıkamış G., Balkaya A., Yanmaz R., 2008. Glucosinolates in kale genotypes from the Black sea region of Turkey. *Biotechn. & Biotechn. Equip.*, 22, 942-946

3. Baldacci G., D'Antuono L.F., Elementi S., Neri R., 2007. Componenti nutrizionali del cavolo nero di Toscana (*Brassica oleracea* L., ssp. *acephala* DC., var. *sabellica* L.). Graduation thesis, Food Science and Technology, University of Bologna

4. Higdon J., Drake V.J., Williams D.E., 2008. Indole-3-carbinol. Linus Pauling Institute. Oregon State University. <http://lpi.oregonstate.edu/infocenter/phytochemicals/i3c/>



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Putative health-promoting compounds of kales

Phenolics

- Phenolic components presently represent the main focus as dietary antioxidant agents
- Kales have been characterised for their phenolic content and profile ¹⁻⁵
- They proved to be comparatively rich sources of these components, and associated antioxidant capacity, with respect to other vegetables ⁴⁻⁵

Carotenoids

- Kales have been recognised as the richest sources of chlorophyll-associated carotenoids (β -carotene and lutein) among leafy vegetables. ⁵⁻⁶

1. Cartea M.E., Francisco M., Soengas P., Velasco P., 2011. Phenolic compounds in *Brassica* vegetables. *Molecules*, 16, 251-280.
2. Lin L.Z., Harnly J.M., 2009. Identification of the phenolic components of collard greens, kale, and chinese broccoli. *J. Agric. Food Chem.*, 57, 7401-7408.
3. Olsen H., Aaby K., Borge G.I.A., 2009. Characterisation and quantification of flavonoids and hydroxyconnamic acids in curly kale (*Brassica oleracea* L. convar. *acephala* var. *sabellica*) by HPLC-DAD-ESI-MS.
4. Ayaz F.A., Hayırlıoğlu-Ayaz S., Aplay-Karaoğlu S., Grúz J., Vanentová K., Ulrichová J., Strnad M., 2008. Phenolic acid contents of kale *Brassica oleracea* L. var. *acephala* DC.) extracts and their antioxidant and antibacterial activities. *Food chemistry*, 107, 19-25.
5. Nilsson J., Olsson K., Engqvist G., Ekvall J., Olsson M., Nyman M., Akesson B., 2006. Variation in the content of glucosinolates, hydroxycinnamic acids, carotenoids, total antioxidant capacity and low-molecular weight carbohydrates in *Brassica* vegetables. *J. Sci. Food Agric.*, 86, 528-538.
6. Lefsrud M., Kopsell D., Wenzel A., Sheehan J., 2007. Changes in kale (*Brassica oleracea* L. var. *acaphala*) carotenoids and chlorophyll pigment concentrations during leaf ontogeny. *Sci. Hortic.*, 112, 136-141.



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Kales in BaSeFood

Kales have been targeted as one of the investigation topics of BaSeFood (Sustainable exploitation of bioactive components from the Black Sea Area traditional foods), because of the following characters:

- they are local crops, widely used as food plants, highly appreciated in the native areas
- they are potential sources of health promoting substances and functional foods

A targeted research has been started in three BaSeFood participant countries: Turkey, Italy and Portugal, aimed at:

- comparing local populations and uses, in a cross country, cross-cultural perspective
- comparing phytochemical content of some populations
- determining bioactive retention in some traditional food preparation schemes

The comparison of glucosinolate and phenolic content of local populations from native areas, or grown in a common environment, is the subject of this contribution



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Materials and methods

Local populations

- 25 samples taken from local crops or markets, winter 2010
- Turkey: 7 (*kara lahana - black cabbage*)
- Portugal: 9 (*couve galega - Galician kale*)
- Italy: 9 (*cavolo nero - black cabbage*)

- mature leaves random sampled, in triplicate
- refrigerated and transported to the lab
- frozen, freeze dried and kept at $-20\text{ }^{\circ}\text{C}$ until extra ction





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Materials and methods

Experimental trial

- 15 seeds samples obtained from local sources (local seeds companies, farmers)
 - Turkey: 6
 - Portugal: 2
 - Italy: 7
- seedlings produced in nursery, sown August 8, 2010
- transplant in the field, Martorano 5 experimental farm, September 2, 2010
- 3 replication, randomised block
- mature leaves harvested December 9, 2010.
- frozen, freeze dried and kept at $-20\text{ }^{\circ}\text{C}$ until extra ction





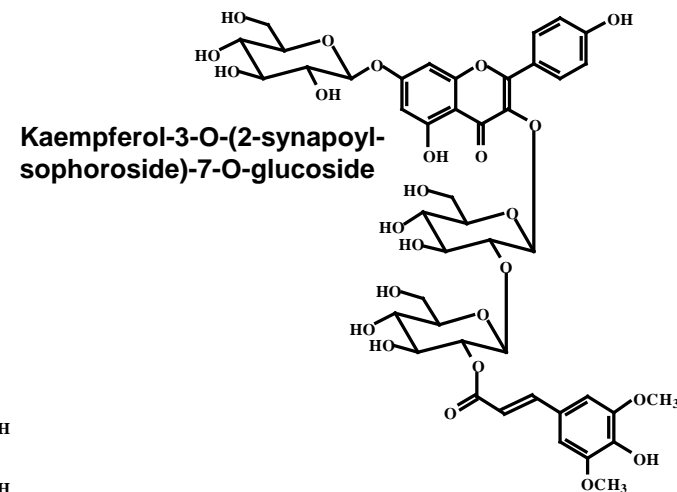
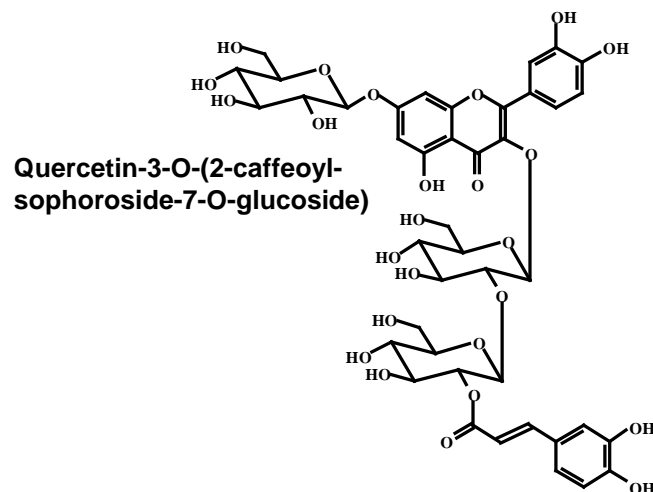
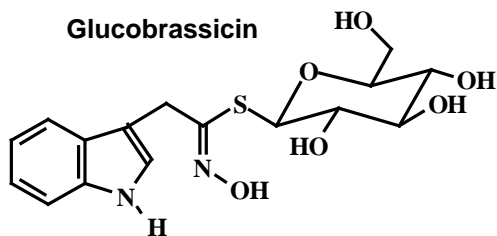
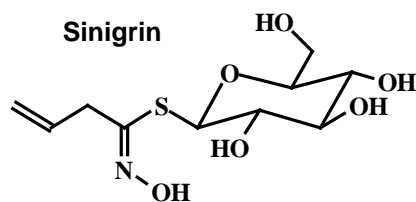
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Materials and methods

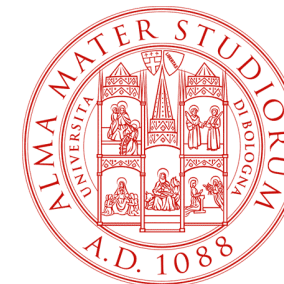
Analytical determination of glucosinolates (GLS) and phenols

- inactivation of endogenous myrosinase in freeze-dried material at 70°C mixtures
- simultaneous extraction of phenolics and glucosinolates by methanol/water
- purification of GLS from phenols by solid phase extraction (stationary phase: DEAE Sephadex anion exchanger)
- desulphation of GLS on SPE cartridge by sulphatase (from *Helix pomatia*)
- elution of desulpho-GLS by water
- quantification of GLS and phenols (on crude extract) by high performance liquid chromatography (HPLC) by external standard method
- identification of compounds by a coupled system HPLC-mass spectrometry



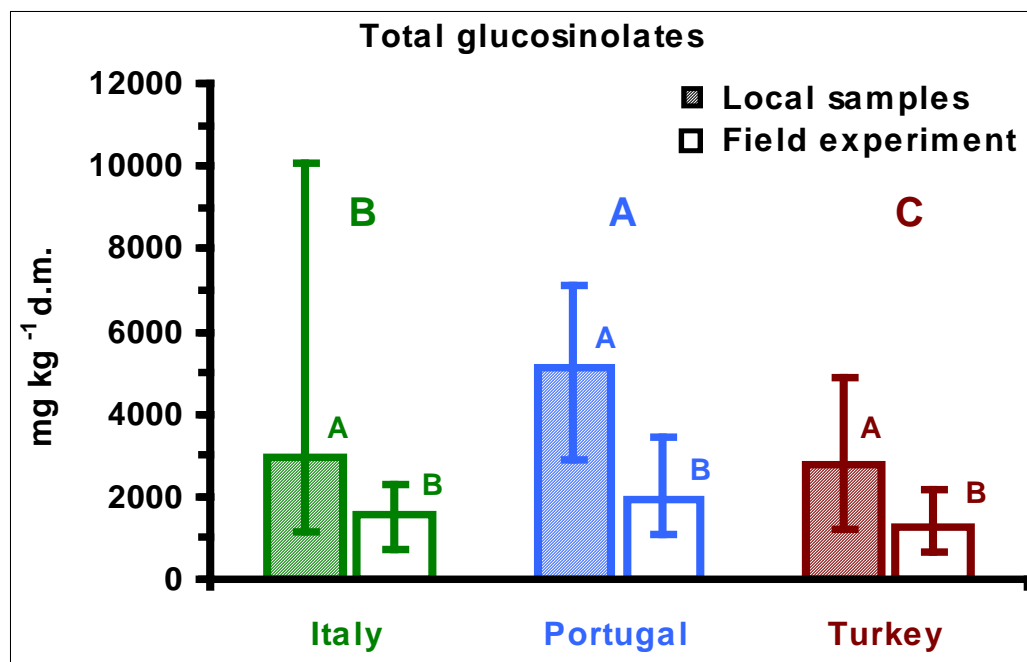


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Results

Total glucosinolates



Analysis of variance	Significance
Origin	**
Source	**
Origin × Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant

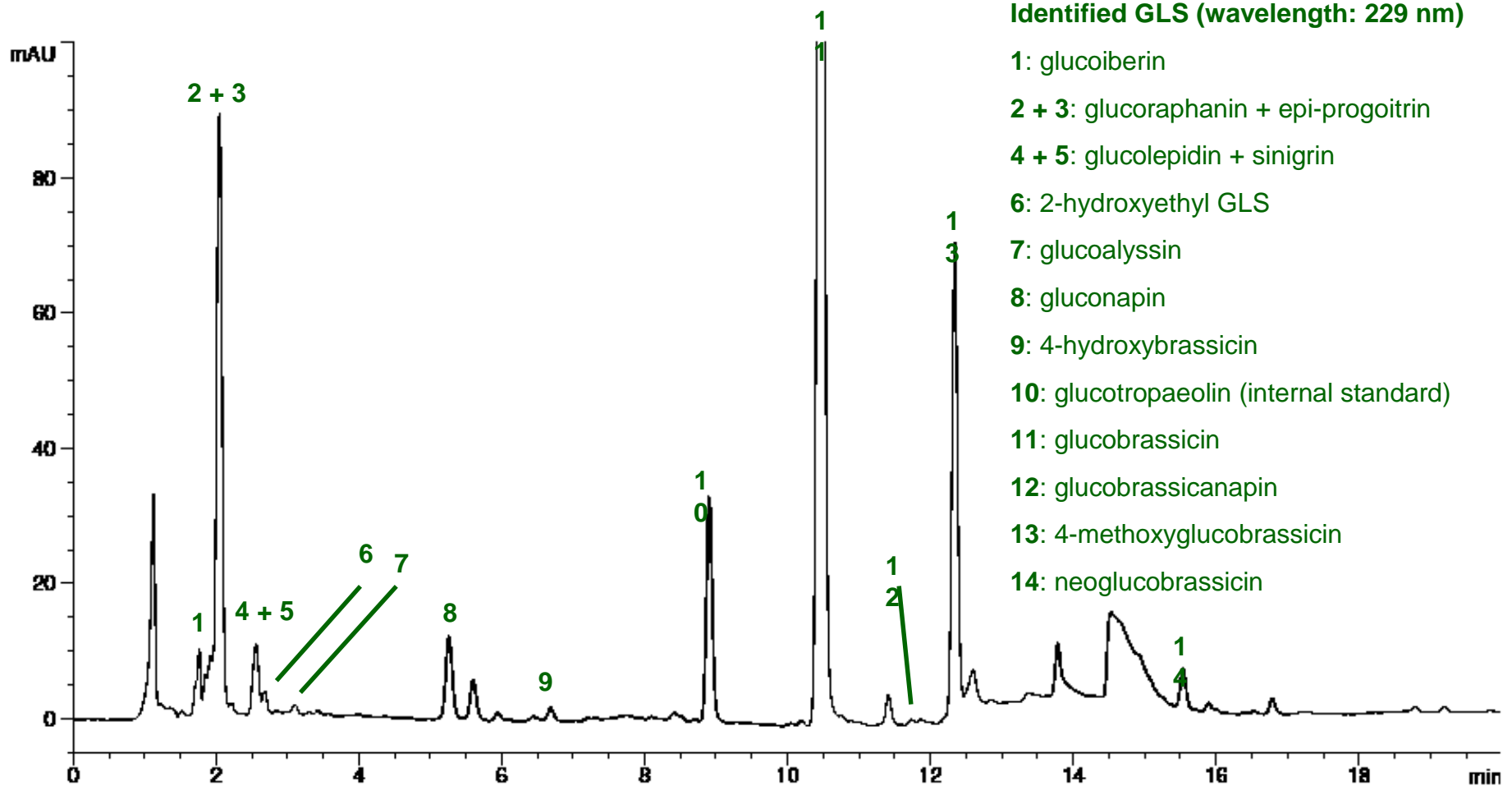
Bars = overall variability range



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HPLC trace of desulpho-GLS extracted from a Turkish kale sample



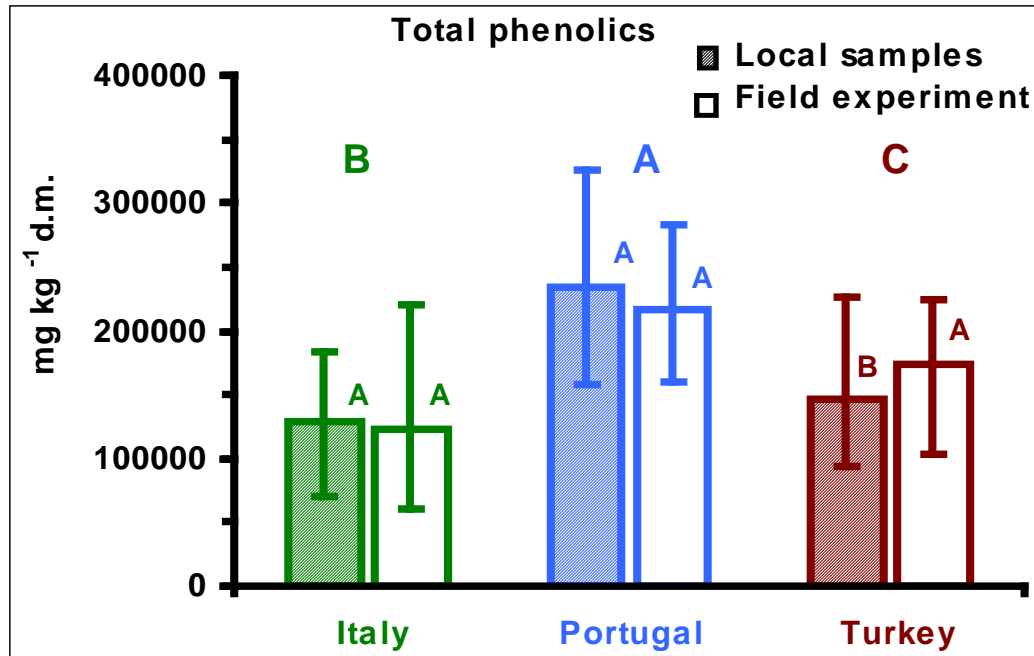


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Results

Total phenolics



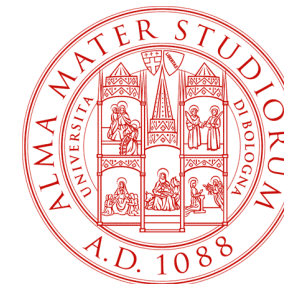
Analysis of variance	Significance
Origin	**
Source	NS
Origin × Source	**
Samples	**

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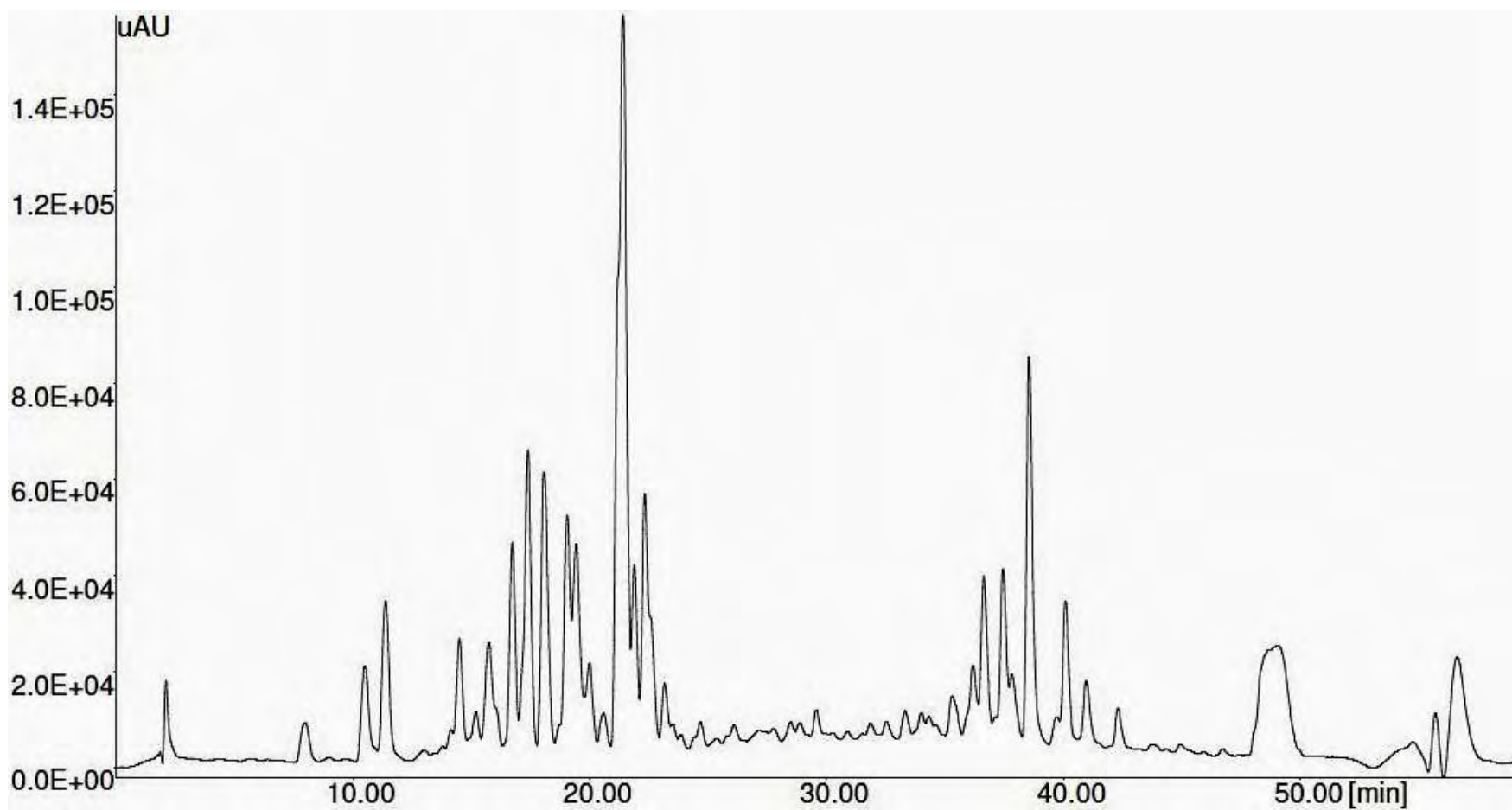
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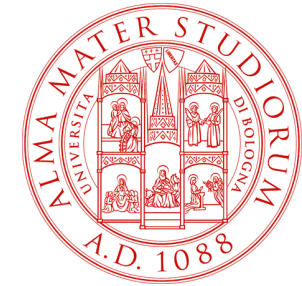


HPLC trace of phenolics extracted from an Italian kale sample





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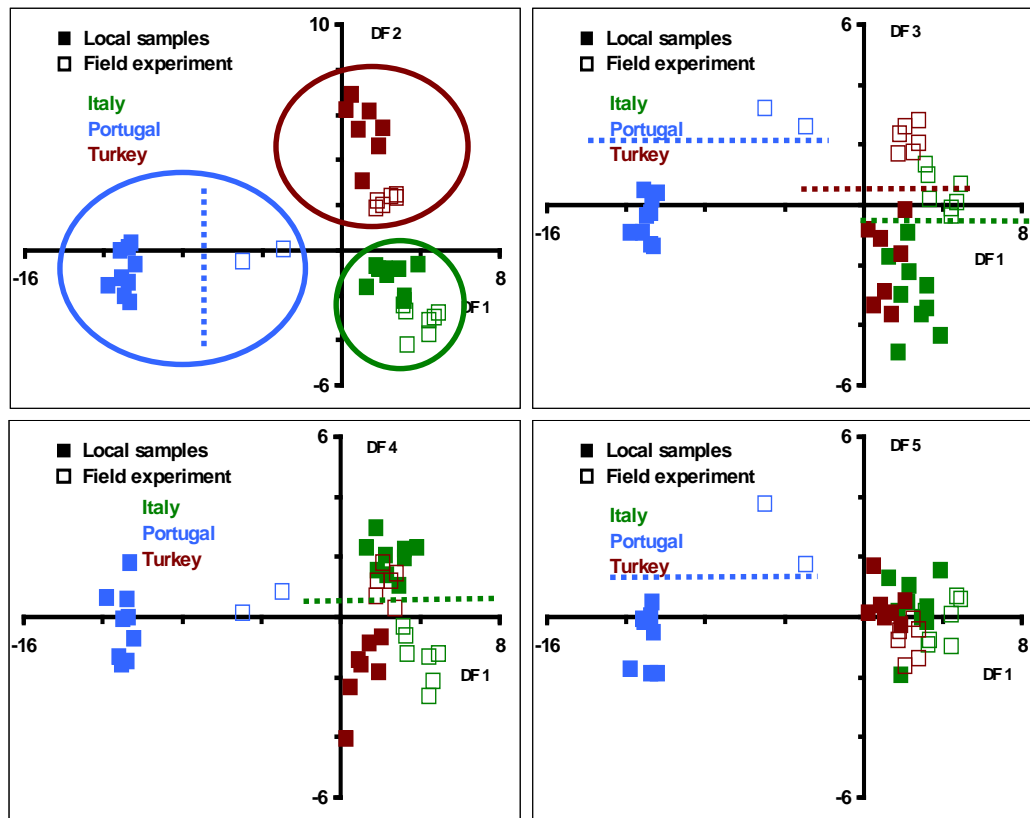


Results

Discriminant analysis of relative content of glucosinolate and phenolic compounds

Distribution of the experimental cases (origin x source) in the space of the five discriminant factors (DF)

Correlations between relative component content and discriminant factors (DF)



	DF1	DF2	DF3	DF4	DF5
1. Glucoiberin	-0.512	-0.148	-0.071	0.282	0.179
2. Glucoraphanin	0.122	-0.136	0.242	-0.052	-0.309
3. Sinigrin	-0.910	-0.152	0.045	0.005	-0.006
4. Gluconapin	0.035	0.279	-0.421	-0.050	0.145
5. 4-Hydroxy-glucobrassicin	-0.048	0.173	-0.181	-0.147	0.173
6. Glucobrassicin	0.894	0.052	0.054	-0.044	-0.032
7. 4-Methoxy-glucobrassicin	0.234	0.367	0.400	-0.223	-0.220
8. Neoglucobrassicin	-0.456	-0.363	-0.201	-0.063	-0.218
1. 3-Caffeoylquinic acid	0.298	-0.187	0.244	-0.123	0.139
2. 3-p-Coumaroylquinic acid	0.236	-0.302	-0.104	-0.050	-0.041
3. 4-p-Coumaroylquinic acid	0.202	-0.303	-0.195	0.026	-0.020
4. 3-Feruloylquinic acid	-0.158	-0.211	-0.141	0.050	-0.025
5. Quer-3-diglu-7-glu	0.041	-0.407	-0.062	0.098	0.012
6. Quer-3-caf-diglu-7-glu + kaemp-diglu	0.113	-0.366	-0.049	-0.004	-0.035
7. Kaemp-3-diglu-7-glu	-0.764	0.214	0.168	0.062	0.007
8. Sin-glu	0.414	-0.230	0.480	-0.115	0.126
9. Kaemp-3-hydrfer-diglu-7-diglu	0.264	-0.112	-0.012	-0.035	0.234
10. Quer-3-sin-diglu-7-glu	0.090	-0.473	-0.184	0.295	-0.119
11. Quer-3-fer-diglu-7-glu + quer-3-diglu-7-sin-diglu	-0.179	0.104	-0.560	0.317	-0.152
12. Kaemp-3-caf-diglu-7-glu + quer-3-diglu-7-fer-diglu	-0.011	-0.080	-0.225	0.083	-0.118
13. Kaemp-3-sin-diglu-7-glu + kaemp-3-sin-diglu-7-diglu	0.248	0.157	0.125	0.155	-0.158
14. Kaemp-3-fer-diglu-7-glu	-0.032	0.490	0.542	-0.243	-0.016
15. Kaemp-3-fer-diglu-7-diglu	0.409	-0.228	-0.122	-0.110	0.077
16. Kaemp-3-fer-triglu-7-diglu	-0.336	0.435	0.196	0.081	0.128
17. Keamp-3-cum-diglu-7-glu	0.005	-0.194	-0.020	0.193	0.078
18. Quer-3-sin-triglu-7-sin-diglu	-0.168	-0.114	-0.206	-0.104	0.382
19. Quer-3-disin-triglu-7-glu	-0.306	-0.030	-0.335	0.010	-0.174
20. Kaemp-3-disin-triglu-7-diglu	0.517	-0.001	-0.645	-0.118	0.133
21. Kaemp-3-fer,sin-triglu-7-diglu	0.129	0.002	0.127	-0.194	-0.116
22. Disin-diglu	0.296	-0.375	-0.441	-0.023	0.039
23. Sin-fer-diglu	0.373	-0.352	0.055	-0.470	0.138
24. Trisin-diglu	0.122	-0.246	-0.497	0.214	0.027
25. Disin-fer-diglu	0.040	0.124	-0.188	-0.233	-0.064

Variability explained

0.660 0.186 0.090 0.045 0.019

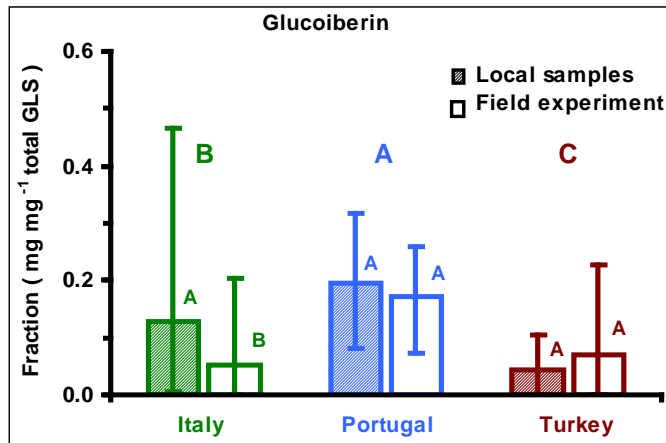


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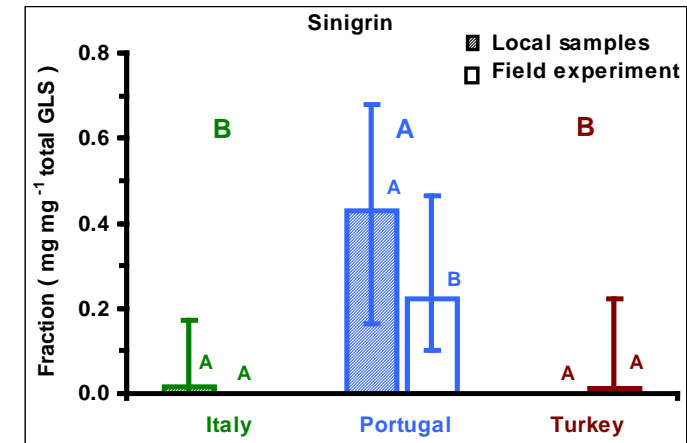
Results

Relative content of relevant glucosinolates



ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant

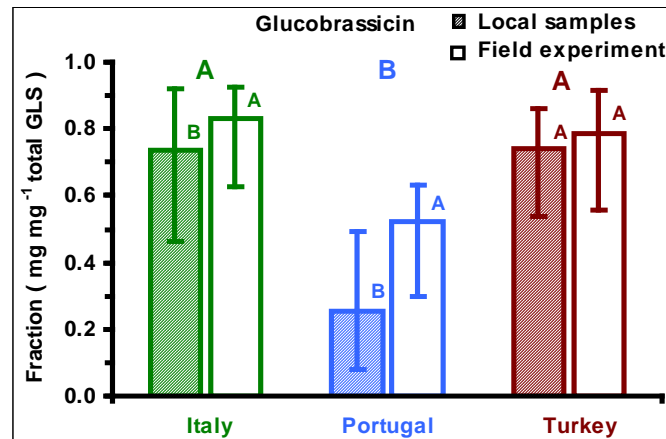


ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

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ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



Bars = overall variability range

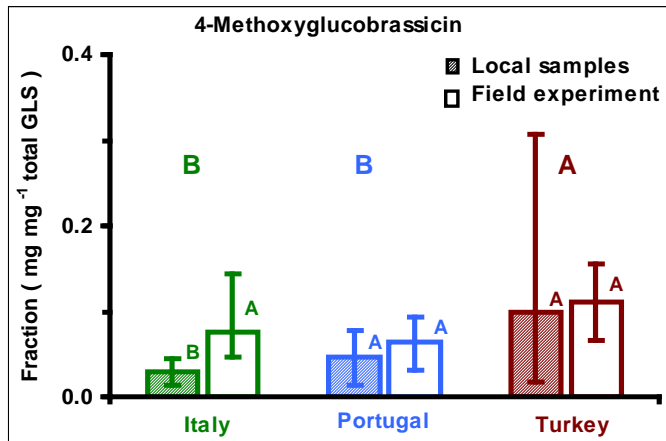


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Results

Relative content of relevant glucosinolates

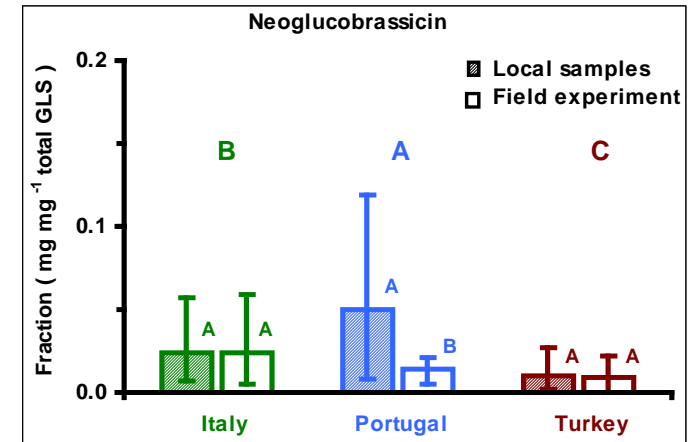


ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

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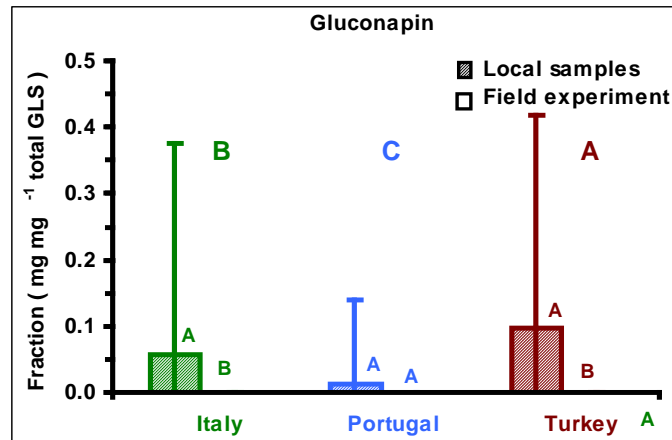
ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

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ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

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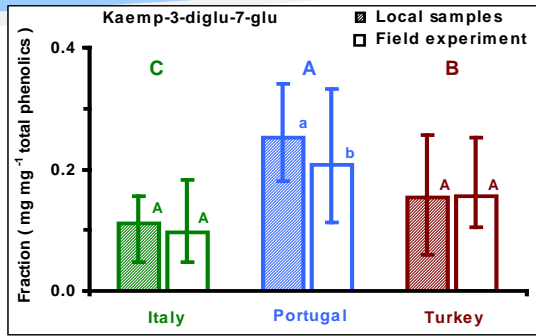


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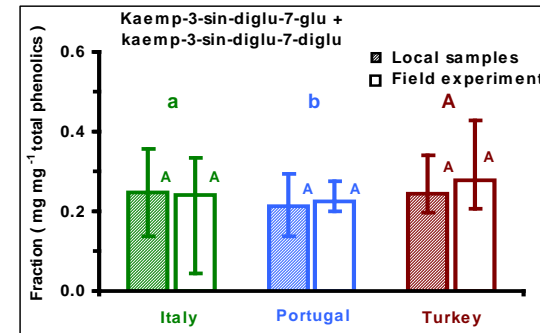
Results

Relative content of relevant phenolics – correlated to DF1



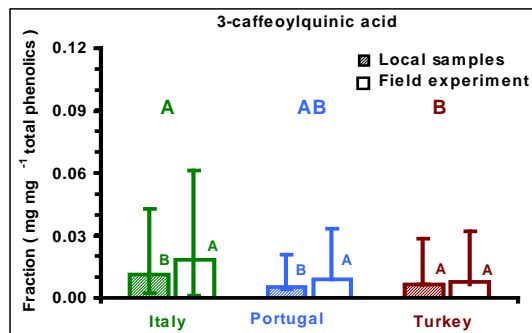
ANOVA	Significance
Origin	**
Source	**
Origin x Source	*
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



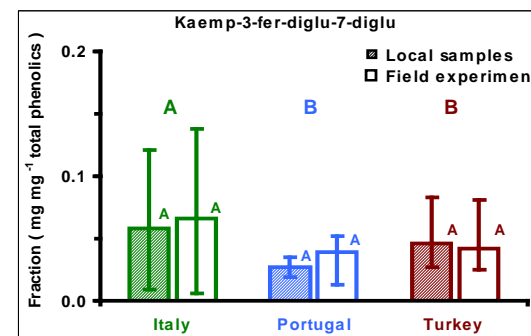
ANOVA	Significance
Origin	**
Source	NS
Origin x Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



ANOVA	Significance
Origin	*
Source	**
Origin x Source	*
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



ANOVA	Significance
Origin	**
Source	NS
Origin x Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant

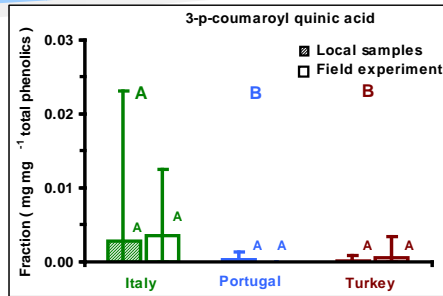


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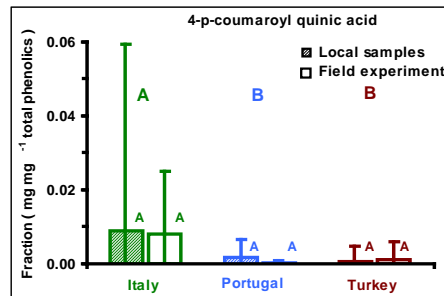
Results

Relative content of relevant phenolics – correlated to DF2



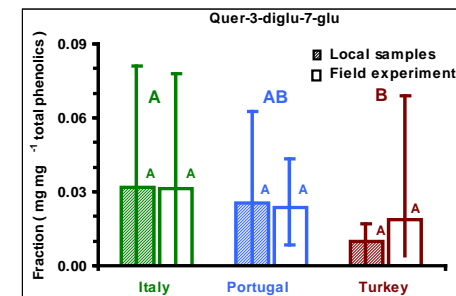
ANOVA	Significance
Origin	**
Source	NS
Origin × Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



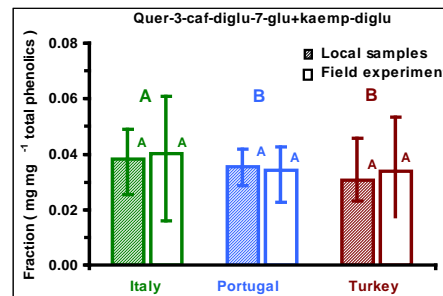
ANOVA	Significance
Origin	**
Source	NS
Origin × Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



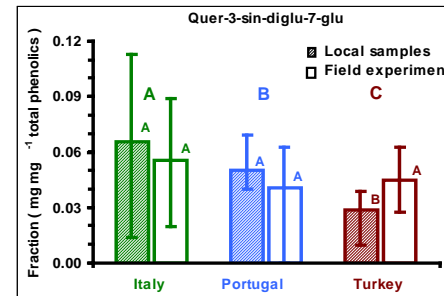
ANOVA	Significance
Origin	**
Source	NS
Origin × Source	NS
Samples	*

** $p < 0.01$; * $p < 0.05$; NS, not significant



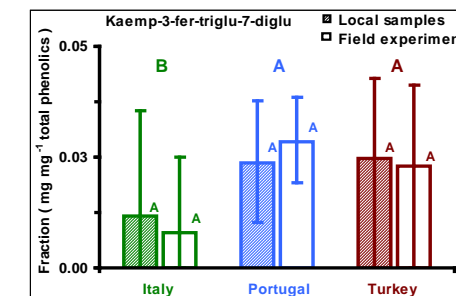
ANOVA	Significance
Origin	**
Source	NS
Origin × Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



ANOVA	Significance
Origin	**
Source	NS
Origin × Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



ANOVA	Significance
Origin	**
Source	NS
Origin × Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant

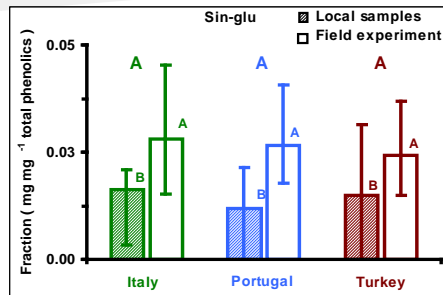


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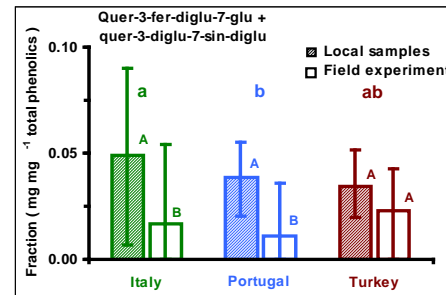
Results

Relative content of relevant phenolics – correlated to DF3



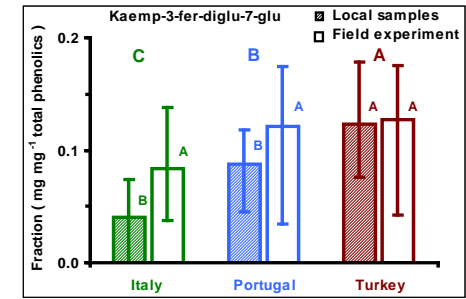
ANOVA	Significance
Origin	NS
Source	**
Origin x Source	NS
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



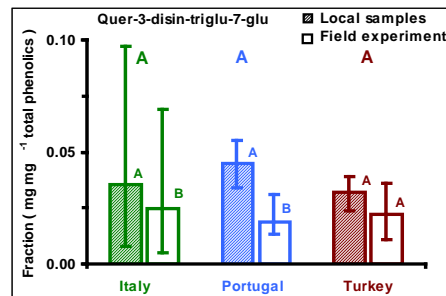
ANOVA	Significance
Origin	*
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



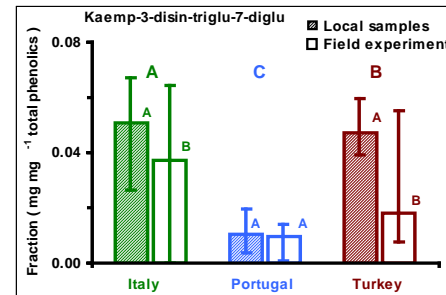
ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



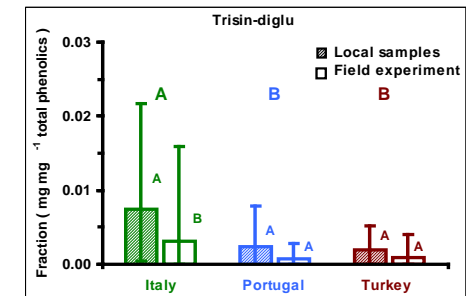
ANOVA	Significance
Origin	NS
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



ANOVA	Significance
Origin	**
Source	**
Origin x Source	**
Samples	**

** $p < 0.01$; * $p < 0.05$; NS, not significant



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Conclusions

- First time exploration of populations from different countries in a common environment
- Glucosinolate content in the range of other experiences
- High variability due to environmental factors
- Portuguese populations show a more different profile (sinigrin)

- Scarce clear references available for phenolics
- Very high content in the populations examined
- Some phenolics are more effective to discriminate growing conditions

- Kales confirmed to be very interesting food crops and potential easy to grow raw materials for food extracts or preparations.