Comprehensive HRMS chemical characterization of an antioxidant drink via a newly developed suspect and target screening workflows

SuperFruits project code AMΘP7-0072324

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Introduction

What is an antioxidant drink?

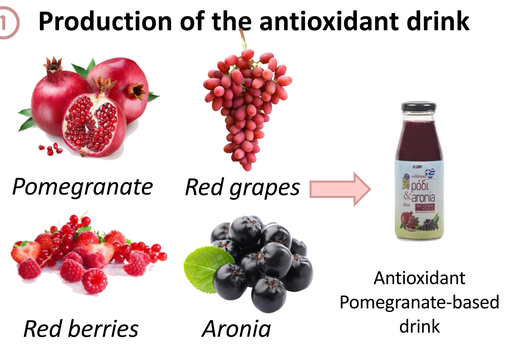
Antioxidant drink is comprised of ingredients with high antioxidant capacity.

Why is this drink beneficial?

- *Anticancer*
- *Anti-inflammatory*
- *Antidiabetic*
- *Anti-aging*
- *Anti-Alzheimer*
- *Cardiovascular disorders*

① Production of the antioxidant drink

Ingredients



Research aims

② Novel Workflows for Target & Suspect screening

- Maximization of the intended chemical space coverage (antioxidants).
- Highlight the suspect list's new role as a searchable database.
- Exploitation of open-source software as an alternative yet efficient tool for the scientific community.
- Exploratory integration in drug discovery pipelines.

③ HRMS Chemical characterization

- Bioactive-based characterization
- Comprehensive characterization
- Virtual chemical space characterization

④ Quantification

- via TASQ 1.4 (BRUKER)

Sample preparation

Weigh of 0.1 g sample

↓

Addition of 0.2 mL MeOH: H_2O, (80:20, v/v)

↓

Filtration through RC syringe filters

↓

5μL injection to LC-QTOF-MS

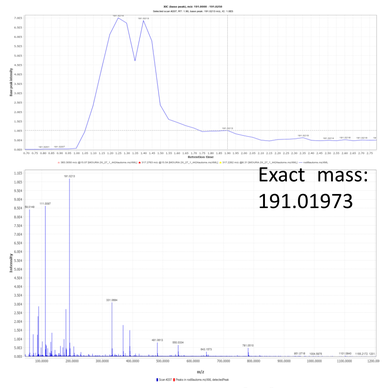
Instrumentation

LC conditions	
Column: Acclaim RSLC 120 C18 (2.2 μm, 100 mm× 2.1 mm)	
(-ESI)	**A:** H_2O (10 mM CH_3COONH_4): MeOH (90:10) **B:** MeOH (10 mM CH_3COONH_4)
(+ESI)	**A:** H_2O (5 mM $HCOONH_4$+ 0.01% HCOOH): MeOH (90:10) **B**: MeOH (5 mM $HCOONH_4$+ 0.01% HCOOH)

UHPLC-QToF-MS

MS conditions		
Range m/z: 50-1200 Da	Scan rate: 2 Hz	
bbCid	Full scan (MS) : CE 4 eV	
	(MS/MS) : CE 25 eV	
autoMS	Full scan (MS): CE 4 eV	
	MS/MS of the 5 most abundant ions: CE 25 eV	

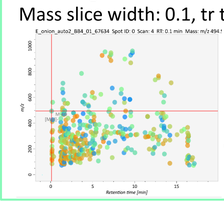
Methodology exemplification employing citric acid MS1 spectrum and chromatogram



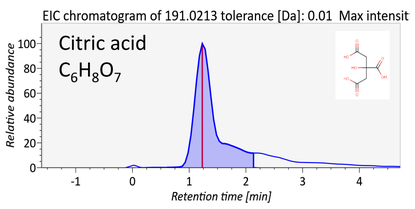
MS2 spectrum and chromatogram

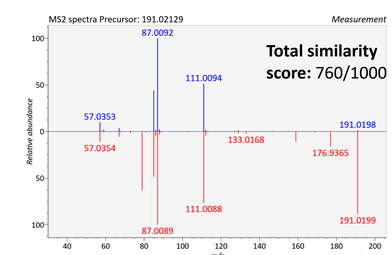
MS-DIAL

Algorithm Deconvolution MS2Dec Parameters:
Peak height >1000, Mass tol. ≤5 mDa
Mass slice width: 0.1, tr tol. ≤0.1 min



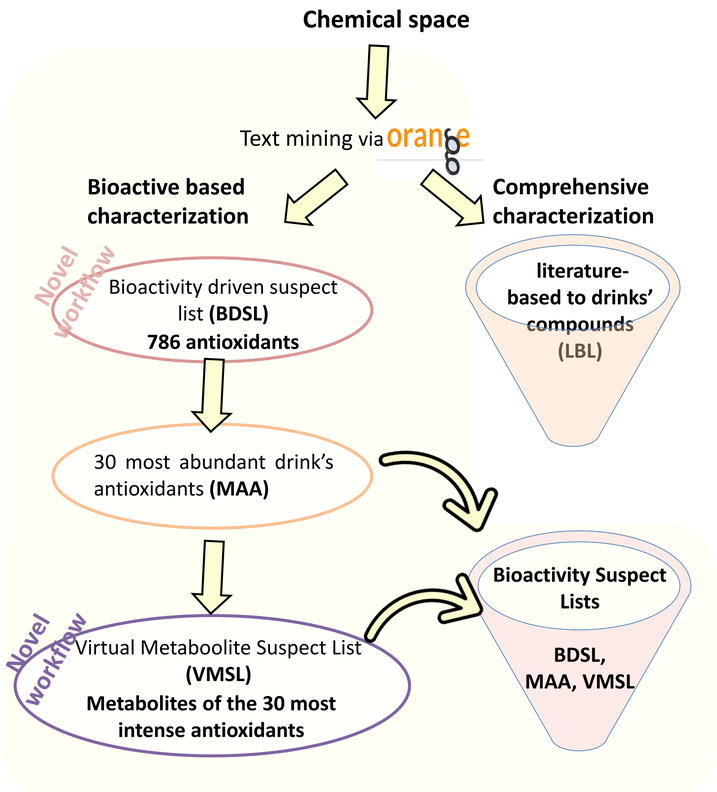
Exclude masses derived from procedure blank



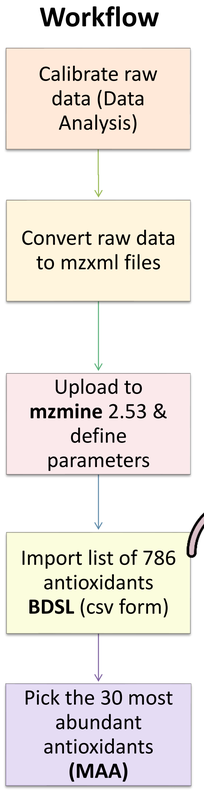


Data Processing and Identification Workflows

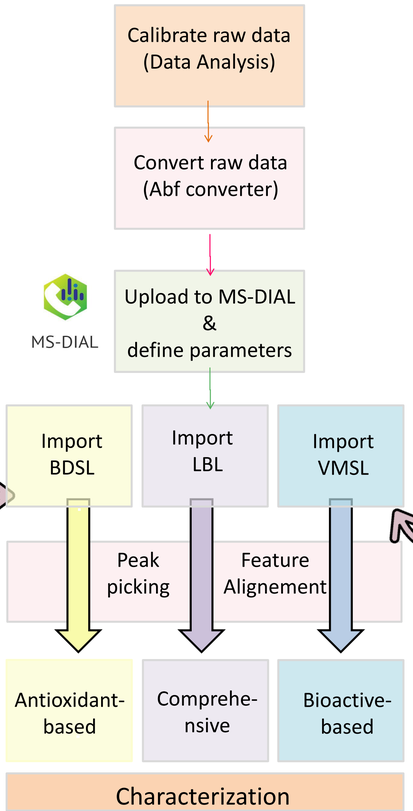
Workflows for the compilation of the suspect lists



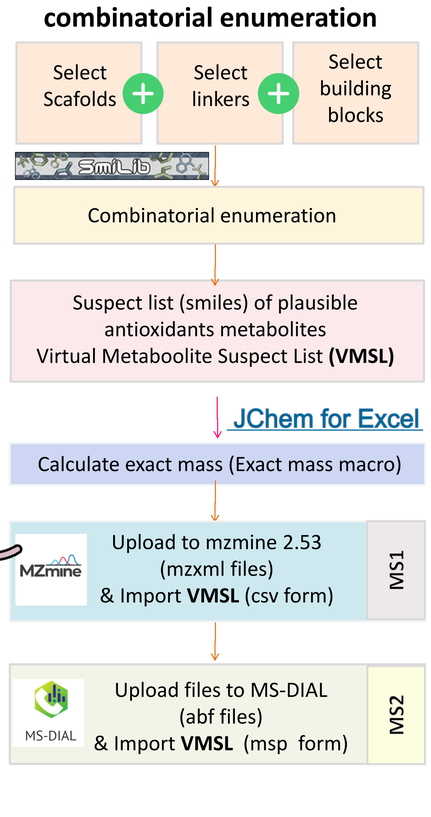
MS1 driven Workflow



MS2 driven Workflow

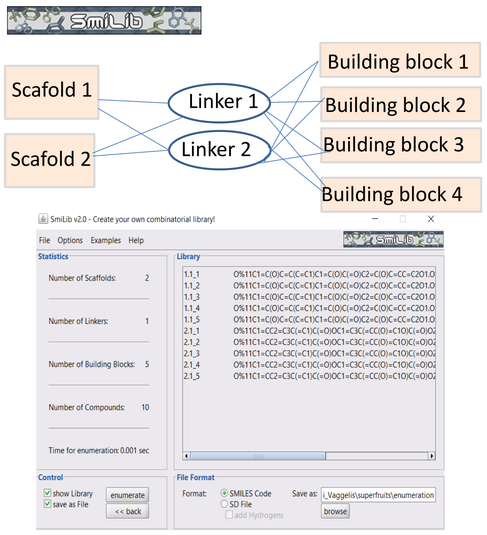


Workflow for MAA's metabolites combinatorial enumeration



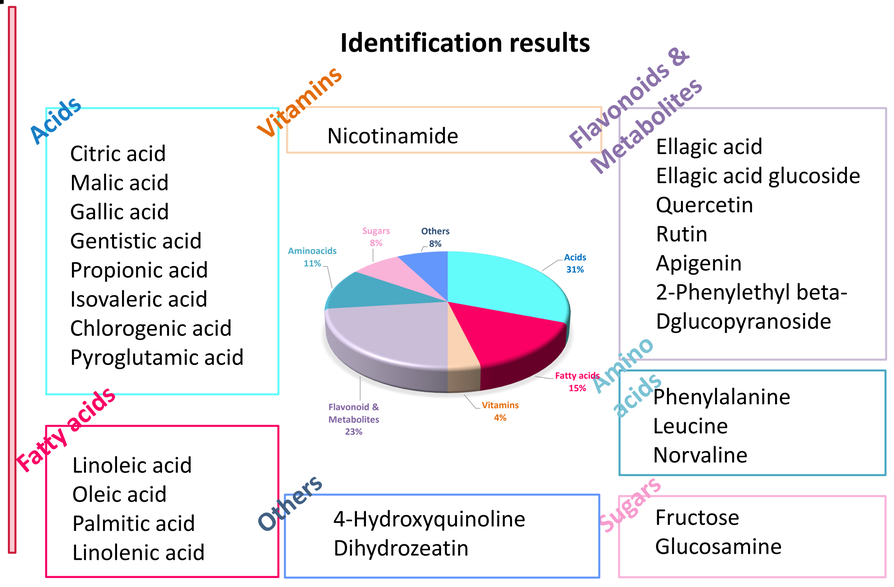
Methodology exemplification for metabolites enumeration

SmiLib



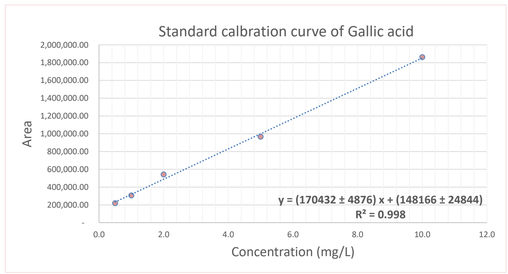
Results

Identification results



Quantification results

TASQ



- Quantification with standards calibration curve was conducted.
- Five-point calibration curve was constructed for each analyte.
- High recoveries were obtained compared with matrix matched for all the investigated analytes.

Analyte	Concentration (mg/kg)
Citric acid	863
Kaempferol	9.94
Fumaric Acid	223
Ethyl gallate	0.27
Quercetin	22.0
Gallic acid	6.11
Phloridzin	2.61
Myricetin	9.67
Quinic acid	1.14
Verbascoside	2.79
Chlorogenic acid	5.26

Conclusions

- Novel suspect and target screening methodologies for drink characterization have been developed using open-source software.
- Bioactivity-based and combinatorial-based suspect list.
- 26 compounds were identified via the developed methodologies in both ionization modes.
- The identified compounds, belonging to fatty acids, organic acids, flavonoids and their metabolites, amino acids and vitamins, enhance the beneficial effects of drink to humans' health.
- Citric acid and fumaric were found in high concentration levels.
- Significant amounts of the antioxidants; quercetin and kaempferol were noticed.

For further information
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