Metabolic survey of Italian vegetables: FTICR-MS and NMR-based untargeted analyses

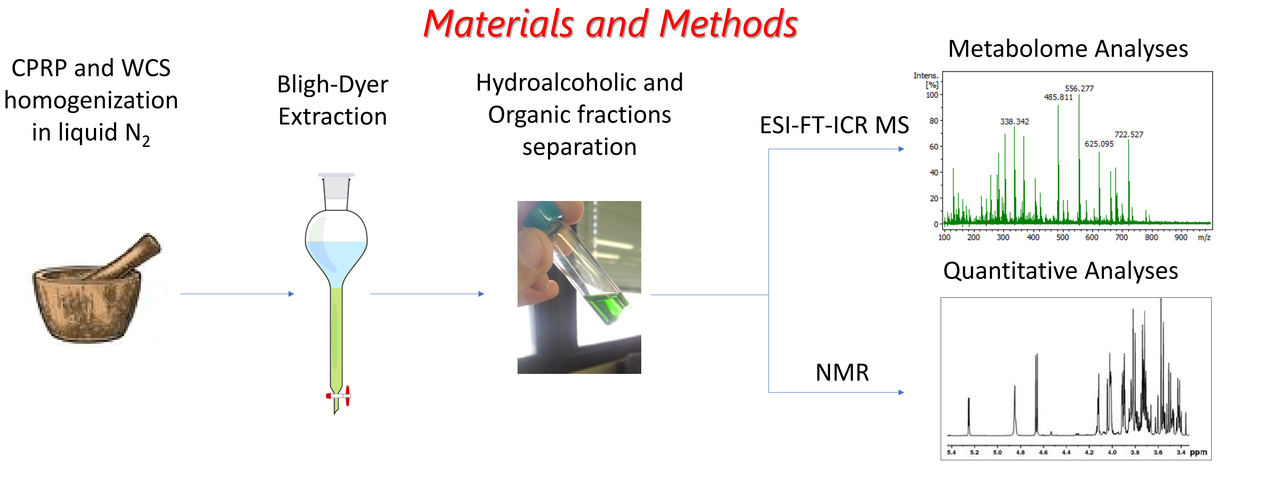
Alessandro Maccelli,[1] Anatoly P. Sobolev,[2] Donatella Capitani,[2] Barbara Chiavarino,[1] Simonetta Fornarini,[1] Luisa Mannina,[1] Maria Elisa Crestoni[1]

[1]Dipartimento di Chimica e Tecnologie del Farmaco, Università degli Studi di Roma La Sapienza, P.le A. Moro 5-00185 Roma, Italy
[2]Istituto di Metodologie Chimiche, Laboratorio di Risonanza Magnetica "Annalaura Segre", CNR, 00015 Monterotondo (Rome), Italy

Introduction

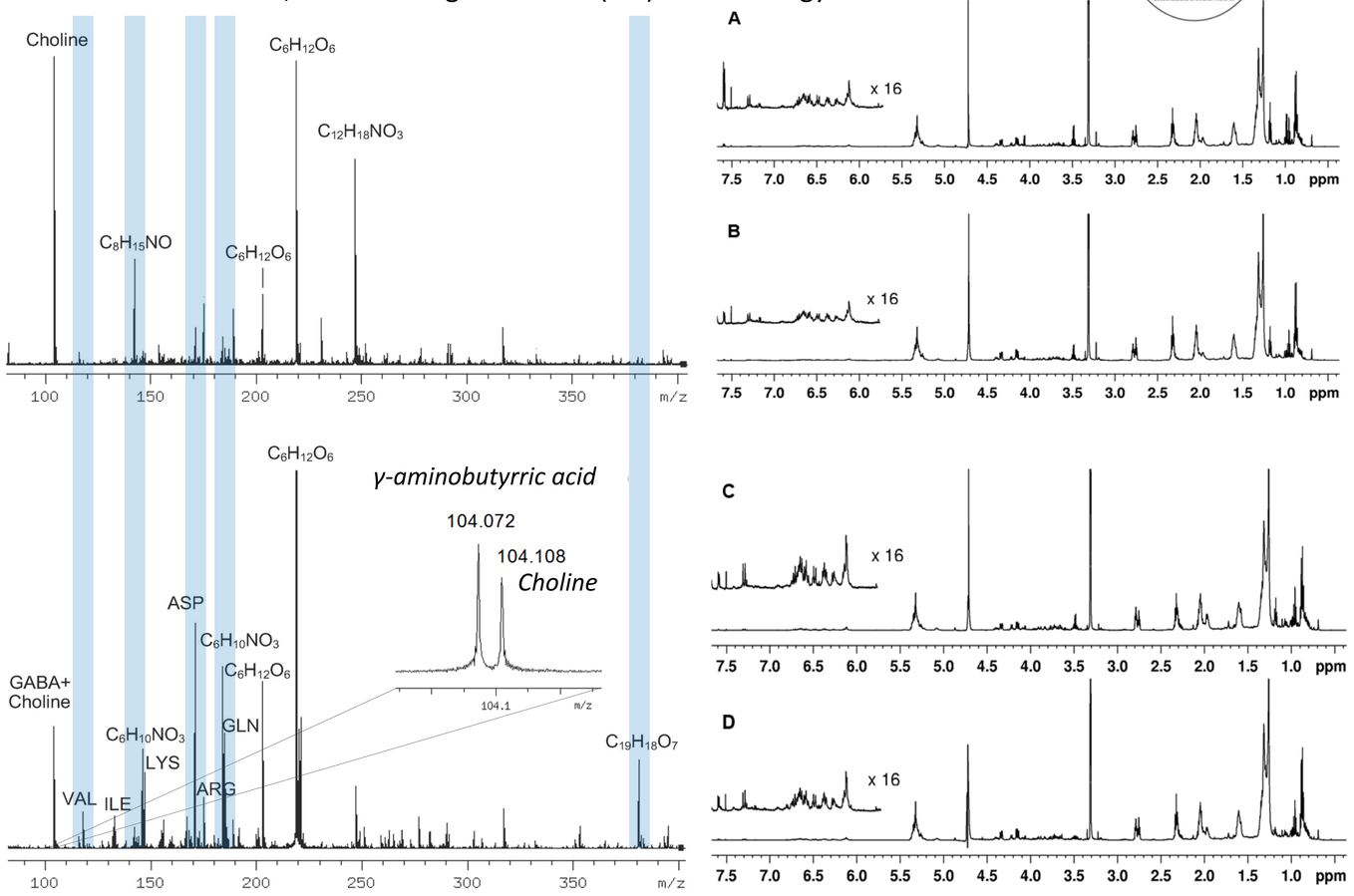
The use of untargeted analyses of complex biological mixtures is a rapidly expanding field. Combined use of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and nuclear magnetic resonance (NMR) spectroscopy allows to increase metabolome coverage and is applied here to characterize white Celery of Sperlonga (WCS) and "Cornetto di Pontecorvo" red pepper (CPRP) samples. In the context of the e-ALIERB project, an open lab with the aim of enhancing and defining foodstuffs and botanicals of Lazio region (Italy), a combined approach exploiting the high sensitivity, accuracy and specificity of high resolution mass spectrometry (ESI HR-MS) and quantitative NMR analyses has allowed the assignment of a large number of metabolites in these complex vegetable extracts.

Materials and Methods



The Italian PDO "Cornetto di Pontecorvo"

The Italian PDO (protected designation of origin) CRPR is a *Capsicum annuum* ecotype, cultivated in the Ciociaria area (G.U. n. 285 del 6/12/2010 – Reg. UE 1021/2010). The volcanic nature of the soil confers to CRPR unique sensorial properties and an intense flavour. The PDO production protocol requires the open air cultivation (OF). However, the increase of fungal infections by *Aspergillus* and *Fusarium* spp. affected the growth and the yield of *Cornetto,* leading to the development of new cultivation strategies under carefully controlled conditions, such as the greenhouse (GH) methodology[1].



Metabolites in hydroalcoholic Bligh-Dyer extracts of Cornetto di Pontecorvo assigned by HR analyses and CID fragmentation based on ESI-MS.

ESI-MS in positive mode

Carbohydrates	*Mass (m/z)*	*CID fragment*
$[C_6H_{12}O_6 + Na]^+$	203.057	-
$[C_{12}H_{22}O_{11} + Na]^+$	365.116	275, 203
Amino acids and other compounds		
$[Glycine + H]^+$	76.046	-
$[\gamma\text{-Aminobutyrate} + H]^+$ (GABA)	104.072	-
$[Choline]^+$	104.108	-
$[Proline + H]^+$ (Pro)	116.072	70
$[Asparagine + H]^+$ (Asn)	133.063	116, 87
$[Arginine + H]^+$ (Arg)	175.119	158, 130, 116, 70, 60
ESI-MS in negative mode		
Organic acids and other compounds		
$[Lactic\ acid - H]^-$	89.025	71
$[3\text{-Furoic acid} - H]^-$	111.009	93, 83, 67
$[Pyroglutamic\ Acid - H]^-$	128.036	84
$[Threonic\ acid - H]^-$	135.030	-
$[Citric\ acid - H]^-$	191.019	173, 111

A comprehensive metabolite profile of pulp, peel and seeds hydroalcoholic and organic extracts of OF and GH CPRP was performed, revealing the presence of sugars, amino acids, organic acids, polyphenols and flavonoids. Interestingly, the main features of CPRP are preserved in either OF or GH cultivation methods.

Figure 1: ESI^+-FTICR spectrum of the hydroalcoholic extract from CPRP, OF seeds (top) and pulp (bottom). ^1H NMR spectra of organic fractions of pulp, grown OF (A) ; pulp, grown GH (B); peel, grown OF (C) and peel, grown GH (D).

The Italian WCS "Sedano Bianco di Sperlonga"

The Italian PGI (Protected Geographical Indication) "Bianco di Sperlonga" (*Apium graveolens* variety dulce, WCS) is strictly cultivated in the Ciociaria area (G.U. L 68 del 18/3/2010 – Reg. UE 222/2010). This is the only area of WCS production specified by law, characterized by a sandy-loamy soil with a high degree of salinity and the best pedoclimatic conditions. This environment bestows WCS special traits such as the specific organoleptic qualities of flavour, the aromatic sweet taste and the unique nutritional properties.

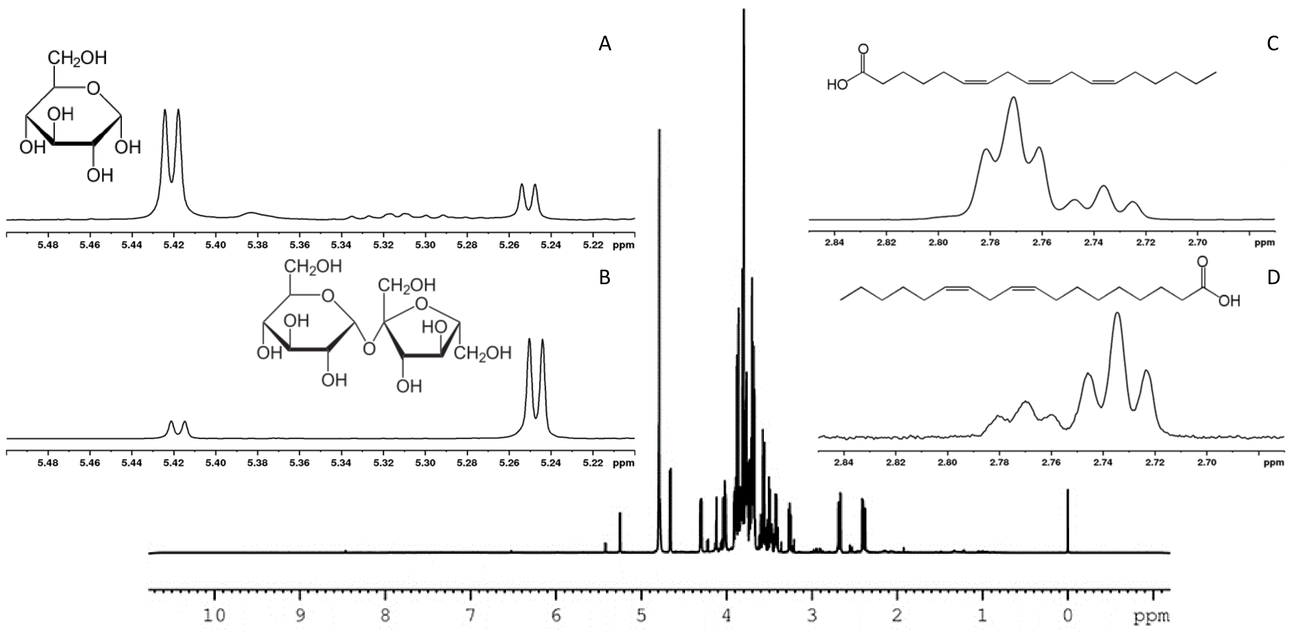


Figure 2: ^1H NMR spectrum of the WCS leaves hydroalcoholic extract. Differences between the amount of glucose and linoleic acid in leaves (insert A and C) respect to amount of sucrose and linolenic acid (insert B and D) in stalks extract, are shown.

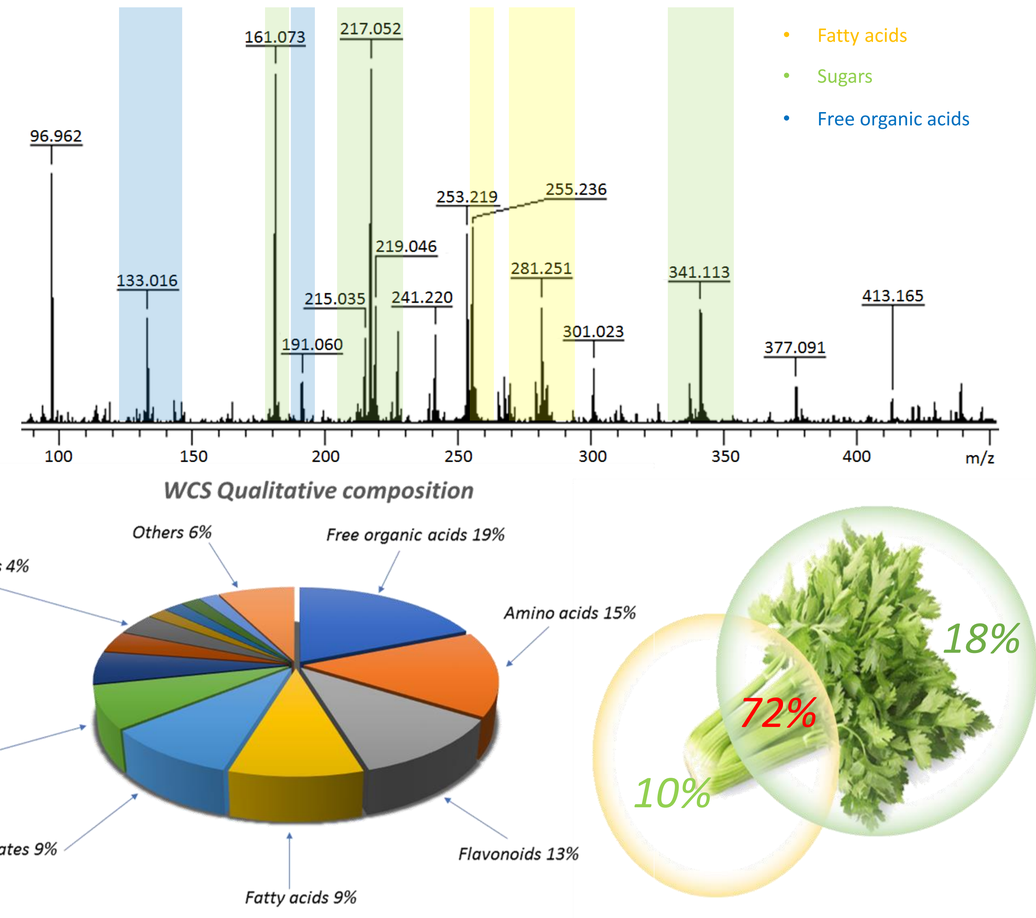


Figure 3: ESI^--FTICR spectrum of the hydroalcoholic Bligh-Dyer extract of the leaves from WCS. The abundances percentage of the assigned metabolites in both organic and hydroalcoholic WCS extracts and differences between the proper leaves and stalk metabolites are shown.

ESI-FTICR and NMR analyses on WCS extracts revealed that leaves and stalks present a similar composition, including sugars, polyalcohols, amino acids, glycosylated compounds and organic acids. In organic extracts, major peaks correspond to phtalides, as senkyunolide, responsible for organoleptic properties[2], chlorophyll a, linoleic and linolenic fatty acids, the latter more abundant in leaves.

Conclusions

This combined approach has allowed an extensive phytochemical characterization of different vegetable extracts, displaying the main metabolites from Bligh-Dyer extracts of CPRD and WCS. No significant differences between the OF and GH CRPR extracts were obtained. WCS leaves extracts generally showed an higher concentration of amino acids, organic acids, sucrose and choline with respect to stalks extracts, excepting for asparagine, glucose and formic acid for which this trend is reversed. These data reassure about the possibility of preserving the biodiversity in favor of the local production adding value to food and vegetables of the Lazio region in the international market, considering the very low content of biogenic amines and mycotoxins as mark of quality and freshness of the vegetables.

Acknowledgements

This work was supported by the Project_EU_FT-ICR_MS, funded by the European Union's Horizon 2020 research and innovation programme under grant agreement nr. 731077 and by the by "e-ALIERB" Project [Regione Lazio LR13/2008 – Dipartimento di Chimica e Tecnologie del Farmaco].

References

[1] Sobolev, A. P.; Mannina, L.; Capitani, D.; Sanzò, G.; Ingallina, C.; Botta, B.; … & Di Sotto, A; (2018). *A multi-methodological approach in the study of Italian PDO "Cornetto di Pontecorvo" red sweet pepper*; Food Chemistry. 2018, 255, 120-131
[2] Lin, L; Shengmin, Lu; Harnly, J,M; *Detection and Quantification of Glycosylated Flavonoid Malonates in Celery, Chinese Celery, and Celery Seed by LC-DAD-ESI/MS*; J. Agric. Food Chem. 2007, 55, 1321-1326