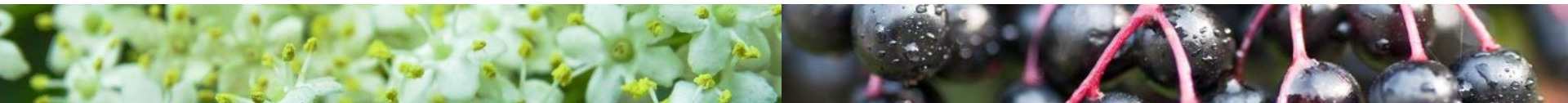


"Czarny bez" (*Sambucus nigra* L.): bioactive compounds' prospection and its potential health benefits

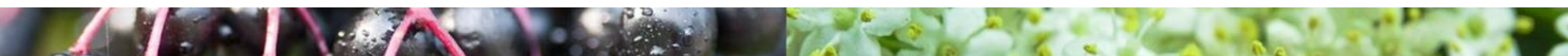


Ângelo C. Salvador, Armando J. D. Silvestre, Sílvia M. Rocha

Emails: angelomcsalvador@ua.pt, armsil@ua.pt, smrocha@ua.pt

***QOPNA and CICECO, University of Aveiro, Portugal
2018***

Presentation Outline



Introduction

Context of the work

Aim of the work

Results

Unveiling *Sambucus nigra* L. chemical profile

Elderberries' phenolic and lipophilic profile

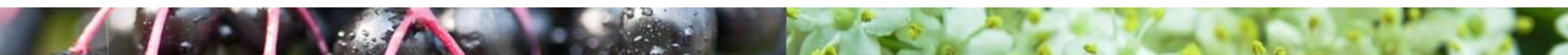
Elderberries and elderflowers' volatile terpenic and norisoprenoids profile

Pre- and postharvest effects

Elderberry dietary supplementation

Effects in a diabetic *in vivo* model

Conclusions and perspectives



Exploitation of natural products as sources of bioactive compounds

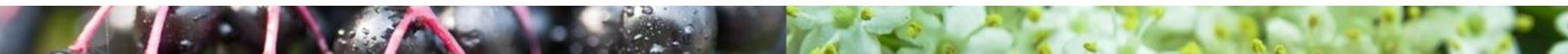
Markets

Functional foods

Nutraceuticals

Dietary supplements





Sambucus nigra L.

- ✓ Widely used **on folk medicine**
- ✓ Available products on the market

Antiviral

Respiratory problems

- ✓ **Ingredients** for jams, juices, pastry



- ✓ **Presence of many bioactive compounds**

Context of the work

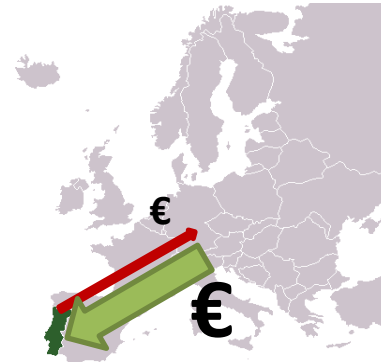
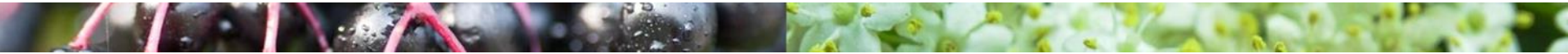


In 1756, **Marquês of Pombal** established the declaration that delineated the **Douro Appellation**, making it the world's oldest established appellation

The aim was to supervise the production of Porto wine in all stages of winemaking

Avoid fraud by ordering that all elderberry plants in the Douro be ripped out

Context of the work



Elderberry ca. 2500 tons/year
Elderflower ca. 0.6 tons/year

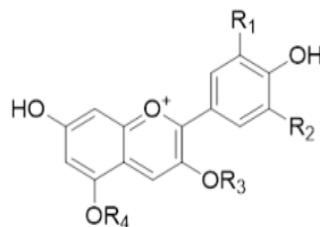


Context of the work

Elderberries

Phenolic compounds

Well studied fraction

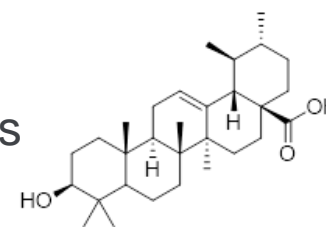


R ₁	R ₂	R ₃	R ₄	Compound
OH	H	Glc	H	Cyanidin 3-glucoside
OH	H	Sam	H	Cyanidin 3-sambubioside
OH	H	Sam	Glc	Cyanidin 3-sambubioside-5-glucoside

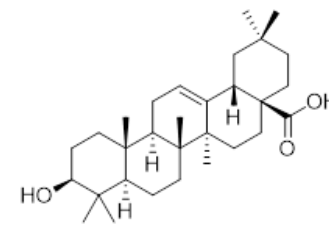
Triterpenic compounds

Only a **study** reported the presence triterpenic acids

No information about preharvest effects



Ursolic acid

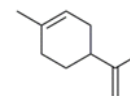


Oleanolic acid

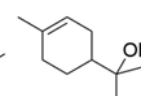
Volatile terpenic and norisoprenoids

Elderberry's **aroma**

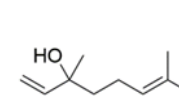
Scarce information about preharvest effects



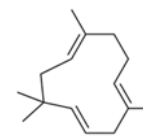
Limonene



α-Terpineol



Linalool



α-Humulene

Ripening stage

Its effect is still **poorly explored** and **understood**

Context of the work

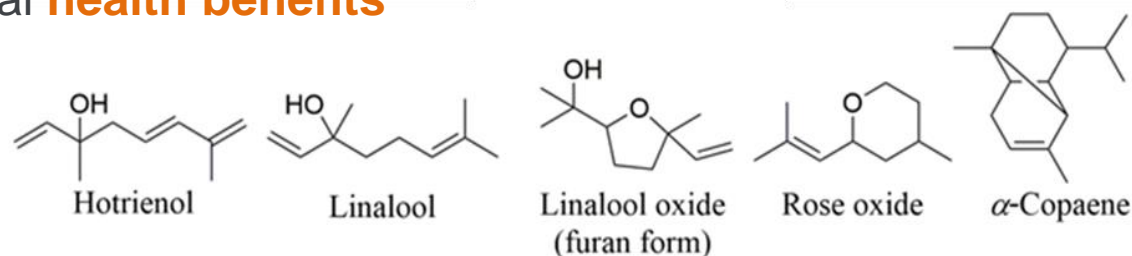


Elderflowers

Terpenic metabolites

Important contributors for the **characteristic elderflower aroma**

Diverse potential **health benefits**



Elderflower formulations

Are normally prepared from **fresh**, **frozen** or **dried** flowers

Postharvest conditions

Different **parameters** may affect *S. nigra* chemical profile
Their effect are still **poorly explored** and **understood**

Aim of the work

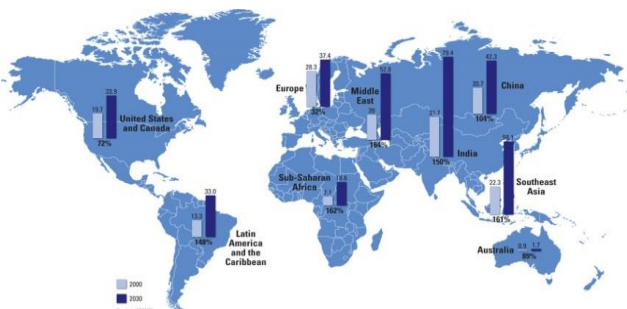


Main Objective:

Chemical characterization of *Sambucus nigra* L. berries and flowers, and the biological evaluation of elderberry extracts, in view of their valorization

Specific objectives:

- ✓ To **establish the volatile terpenic and norisoprenoids profile** from two **elderflowers** cultivars and evaluate the impact of different **postharvest** conditions
- ✓ To **establish the lipophilic (dichloromethane extractives) profile** from three **elderberries** cultivars and evaluate the effect of **ripening** during two harvesting seasons
- ✓ To establish a metabolomic-based strategy for fingerprinting of **elderberries volatile terpenic and norisoprenoids** from three cultivars through **ripening**



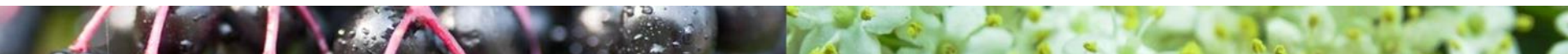
Diabetics ~300 million people up to 2025

WHO considers that **further research** is needed:

- ✓ **healthy diet (fruits and vegetables)**
- ✓ **regular physical activity**

- ✓ To evaluate the effect of the **elderberry extracts dietary supplementation** on high fat fed **diabetic rats**

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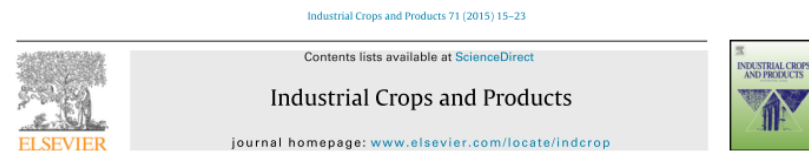
Elderberries and elderflowers' volatile terpenic and norisoprenoids profile

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Conclusions and perspectives



Lipophilic phytochemicals from elderberries (*Sambucus nigra* L.):
Influence of ripening, cultivar and season

Ângelo C. Salvador^{a,b}, Sílvia M. Rocha^{a,*,*}, Armando J.D. Silvestre^{b,*}

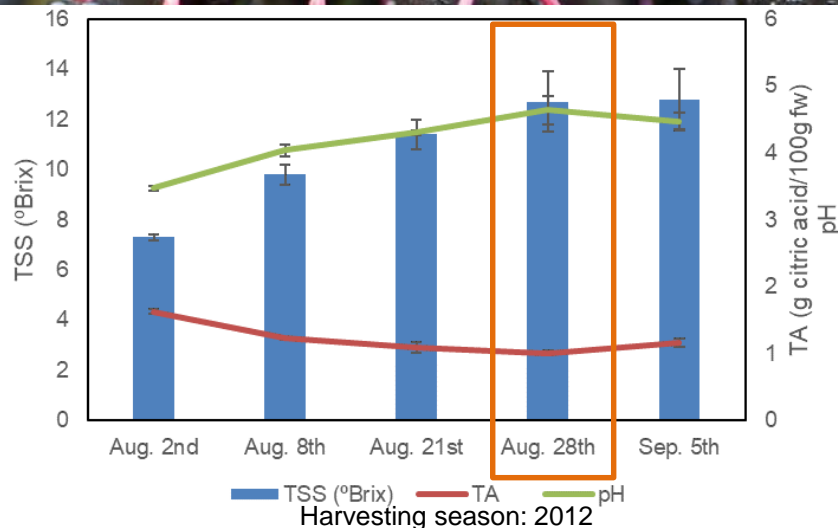
^a Department of Chemistry, QOPNA, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

^b Department of Chemistry, CICECO, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal



Unveiling *Sambucus nigra* L. chemical profile:

Elderberries' lipophilic and phenolic profile



Mature stage:

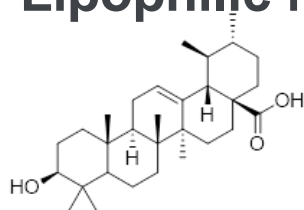
Brix degree

pH

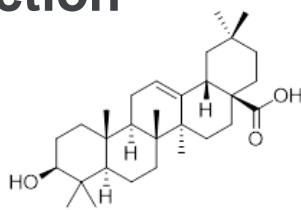
Titrateable acidity

Cluster homogeneous pigmentation

Lipophilic fraction



Ursolic acid



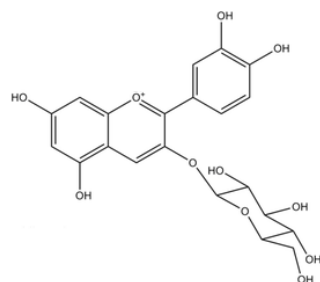
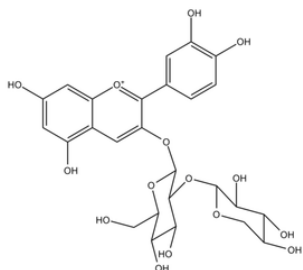
Oleanolic acid

19 reported components

Triterpenoids up to 94%

Smaller amounts of **fatty acids**, **long chain aliphatic alcohols** and **sterols**

Polar fraction



8 reported phenolic compounds

Anthocyanins prevailed with **cyanidin 3-glucoside** and **cyanidin 3-sambubioside** representing up to **73%**

Flavonols and a phenolic acid also reported

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Unveiling elderflowers (*Sambucus nigra* L.) volatile terpenic and norisoprenoids profile: Effects of different postharvest conditions

Ângelo C. Salvador^{a,b}, Armando J.D. Silvestre^b, Sílvia M. Rocha^{a,*}



JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Article

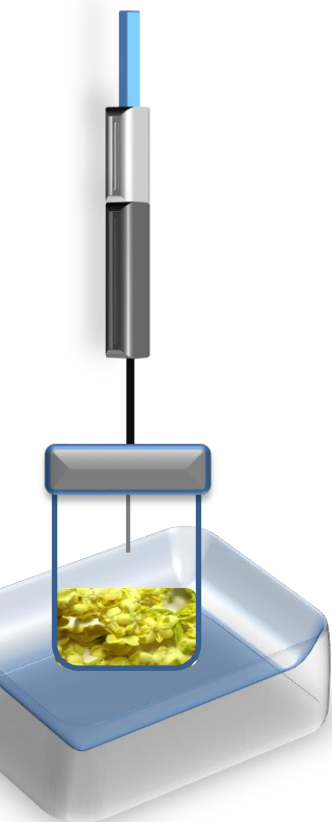
pubs.acs.org/JAFC

Metabolomic-Based Strategy for Fingerprinting of *Sambucus nigra* L. Berry Volatile Terpenoids and Norisoprenoids: Influence of Ripening and Cultivar

Ângelo C. Salvador,^{†,‡} Alisa Rudnitskaya,[§] Armando J. D. Silvestre,^{†,‡} and Sílvia M. Rocha^{*,†}

Why Headspace solid-phase microextraction (**HS-SPME**)?

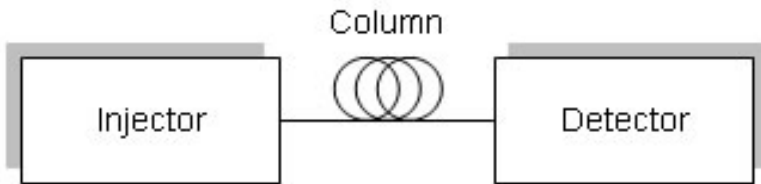
- ✓ Solvent free technique
- ✓ Requires low extraction times and temperatures
- ✓ Very easy to use
- ✓ **Selectively extracts the free volatile (and semi-volatile) compounds**



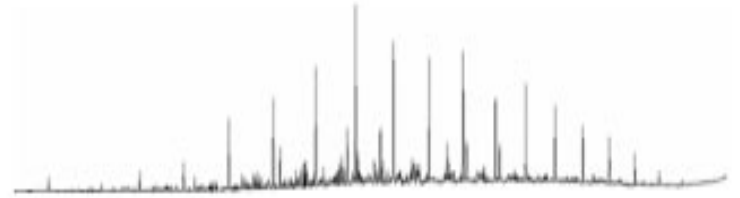
Water bath
(controlled temperature)

GC analysis
Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry
(GCxGC-ToFMS)

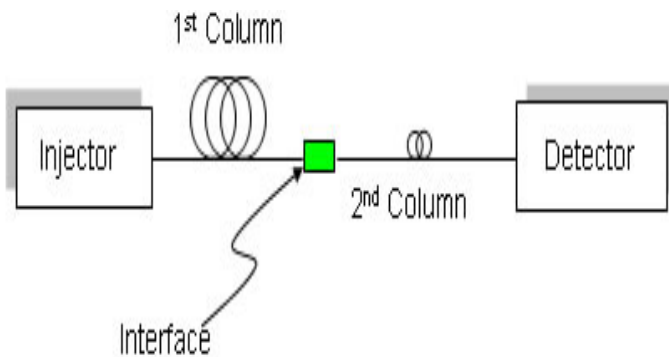
Conventional Gas Chromatograph



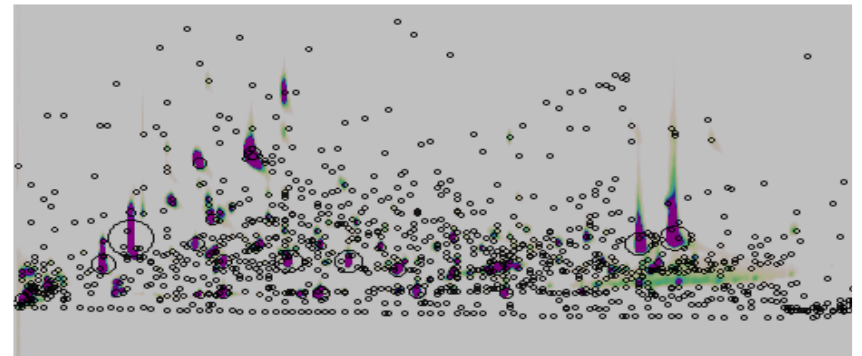
Conventional Gas Chromatogram



GC x GC system



Additional independent
separation → 2D



Conventional 1D Separation

1. **Couple another GC column:**

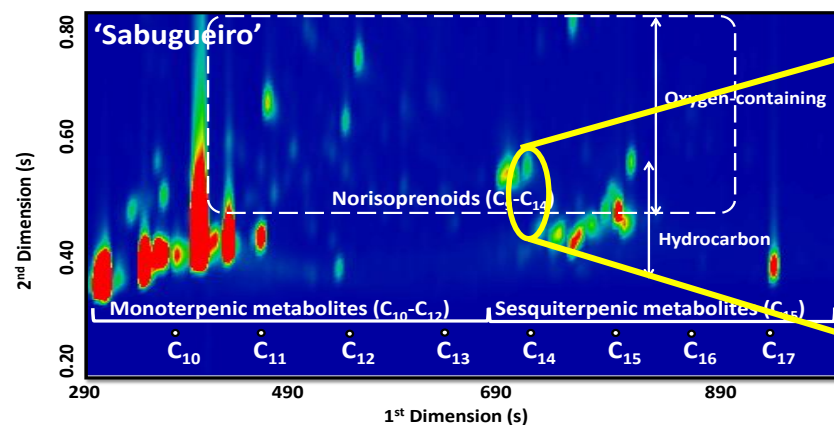
2D Orthogonal separation on GCxGC is significantly higher than conventional 1D GC

2. **Better sensitivity** than 1D GC due to the peak compression during modulation process.

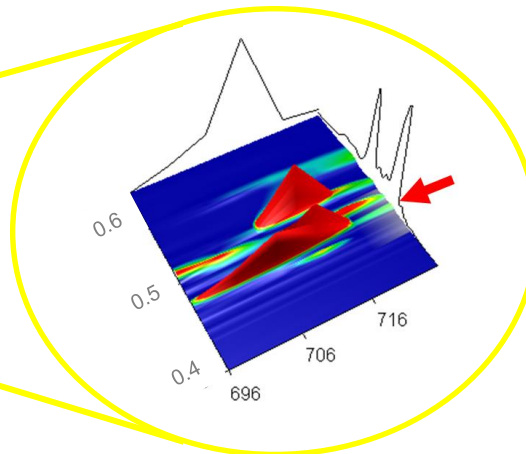
Unveiling *Sambucus nigra* L. chemical profile:

Elderberries' volatile terpenic and norisoprenoids profile

Mature elderberries



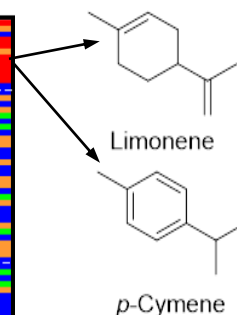
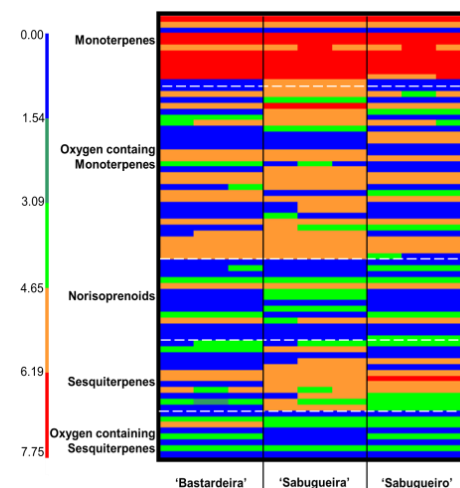
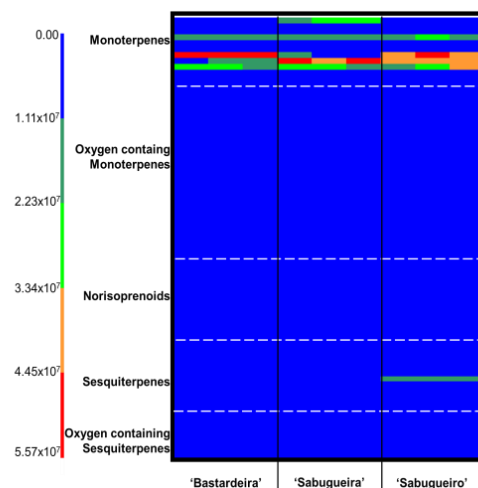
Structured chromatogram



- 42 monoterpene compounds
- 20 sesquiterpene compounds
- 14 norisoprenoids



48 compounds
reported for the **first time** on
elderberries



Unveiling *Sambucus nigra* L. chemical profile:

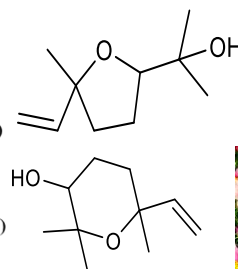
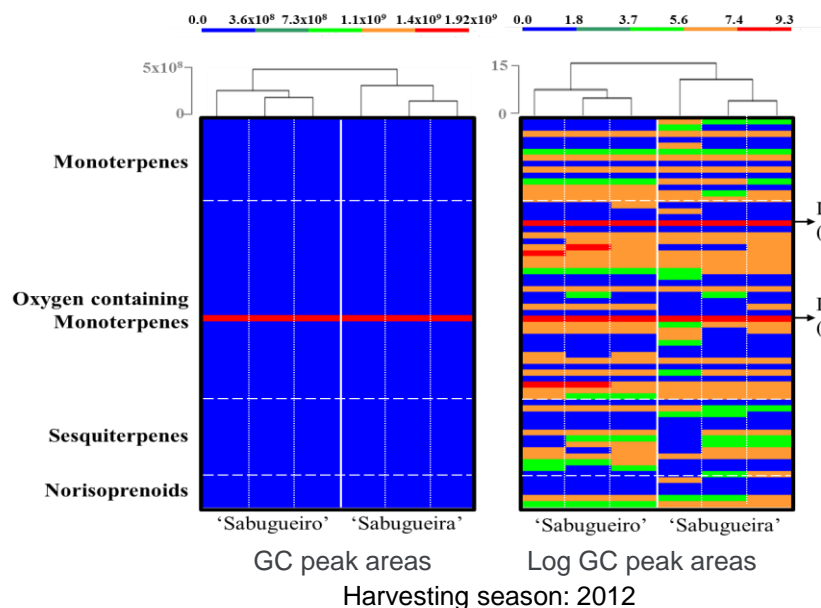
Elderflowers' volatile terpenic and norisoprenoids profile

Fresh elderflowers

- 47 monoterpene compounds
- 13 sesquiterpenes
- 5 norisoprenoids



38 compounds
reported for the **first time** on
elderflowers



'Sabugueiro'

Ocimene

2,6-Dimethyl-2,6-octadiene

Cosmene

Hotrienol

Linalool

'Sabugueira'

α -Pinene

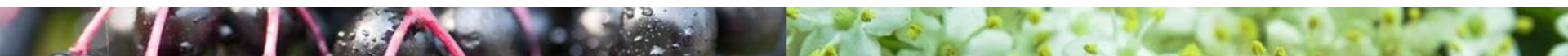
p-Cymenene

Citronellal

β -Bourbonene



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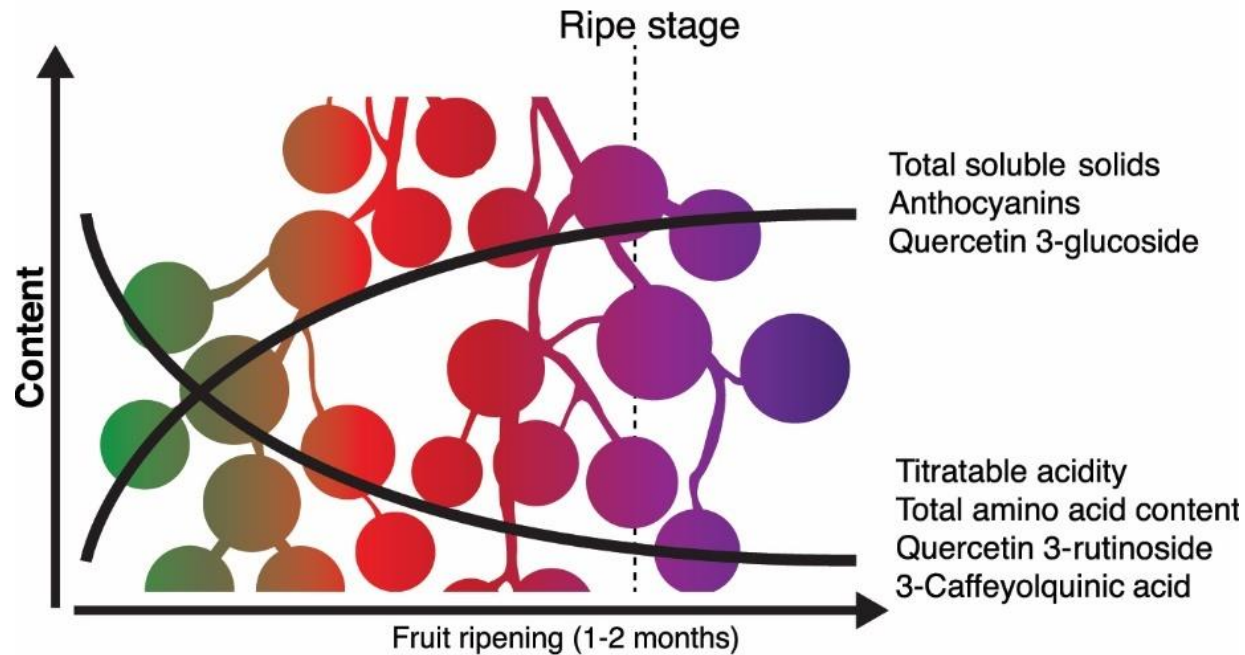
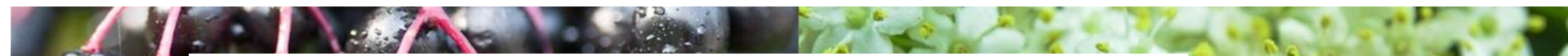
Elderberry dietary supplementation

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Unveiling *Sambucus nigra* L. chemical profile:

Preharvest effects



Elderberries

Understand the chemical variations under the preharvest factor of:

Ripening

As case study:

3 Cultivars

2 Harvesting seasons

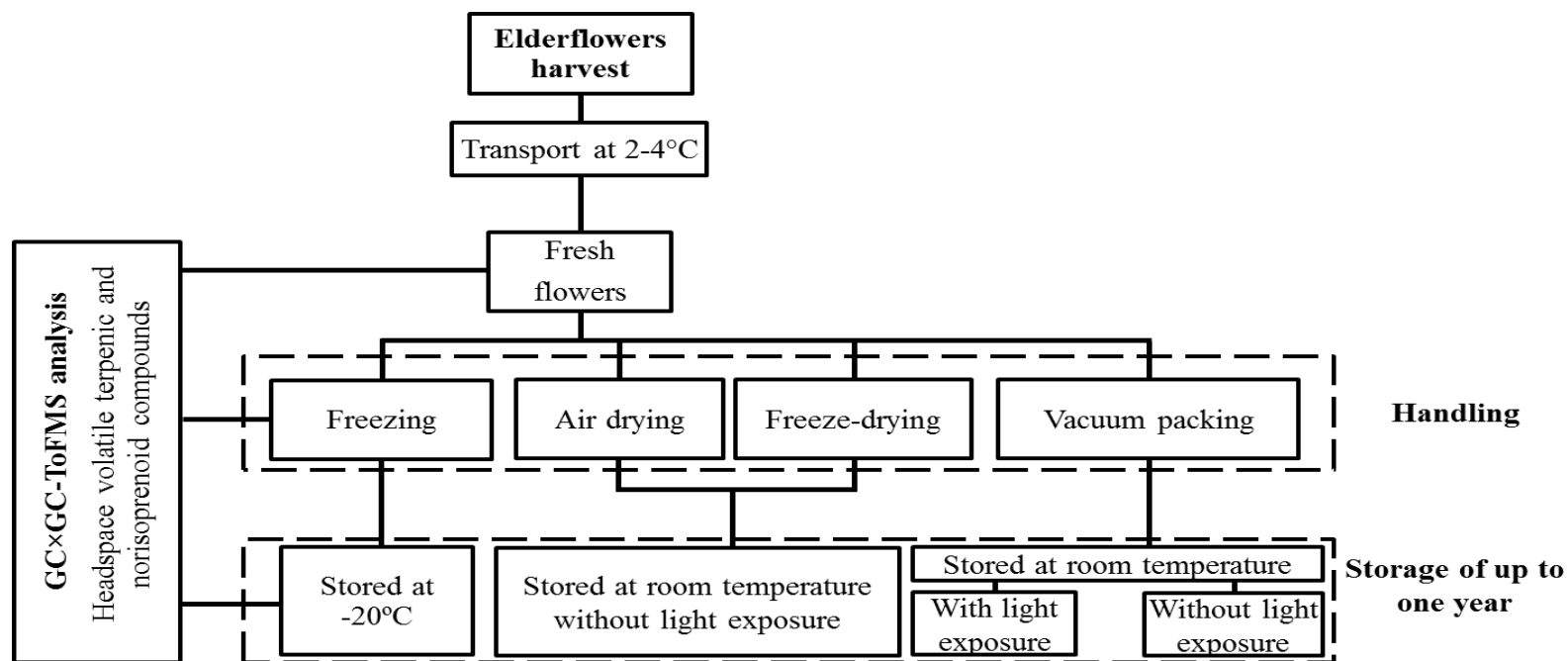
Unveiling *Sambucus nigra* L. chemical profile:

Postharvest effects

Elderflowers

Seasonal and perishable matrix

2 Cultivars were analyzed

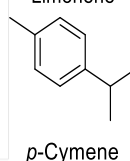
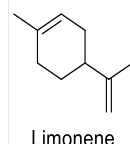
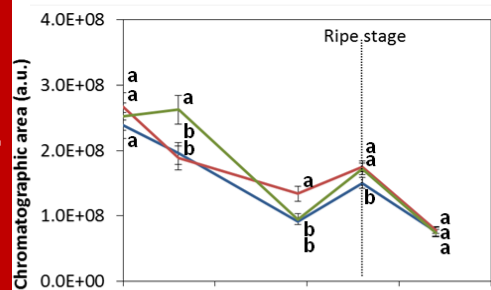


Unveiling *Sambucus nigra* L. chemical profile:

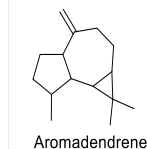
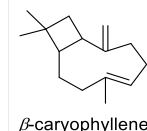
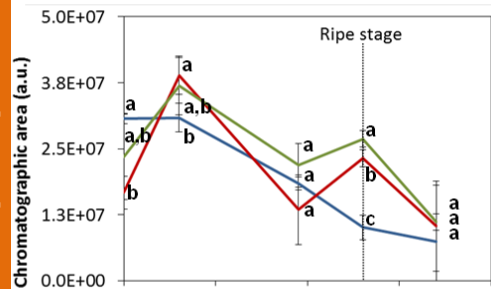
Preharvest effects

Elderberries

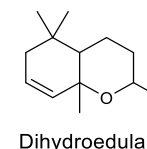
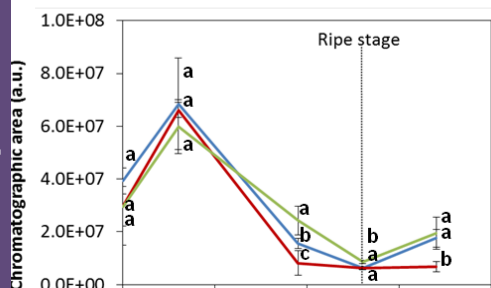
Monoterpenes



Sesquiterpenes

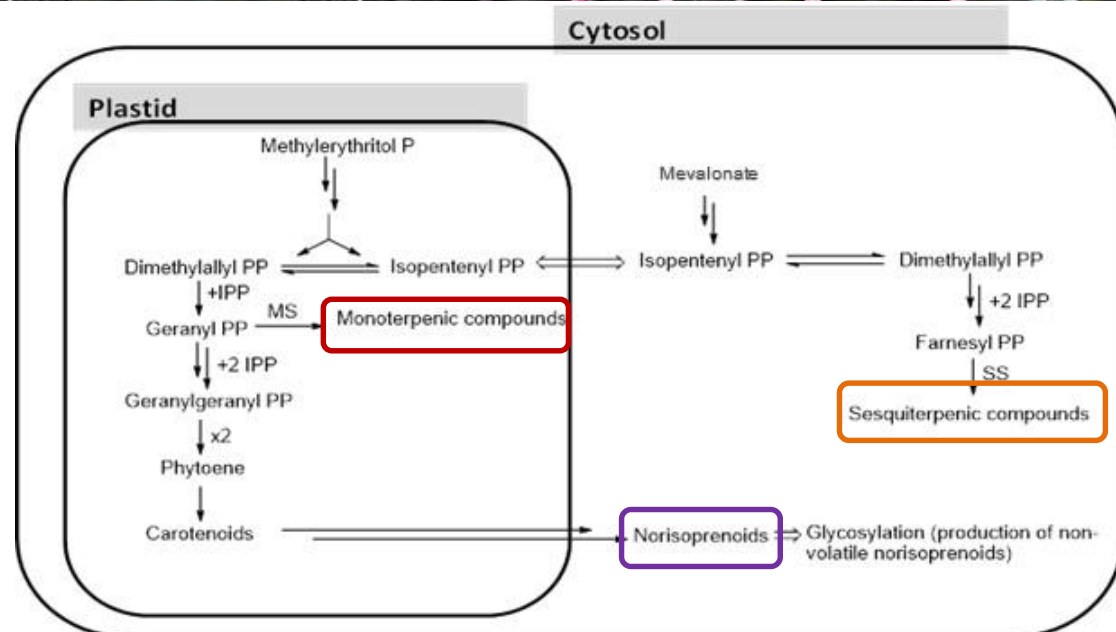


Norisoprenoids



— 'Bastardeira' — 'Sabugueira' — 'Sabugueiro'

Harvesting season: 2012



Pandit, S.S.; Kulkarni, R.S.; Giri, A.P.; Köllner, T.G.; Degenhardt, J.; Gershenzon, J.; Gupta, V.S. Expression profiling of various genes during the fruit development and ripening of mango. *Plant Physiol. Biochem.*, 2010, 48, 426–33.

Baumes, R.; Wirth, J.; Bureau, S.; Gunata, Y.; Razungles, A. Biogenesis of C_{13} -norisoprenoid compounds: Experiments supportive for an apo-carotenoid pathway in grapevines. *Anal. Chim. Acta*, 2002, 458, 3–14.

Analysis of Variance – Simultaneous Component Analysis

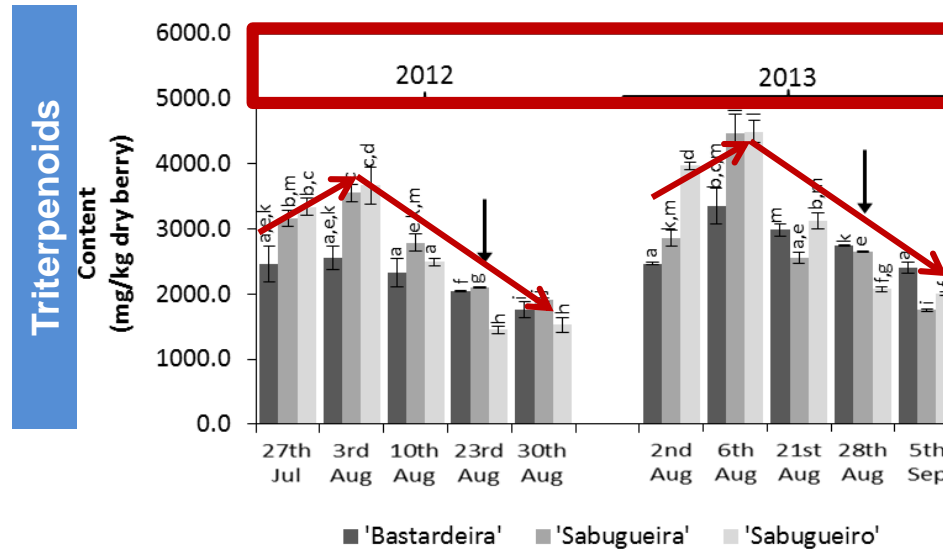
Ripening stage factor: 40.0%, $p < 0.0005$

Cultivar factor: 10.3%, $p < 0.001$

Unveiling *Sambucus nigra* L. chemical profile:

Preharvest effects

Elderberries



Higher lipophilic content for the **2013** compared to **2012**

During **ripening**: an **initial growth** of their content followed by a **systematic decrease** until **maturity**

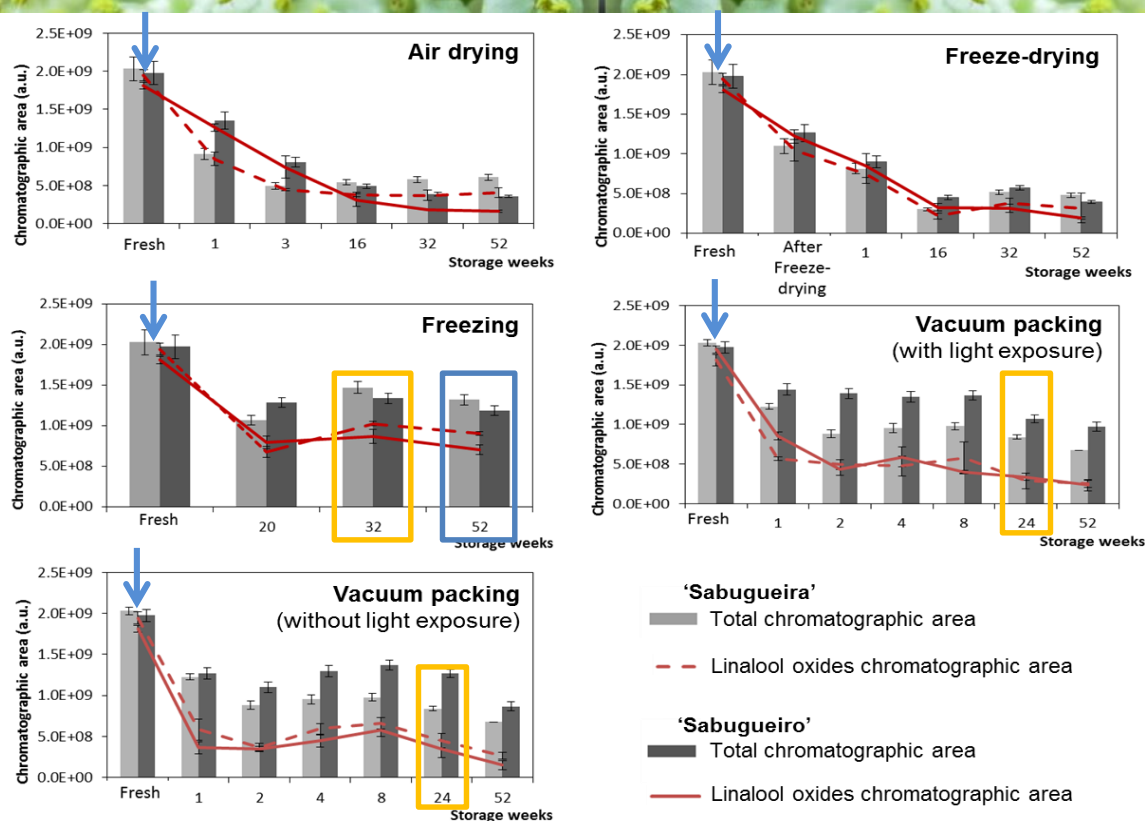
Mature elderberries:

'**Sabugueira**' and '**Bastardeira**' showed **higher contents** of **triterpenic acids**

Unveiling *Sambucus nigra* L. chemical profile:

Postharvest effects

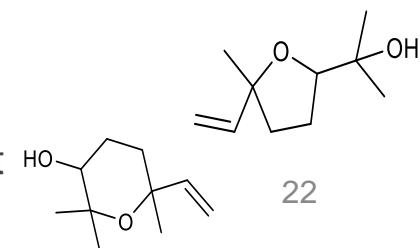
Elderflowers



Vacuum packing and **freezing** were the most suitable methods for intermediary storage times (24-32 weeks)

Freezing, for longer period (52 weeks)

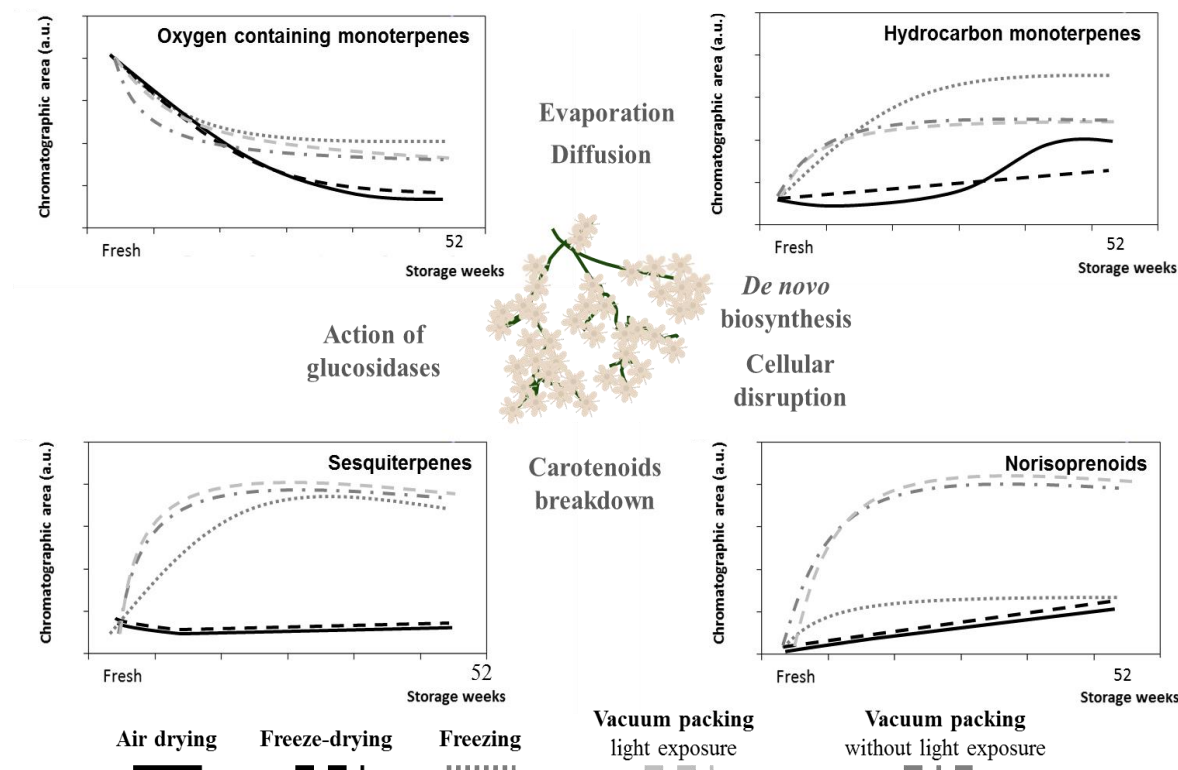
Linalool oxides are proposed as markers to assess the postharvest impact



Unveiling *Sambucus nigra* L. chemical profile:

Postharvest effects

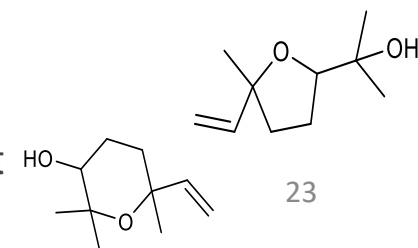
Elderflowers



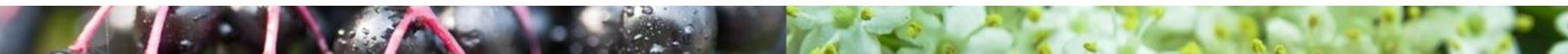
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Uniwersytet Przyrodniczy w Poznaniu



International Journal of
Molecular Sciences



Article

**Effect of Elderberry (*Sambucus nigra* L.) Extract
Supplementation in STZ-Induced Diabetic Rats Fed
with a High-Fat Diet**

Ângelo C. Salvador ^{1,2}, Ewelina Król ³, Virgínia C. Lemos ¹, Sónia A. O. Santos ²,
Fernanda P. M. S. Bento ^{1,4}, Carina P. Costa ⁴, Adelaide Almeida ⁵, Dawid Szczepankiewicz ⁶,
Bartosz Kulczyński ³, Zbigniew Krejpcio ³, Armando J. D. Silvestre ² and Sílvia M. Rocha ^{1,*}

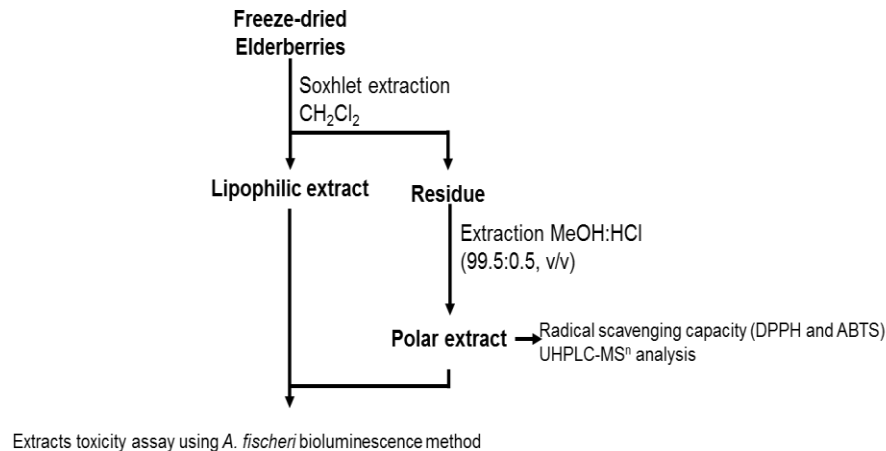
Elderberry dietary supplementation: Effects in a **diabetic** in vivo model

Extracts preparation and characterization

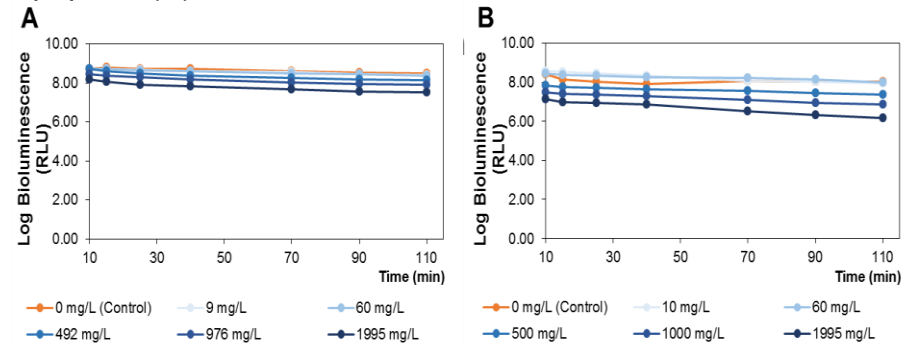
Extracts toxicity evaluation

Animals and Diets

Biofluid and tissue analysis



Aliivibrio fischeri bioluminescence of polar (A) and lipophilic (B) extracts



For both extracts:

Innocuous for 9–60 mg/L

Applying interspecies conversion equation:

$$\log_{\text{extract concentration } A. \text{ fischeri}} = 0.55 \log_{\text{extract dose mouse}} - 0.13$$

And mouse to rat oral administration dosage conversion



A dosage of up to ~5.2 g/kg of body weight.

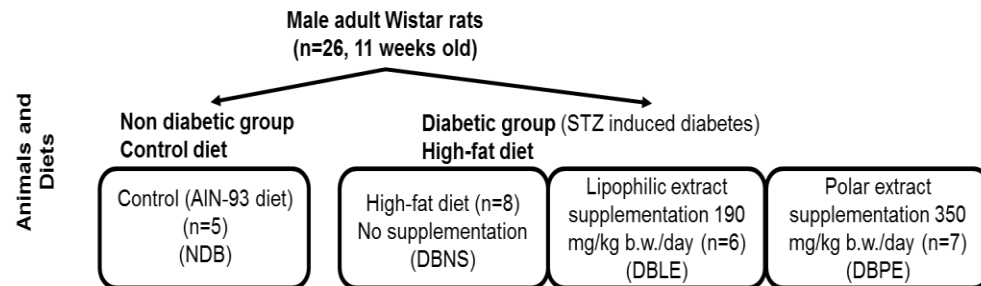
Diets were prepared ensuring that both extracts' doses did not exceed this value

In vivo blood sera toxicity markers:

ALT, AST, ALP enzymes were not altered after elderberries extracts dietary supplementation

Elderberry dietary supplementation:

Effects in a **diabetic** in vivo model

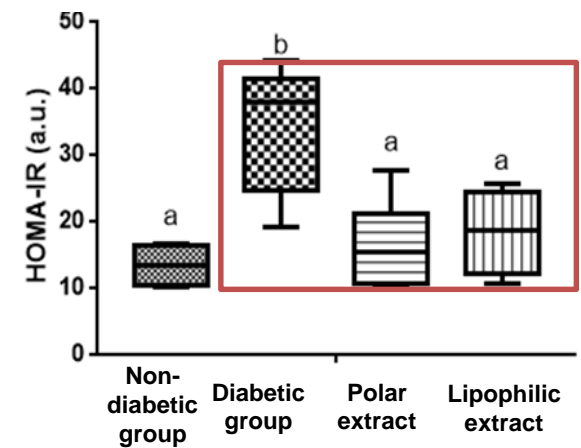
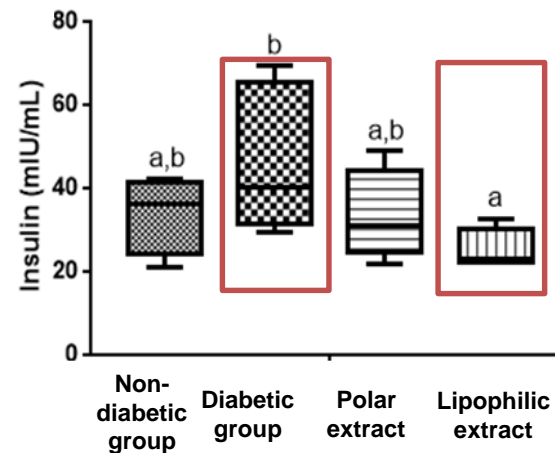
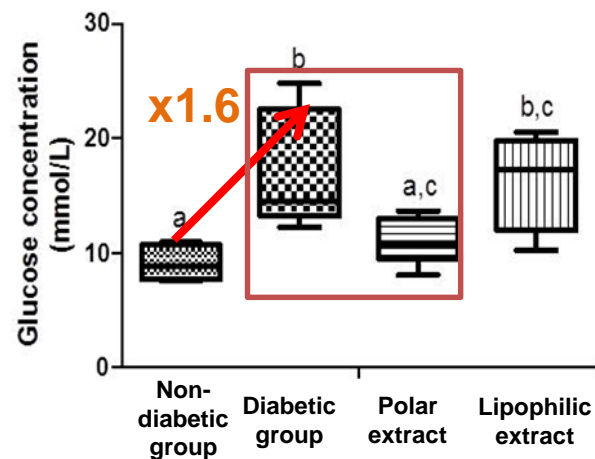


Supplementation length: 4 weeks

Biofluid and tissue analysis

- Rats' growth indices and blood morphological and hematological analysis
- Blood glucose and insulin analysis
- Lipids, inflammatory and toxicity markers analysis
- Microelement determinations

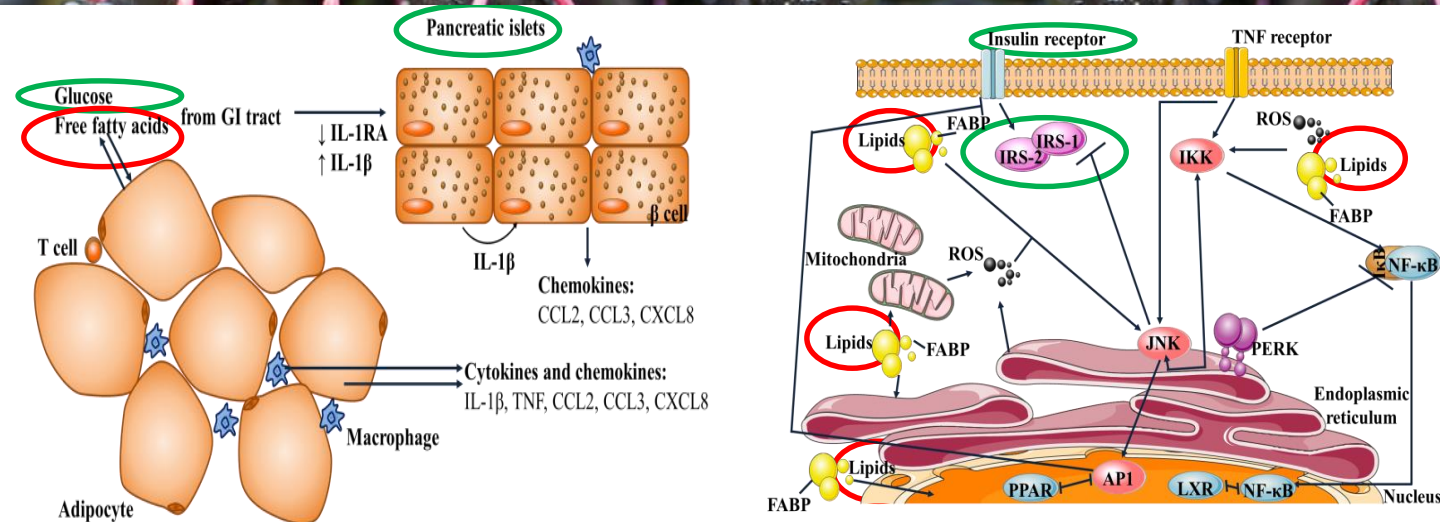
Donath, M.Y.; Shoelson, S.E. Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.*, 2011, 11, 98–107.
Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature*, 2006, 444, 860–867.



Both extracts lowered **insulin resistance**

Elderberry dietary supplementation:

Effects in a **diabetic** in vivo model



Modulation of diabetic status though:

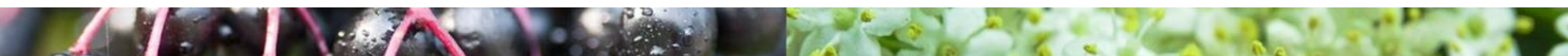
✓ **Blood glucose and insulin modulation**

✗ **Lipidic pattern**

Donath, M.Y.; Shoelson, S.E. Type 2 diabetes as an inflammatory disease. *Nat. Rev. Immunol.*, 2011, 11, 98–107.
Hotamisligil, G.S. Inflammation and metabolic disorders. *Nature*, 2006, 444, 860–867.

Index		Non-Diabetic rats (n=5)	Diabetic rats with high-fat diets		
			Not supplemented (n=8)	Polar extract (n=7)	Lipophilic extract (n=6)
Total	cholesterol concentration (mg/dL)	99.34 ± 15.01 ^a	83.34 ± 17.04 ^a	90.79 ± 11.16 ^a	90.92 ± 14.25 ^a
HDL	cholesterol concentration (mg/dL)	72.80 ± 3.19 ^a	64.69 ± 13.32 ^a	71.37 ± 10.09 ^a	71.28 ± 11.25 ^a
LDL	cholesterol concentration (mg/dL)	12.15 ± 8.86 ^a	4.36 ± 1.89 ^a	7.13 ± 5.03 ^a	6.90 ± 5.45 ^a
Triacylglycerol	(mg/dL)	88.44 ± 45.21 ^a	110.79 ± 77.90 ^a	65.52 ± 14.91 ^a	82.50 ± 28.87 ^a

Presentation Outline



Introduction

Context of the work

Aim of the work

Results

Unveiling *Sambucus nigra* L. chemical profile

Elderberries' phenolic and lipophilic profile

Elderberries and elderflowers' volatile terpenic and norisoprenoids profile

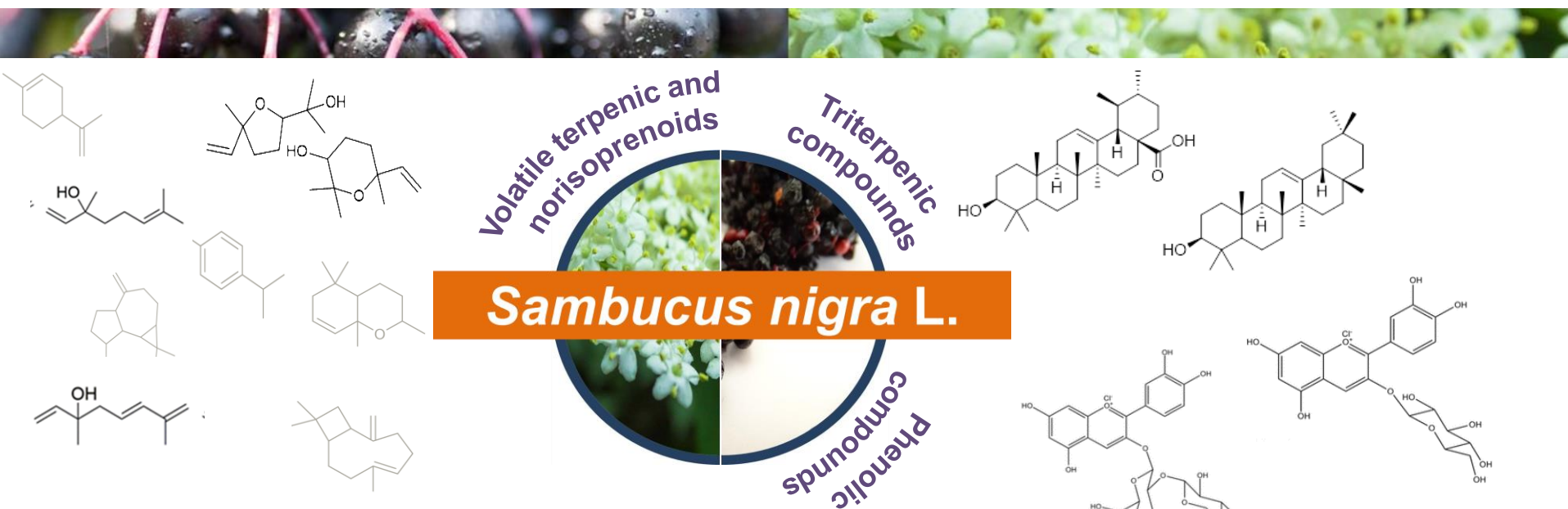
Pre- and postharvest effects

Elderberry dietary supplementation

Effects in a diabetic *in vivo* model

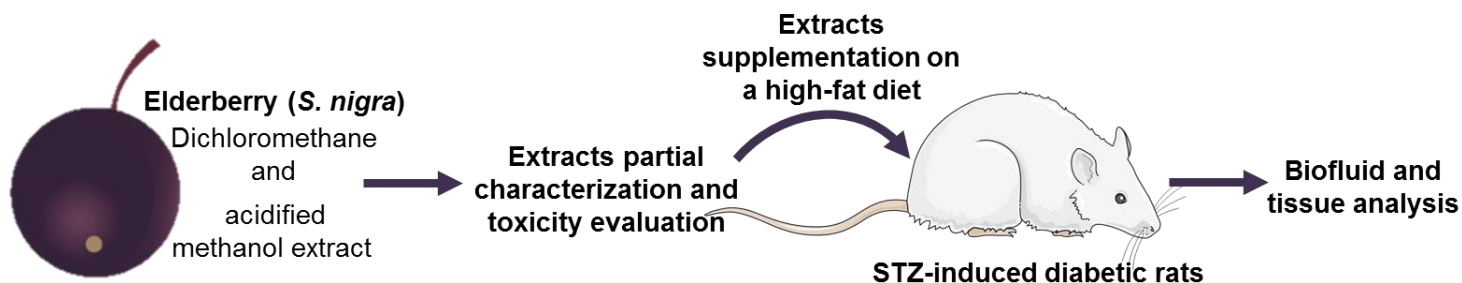
Conclusions and perspectives

Conclusions



Pre- and postharvest parameters modulated *S. nigra* L. chemical profile:

- Ripening
- Handling and Storage



- **Dietary adjuncts** could help to alleviate the metabolic disorder in **diabetes type 2**
- Diabetes management through **shorter administration periods** (4 weeks) and **higher doses** (5-fold) compared to literature
- **Complementary action** through modulation of **glucose** and **insulin** levels

Comprehensive Insight into the Elderflowers and Elderberries (*Sambucus nigra* L.) Mono and Sesquiterpenic Metabolites: Factors that Modulate Their Composition

Ângelo C. Salvador, Armando J. D. Silvestre and

Chapter 1

**ELDERBERRY JUICE COMPOSITION
AND HEALTH BENEFITS**

Robert E. Smith^{1,2,}, Kevin Tran², Kristy M. Richards², Sean Ryan²,
Rensheng Luo³, Ângelo C. Salvador⁴, Armando J. D. Silvestre⁵
and Silvia M. Rocha⁴*

Frontiers in Natural Product Chemistry, 2016, Vol. 2, 343-392

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CHAPTER 6

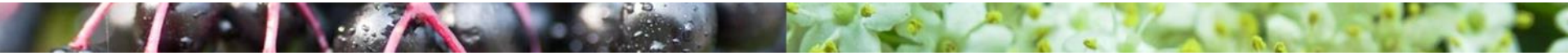
***Sambucus nigra* L.: A Potential Source of Health-promoting Components**

Ângelo C. Salvador^{a,b}, Armando J. D. Silvestre^b and Silvia M. Rocha^{a,*}

Department of Chemistry, ^aQOPNA and ^bCICECO, University of Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

***Sambucus nigra* Berries and Flowers Health Benefits: From Lab Testing to Human Consumption**

Ângelo C. Salvador, Ricardo J. R. Guilherme, Armando J. D. Silvestre, and Sílvia M. Rocha



Polish-Portugal Research Joint Project 2017-2018; Evaluation of plant resources as candidates for antidiabetic functional food prototypes

NEW

***Project Sambucus Valor** - Valorization of elderberry plant according to the patterns of healthy consumption: from the plant to the creation of new value-added food products*

2018-2021



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Ewelina Król

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Joint Project 2017-2018

Research units QOPNA and CICECO

Régiefrutas





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<http://xchromatographylab.x10.mx>